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Research Article

Physicochemical Properties of Amorphous Roxithromycin Prepared by Quench Cooling of the Melt or Desolvation of a Chloroform Solvate

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ABSTRACT. Roxithromycin is a poorly soluble antibacterial drug. The aim of this study was to prepare and characterize an amorphous form of roxithromycin. The amorphous form was prepared by desolvation of its chloroform solvate, and by quench cooling a melt of the crystalline monohydrated solid. The X-ray powder diffraction pattern of the desolvated chloroform solvate was indistinguishable from that of the glass prepared by melting, which indicated that it was amorphous. The roxithromycin glass was determined to be a fragile glass, but due to its high Kauzmann temperature (approximately 8°C), it should remain fairly stable upon refrigeration or even at room temperature. It was also determined that this glass remains stable in the presence of moisture with no indication of crystallization.

KEY WORDS: amorphous; desolvation; roxithromycin; solvate; stability.

INTRODUCTION

Roxithromycin is a semi-synthetic 14-membered-ring macrolide antibacterial drug, and it is clinically used for the treatment of respiratory infections caused by Gram-positive and Gram-negative cocci and bacilli, and a variety of other atypical microorganisms (1–3). The chemical structure of roxithromycin (Fig. 1) is modified from that of erythromycin, such that it has improved stability in acidic media and an improved pharmacokinetic profile (1). However, the macrolide group of drugs is poorly water soluble, with the solubility ranging from 2 mg mL^{-1} for erythromycin to 0.1 mg mL^{-1} for azithromycin (4). Roxithromycin (monohydrate) is poorly water soluble (it is even less soluble than azithromycin), and an improvement in its solubility could result in an improvement in its bioavailability (5).

It is known that amorphous solids have an increased free energy, which results in an enhanced solubility when compared with crystalline solids (6). 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 (7). This means that if an amorphous solid of roxithromycin can be prepared, it could result in an increase in the solubility and bioavailability of this drug (bearing in mind the effect on stability). Three methods have previously been used to prepare a more soluble solid of roxithromycin, including homogenization, freeze and spray drying (5). Two other methods that might also be used to prepare an amorphous form include supercooling of the melt and desolvation of a solvate (7,8). The melting and quenching method of preparing an amorphous solid is not always feasible since it exposes the solid to high temperatures which might adversely affect the stability due to possible degradation. However, during desolvation of a solvate, the loss of the solvent from the crystal structure occurs at a temperature lower than the melting point of the solid, and this results in the collapse of the crystal lattice with the formation of an amorphous solid (9).

Both acetonitrile and water (monohydrate) solvates of roxithromycin have been reported previously, however little is known about the solvated form obtained through crystallization with chloroform and the nature of the solid that forms after desolvation of this solvate (10). In this study, we report the preparation of a chloroform solvate of roxithromycin, and show that desolvation of this solvate results in the formation of a stable amorphous form of roxithromycin. The stability of this desolvated solid was also compared with the roxithromycin glass prepared by fusion and quenching.

MATERIALS AND METHODS

Materials

Crystalline roxithromycin (believed to be the monohydrate due to a moisture content of 2.06% according to its certificate of analysis (11); Fig. 1) was purchased from Alembic Ltd. (Vadodara, Gujarat, India), and chloroform was purchased from Fischer Scientific, Fair Lawn, NJ. Milli-Q water with a resistivity

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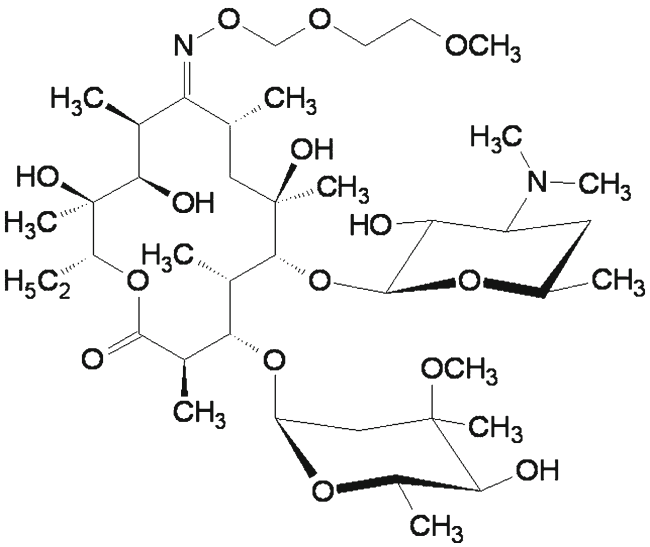

Fig. 1. The chemical structure of roxithromycin

of 18.2 MΩ cm was used throughout this study where mentioned, and all other reagents were analytical grade.

Preparation of Roxithromycin Solid Forms

The chloroform solvate of roxithromycin was prepared by recrystallization of the raw material from chloroform. Approximately 15 g of roxithromycin was added to 200 mL of chloroform while stirring continuously and heating the solution to approximately 60°C. The beaker containing the resulting solution was covered with Parafilm®. After slow evaporation of the chloroform, a dense mass was obtained. This mass was dry but tend to stick to the surfaces of containers. The desolvated solid was prepared by placing the chloroform solvate in a vacuum oven (VWR model 1410, West Chester PA) at room temperature for 24 h, with a vacuum of 1.3 kPa. The roxithromycin glass was prepared by melting the raw material on a glass microscope slide, with a hotplate at approximately 120°C, and subsequently quenching it to room temperature on an aluminum block.

HPLC Analysis of Roxithromycin Solid Forms

HPLC assays were performed on roxithromycin raw material, the chloroform solvated and desolvated form as well as the glass form. The mobile phase used consisted of 30 g/L ammonium dihydrogen phosphate buffer, pH adjusted to 5.3 with sodium hydroxide solution. The buffer phase was mixed with acetonitrile in the ratio 60:40. A Luna C18 150×4.6 mm column was used with a flow rate set to 1.0 mL/min and a wavelength of 205 nm. Validation of this method provided a linear regression (R^2) of 0.9998.

Characterization of Roxithromycin Solid Forms

Thermal techniques, including differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA), as well as X-ray powder diffraction (XRPD) were used to characterize the various solid states of roxithromycin. The chloroform solvate was blotted on a piece of filter paper to remove any chloroform that might be left on the surface, prior to analysis. In order to obtain the roxithromycin chloroform desolvated form, the chloroform solvated form was desolvated under vacuum prior to analysis. A TA Instruments DSC Q2000 (for DSC analyses) and an SDT Q600 (for TGA) with Universal TA Analysis 2000 software (New Castle, DE) were used for all the thermal analyses. Approximately 4–5 mg of each sample was placed in an aluminum pan and either hermetically sealed with an aluminum lid (DSC analyses) or left uncovered (TGA analyses). For the roxithromycin chloroform solvate, approximately 4–5 mg of sample was weighed into an aluminum pan and dried under vacuum of 1.3 kPa at ambient temperature for a period of 24 h. Samples were scanned at a heating rate of 10°C.min^{-1} (unless otherwise stated) from 25 to 140°C for both DSC and TGA analyses. The purge gas was nitrogen, and it had a flow rate of 50 ml min^{-1} for DSC analyses and 100 ml min^{-1} for TGA analyses.

XRPD patterns were recorded using a Bruker D8 Advance diffractometer (Bruker, Germany), fitted with a Linxeye™ one-dimensional detector, 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^{-1} with a step size of 0.02° 2θ and a step time of 3.0 s. Approximately 10–20 mg of the chloroform solvated form were weighed onto an aluminium sample holder and dried under vacuum of 1.3 kPa at ambient temperature for a period of 24 h, prior to XRPD analysis.

Solid-State Transformation During Desolvation

The stability of the roxithromycin-chloroform solvate was studied with increasing temperature, by placing approximately 50 mg of the solid in an aluminum sample holder, and keeping the sample at a constant temperature for 12 h (starting at 25°C) using a VWR oven (model 1410, West Chester PA). After the allotted time, the sample was subjected to XRPD analyses, after which the temperature was increased by 10°C and the sample aged at this new temperature, and again analyzed using XRPD. This process was repeated until the temperature reached 85–95°C, at which point the sample melted, to form the amorphous solid.

Effect of Moisture on Amorphous Roxithromycin

The effect of moisture on the amorphous form of roxithromycin was also studied. Roxithromycin glass samples containing 1–15% (*w*/*w*) water were prepared by weighing equal amounts of the roxithromycin glass followed by the addition of known quantities of water. The samples were then thoroughly mixed, for a period of 5 min, using a mortar and pestle followed by subsequent DSC analyses at a heating rate of 10°C min^{-1}.

Vapor sorption studies were also performed on the amorphous sample and the crystalline raw material, by placing approximately 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.

The effect of moisture and temperature on these samples was also visually studied by preparing two samples of the roxithromycin glass on a microscope cover glass. One sample was placed in a desiccator at 50°C containing $CaSO_4$ (0% RH), and the other in a desiccator at 50°C containing a saturated solution of NaCl (75% RH). Each sample was observed over 18 days for the presence of possible crystal growth using an optical polarizing microscope (Lomo, Northbrook IL) with an attached camera (Ken-A-Vision, Kansas City MO).

Scanning electron microscopy (SEM) images of the crystalline raw material, the chloroform solvate and the glass were also gathered in order to observe possible morphological differences between the samples. For SEM, 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 SEM (Quanta 200 ESEM, FEI Corporation, Hillsboro OR, USA).

RESULTS AND DISCUSSION

Characterization of the Roxithromycin-Chloroform Solvate

DSC analyses (Fig. 2) showed that the onset temperature of the crystalline raw material was 116.2°C, which suggested that it was the monohydrate, since its melting onset temperature is 114.5°C (10). In addition, the moisture content of the raw material was 2.06%, which agrees with the theoretical moisture content of 2.11% for the roxithromycin monohydrate. The X-ray pattern of the raw material also agreed with that reported for the monohydrate (10).

Amorphous solids tend to exhibit characteristics, i.e., improved solubility which could result in improved bioavailability in comparison with their crystalline counterparts (7), previously, various techniques have been used to prepare amorphous solids of roxithromycin, which include freeze and spray drying (5). Since it is also possible to prepare an amorphous phase by desolvation of a solvate (8,12), it was decided to prepare an amorphous form of roxithromycin using this technique, and compare this with the amorphous solid prepared by fusion and quenching. Roxithromycin is known to form solvates with acetonitrile and water (a monohydrate) (10). For this study, chloroform was chosen as a possible solvent for the preparation of the solvate since it has a low boiling point (60.5–61.5°C), which would enable desolvation to occur at low temperatures, avoiding unnecessary exposure of the solid to high temperatures. Thermal analyses confirmed that the solid that recrystallized from chloroform was indeed a solvate. TGA analysis showed a weight loss of 6.04% (Fig. 3), which agreed with the theoretical predicted weight loss of 6.66% for a roxithromycin-chloroform solvate with a stoichiometry of 2:1 (roxithromycin/chloroform). As seen in Fig. 2, only one endothermic peak is observed in the DSC thermogram of the chloroform solvate; however, this peak has two steps with two different onset temperatures. The first onset temperature is at 57.1°C and the second onset is at 67.1°C. This phenomenon (a broad endotherm with two onset temperatures) was also previously observed for the triamcinolone

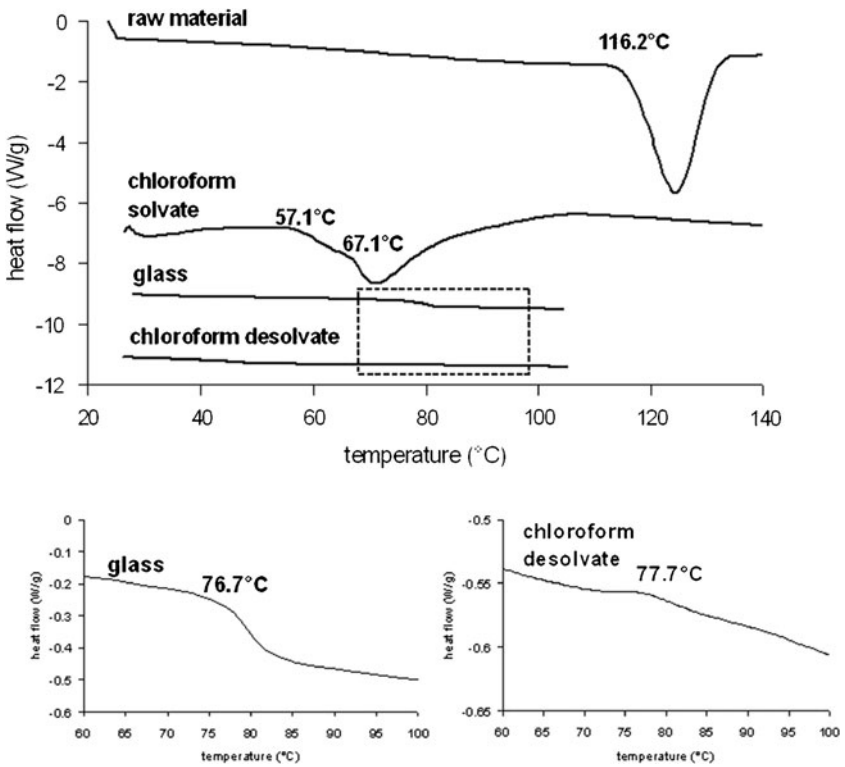

Fig. 2. The DSC thermograms of the roxithromycin raw material, chloroform solvate, glass, and desolvated solid, with the corresponding onset temperatures for the endothermic peaks of each thermogram. The DSC thermograms of the glass and the desolvated solid were increased in scale (the demarcated area) in order to clearly show the T_g

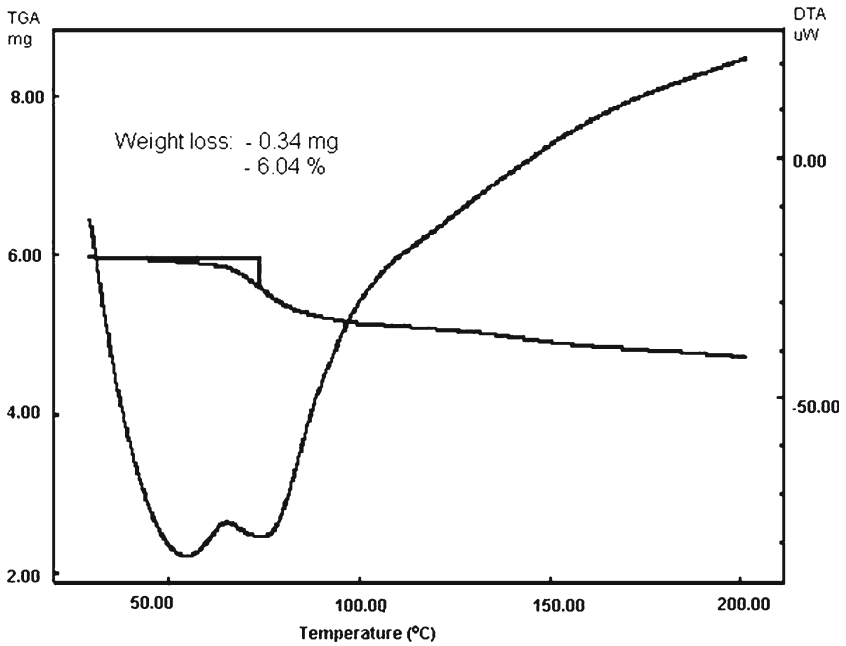

Fig. 3. The TG thermogram of roxithromycin chloroform solvate

acetate-methylene chloride solvate (13). It is assumed that the first onset temperature, which is closer to the boiling point of chloroform than the second onset, corresponds to the desolvation temperature of the solid, whereas the second onset temperature corresponds to the glass transition temperature (T_g) for the resultant amorphous solid that formed after desolvation. However, this T_g is lower than that of the amorphous solid prepared from melting, and it is suggested that the presence of chloroform in the solvate has a plasticizing effect, resulting in a lowering of the T_g. The X-ray powder diffractograms for the various roxithromycin solids studied in this investigation are shown in Fig. 4. It is noticed that the diffraction pattern of the roxithromycin raw material (monohydrate) and chloroform solvate are different, with the raw material being more crystalline in nature due to the higher number of high intensity diffraction peaks compared with the chloroform solvate. In addition, the raw material has unique diffraction peaks at 9.93°, 11.78°, and 14.63° 2θ, whereas the chloroform solvate has unique peaks at 6.59°, 7.58°, and 10.97° 2θ. It was shown that the crystal structure of the roxithromycin monohydrate consists of channels filled with the solvent, and various solvates of roxithromycin can be formed by solvent exchange with the solvent already in these channels (10). When this solvent exchange takes place to form a new solvate, it does not significantly alter the X-ray diffraction pattern of the newly formed solid (10). Complete desolvation (dehydration) of the monohydrate in a vacuum oven also did not change the X-ray diffraction pattern, indicating that the crystal structure remained intact when the solvent was removed from the channels. This is thus an example of the formation of an isomorphic desolvate (14). Since the X-ray patterns of the raw material (monohydrate) and chloroform solvate show no agreement, it is suggested that the chloroform solvate does not have this channel structure. In addition, on desolvation, the X-ray diffraction pattern of the chloroform solvate resembles that of the amorphous solid (Fig. 4). Thus, during desolvation, the crystal lattice of the chloroform solvate collapses to form an amorphous solid.

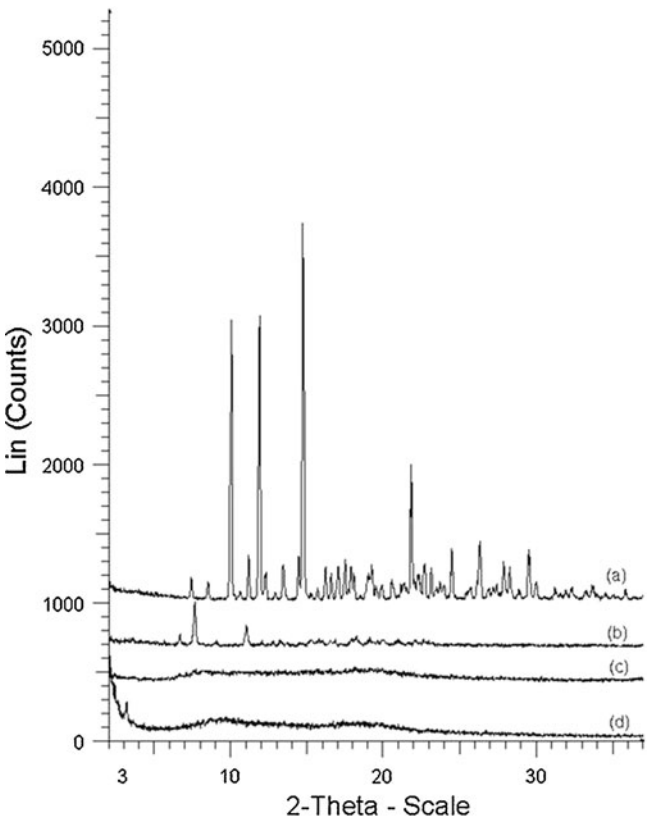

Fig. 4. The X-ray powder diffraction patterns of **a** the roxithromycin raw material (the monohydrate), **b** the chloroform solvate, **c** the roxithromycin glass, and **d** the desolvated solid

Roxithromycin-Chloroform Desolvation Kinetics

Since a change in crystallinity of the chloroform solvate was observed on desolvation, two methods based on DSC and TGA analyses were used for studying the kinetics of desolvation of the roxithromycin-chloroform solvate. Firstly, four samples of equal size were analyzed at four different heating rates (3, 5, 7, and 10°C min^{-1}) using DSC analyses. A Kissinger plot (Fig. 5a) was constructed using the first onset temperature observed with DSC analyses, as this was previously determined to be the temperature of desolvation. The activation energy (E_a) of desolvation was calculated from this linear (R^2=0.9963) plot, and was 181.6±7.8 kJ mol^{-1}, with the pre-exponential factor (*ln A*) equal to 66.8±12.8. It should be mentioned that the Kissinger method normally makes use of the peak maximum of the DSC endotherm for constructing of the plot (15). However, since the desolvation peak and transformation peak values are indistinguishable from the DSC thermograms of this solvate, the onset temperatures were used. Furthermore, the Kissinger method is a model-free method and does not require the assumption of a specific model for the calculation of E_a (16). The use of the Kissinger method enables the determination of activation energy (E_a), without taking into account the mechanism that result in the transformation. Since the Kissinger method assume constant activation energy (E_a) values and does not allow calculation of E_a at progressive α values, this method cannot detect complexities within the reaction being measured (16,17).

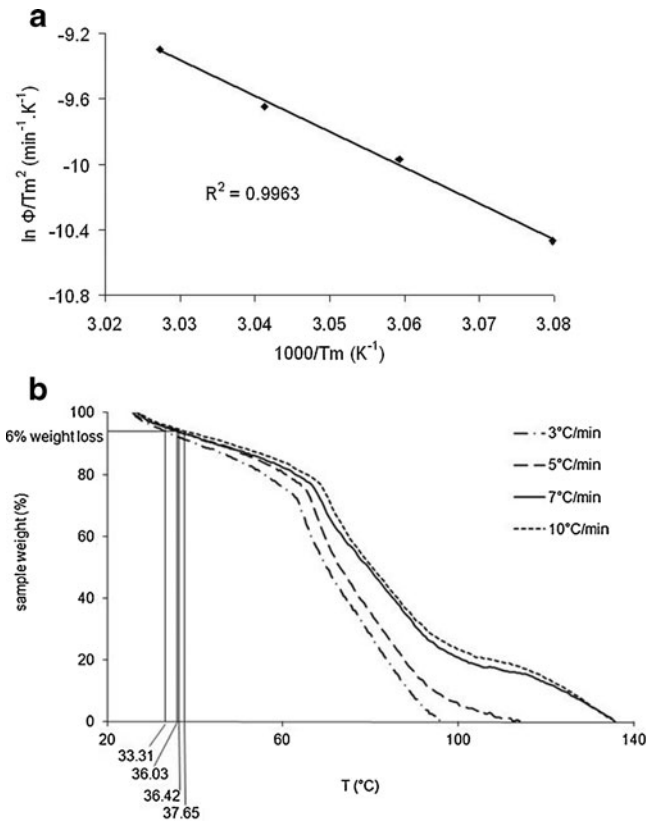

Fig. 5. **a** The Kissinger plot constructed using the onset temperatures of desolvation, obtained from DSC analyses. **b** The TGA weight loss curves and an example of the 6% weight loss data points used for constructing the log heating rate *vs.* 1/T plots for calculating E_a of desolvation

To validate this approach a second thermal analytical technique (18) was used to determine the activation energy, in order to compare the results obtained. Flynn and Wall (19) first proposed this method. Four samples of approximately equal weight were analyzed using TGA, at heating rates of 3, 5, 7, and 10°C min^{-1} respectively (Fig. 5b). The temperature, corresponding to a weight loss from 2 to 9%, was determined for each heating rate. Plots of the logarithm of the heating rate *vs.* the reciprocal of these temperatures yielded straight lines with a mean R^2 of 0.8960±0.0442. The linear lines drawn from the corresponding temperatures at weight losses of 4, 5, and 6% were the most parallel compared with the other lines. The activation energy of desolvation was thus calculated from the slopes of these three lines, and yielded an average value of 217.7± 27.5 kJ mol^{-1}, with the average *ln A* calculated to be 84.0±10.9. It is noticed that the E_a calculated using this method is greater compared with the value calculated from the Kissinger plot. A possible explanation for this could be the fact that the Kissinger plot was constructed using the onset temperatures of the DSC endothermic peaks instead of the peak values. However, statistically the E_a values and *ln A* values for these two methods were not significantly different (p>0.05).

Comparison of Desolvated and Amorphous Roxithromycin

In order to determine if the solid formed after desolvation of the roxithromycin-chloroform solvate was in fact amorphous, its XRPD pattern and DSC thermogram was compared with that of the glassy roxithromycin, prepared by fusion and quenching. SEM and microscopy images of the solids were also compared with that of the crystalline raw material (Fig. 6a–d). It is seen that the surface of the raw material has a striated appearance, which is assumed to be the solvent channels (Fig. 6a), whereas the chloroform solvate (Fig. 6b) has a smooth surface, indicating a lack of any channels (10). The amorphous solid (Fig. 6c) does not have a specific morphological shape, but it does show the appearance of air bubbles. The X-ray diffractogram of the desolvated solid and the glassy roxithromycin are shown in Fig. 4, and it can be seen that both patterns have the characteristic amorphous halo appearance with no high intensity peaks (Fig. 4). XRPD analyses of a chloroform solvate sample exposed to increasing temperatures indicated that the crystalline-to-amorphous transformation (because of desolvation) occurs between 55 and 65°C. This agrees with the temperatures of desolvation observed with DSC analysis (Fig. 2). This crystalline-to-amorphous transformation during desolvation occurs due to the collapse of the crystal structure, as was also observed for the solvates of erythromycin (another 14-membered macrolide antibiotic drug) formed from organic solvents (2).

To compare and characterize the two amorphous solids further, DSC analyses were performed on the desolvated and glassy solids (Fig. 2). A T_g at 76.7°C was observed for the amorphous solid prepared by melting and cooling. Since no other recrystallization or melting peaks are observed, the glass is thus considered to be a supercooled liquid above T_g and a glass below T_g, and this DSC thermogram is similar in appearance (except for the value of T_g) to that of amorphous erythromycin (20). The thermogram of the desolvated solid shows a glass transition with the onset temperature of the T_g at 77.7°C. Although the shape of the glass transition step differs from

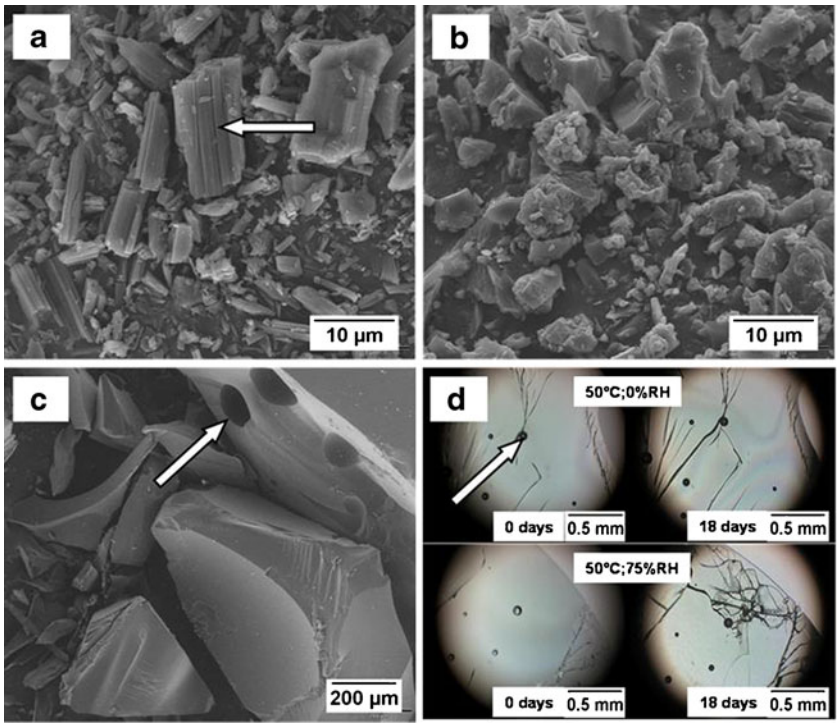

Fig. 6. SEM photos of **a** the roxithromycin raw material, **b** the roxithromycin-chloroform solvate, and **c** the amorphous solid. **d** Microscopy images of the glassy solid exposed to 50°C; 0% RH and 50°C; 75% RH, indicating the absence of crystallization after 18 days of exposure to these conditions. The *arrow* in (**a**) indicates the presence of the solvent channels in the raw material (monohydrate), and the *arrows* in (**c**) and (**d**) indicate the presence of air bubbles in the amorphous solid which could increase its porosity

that obtained with the glass prepared through fusion and quench cooling, the glass T_g of the quenched cooled glass and the desolvated solid still indicate similarity. It is worth noting that an amorphous form with different short-range order could be obtained through the desolvation of a solvate with comparison to an amorphous form obtained with quench cooling of the melt. The results obtained from the XRPD and DSC analyses, confirm that indeed an amorphous solid is formed after desolvation of the chloroform solvate. Furthermore, it should be noted that after cooling of the desolvated form and subsequent reheating, a similar step change with a similar T_g was obtained. The chemical stability of the amorphous desolvated form was confirmed through HPLC analysis. The analysis proved that roxithromycin remained stable after the desolvation process. Although no residual solvent analysis was performed in order to determine if any chloroform was still present after desolvation this should be considered and should be investigated further, especially in future studies that might involve product formulation.

Characterization of Amorphous Roxithromycin

The amorphous form of roxithromycin, prepared by melting and quench cooling, has not been described in the literature. In this section some properties of the glassy roxithromycin is described. These include T_g, the fragility parameter (m), the strength parameter (D) and the zero mobility temperature (T_0). The Kauzmann temperature (T_0) is often defined as the temperature of zero molecular mobility, and is the temperature where the glass is probably physically and chemically most stable (7). It should however be noted that the T_0 is actually the temperature of zero mobility of an ideal glass and in a strict sense this is not possible. However, the T_0 could still be considered as a guide to determine a temperature where the glass will indicate physico-chemical stability. Furthermore, it should be considered advantageous to conduct extensive studies in order to determine the accurate storage temperature of a glass. D is an indication of the fragility of the glass, where low D values (<10) are indicative of fragile glasses and glasses with D>100 are classified as strong glasses (7). When heated, strong glasses maintain their structure and have a gradual loss in rigidity at the T_g, whereas fragile glasses have a sudden structural change at T_g (12). This means that fragile glasses will have a large change in heat capacity (ΔCp) at T_g, whereas the ΔCp of strong glasses are often undetectable with standard calorimetric techniques (7). The relatively large ΔCp of 295.2 J K^{-1} mol^{-1} for amorphous roxithromycin, suggests that this is a fragile glass, however further analyses was done in order to confirm this.

The fragility can be determined by measuring the temperature dependence of viscosity above T_g and by applying the Vogel–Tammann–Fulcher equation (Eq. 1):

$$\eta = \eta_0 \exp\left[\frac{DT_0}{T - T_0}\right] \quad (1)$$

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 and 50°C

lower than the measured T_g (12,21,22). The fragility parameter (m) can be defined as:

$$m = \left[\frac{d \log \eta}{d(T_g/T)}\right]_{T=T_g} \quad (2)$$

and thus

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

where $\Delta H\eta*$ is the activation enthalpy for viscous flow and R is the gas constant (21,23,24). Using Eqs. 1–3, the following relationship between m and D can be set up:

$$m = \frac{D(T_g/T_0)}{(\ln 10)\,(T_g/T_0 - 1)^2} \quad (4)$$

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:

$$D = \frac{666}{m - 17} \quad (5)$$

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 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 ($\Delta H*$) (21,25). The T_g was determined for a sample of glassy roxithromycin using DSC analysis at heating rates of 2, 4, 6, 8, and 10°C min^{-1} (Fig. 7a). From this it was possible to calculate $\Delta H*$ from the slope of the plot of *ln* heating rate (ϕ) *vs.* $1/T_g$ (Fig. 7b; Eq. 6), which subsequently enabled the calculation of m and D using Eqs. 3 and 5, respectively (21).

$$\frac{d \ln \phi}{d\,(1/T_g)} = \frac{-\Delta H*}{R} \quad (6)$$

The $\Delta H*$ of the glassy roxithromycin was calculated to be 575±27 kJ mol^{-1}. Table I provides a summary of the different parameters that describes its fragility. The fragility (m=85.5) and strength parameters (D=9.7) indicate that this is a fragile glass former. These parameters were compared with that of other organic glasses, and it was noticed that the fragility parameters of sucrose has the closest agreement with that of roxithromycin (Table I). The reason might be inherent in the number of monosaccharides in each of these molecules since sucrose is a disaccharide and roxithromycin also contains two sugar molecules (D-desosamine and L-cladinose) in its structure (Fig. 1) (27). Also, the fragility of amorphous roxithromycin predicted by m and D, agrees with the fragility prediction made on the change in ΔCp at T_g. It should be noticed that, even though it is a fragile glass, amorphous roxithromycin has a T_0 of 8.5°C and would thus remain fairly stable at room temperature or when stored in a refrigerator.

The Effect of Moisture on Amorphous Roxithromycin

The T_g of a solid determines its physical-chemical and viscoelastic properties, and any change in the T_g will thus also influence the stability of the solid (29). An additive can either lower or raise the T_g of a substance, and this is referred to as plasticization or antiplasticization respectively (29). Water (with a T_g of −138°C) has a plasticizing effect when it is added to amorphous solids, which increases the possibility of amorphous-crystal transformations (29,30). When an amorphous to crystalline transformation occurs in the presence of moisture, the water sorption capacity decreases or slows down (31). Vapor sorption and calorimetric studies were subsequently performed on the glassy solid of roxithromycin in order to determine the effect of moisture on its stability and how it affects the T_g of this solid.

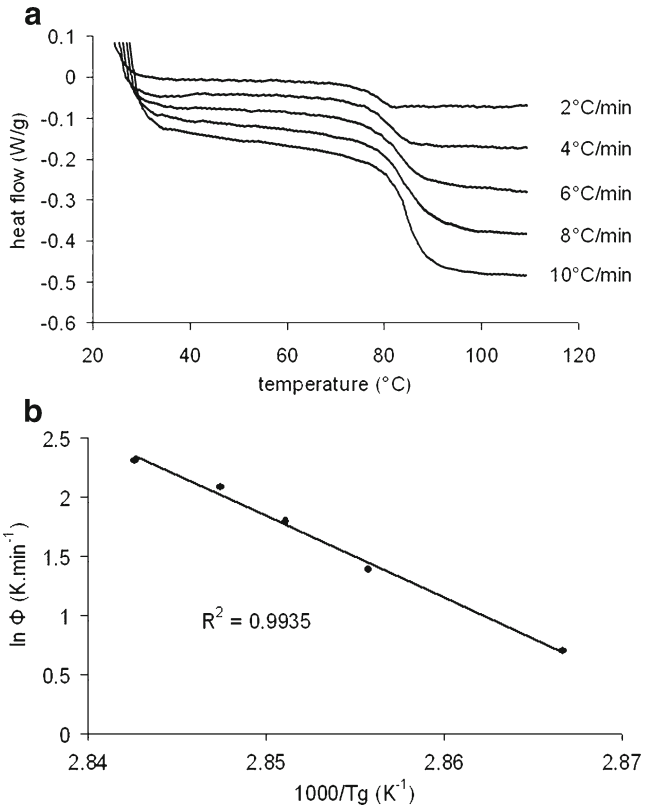

Fig. 7. **a** The glass transition of amorphous roxithromycin obtained from DSC analyses, at the indicated heating rates. **b** The plot of ln heating rate *vs.* $1/T_g$, of which the slope was used to calculate the activation enthalpy for relaxation ($\Delta H*$)

The results of the vapor sorption experiments of the glassy solid and the crystalline raw material are shown in Fig. 8a, b, respectively. According to the moisture isotherm, the amorphous solid showed an insignificant increase in weight (approximately 1.5%) up to 60% RH. The sharp increase in weight up to 4% from 70 to 90% RH is attributed to condensation of water on the sample holder. When the humidity was decreased from 90 to 0% RH, the sample showed a weight loss of 4% and returned to its starting weight. Thus, an increased humidity did not change the solid-state properties of the amorphous form since there is no change in the sorption profile, *i.e.* it did not transform into a crystalline solid but remained amorphous, and high levels of moisture did not induce crystallization. X-ray diffraction analysis of the sample recovered after vapor sorption confirmed this, since its diffraction pattern was super-imposable on that of roxithromycin glass stored in a desiccator. However, the moisture sorption isotherm forms a hysteresis since

Table I. Properties of the Amorphous Solid of Roxithromycin (ROX) and Sucrose (24–26)

Drug	T_g (K)	m (fragility parameter)	D (strength parameter)	T_0 (K)	ΔCp (T_g) (J K^{-1} mol^{-1})
ROX	351.4	85.5	9.7	281.5	295.2
Sucrose	350	93.3	8.6	276.5	215

adsorption and desorption do not follow the same pattern. The most common cause of hysteresis is the condensation of water in capillaries in porous solids during adsorption, resulting in a slower decline in sample moisture during desorption (32). Since a glass is not expected to be porous, this explanation would not be valid for explaining the hysteresis seen for amorphous roxithromycin. However, it is noticed that amorphous roxithromycin forms bubbles during preparation, and cracks on its surface at room temperature (Fig. 6c, d). It is thus possible that during adsorption, water penetrates and condenses in these cavities, resulting in the rate of desorption being different from that of adsorption. The moisture isotherm of the crystalline raw material (monohydrate) shows a sharp increase in weight of approximately 0.8% when the humidity was increased from 0 to 10% RH. Further increasing the humidity up to 90% RH resulted in the weight of the sample increasing by an additional 1.2% up to 2%. Since the adsorption isotherm starts at 0% RH, it is possible that at this low humidity most of the water in the channels in the crystal lattice is removed. As the humidity is increased up to 90% RH, the channels are filled with water again, which would explain the 2% increase in weight (the theoretical moisture content of the monohydrate is 2.11%). When the humidity was decreased to 0% RH again, the sample lost all of the moisture that it took up, indicating that water is not tightly bound in the channels. In addition, since no change in the crystal structure was observed it confirmed the formation of an isomorphic desolvate (10). This means the crystal structure of the crystalline raw material (monohydrate) shows no transformation with increasing or decreasing moisture.

The effect of water on the amorphous solid was also visually studied (Fig. 6d). No surface or bulk crystallization was observed under polarized light when amorphous samples were kept at either 50°C; 0% RH or 50°C; 75% RH for 18 days, and the samples thus remained stable. A similar observation was made for amorphous sucrose stored at 25°C; 23% RH for 30 days (33). This again shows the similarity in properties between amorphous roxithromycin and sucrose.

The interaction between an amorphous solid and water can also be determined calorimetrically. Various relationships between T_g and moisture content are described, which were originally described for polymer mixtures (34,35). The three relationships that are often used to describe the relationship between T_g and moisture are the linear (Eq. 7), Gordon–Taylor (Eq. 9), and Fox (Eq. 11) equations (29). The linear equation is written as:

$$T_{g_{mix}} = w_1 T_{g1} + w_2 T_{g2} \tag{7}$$

where w is the weight fraction for each component (35). It should be noticed that the linear equation implies that there is

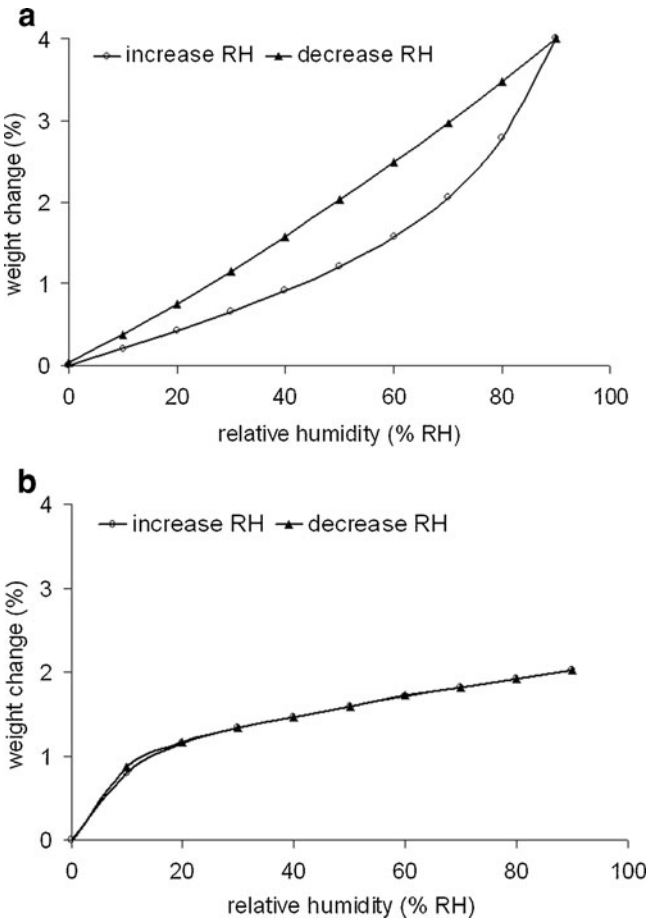

Fig. 8. The results of the vapor sorption experiments for **a** the amorphous solid and **b** the crystalline raw material of roxithromycin

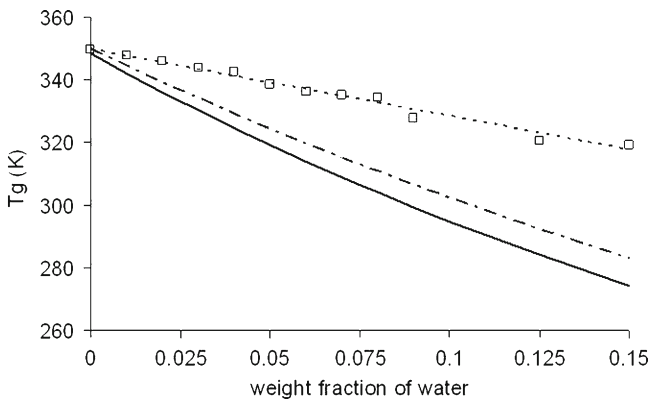

Fig. 9. Plots showing the change in the T_g of amorphous roxithromycin with increasing amounts of water. The *solid line* is the Gordon–Taylor plot, the *solid/dashed line* is the Fox plot, and the *dashed line* is a linear plot

no interaction between the two mixed substances (28). If the volume fraction (ϕ) is used, instead of the weight fraction, the Eq. 7 becomes:

$$T_{g_{mix}} = \phi_1 T_{g_1} + \phi_2 T_{g_2} \tag{8}$$

and since $\phi=[(\Delta\alpha \cdot w)/\rho]$, with $\Delta\alpha$ being the thermal expansivity of T_g, w the weight fraction, and ρ the true density of the material, it is possible to write Eq. 9, also known as the Gordon–Taylor equation (29,36):

$$T_{g_{mix}} = \frac{(w_1 T_{g_1}) + (K \cdot w_2 T_{g_2})}{w_1 + (K \cdot w_2)} \tag{9}$$

where

$$K = \frac{(\rho_1 T_{g_1})}{(\rho_2 T_{g_2})} \tag{10}$$

If the densities of the two substances are equal, Eq. (9) simplifies to:

$$\frac{1}{T_{g_{mix}}} = \frac{w_1}{T_{g_1}} + \frac{w_2}{T_{g_2}} \tag{11}$$

which is also known as the Fox equation (29,37). However, this relation is more applicable to polymeric systems because of their similar densities compared with the significant differences in densities between low molecular weight organic glasses (28).

Figure 9 shows the change in T_g of the roxithromycin amorphous solid with increased water content, predicted using Eqs. 7, 9 (assuming a roxithromycin density of 1.184 g cm^{-3}, (11)), and 11. It is noticed that the experimental data (open squares in Fig. 9) are well distributed around the linear plot (dashed line), compared with the Gordon–Taylor (solid) and Fox (solid/dashed) plots. A linear plot (with r^2=0.9824) was fitted through these experimental data points, and it was super-imposable on the theoretical linear plot. This suggests that the interaction between amorphous roxithromycin and water, or lack thereof, is best described by the linear equation (Eq. 7), i.e., there was little interaction between these two substances when mixed.

CONCLUSIONS

During this study, an amorphous form of roxithromycin was successfully prepared, both by desolvation of the roxithromycin-chloroform solvate and by fusion and quenching of the crystalline solid. The thermal and X-ray diffraction patterns of these two amorphous solids agreed with each other, indicating that both techniques results in the formation of an amorphous solid with similar properties. It was also shown that the roxithromycin glass can be classified as a fragile glass. The favorable thermal stability at pharmaceutically acceptable storage conditions, chemical stability at ambient temperatures, coupled with the negligible effect that moisture has on the stability of this glass (i.e., it does not induce crystallization even at high humidity), suggests that this amorphous form might be an alternative solid to use in commercially available products containing roxithromycin.

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REFERENCES

1. Bryskier A. Roxithromycin: review of its antimicrobial activity. J Antimicrob Chemother. 1998;41(Suppl B):1–21.
2. Miroshnyk I, Mirza S, Zorky PM, Heinämäki J, Yli-Kauhaluoma J, Yliruusi J. A new insight into solid-state conformation of macrolide antibiotics. Bioorgan Med Chem. 2008;16:232–9.
3. Qi M, Wang P, Cong R, Yang J. Simultaneous determination of roxithromycin and ambroxol hydrochloride in a new tablet formulation by liquid chromatography. J Pharm Biomed Anal. 2004;35:1287–91.
4. Nakajima Y. Mode of action and resistance mechanisms of antimicrobial macrolides. In: Omura S, editor. Macrolide antibiotics, second edition: chemistry, biology, and practice. San Diego: Academic Press; 2002. p. 453–500.
5. Biradar SV, Patil AR, Sudarsan GV, Pokharkar VB. A comparative study of approaches used to improve solubility of roxithromycin. Powder Technol. 2006;169:22–32.
6. Hancock BC, Parks M. What is the true solubility advantage for amorphous pharmaceuticals? Pharm Res. 2000;17:397–404.
7. Hancock BC, Zografi G. Characteristics and significance of the amorphous state in pharmaceutical systems. J Pharm Sci. 1997;86(1):1–12.
8. Henwood SQ, Liebenberg W, Tiedt LR, Lötter AP, de Villiers MM. Characterization of the solubility and dissolution properties of several new rifampicin polymorphs, solvates, and hydrates. Drug Dev Ind Pharm. 2001;27(10):1017–30.
9. Byrn SR, Pfeiffer RR, Stowel JG. Solid-state chemistry of drugs. 2nd ed. West Lafayette: SSCI Inc.; 1999.
10. Mallet F, Petit S, Lafont S, Billot P, Lemarchand D, Coquerel G. Solvent exchanges among molecular compounds. J Therm Anal Calorim. 2003;73:459–71.
11. Bachet PB, Brassy C, Mornon JP. [*O*-(dioxa-2,5 hexyl) oxime]-9 de l'Erythromycine A Hydratée. Acta Cryst. 1988;C44:112–6.
12. 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.
13. Suitchmezian V, Jeß I, Näther C. Crystal structures and properties of two new pseudopolymorphic modifications of the glucocorticoide triamcinolone diacetate. Solid State Sci. 2006;8:1373–9.
14. Byrn SR, Lin CT, Perrier P, Clay GG, Sutton PA. Solid state photooxidation of 21-cortisol tert-butylacetate to 21-cortisone. J Org Chem. 1982;47:2978–81.
15. Ford JL, Timmins P. Pharmaceutical thermal analysis. Chichester: Ellis Horwood Ltd.; 1989. p. 85–107.
16. Khawam A, Flanagan DR. Basics and applications of solid-state kinetics: a pharmaceutical perspective. J Pharm Sci. 2006;95(3):472–98.
17. Farjas J, Butchosa N, Roura P. A simple kinetic method for the determination of the reaction model from non-isothermal experiments. J Therm Anal Calorim. 2010;102:615–25.
18. ASTM International, Standard test method for decomposition kinetics by thermogravimetry. E 1641–04 (2004). Available from www.astm.org
19. Flynn JH, Wall LA. A quick, direct method for the determination of activation energy from thermogravimetric data. Polym Lett. 1966;4:323–8.
20. Fukumori Y, Fukuda T, Yamamoto Y, Shigitani Y, Hanyu Y, Takeuchi Y, *et al.* Physical characterization of erythromycin dehydrate, anhydrate and amorphous solid and their dissolution properties. Chem Pharm Bull. 1983;31(11):4029–39.
21. Lu Q, Zografi G. Properties of citric acid at the glass transition. J Pharm Sci. 1997;86(12):1374–8.

22. Angell CA. Why C1=16–17 in the WLF equation is physical—and the fragility of polymers. Polymer. 1997;38(26):6261–6.
23. Bohmer R, Angell CA. Correlations of the nonexponentiality and state dependence of mechanical relaxations with bond connectivity in germanium-arsenic-selenium supercooled liquids. Phys Rev B. 1992;45(17):10091–4.
24. Angell CA, Alba C, Arzmanoglou A, Bohmer R, Fan J, Lu Q, *et al*. 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. 1992;256:3–19.
25. Moynihan CT, Easteal AJ, Wilder J, Tucker J. Dependence of the glass transition temperature on heating and cooling rate. J Phys Chem. 1974;78(26):2673–7.
26. Hatley RSM. Glass fragility and the stability of pharmaceutical preparations-excipient selection. Pharm Dev Tech. 1997;2 (3):257–64.
27. Wang LM, Angell CA, Richert R. Fragility and thermodynamics in nonpolymeric glass-forming liquids. J Chem Phys. 2006;125 (7):074505/1–8.
28. Bertho G, Ladam P, Gharbi-Benarous J, Delaforge M, Girault JP. Solution conformation of methylated macrolide antibiotics roxithromycin and erythromycin using NMR and molecular modeling. Ribosome-bound conformation determined by TRNOE and formation of cytochrome P450-metabolite complex. Int J Biol Macromol. 1998;22:103–27.
29. Hancock BC, Zografi G. The relationship between the glass transition temperature and the water content of amorphous pharmaceuticals. Pharm Res. 1994;11(4):471–7.
30. Strydom SJ, Liebenberg W, Yu L, de Villiers MM. The effect of temperature and moisture on the amorphous-to-crystalline transformation of stavudine. Int J Pharm. 2009;379:72–81.
31. Burnett DJ, Thielmann F, Booth J. Determining the critical relative humidity for moisture-induced phase transitions. Int J Pharm. 2004;287:123–33.
32. Wolf M, Walker JE, Kapsalis JG. Water vapor sorption hysteresis in dehydrated food. J Agr Food Chem. 1972;20(5):1073–7.
33. Naini V, Byron PR, Phillips EM. Physicochemical stability of crystalline sugars and their spray-dried forms: dependence upon relative humidity and suitability for use in powder inhalers. Drug Dev Ind Pharm. 1998;24(10):895–909.
34. Pochan JM, Beatty CL, Pochan DF. Different approach for the correlation of the T_g of mixed amorphous systems. Polymer. 1979;20:879–86.
35. Schneider HA. Glass transition behaviour of compatible polymer blends. Polymer. 1989;30:2143.
36. Gordon M, Taylor JS. Ideal copolymers and the second-order transitions of synthetic rubbers. I. Noncrystalline copolymers. J App Chem. 1952;2:493–500.
37. Fox TG, Flory PJ. Second-order transition temperatures and related properties of polystyrene. I. Influence of molecular weight. J App Phys. 1950;21:581–91.