The physico-chemical properties and recrystallisation kinetics of selected amorphous active ingredients

Marnus Milne

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Promoter: Dr. M.E. Aucamp

Co-promoter: Prof. W. Liebenberg

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Abstract

During the last two to three decades the application of amorphous solid-state forms within the pharmaceutical industry gained much interest. The rationale for this heightened interest is the increased aqueous solubility, increased dissolution rate and subsequent possible improved bioavailability offered by the amorphous form of a drug. Although amorphous solid-state forms of drugs are not considered something new within the pharmaceutical industry, thorough reviewing of current and older literature on this topic, shows that much is still to be discovered, learned and understood about this very interesting field within the solid-state chemistry of drugs. In order to gain a better understanding of amorphous solid-state behaviour, three structurally unrelated active pharmaceutical ingredients, namely zopiclone, sulfadoxine and roxithromycin have been selected for this study.

In the first study, zopiclone was investigated for glass-forming ability. The well-known method of quench cooling of the melt proved to be somewhat of a challenge for the preparation of amorphous zopiclone. The purchased crystalline anhydrate form of zopiclone melted at ≅ 177°C, followed by the rapid degradation of the drug. Therefore, an additional step was added to the preparation method of amorphous zopiclone. A dihydrated form (Form B, already described in literature) was recrystallised from toluene. This form was subsequently dehydrated to form an anhydrous form with a lower melting point (± 150°C). Therefore, a lower melting temperature was used to obtain molten zopiclone. Although amorphous zopiclone was successfully prepared through a quench cooling of the melt method, it was determined that this method produced a fragile amorphous form with poor physical stability. The stability of this amorphous form was influenced by temperature, moisture as well as physical agitation. Further studies focused on successfully stabilising the amorphous form of zopiclone, this lead to the formulation of an amorphous solid dispersion. The advantage of the amorphous solid dispersion lies within the improved dissolution rate, as well as the inhibition of the recrystallisation of the amorphous zopiclone.

In the second study an amorphous form of sulfadoxine was successfully prepared through quench cooling of the melt. The study illustrated that in contrast to literatures' dictation that good glass-forming ability and strong strength parameters infer good stability, is not always the case. The recrystallisation behaviour was studied isothermally by applying the Johnson-Mehl-Avrami model and non-isothermally by applying the Kissinger model. The study illustrated that the nucleation and crystal growth rate is temperature dependent and that the presence of crystal seeds significantly decreases the amount of activation energy that is necessary for the recrystallisation process to start. The physical stabilisation of the

amorphous form was investigated using physical mixtures of amorphous sulfadoxine with polyvinylpyrrolidone-25 (PVP-25). This proved that a drug: polymer physical mixture of 1 : 4 inhibited the thermally induced recrystallisation of amorphous sulfadoxine completely. From this it was deduced that in some instances the preparation of amorphous solid dispersions to stabilise the metastable amorphous form of a drug is not always necessary. A mere physical mixture of the amorphous drug with a stabilising agent could suffice.

In the third study, the complex and highly controversial concept of the existence of "polyamorphism" within pharmaceutical compounds have been addressed. The influence of different preparative techniques on the thermodynamic and morphological properties of amorphous roxithromycin was investigated. The outcome of this study showed that the preparation route has a pronounced effect on the thermodynamic and morphological properties of the resulting amorphous solid-state forms. Subsequently, such differences have a mentionable effect on the drug performance, either during pharmaceutical processes or after patient administration. Findings and results of other studies on the topic of "polyamorphism" were correlated with this study and it was concluded that different amorphous forms of the same drug do indeed exist. However, it was evident from other literature reviews and original research that a well-defined definition for this phenomenon is still being evasive. Through a combination of this study on different amorphous forms of roxithromycin as well as other studies on an array of other drugs, the proposed terms of pseudo-polyamorphism or atypical polyamorphism were explored.

Forthcoming from the first study on amorphous zopiclone, the fourth study developed. During the initial investigation of zopiclone dihydrate, recrystallised from toluene, it became apparent that the dihydrate easily dehydrates to form an anhydrous form with a lower melting point as that of a commercially available anhydrate form of zopiclone. This prompted the investigation of the dehydration kinetics of zopiclone dihydrate (Form B). The dehydration kinetics was investigated by applying two model-free methods, namely the Kissinger and the Ozawa-Flynn-Wall methods. The application of both methods correlated very well with one another. It was deduced that zopiclone Form B dehydrates relatively easy and that disruption of the crystal structure is not necessary for the dehydration process to complete.

Keywords: amorphous; recrystallisation; stability; zopiclone; sulfadoxine; roxithromycin.

Uittreksel

Gedurende die laaste twee tot drie dekades het navorsing en die gebruik van amorfe vastestofvorme baie aandag in die farmaseutiese bedryf geniet. Die amorfe vastestofvorm van 'n geneesmiddel het meestal verbeterde wateroplosbaarheid, verhoogde dissolusietempo en moontlike verbeterde biobeskikbaarheid tot gevolg. Gesien in die lig hiervan is dit te verstane dat dit die dryfveer is vir die toename in die belangstelling in amorfe vorme van geneesmiddels. Alhoewel amorfe vastestofvorme nie gesien kan word as 'n nuwe verskynsel in die farmaseutiese bedryf nie, het deeglike studies rakende huidige en ouer literatuur aangaande hierdie onderwerp daarop gewys dat daar nog baie navorsing gedoen moet word om meer te verstaan rakende hierdie baie interessante veld. Tydens hierdie studie is drie nie-verwante geneesmiddels gekies om te bestudeer in terme van amorfisiteit. Hierdie geneesmiddels sluit in: sopikloon, sulfadoksien en roksitromisien.

Tydens die eerste studie is sopikloon ondersoek vir sy glasvormende vermoë. Die bekende metode van vinnige afkoeling na smelting was 'n uitdaging vir die voorbereiding van amorfe sopikloon. Die kommersiële, anhidriese vorm van sopikloon smelt by ≅ 177°C gevolg deur vinnige termiese afbraak van die geneesmiddel. Gevolglik is 'n addisionele voorbereiding stap by die vervaardigingsmetode van amorfe sopikloon gevoeg. Hierdie stap sluit in dat 'n dihidraat (soos reeds beskryf in literatuur) gekristalliseer is vanuit tolueen. Hierdie vastestof vorm is gevolglik gedehidreer sodat 'n anhidriese vorm met 'n laer smeltpunt verkry (omtrent 150°C) is.

Gevolglik is 'n laer temperatuur gebruik om gesmelte sopikloon te verkry. Alhoewel amorfe sopikloon suksesvol voorberei is deur die vinnige afkoeling na smelting tegniek, is daar vasgestel dat hierdie metode 'n relatief onstabiele amorf tot gevolg het. Die stabiliteit van hierdie amorfe vorm is deur temperatuur, vog, sowel as fisiese steuring beïnvloed. Verdere studies het daarop gefokus om hierdie amorfe vorm as 'n stabiele vastestofvorm te berei. Dit het gelei tot die formulering van 'n soliede amorfe dispersie. Die gevolglike soliede amorfe dispersie het die voordeel van 'n verbeterde dissolusietempo, sowel as die onderdrukking van die rekristallisasie van amorfe sopikloon getoon.

In die tweede studie is 'n amorfe vorm van sulfadoksien deur die metode van vinnige afkoeling na smelting, suksesvol voorberei. Die studie het geïllustreer dat in teenstelling met die literatuur se voorskrifte, dat indien 'n geneesmiddel oor 'n goeie glasvormende vermoë beskik en indien dit 'n sterk amorf vorm dit 'n stabiele amorfe vorm tot gevolg sal hê, is nie noodwendig altyd die geval nie. Die rekristallisasie gedrag van amorfe sulfadoksien is isotermies bestudeer deur die Johnson-Mehl-Avrami model toe te pas en nie-isotermies deur

die Kissinger model toe te pas. Die studie het getoon dat die kernvorming en kristal groeitempo van amorfe sulfadoksien, temperatuur afhanklik is en dat die teenwoordigheid van kristallyne kerne die hoeveelheid aktiveringsenergie wat nodig is vir die rekristallisasie proses aansienlik verminder, in vergelyking met die amorfe vorm sonder kerne teenwoordig.

Die onstabiliteit van hierdie amorfe vorm is ondersoek deur gebruik te maak van fisiese mengsels van amorfe sulfadoksien en polivinielpirrolidoon-25 (PVP-25). Dit het bewys dat 'n fisiese mengsel van die amorfe geneesmiddel en 'n polimeer in 'n verhouding van 1 : 4 die termies geïnduseerde rekristallisasie van amorfe sulfadoksien kan inhibeer. Hiervolgens is daar tot die gevolgtrekking gekom dat die voorbereiding van soliede amorfe dispersies om die metastabiele vorm van 'n geneesmiddel stabiel te laat, nie altyd noodsaaklik is nie. Hieruit blyk dit dat, in sommige gevalle, 'n fisiese mengsel van die amorfe geneesmiddel met 'n polimeer voldoende kan wees.

Tydens die derde studie is die komplekse en hoogs kontroversiële onderwerp oor die bestaan van poli-amorfisme in geneesmiddels ondersoek. Die invloed van verskillende voorbereidingsmetodes op die termodinamiese en morfologiese eienskappe van amorfe roksitromisien is ondersoek. Die resultate van hierdie studie het aangedui dat die voorbereidingsroete 'n beduidende invloed op die termodinamiese en morfologiese eienskappe van die gevolglike amorfe vastestofvorme het. Gevolglik het sulke verskille 'n noemenswaardige effek op die geneesmiddel se werking, hetsy tydens farmaseutiese vervaardigingsprosesse, of na pasiënt toediening.

Bevindinge en resultate van ander studies aangaande die onderwerp van poli-amorfisme is vergelyk met hierdie studie en daar is tot die gevolgtrekking gekom dat verkillende amorfe vorme van dieselfde geneesmiddel 'n algemene verskynsel is. Dit is egter duidelik vanuit ander literatuuroorsigte en oorspronklike navorsing dat 'n goed uiteengesette definisie van hierdie verskynsel steeds ontbrekend is. Deur middel van 'n studie aangaande die verskillende amorfe vorme van roksitromisien, sowel as ander studies wat 'n verskeidenheid van amorfe geneesmiddels insluit, is die voorgestelde terme van pseudo-poli-amorfisme of atipiese poli-amorfisme ondersoek.

Voortvloeiend uit die eerste studie op amorfe sopikloon het die vierde studie ontwikkel. Gedurende die aanvanklike ondersoek op sopikloon dihidraat, wat uit tolueen gerekristaliseer is, het dit duidelik geword dat hierdie dihidraat maklik dehidreer. Die gevolglike anhidriese vastestofvorm toon 'n laer smeltpunt in vergelyking met die van kommersieel beskikbare sopikloon. Hierdie verskynsel het daartoe gelei dat die dehidrasie kinetika van sopikloon dihidraat ondersoek is. Die dehidrasie kinetika is ondersoek deur twee modelvrye metodes toe te pas. Hierdie metodes het ingesluit die Kissinger metode

sowel as die Ozawa-Flynn-Wall metode. Die resultate verkry met beide metodes het goed met mekaar gekorreleer. Hieruit is ook afgelei dat sopikloon vorm B redelik maklik dehidreer sonder dat dit noodwendig nodig is dat die kristalstruktuur verlore gaan.

Sleutelwoorde: amorfe; rekristallisasie; stabiliteit; sopikloon; sulfadoksien; roksitromisien.

Preface

The article format has been chosen for this PhD study. Chapter 1 presents a literature study on solid-state properties of pharmaceuticals, including the amorphous state. Chapter 2 of the thesis reports on amorphous zopiclone and the stabilisation thereof through preparation of an amorphous solid dispersion. Chapter 3 describes the physical stability and crystallisation kinetics of amorphous sulfadoxine. In Chapter 4 the physico-chemical properties, focussing especially on thermodynamic and morphological properties of different amorphous forms of the same drug, namely roxithromycin has been investigated. In Chapter 5 the dehydration kinetics of zopiclone dihydrate (Form B) was investigated. The first two chapters have already been accepted in the respective leading pharmaceutical journals. The last two chapters have been submitted to the respective pharmaceutical journals and are currently in the peer-reviewing process. The composition of the thesis is as follows:

- Chapter 1 – Overview- Solid-state properties
- Chapter 2 – The stabilization of amorphous zopiclone in an amorphous solid dispersion. *AAPS PharmSciTech, accepted for publication, 2015.*
- Chapter 3 - Amorphous sulfadoxine: a physical stability and crystallization kinetics study. *AAPS PharmSciTech.*
- Chapter 4 – Different amorphous solid-state forms of roxithromycin: a thermodynamic and morphological study. *International Journal of pharmaceutics.*
- Chapter 5 – A non-isothermal dehydration study of zopiclone dihydrate. *Die Pharmazie.*
- Chapter 6 - Concluding remarks.

CHAPTER 1

Overview: Solid state properties

1.1 Introduction

The study of the Solid state properties of an active pharmaceutical ingredient (API) or excipient is of great pharmaceutical importance, as the majority of drugs and excipients exist as solids (Aulton and Taylor, 2013). The physical and chemical properties of solid pharmaceuticals are significantly influenced by differences in the molecular arrangement of a pharmaceutical solid (Datta and Grant, 2004). The *in vivo* performance of a dosage form can be influenced by the solid-state properties of pharmaceutical actives (Han and Suryanarayanan, 1999). Other important factors to reckon with is the decrease in the number of new drug molecules that reaches the market, posing a challenge to the pharmaceutical research and development environment, but also the new molecules that tend to be less soluble (Almog, 2005; Subramaniam, 2003). This decrease of new drug molecules on the market could lead to new investigations into "old" drugs which could reinvent itself, especially those with poor physico-chemical properties such as poor water solubility. To improve the physico-chemical properties of such drugs is considered more cost-effective than the development of new drugs. To minimise drug degradation and loss, to prevent harmful side effects and to increase drug solubility, bioavailability and the fraction of the drug accumulated in the required region remains the top priority of the pharmaceutical scientist. Various drug delivery and drug targeting systems are currently under investigation. Among the drug carriers are soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes and micelles (Kaparissides *et al*., 2006).

1.2 Solid-state pharmaceutical chemistry

Matter consists of three states, namely: solid, liquid and gas (or vapour). Solids will retain their original shape unless a compressive force is applied to them. Solids are for this reason unique, because their physical form (the packing of the molecules and the size and shape of the particles) can influence the behaviour of the material. Materials in the solid-state can be crystalline, amorphous or a combination of both. Figure 1.1 represents a classification of the solid-state properties of pharmaceutical solids (Datta and Grant, 2004; Vippagunta *et al*., 2001).

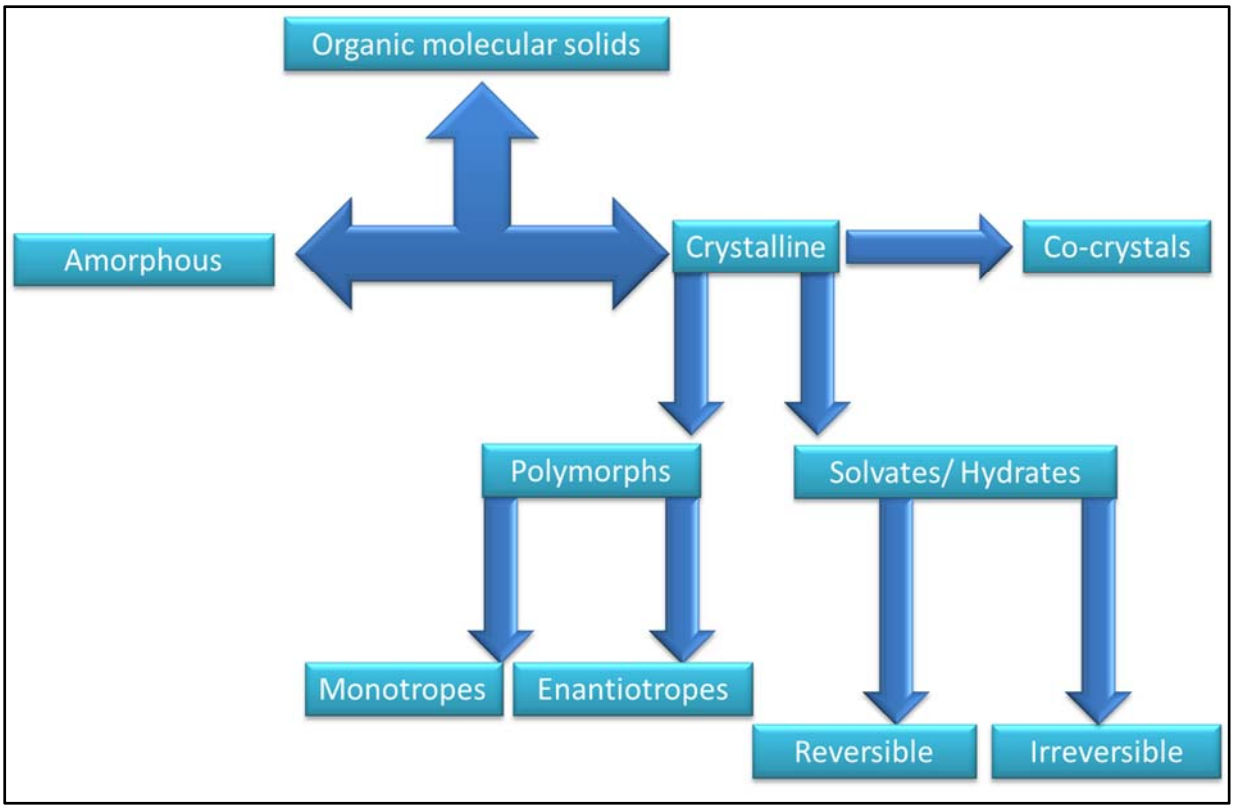

Figure 1.1: Classification of the solid-state properties of pharmaceutical solids (As adapted from Datta and Grant, 2004; Vippagunta *et al*., 2001).

1.3 Principles of the crystalline state

Crystalline materials are characterised as those in which the molecules are packed in a defined order (structural unit), and the same order (structural unit) repeats over and over again throughout the material. A unit cell is the simplest repeating unit in a crystal (Figure 1.2). Each unit cell is defined in terms of lattice points. Lattice points are the points in space about which the particles are free to vibrate in a crystal (Datta and Grant, 2004; Vippagunta *et al*., 2001).

The unit cell dimensions are characterised by six quantities namely: the three axial lengths a, b, c and the three interaxial angles α, β, γ. Each unit cell contains at least one molecule and classification can occur through one of the seven three dimensional coordinate systems that exist. Each of these crystal systems has one or more symmetry elements that enables the description of the internal symmetry of the unit cell (Datta and Grant, 2004; Vippagunta *et al*., 2001).

The symmetry elements consist of:

1. **Rotation axis** - the crystal contains a *n*- fold rotation axis if a rotation of 360°/ *n* gives the same structure. For crystals *n* is restricted to 1, 2, 3, 4 and 6;
2. **Mirror plane** - if a deflection through a given plane results in the same structure;
3. **Screw axis** when a 360°/ *n* followed by a translation parallel to the axis of rotation brings the structure into coincidence;

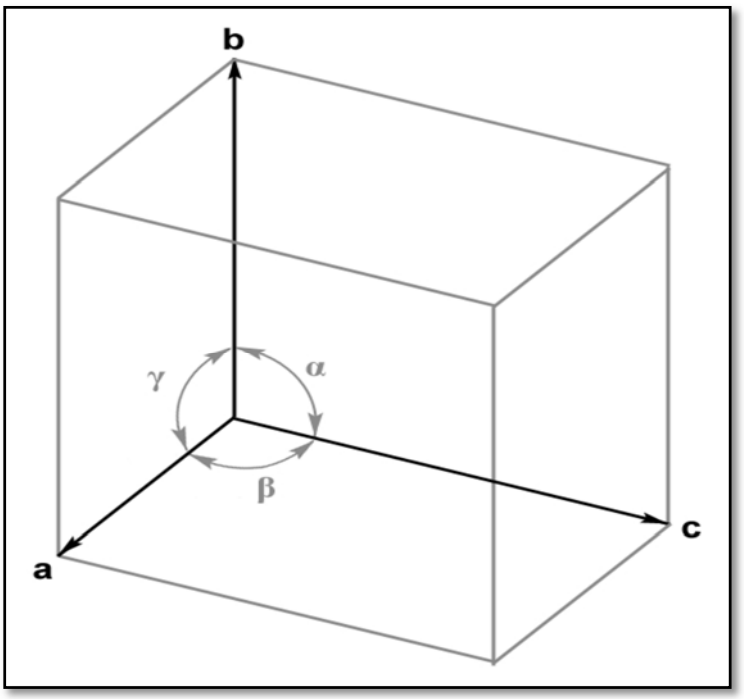

Figure 1.2: Representation of a basic unit cell (Rodríguez-Homedo *et al*., 2006).

4. **Glade plane-reflection** through a mirror plane followed by translation brings structure in coincidence;
5. **Rotation-inversion** operations-exists when a rotation of 360°/ *n* followed by inversion results in the same structure (Byrn, 2006). Bravias proved that fourteen distinct types of space lattices exist. These lattices are known as the Bravias lattices. The fourteen Bravias lattices include: cubic-P, cubic-I, cubic-F, orthorhombic-P, orthorhombic-I, orthorhombic-F, orthorhombic-C, tetragonal-P, tetragonal-I, monoclinic-P, monoclinic-B, trigonal-R, hexagonal-P, and triclinic-P. These fourteen Bravais lattices consists of seven primitive and seven non-primitive lattices and since there exists thirty two possible unique combinations of the different crystallographic symmetry elements there are two hundred and thirty unique arrangements of points in space, termed space groups (Bernstein, 2002). From the above section it is clear that crystals can exist in several lattice forms. The lattice energy of a crystal consists

of a large number of relatively weak inter-molecular interactions (0.5-2 KJ/mol), relatively strong inter-molecular interactions (~ 30 KJ/mol) and especially strong intra-molecular and inter-ionic interactions (~ 150 KJ/mol). The inter-molecular interactions consist of hydrogen bonding, van der Waals forces (non-bonded, non-electrostatic) and electrostatic forces. The van der Waals forces are sub-divided into three categories namely: dipole-dipole, dipole-induced dipole and induced dipole-induced dipole. The major forces that play a role in the packing of pharmaceutical crystals are the non-covalent interactions (van der Waals forces) and hydrogen bonding, which are attractive interactions (Datta and Grant, 2004). Hydrogen bonding is the most important type of force out of the above mentioned forces holding organic solids together. Hydrogen bonds are strong, directional, non-covalent bonds that determine the configuration of the molecules (Etter *et al*., 1990). Polar organic molecules in solution tend to form hydrogen-bonded aggregates. These hydrogen-bonded aggregates act as precursors to the crystals which form when the solution is supersaturated. A system which entails a graph-theory-based approach to classify and symbolically represent the different types of hydrogen bonds that can be formed, were developed by Etter, MacDonald and Bernstein. Etter further developed rules to control hydrogen bonding in solid organic compounds. These rules apply well to hydrogen bonding of especially small molecules; however steric factors make it impossible to satisfy all of the possible hydrogen bonded interactions, resulting in some donors and acceptors not being involved in any hydrogen bonds as in the case of erythromycin (a larger molecule). These rules require a classification of hydrogen bond donors and acceptors into “reliable” hydrogen-bond donors and “occasional” hydrogen-bond donors and acceptors (Byrn *et al*., 1994, Byrn *et al*., 1999; Etter *et al*., 1990). Table 1.1 summarises the reliable and occasional hydrogen-bond donors and acceptors.

Table 1.1: Reliable and occasional hydrogen-bond donors and acceptors (Byrn *et al*., 1994)

Type	**Functional group involved**
Reliable donor	-OH, -NH_2, -NHR, $CONH_2$, -CONHR, -COOH
Occasional donor	-COH, -XH, -SH, -CH
Reliable acceptors	-COOH, -CONHCO, -NHCONH, -CON_i (1-3°), P=0, >S=0, -OH
Occasional acceptors	>O, -NO_2, -CN, -CO, COOR, -N<, -Cl

The rules can be summarised as follows:

1. All reliable proton donors and acceptors are used in hydrogen bonding;
2. Six-membered ring intramolecular hydrogen bonds form in preference to intermolecular hydrogen bonds;
3. The best proton donors and acceptors remaining after intramolecular hydrogen bond formation will form intermolecular hydrogen bonds (Byrn *et al*., 1994).

A characteristic property of a crystal is that it has a melting point. This is the temperature, at which the crystal lattice breaks down, owing to the molecules having gained sufficient energy from the heating process to overcome the attractive forces that hold the crystal together. Crystals with weak forces holding the molecules together have low melting points; in contrast crystals with strong lattices have high melting points (Aulton and Taylor, 2013).

1.3.1 Crystal formation methods

Crystals are produced by inducing a change from the liquid to the solid state. This includes:

1. **Cooling a molten sample to below its melting point:** This method involves the cooling of a saturated solution of the substance being crystallised. A saturated solution is the maximum amount of solute that can be dissolved in any liquid, at a given temperature and pressure. This process can be viewed as two separate events, starting with the dissolution of the initial phase followed by the nucleation of the final phase (Vippagunta *et al*., 2001).
2. **Seeding a supersaturated solution of crystals of the desired form:** In this method nuclei or seeds of the relevant substance are incorporated into a saturated

solution resulting in the promotion of crystallisation of the substance. Various factors may affect the formation of crystals including, solubility, supersaturation, rate of supersaturation, desupersaturation occurrence, diffusivity, temperature and reactivity of the surfaces towards nucleation (Vippagunta *et al*., 2001).

3. **Vapour diffusion:** This method involves the placing of a solution into a desiccator which is then tightly closed. As solvent equilibrium is reached the miscible non-solvent in the desiccator diffuses through the vapour phase into the solution, resulting in saturation or supersaturation of the solution. This technique is ideally applied when single crystals should be prepared for crystallographic analysis (Vippagunta *et al*., 2001).

4. **Sublimation:** In this method a solid is heated, such that the phase changes from the solid to vapour occur without the intervention of the liquid phase. During sublimation it often occurs that crystals form on cooler surfaces in close proximity of the melt of the compound. The sublimation temperature and the distance of the collecting surface from the material undergoing sublimation have a significant effect on the type of crystals that forms (Vippagunta *et al*., 2001).

5. **Changing pH/salting out:** For the reason that many APIs (active pharmaceutical ingredients) are either slightly soluble weak acids, or slightly soluble weak bases, of which the salt forms are significantly more soluble in water, in this method crystallisation is facilitated through the addition of either an acid in the case of an aqueous solution of salt of a weak acid or of an alkali to a solution of a soluble salt of a weak base (Vippagunta *et al*., 2001).

6. **Thermal interaction:** One form could have the ability to transform to another polymorphic form only by means of thermal manipulation. Differential scanning calorimetry (DSC) can be utilised to observe this. During the analysis an endothermic peak corresponding to a phase transition, followed by a second endothermic peak corresponding to a melting could be observed. Sometimes an exothermic peak exists between the two endothermic peaks, representing a crystallisation step. For these reasons a higher melting polymorph could be prepared by thermal treatment. (Vippagunta *et al*., 2001).

7. **Binary mixtures of solvents:** This method offers a possible solution where single solvent solutions do not facilitate the formation of crystals. During this approach the addition of a second solvent in which the substance is sparingly soluble to the saturated solution of the compound in a good solvent, takes place. Most often a solvent system is selected in which the solute is more soluble in the component with

the higher vapour pressure. As the solution evaporates, the volume of the solution is reduced and since the solvents evaporate at different rates the composition of the solvent mixture will change. The second liquid is referred to as an antisolvent. Many drugs are crystallised by adding water as an antisolvent to a solution of the drug in an organic liquid. For example, if a drug is almost insoluble in water but freely soluble in ethanol, the drug could be crystallised by adding water to a near-saturated solution of the drug in ethanol (Vippagunta *et al*., 2001; Aulton and Taylor, 2013). Nucleation is formation of a small mass on to which a crystal can grow. Growth is the addition of more solute molecules to the nucleation site. A supersaturated solution, i.e. one where the amount of solute dissolved in the liquid is greater than the true solubility is required in order to achieve nucleation and growth. Factors effecting the rate and mechanisms by which crystals are formed includes: solubility, supersaturation, rate at which supersaturation and desupersaturation occur, diffusivity, temperature, the reactivity of surfaces towards nucleation as well as the forces responsible for holding the organic crystalline solids together (Vippagunta *et al*., 2001).

1.4 Polymorphism

A polymorph can be defined as crystals of the same chemical compound, but which differ in the internal arrangement of the molecules of the given compound. It is therefore a solid material with at least two different molecular arrangements that give distinct crystal species. Polymorphs have the same liquid or gaseous state, but behave differently in the solid-state, indicating that these differences disappear in the liquid or the vapour state. Alteration of the polymorph(s) may influence the bulk properties, dissolution rate, bioavailability, chemical stability, physical stability as well as affect the mechanical properties of pharmaceutical solids and therefore influence the manufacturability and physical attributes of the dosage forms. For this reason the understanding as well as the control of polymorphs in the pharmaceutical industry is extremely important. The alteration of flow properties due to the difference in partial morphology of polymorphs is a common effect of polymorphism on mechanical properties. Polymorphism, where crystal habits also differed for instance particles with needle- or rod-shaped may exhibit poor flow, compared to polymorphs with cubic or irregular shapes (Singhal and Curatolo, 2004).

1.4.1 Enantiotropic and monotropic systems

Monotropic polymorphism means that only one polymorphic form is stable and any other polymorph that is formed will eventually convert to the stable form. In other words, no reversible transition is observed between the polymorphs below the melting point. The monotropic polymorph has the highest melting point. The polymorph with the highest

melting point will have a strong lattice and it would thus be hard to remove molecules resulting in a low dissolution rate, in contrast enantiotropic polymorphism means that under different conditions (temperature and pressure) the material can reversibly transform between alternative stable forms, meaning the other forms exist for a period of time, and appear stable, but given a chance they will convert to the true stable form. The polymorph with the lowest melting point will have a weak lattice and it would thus be easier to remove molecules resulting in a high dissolution rate. Each polymorph differs with respect to physical properties, solubility, melting point, density, crystal shape, optical, electrical properties and vapour pressure. Polymorphs are conventionally numbered in order of stability, at room temperature, starting with form I using Roman numerals. Form I usually has the highest melting point and the lowest solubility. In suspension formulation it is essential to use the least soluble polymorph, because of Ostwald ripening. See figure 1.3, illustrating the relationship between melting point (°C) and solubility (mg.mL^{-1}) for three polymorphs of riboflavin (Aulton and Taylor, 2013).

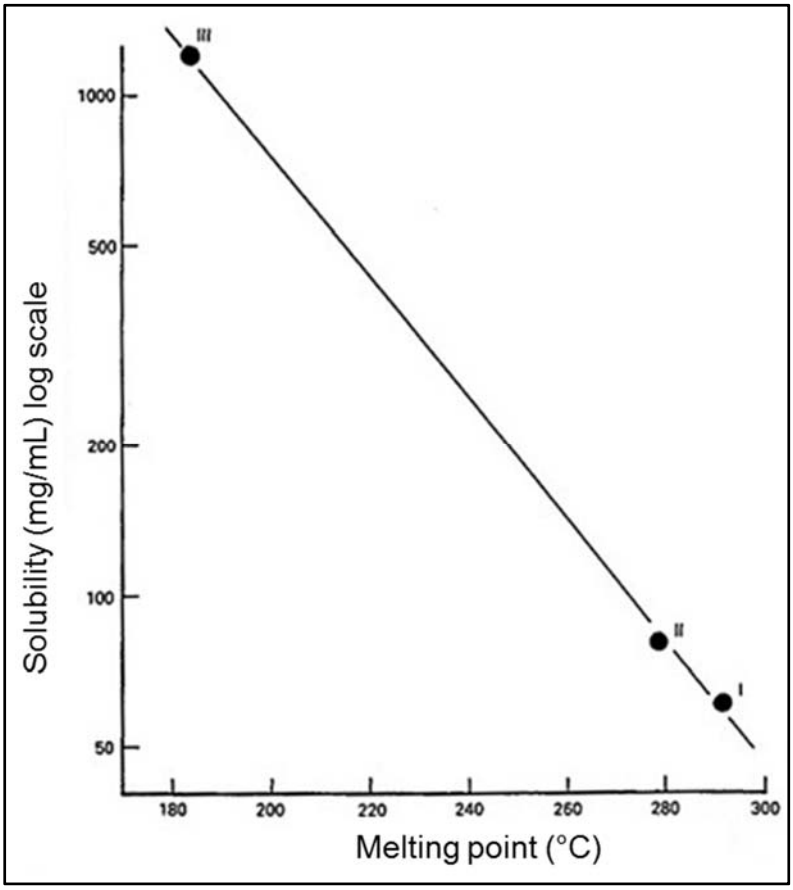

Figure 1.3: The relationship between melting point (°C) and solubility (mg/mL^{-1}) for three polymorphs of riboflavin (Adapted from Aulton and Taylor, 2013).

Several rules have been developed to facilitate the qualitative determination of the enantiotropic or monotropic nature of the relationship between polymorphs and to predict the

relative thermodynamic stability of polymorphs. These rules include: heat of fusion rule, heat of transition rule, infrared rule and density rule. After the identification of enantiotropism or monotropism, the following step is to define the thermodynamically stable (metastable) domain of each crystalline phase a function of temperature. For this determination the plot of Gibbs free energy difference gives the most complete information regarding the stability relationships of polymorphs (see figure 1.4) (Datta and Grant, 2004; Lohani and Grant, 2006).

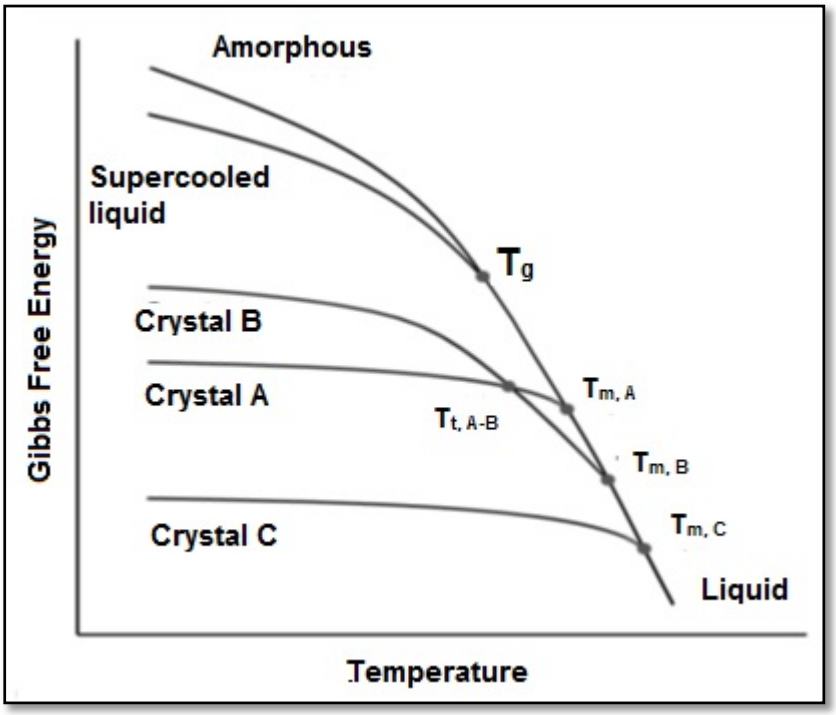

Figure 1.4: Schematic representation of Gibbs free energy curves for a component that exhibits crystalline and amorphous phase transitions (Adapted from Rodriquez-Spong *et al*., 2004).

1. Heat of fusion rule: Indicates that in an enantiotropic system the higher melting point polymorph will have the lower heat of fusion. The two polymorphic forms are related monotropically if the higher melting point polymorph has a higher heat of fusion. In the case where the rate of polymorphic transition is too slow to allow for an accurate measurement of the heat of transition, the heat of fusion rule may be applied. This rule is based on the assumption that the heat of transition can be approximated by the difference between the heats of fusion of the polymorphs. However, Burger and Ramberger stated that this difference alone is not accurate enough to indicate the heat of transition between two polymorphs, but that the difference between heat capacity should also be utilised to calculate the heat of transition of the two polymorphs (Bernstein, 2002; Lohani and Grant, 2006; Burger and Ramberger, 1979).

2. Heat of transition rule: The heat of transition rule could be explained as follows: if an endothermic phase change is observed at a particular temperature, the transition point lies below that temperature and the polymorphic forms are enantiotropes. If an exothermic phase transition is observed, there is no thermodynamic transition point below that transition temperature. This occurs when the polymorphic forms are monotropically related or when they are enantiotropically related, but the thermodynamic transition point is higher than the measured transition temperature (Bernstein, 2002).

3. Entropy of fusion rule: If the polymorph with the higher melting point has higher entropy of fusion, then the two polymorphs are monotropes. If the polymorph with the higher melting point has a lower entropy of fusion, the two polymorphs are enantiotropically related to each other. The entropy of fusion (ΔS_f) of a polymorph can be obtained from the heat of fusion (ΔH_f) and melting point (T_f) (Lohani and Grant, 2006).

$$\Delta S_f = \frac{\Delta H_f}{T_f} \quad (1)$$

4. Heat capacity rule: Two polymorphs are related enantiotropically if the polymorph with the higher melting point also has the higher heat capacity at a given temperature. In contrast, monotropes exhibit a lower heat capacity at the higher melting point at a given temperature (Lohani and Grant, 2006).

5. Infrared rule: Hydrogen-bonded polymorphic forms with a higher frequency in the bond stretching modes may be assumed to have a higher entropy (Lohani and Grant, 2006).

6. Density rule: The more thermodynamically stable polymorph is more chemically stable than the metastable polymorph. This could be attributed to the higher crystal packing density of the thermodynamically stable polymorph. This rule is generally applied to ordered molecular solids that are dominated by van der Waals interactions (Bernstein, 2002).

1.4.2 Packing polymorphism and conformational polymorphism

Two different mechanisms are involved in the formation of different crystal lattices from organic molecules. The first mechanism, termed packing polymorphism or orientational polymorphism, occurs when molecules that are conformationally relatively rigid can be assembled into different three-dimensional structures through different intermolecular juxtapositions. In the second mechanism, known as conformational polymorphism, a flexible molecule bends into different conformations, which subsequently can be packed into alternative crystal structures (Datta and Grant, 2004; Vippagunta *et al*., 2001).

The presence solvates should be identified as most polymorphs can be obtained by changing the recrystallising solvent. Hydrates (water) and solvates (e.g. methanolate,

ethanolate) have been confused with true polymorphism. The distinction between solvates / hydrates and true polymorphs can be established by observing the melting behaviour of the compound dispersed in silicone oil using hot-stage microscopy. Solvates or hydrates will evoke a gas (steam or solvent vapour), causing the oil to bubble. True polymorphs merely melt, forming a second globular phase. The temperature at which the solvent volatilises will be close to the boiling point of the solvent. Solvates and hydrates will be discussed in more detail in section 1.5 of this chapter.

1.4.3 Polymorphism and bioavailability

Owing to the fact that many drugs are hydrophobic, the low water solubility renders a slow dissolution rate, resulting in only a small percentage of the administered drug actually being available to the patient (low bioavailability). The polymorphic form must be well controlled to ensure that the bioavailability is the same each time the product is manufactured and throughout the shelf life of the product, for drugs with low aqueous solubility. The stable polymorphic form will have the slowest dissolution rate. The metastable form can be used to speed up the dissolution, where required. However, the risk associated with using the metastable form, is that it will convert back to the stable form during the product's life, resulting in reduction of bioavailability and hence therapeutic effect of the product (Aulton and Taylor, 2013).

1.5 Hydrates and solvates

1.5.1 Hydrates

A hydrate is a solid adduct containing both the parent compound and water. A monohydrate will have one molecule of water for each of the crystallising material, and a dihydrate and trihydrate two and three molecules of water, respectively, to each molecule of drug (Aulton and Taylor, 2013). Water behaves as if it consists of a tetrahedral distribution of two positive and two negative regions of charge. On each negatively charged region the water molecule interacts with its neighbours via a coordinate covalent (dative) bond or by accepting a hydrogen bond. On each positively charged region the water molecules interact with its neighbours via a donated hydrogen bond. The neighbours of a water molecule in a hydrate, therefore includes electron acceptor groups (or proton donors), such as M^{n+}, R-OH, R_1R_2NH, and electron donor groups (or proton acceptors), such as $R\text{-}COO^-$, $R\text{-}O^-$, Cl^-. The neighbours of a water molecule may include other water molecules suitably orientated for hydrogen bond formation. The water molecule may also participate in various types of van der Waals interactions. The hydration of a solid may altered the pharmaceutical important physico-chemical properties of a compound, due to a change in the thermodynamic activity

of a solid. The change in the solubility of an API usually changes its dissolution rate. The alterations in the dissolution rate and the stability of a drug may ultimately modify its bioavailability and product performance. During preformulation, some hydrated compounds may convert to amorphous solids when dehydration occurs or some may become chemically liable. Other pharmaceuticals may convert from a lower state of hydration to a higher state which could result in lower solubility (Morris, 1999). The anhydrous form of a substance is always more soluble in water than the corresponding hydrates which crystallised from water at the same temperature (Shefter and Higuchi, 1963). Techniques for the characterisation of hydrates include hot stage microscopy, X-ray powder diffraction, differential scanning calorimetry, thermogravimetric analysis, Karl Fischer titrimetry, Infrared spectroscopy, single crystal X-ray analysis and solution calorimetry.

Hydrates can be classified into three categories, based on the location of water in their structure:

Class I: Isolated site hydrates: The water molecule is isolated from direct contact with other water molecules by an intervening molecule of the major component. This class is often stoichiometric.

Class II: Channel hydrates: the water molecules included in the crystal lattice lie next to other water molecules of adjoining unit cells. This placement of water molecules along an axis of the lattice structure forms channels through the crystal. This class can further be subdivided into two categories, namely:

1. The expanded channel hydrates (non-stoichiometric hydrates) are characterised by the additional uptake of moisture in the formed channels. This occurs when the crystal is exposed to relative high humidity and for which the crystal lattice may expand or contract as the hydration or dehydration effect the change in the dimensions of the unit cells.

2. In the planar hydrates: the water is localised in a two-dimensional order, forming a plane within the channel.

Class III: ion associated hydrates: metal ions are co-ordinated with water. This class can be stoichiometric or non- stoichiometric.

Some hydrates will be able to comprise more than one category, due to the diverse roles that water play in molecular bonding (Datta and Grant, 2004; Vippagunta *et al*., 2001; Authelin, 2005).

Preparation of hydrates

Hydrates can be prepared through recrystallisation from water, slow evaporation from mixed aqueous solvents (binary mixtures) or exposure of crystal solvates to atmospheric moisture (Guillory, 1999).

Significant differences between polymorphs and hydrates

Polymorphs are different crystal structures with the same molecule (s) as basis. Hydrates are crystals of the drug molecule with different numbers of water molecules. The hydration state of a crystalline hydrate is a function of the water vapour pressure (water activity above the solid). Polymorphs are only affected by changes in water vapour pressure if water sorption allows organisation into a different polymorph (Morris, 1999).

1.5.2 Solvates

When a solvent other than water, for example ethanol are incorporated into the crystal lattice of the compound, either entrapped within empty spaces within the lattice, interacting through hydrogen bonding or van der Waals forces with the molecules of the crystal structure, in stoichiometric proportions, the molecule adducts are known as solvates (Aulton and Taylor, 2013). These forms can also be seen as a type of cocrystal (Rodriquez-Spong *et al*., 2004). Solvent molecules within a crystal lattice will significantly influence the intermolecular interactions, and will exhibit unique physical properties. A solvate will for this reason poses its own characteristic internal energy, entropy, enthalpy, Gibbs free energy and thermodynamic activity (Lohani and Grant, 2006). Solvates in which the solvent fill only empty spaces in the lattice are usually non-stoichiometric and typically the solvate and the non-solvated compound share similar X-ray diffraction patterns (Guillory, 1999). The stability of the compound will be enhanced, when the inclusion of the solvent molecules increase the strength and flexibility of the crystal lattice. Solvates also demonstrates significant differences in solubility, responses to atmospheric pressure and solvent loss. Differences in solubility and dissolution of solvates could result in differences in bioavailability (Lohani and Grant, 2006; Vippagunta *et al*., 2001).

1.5.3 Desolvated solvates

When the included solvent molecule gets removed from a specific solvate, while the initial crystal structure stays retained, the form is known as a desolvated solvate. Desolvated solvates are less ordered than their crystalline counterparts and are difficult to characterise, since analytical studies indicate that they are unsolvated materials, but in truth, they possess the structure of the solvated crystal form from which they were derived. The

following experiments might facilitate in the investigation of whether a solid is a solvate, desolvated solvate or a true anhydrate:

1. Single crystal X-ray structure determination in the presence of mother liquor from the crystallisation;
2. Comparison of the X-ray powder diffraction patterns of the solvated and desolvated crystal forms;
3. Determination of the vapour pressure isotherm by varying the vapour pressure of the solvent involved in the solvate formation (Vippagunta et al., 2001; Byrn *et al*., 1994).

1.6 Amorphous state

An amorphous state is defined as a non-crystalline solid. Amorphous materials possess no clearly defined molecular structure. They lack repeating long-distance order, molecular mobility and intermolecular distances (Cui, 2007). The amorphous state is a high energy state compared to the crystalline state that leads to a higher dissolution rate, improved solubility and in some instances improved compression abilities and oral bioavailability in correspondence with their crystalline counterparts. Factors that affect the drug dissolution rate can be expressed by the modified Noyes-Whitney equation:

$$\frac{dc}{dt} = AD\frac{C_s - C}{h} \quad (2)$$

Where dc/dt is the dissolution rate;

A is the surface area available for dissolution;

D is the diffusion coefficient;

C_s is the saturation solubility of the drug;

C is the concentration of the drug in dissolution medium at time t and

h is the thickness of the diffusion layer.

The higher the saturation solubility of the amorphous form compared to the crystalline form is, the higher the driving force that leads to the higher dissolution rate from the amorphous form (Leuner and Dressman, 2000).

Although the production of amorphous phases may pose advantageous in some instances, a number of difficulties are also associated with their use. The higher potential energy of the amorphous sample renders them more physically unstable compared to the crystalline forms, and they tend to crystallise to a more stable crystalline form (Cui, 2007; Yu, 2001). Increase in molecular mobility decrease chemical stability (Guo *et al*., 2000). An increase in either temperature or humidity increases the molecular mobility. Amorphous samples are

hygroscopic and, therefore, the absorbed moisture acts as a plasticiser. A plasticiser is a small molecule added to an amorphous sample to lower the glass transition temperature. The plasticiser fits between the glassy molecules, giving them greater mobility (Aulton and Taylor, 2013). An increase in molecular mobility can lead to crystallisation of the sample (Gardner *et al*., 2004). Amorphous solids contain an excess of Gibbs free energy, compared to the crystalline phases. Therefore, thermodynamically, amorphous solids are seen as out of equilibrium states (Singhal and Curatolo, 2004; Petit and Coquerel, 2006).

1.6.1 Thermal behaviour of material in the amorphous state

The phase transitions that occur upon heating or cooling an amorphous sample can be classified into first and second order transitions. First order phase transitions involve latent heat, i.e. release or absorption of energy. Such transitions occur during crystallisation (T_c) of an amorphous sample or melting (T_m) of crystalline material. Figures 1.5 and 1.6 represent differential scanning calorimetry (DSC) thermograms, illustrating the release of energy due to crystallisation or absorption of energy due to melting as an exothermic or endothermic peak, respectively.

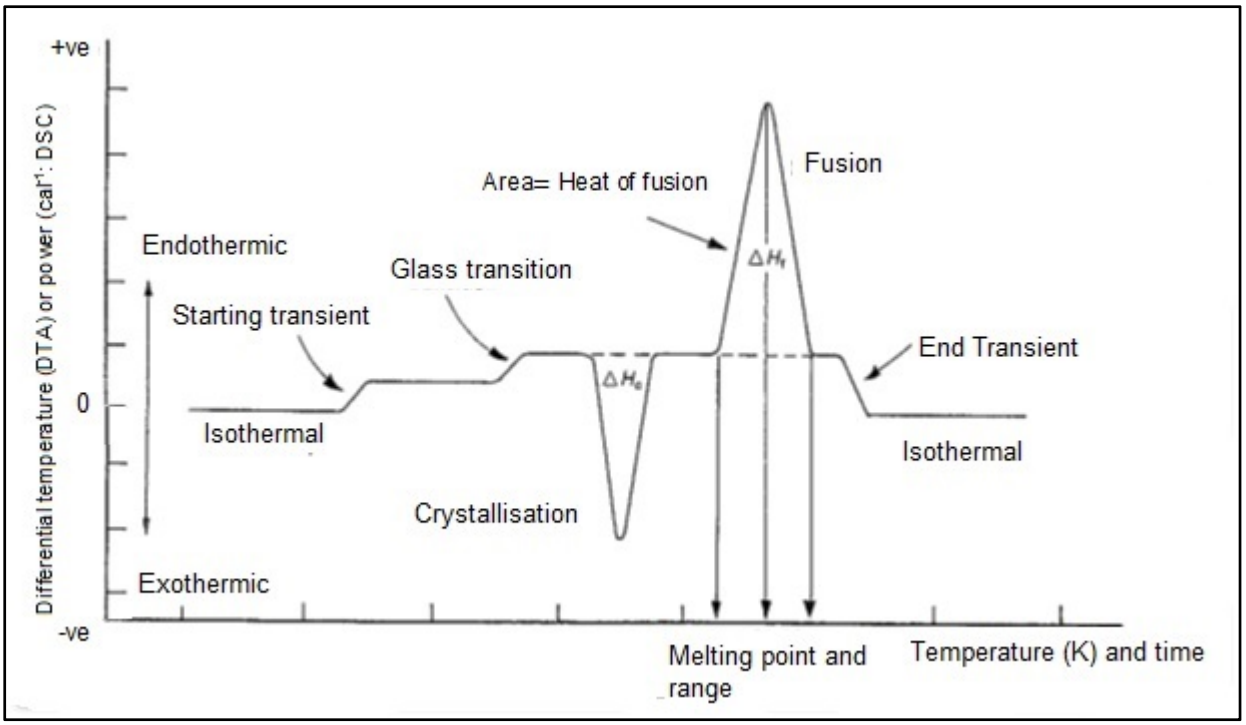

Figure 1.5: Schematic differential scanning calorimeter thermogram (Adapted from Aulton and Taylor, 2013).

In second order phase transitions such as that at the glass transition temperature (T_g), there is no release or absorption of energy, but rather represents a step change in heat flow in the DSC thermogram. In Figure 1.6 a first order phase transition can be seen at the crystallisation temperature (T_c) and the melting temperature (T_m). A second order phase transition can be observed at the glass transition temperature (T_g) (Zhang *et al*., 2004). The glass transition temperature (T_g), of an amorphous solid is a critical physical property and could determine the chemical and physical stability of a particular amorphous solid. Several methods are utilised to determine the T_g of an amorphous sample. These methods include thermomechanical, calorimetric and volumetric determinations (Zhang *et al*., 2004). Figure 1.7 represents the variation of thermodynamic properties with temperature.

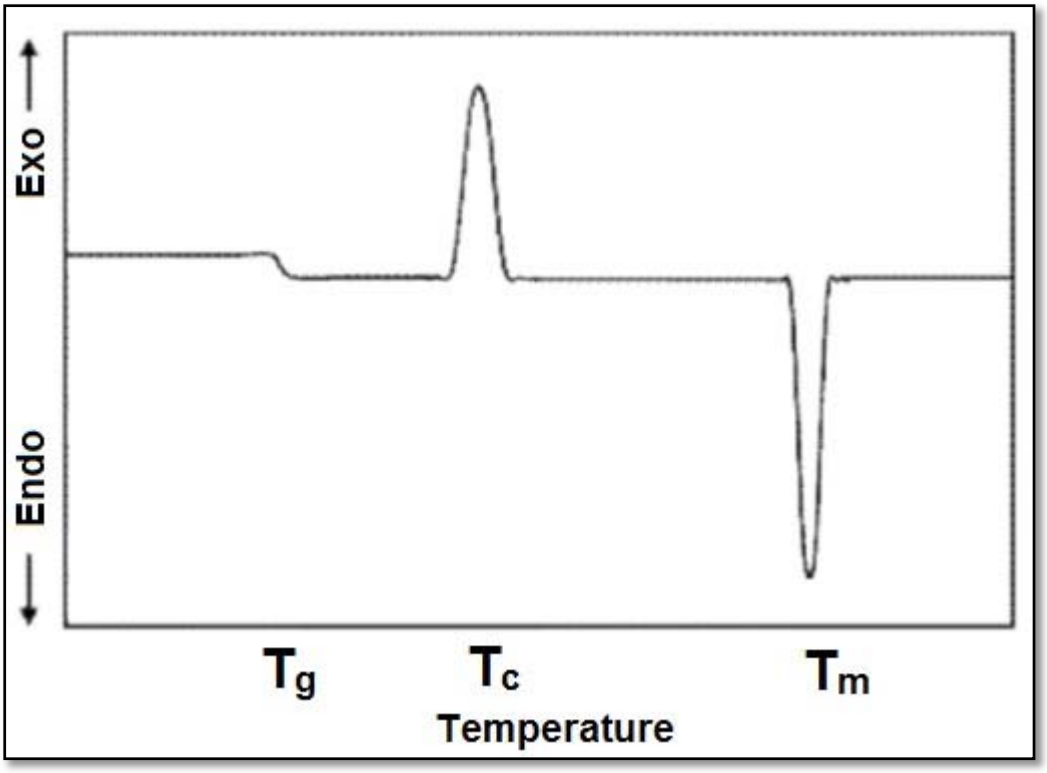

Figure 1.6: Schematic representation of a typical DSC trace obtained, when heating amorphous material (Adapted from Zhang *et al*., 2004).

When a sample is heated, several properties including volume, heat capacity, viscosity and dielectric relaxation, change at the temperature (Figure 1.7)

At the (T_g), the sample undergoes a change in heat capacity (C_p) due to changes in physical properties. This is illustrated in Figures 1.5 and 1.6 as a step change in heat flow. At the T_g the molecular mobility increase and the sample changes from a glass to a supercooled liquid (from the glassy to the rubbery state). Studies have however shown that significant molecular mobility also exists below the T_g allowing the amorphous sample to crystallise (Hancock *et al*., 1995). Samples may have to be cooled at least 50°C below the T_g for the molecular motions negligible during the product life-time (Hancock *et al*., 1995). This region corresponds to the Kauzmann temperature (T_k), where the configurational entropy of the amorphous sample approaches zero.

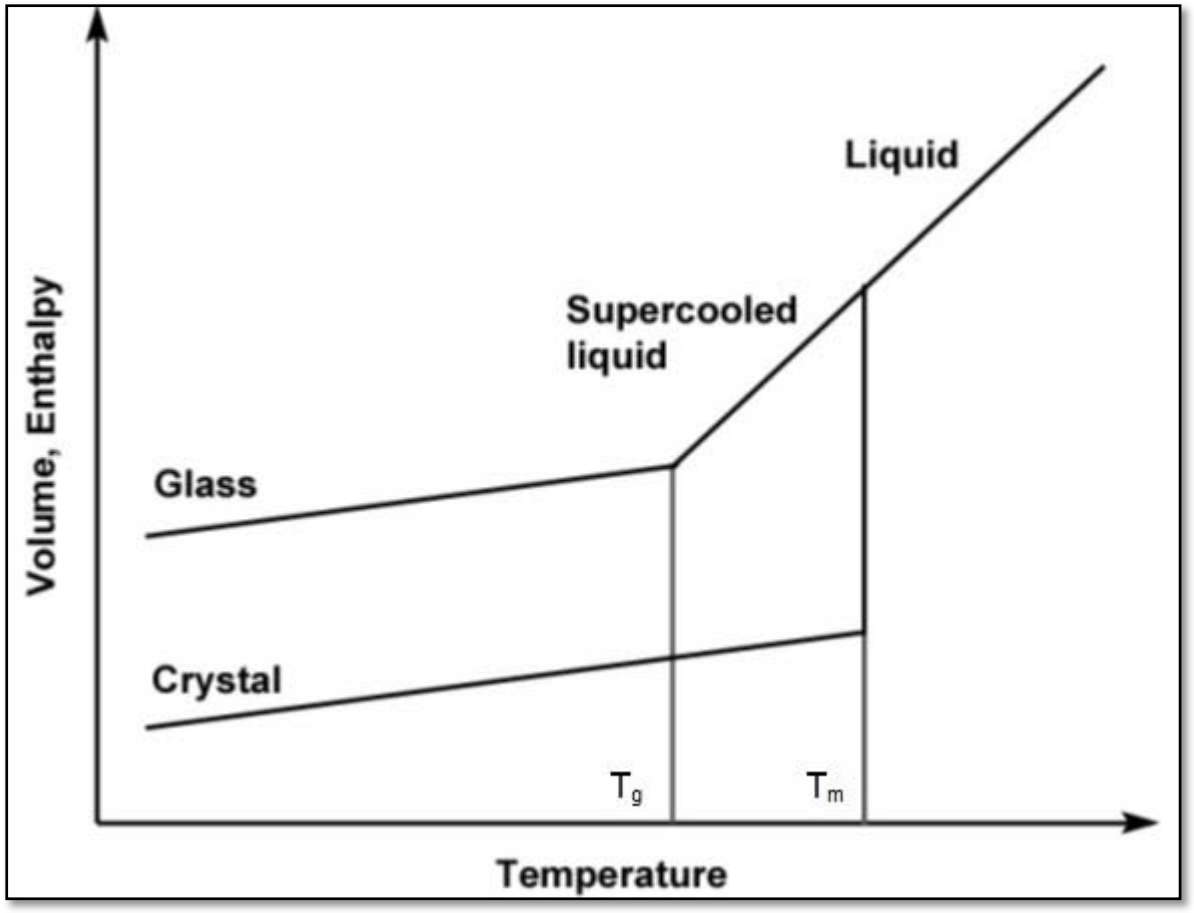

Figure 1.7: Variation of thermodynamic properties with temperature (Adapted from Zhang *et al*., 2004).

1.6.2 Influence of water on amorphous material

Water has a reported T_g of 135 K and could act as a plasticiser for amorphous pharmaceutical solids. At a temperature above the glass transition the molecular mobility increases, due to the fact that the viscosity decreases, resulting in increased flow. The presence of additional materials, especially water, could significantly influence the glass transition of an amorphous material. In binary or mixed systems the T_g value will therefore be affected. Water acts as a plasticiser by means of increasing the free volume of the product, and would therefore have an effect on the glass transition of amorphous material (Craig *et al*., 1999).

Relative to the crystalline form of a given drug, the amorphous state could take up more water; this can be ascribed to the absorption of water into the solid. In the crystalline systems, however, the uptake of water tends to be dependent on sample mass rather than surface area (Craig *et al*., 1999).

This absorbed water could have a plasticising effect, therefore lowering the glass transition temperature (T_g), below the glass transition; water sorption will be limited to surface absorption. As the material passes through the glass transition, molecular mobility increases, allowing water absorption into the bulk structure (Burnett *et al*., 2004).

1.6.3 Preparation of amorphous material

Figure 1.8 summarises the different pathways through which amorphous materials can be prepared.

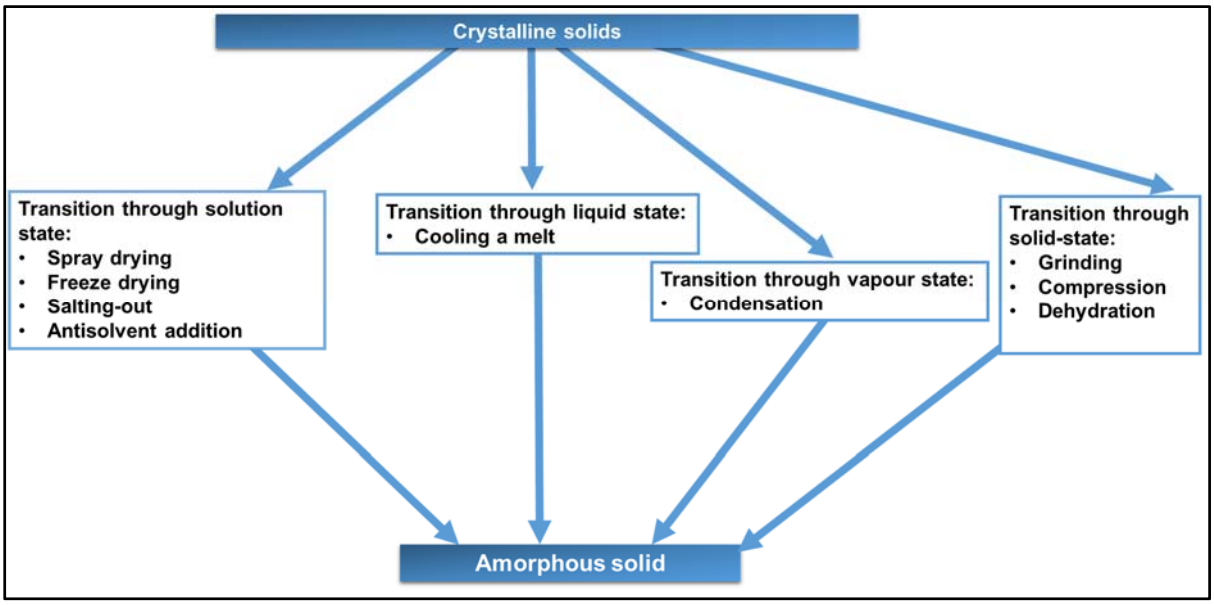

Figure 1.8: Different pathways through which amorphous materials can be prepared (Adapted from Zhang *et al*., 2004).

Amorphous materials can be prepared through a solution, a liquid, a vapour, and a solid state (Zhang *et al*., 2004). If the transition from the liquid state (molten or solution) through the melting point to a solid is fast enough for the molecules to instantly freeze in a random order, an amorphous solid is obtained. Crystallisation requires time to overcome the energy barrier between the crystal-liquid interface for the molecules to rearrange themselves before nuclei formation and crystal growth can begin (Cui, 2007). The most typical way to prepare amorphous material is through a liquid transition by quench cooling a melt. This method is also known as vitrification (Savolainen *et al*., 2009). The formation of amorphous material through a solution state is based on rapid precipitation (Zhang *et al*., 2004). In spray drying, the solution is sprayed into hot air and the solvent is evaporated so fast that the molecules remain unorganised. In freeze drying the solution is rapidly frozen and the solvent is sublimed in low temperature and pressure (primary drying). As the solvent is removed the solute molecules remain in the unordered structure they were frozen in. This phenomenon is also involved in preparation of amorphous material through the vapour state. If the

condensation of the material from the vapour state to the solid state occurs fast enough, the molecules remain unorganised. Secondary drying involves the desorption of residual water/ solvent at low pressure and high temperature. Amorphisation occurs when the freezing step is rapid and performed at liquid nitrogen temperature, so that nucleation can be avoided (Savolainen *et al*., 2009). Mechanical activation includes grinding (and especially high-energy grinding), milling, compression, dehydration and wet granulation. This is the fourth way to prepare amorphous material. In solid-state transitions, the amorphous state is formed through the disruption of the crystal lattice (Feng *et al*., 2008). The amorphous solid is formed, when the amount of crystal defects accumulates gradually above a critical level. For this reason the amorphous form is more likely to possess some "memory" of the long-range order of the original polymorph and to have some seeds or nuclei of the original polymorph left. The physical properties of the end product can however be affected by parameters such as, type of milling apparatus, milling intensity, duration, temperature and the use of excipients (Sheths *et al*., 2004; Sheths *et al*., 2005). Dehydration of crystalline hydrates has been demonstrated as a feasible and gentile route to the amorphous state of organic solids. Saleki-Gerhardt *et al*. (1995) showed that heating the crystalline raffinose pentahydrate at 60°C in a vacuum converts the material to an amorphous form identical to one produced by freeze-drying. The drying of crystalline hydrates may reduce their physiochemical stability through the loss of crystallinity (Yu, 2001).

Amorphous material is often created unintentionally during several pharmaceutical processes, including milling, compression and drying (see Figure 1.9).

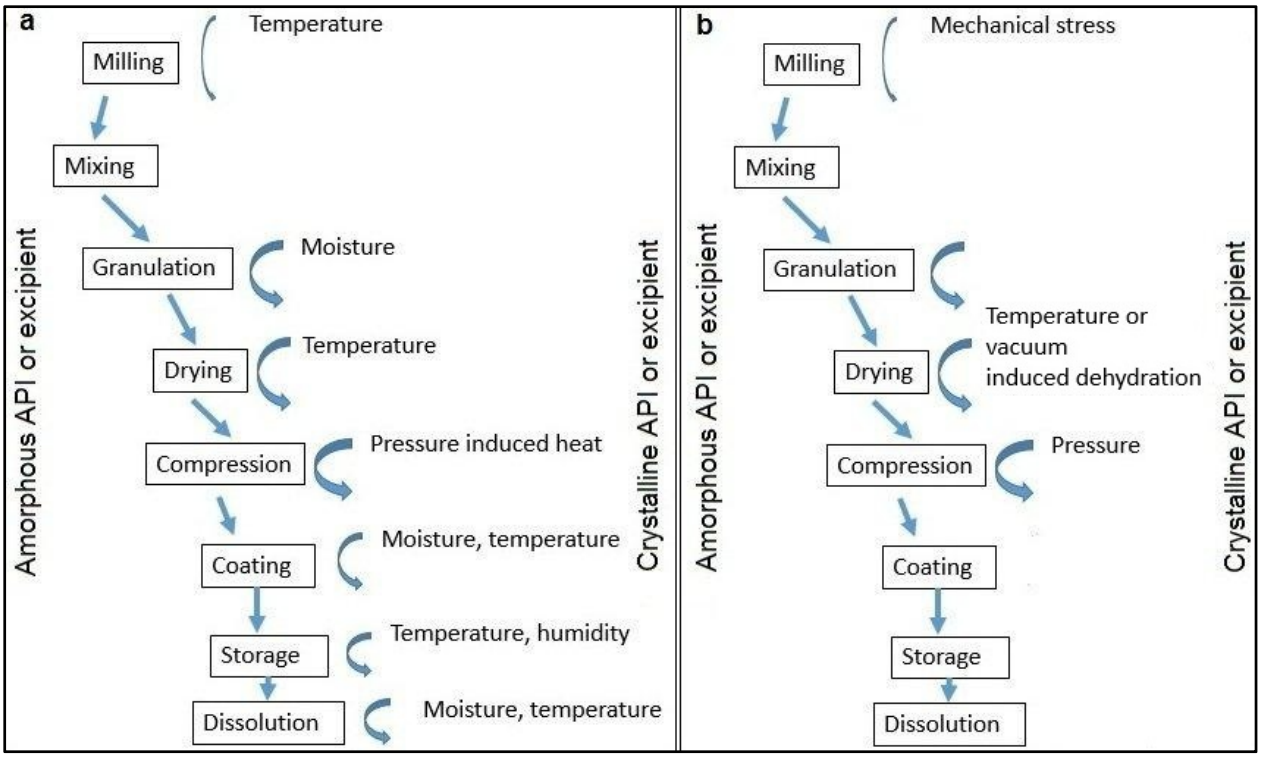

Figure 1.9: A simplified presentation of a lifecycle of an oral solid dosage form. The typical steps involved in the development of oral solid dosage forms as well as the factors that can induce solid-state transformations: (a) crystallisation of amorphous material and (b) amorphisation of crystalline material. The manufacturing process might involve all or only some of the unit operations presented as well as multiple solid-state transformations (Zhang *et al*., 2004; Vippagunta *et al*., 2001).

Amorphous material can however also crystallise to either anhydrate or hydrate forms due to process induced transformations (PITs). During processes such as granulation or coating, where it is possible to heat the sample above T_g or in the presence of water to plasticise the sample, crystallisation can occur. The underlying mechanism is either a solid-state, solution or solution-mediated transformation (Zhang *et al*., 2004). In the solid state mechanism the transformation occurs in the solid state without going through any intermediate liquid or vapour phases. Such a mechanism could occur during dehydration or compression (Li *et al*., 2000). Transformation via solution is caused following the removal of the solvent. This mechanism occurs during processes such as drying. The final solid can either be crystalline, amorphous or a mixture of several forms (Kogermann *et al*., 2007). A solution-mediated transformation is caused by the solubility difference between the solid-state forms. It can therefore only happen from the metastable amorphous phase towards the stability crystalline phase. This type or transformation occurs during dissolution and solubility experiments (Hancock and Parks, 2000). Transformational changes in the solid-state forms during

manufacturing can affect both the physical and chemical stability as well as the bioavailability of the product (Savolainen *et al*., 2009).

1.6.4 Determination of amorphous content

Determination of amorphous or crystalline content is based on the various differences in physical properties noted between the solid-state forms. It is customary to characterise an amorphous material both below and above the glass transition temperature. In other words, both as the frozen solid and as the super cooled viscous liquid (Yu, 2001). The methods can be divided into properties associated with individual molecules, properties related to individual solid particles and properties linked to a mass of particles (Brittain *et al*., 1991).

The method of choice depends on the amount of sample available, the amount of amorphous or crystalline content necessary to determine, whether the determination has to be done during processing or whether the technique has to be surface sensitive. When there is a need for surface sensitive methods or detection of very low amorphous content, methods based on vapour sorption, such as dynamic vapour sorption (DVS), or isothermal microcalorimetry are preferred (Mackin *et al*., 2002). In isothermal microcalorimetry, the heat change caused by crystallisation due to sorption of water in a specific relative humidity is measured. Amorphous levels as low as 0.2% could be measured. High speed DSC (Hyper DSC) has also been proven successful in determining very small amounts of amorphous material (Lappalainen *et al*., 2006). Process analytical technology tools require techniques that are fast, non-destructive, and preferably non-invasive to enable in-line or on-line monitoring, and therefore these methods cannot be used. In order to obtain molecular level information of the sample, vibrational spectroscopy, such as mid infrared (MIR), near infrared (NIR), and Raman spectroscopy can be used (Siesler *et al*., 2002). They are sensitive to changes in the intramolecular interactions and can be used to obtain complimentary information about the molecular interactions in the solid state. MIR and NIR spectroscopy are absorption techniques whereas Raman spectroscopy is a scattering technique. For quantitative purposes MIR and NIR spectroscopy follows the Lambert-Beer law:

$$Log\frac{I_0}{I} = A = abc \qquad (3)$$

Where I_0 = the intensity of the incident light

I = the intensity after passing through the sample

A = absorbance

a = the absorption coefficient or the molar absorptive

b = the path length

c = the concentration of the absorbing material

In contrast, in Raman spectroscopy as long as the sample is not significantly absorbing the incident light, the intensity due to Raman scattering (I_{Raman}) is directly proportional to concentration (C)

$$I_{Raman} \sim C$$

The physical characterisation of amorphous contents offers several types of information as discussed below (Siesler *et al*., 2002; Aulton and Taylor, 2013).

1.6.4.1 Structure

An amorphous solid is described as possessing crystal-like short range molecular arrangement, but lacking long-range orders (see figure 1.10) (Yu, 2001).

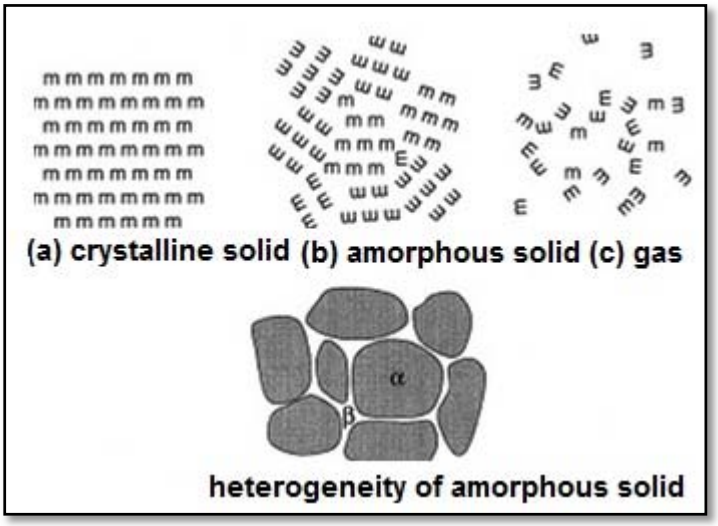

Figure 1.10: Schematic representation of a structure of a crystalline solid (a) amorphous solid (b), gas (c) and heterogeneity of amorphous solid (d). (Adapted from Yu, 2001).

1.6.4.2 Truly amorphous or microcrystalline

XRPD has shown that grinding or milling of crystals can remove all traces of crystallinity. A possibility exists that material can possess crystals so small that they pass the detection of the XRPD, the material is then said to be in the microcrystalline state (Yu, 2001). Johari *et al*. (1990) used DSC to distinguish between amorphous and microcrystalline states, based on the presence and absence of glass transition.

1.6.4.3 Degree of crystallinity

Amorphous solids may co-exist with and have the potential to convert to crystalline solids. XRD, DSC, SC (solution calorimetry), water sorption, isothermal calorimetry, and thermally stimulated current are techniques for determining the degree of crystallinity (Yu, 2001).

1.6.4.4 Microheterogeneity

Dielectric studies of secondary relaxation in amorphous solids have shown that a glass may have different regions: the glass transition (primarily relaxation) involves cooperative motions in high density regions, whereas secondary relaxation involves low-density regions lying between high-density regions (see Figure 1.10 (d)) (Yu, 2001; Johari, 1982).

1.6.5 Thermodynamics

Thermodynamic properties of an amorphous solid are often presented as *excess properties* relative to the crystalline state. Excess entropy, enthalpy and free energy can be obtained from heat capacities of the crystalline and amorphous phases as a function of temperature (Westrum and McCullough, 1963). Excess enthalpy can also be obtained from heats of solution by solution calorimetry, or crystallisation by scanning or isothermal calorimetry. Provided that the equilibrium solubility of the amorphous solid can be measured, excess free energy can be calculated from the solubility of crystalline and amorphous phases (Yu, 2001).

1.6.6 Changes

1.6.6.1 The glass transition temperature (T_g)

Quantitative measurement of T_g takes into account the effect of impurities (often water), scanning rate, and annealing, and distinguishes between onset, midpoint, and end point temperatures. DSC has recently become a principal source of T_g data (Kerč and Srčič, 1995).

1.6.6.2 Crystallisation

If a more stable crystalline state exists, an amorphous material can crystallise when sufficient molecular mobility exists, especially on exposure to heat and humidity. The nucleation growth model recognises two distinct steps in crystallisation that have different temperature dependence: lower temperature favours nucleation and higher temperature favours growth (Jolley, 1970; Yu, 2001). Cooling rate also has an influence on the rate of nucleation. Slow cooling allows the maintenance of a steady-state nucleation rate, whereas rapid cooling prevents a full development of viable nuclei. Rapid cooling, therefore not only facilitates glass formation, but also enhances glass stability against crystallisation (Kelton, 1998).

Nucleation is the first step of crystallisation. Nucleation can be subdivided into primary nucleation and secondary nucleation. Primary nucleation is the first step in crystallisation from a supersaturated solution, and requires the assembly of a critical number of ordered molecules into viable nuclei. This critical number is the point of equilibrium and any assembly below, or above, will continue to dissolve or grow respectively (Byrn *et al*., 1999). Secondary nucleation involves further crystallisation, after initial crystals are formed. Once the nuclei are formed, an equilibrium process exists between the solution and the solid-state.

The next step is termed growth and here the nuclei grow into crystals by deposition of molecules on the crystal faces. The concentration of the solution, the temperature, the degree of agitation, or stirring of the solution is parameters that control the rate of crystallisation (Byrn *et al*., 1999).

At the equilibrium the solid is neither dissolving, nor continuing to crystallise. The equilibrium therefore pertains to a state of saturation. Solubility is the concentration of a given solid substance, at which the solution of the substance is in equilibrium (Byrn *et al*., 1999).

1.6.6.3 Structural relaxation

Structural relaxation, physical aging, or annealing is the phenomena known when an amorphous solid behave as if it always recognises the presence of the more stable equilibrium glassy state and continuously evolves towards it. In contrast, when a material is isolated in a metastable crystalline state, it may behave as if it is independent from the stable crystalline form, until a ‘catastrophic’ first-order polymorphic transition takes place. If structural relaxation occurs exponentially, a characteristic time, T, can be defined, which is a measure of the ‘mobility’ in material (Yu, 2001).

1.6.7 Dissolution

Since a several fold increase in solubility can be obtained, when using the amorphous form compared to the crystalline counterparts, the amorphous form is often presented as a possibility to improve the solubility and dissolution rate of poorly water soluble drugs (Hancock and Parks, 2000). In order to prevent the lowering of the dissolution rate through the gradual change of the amorphous form to that of the stable crystalline form, the drug has to remain amorphous during the entire storage time as well as during the dissolution, in other words, the conversion rate of the metastable form has to be slower than the dissolution rate of the metastable form (Debnath *et al*., 2004).

1.6.8 Pharmaceutical importance of the amorphous form

Amorphous forms, due to their low packing efficiency and lack of long range order, present higher potential energy than their crystalline counter parts (Yu, 2001). This higher potential energy means physical instability and potential conversion to a thermodynamically more stable crystalline form may occur over time. This conversion time is dictated by kinetics. If the kinetics is sufficiently slow relative to the pharmaceutically significant time frame, the amorphous state may still be utilised in drug products (Dannenfelser *et al*., 2004).

Due to their higher molecular mobility amorphous forms often exhibit stronger chemical reactivity and thereby faster degradation rate. The chemical degradation rate is dependent on the energy state of the glass and the scale of the molecular movement that is involved in the particular degradation reaction (Xiang and Anderson, 2004).

Amorphous forms often represent higher solubility. This offers a technique for pharmaceutical scientist to enhance the bioavailability for those poorly water-soluble compounds (Cui, 2007).

1.7 Conclusion

During the last two to three decades the application of amorphous solid-state forms within the pharmaceutical industry gained much interest. The rationale for this heightened interest is the increased aqueous solubility, increased dissolution rate and subsequent possible improved bioavailability offered by the amorphous form of a drug. Although amorphous solid-state forms of drugs are not considered something new within the pharmaceutical industry, thorough reviewing of current and older literature on this topic, shows that much is still to be discovered, learned and understood about this very interesting field within the solid-state chemistry of drugs. In order to gain a better understanding of amorphous solid-state behaviour, three structurally unrelated active pharmaceutical ingredients, namely zopiclone, sulfadoxine and roxithromycin have been selected for this study.

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CHAPTER 2

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The stabilization of amorphous zopiclone in an amorphous solid dispersion

Marnus Milne[1], Wilna Liebenberg[1], Marique Aucamp[1*]

[1]Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, Potchefstroom Campus, North-West University, 2520, Potchefstroom, South Africa

[*]Address for correspondence: Marique Aucamp, Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, Potchefstroom Campus, North-West University, 2520, South Africa, Tel: +27 182994472, Fax: +27 872315432, Email: Marique.Aucamp@nwu.ac.za

Abstract

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

Key words: amorphous; dihydrated; zopiclone; fragile; stability; solid dispersion.

Introduction

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

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

Zopiclone has zero number of rotatable bonds in its chemical structure, and is therefore said to have a rigid structure. According to literature, this together with the low molecular weight of zopiclone, suggests it to have a low glass forming ability (GFA) (13). Considering all mentioned aspects of zopiclone, the successful preparation of amorphous zopiclone could result in an increased solubility and bioavailability of this drug, but the effect of stability needs to be considered (8). In this study, we report on the preparation of amorphous zopiclone through quench-cooling of an anhydrous form of zopiclone, as well as the preparation of a stable solid dispersion and the physico-chemical and recrystallization properties thereof.

Materials and methods

Materials

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

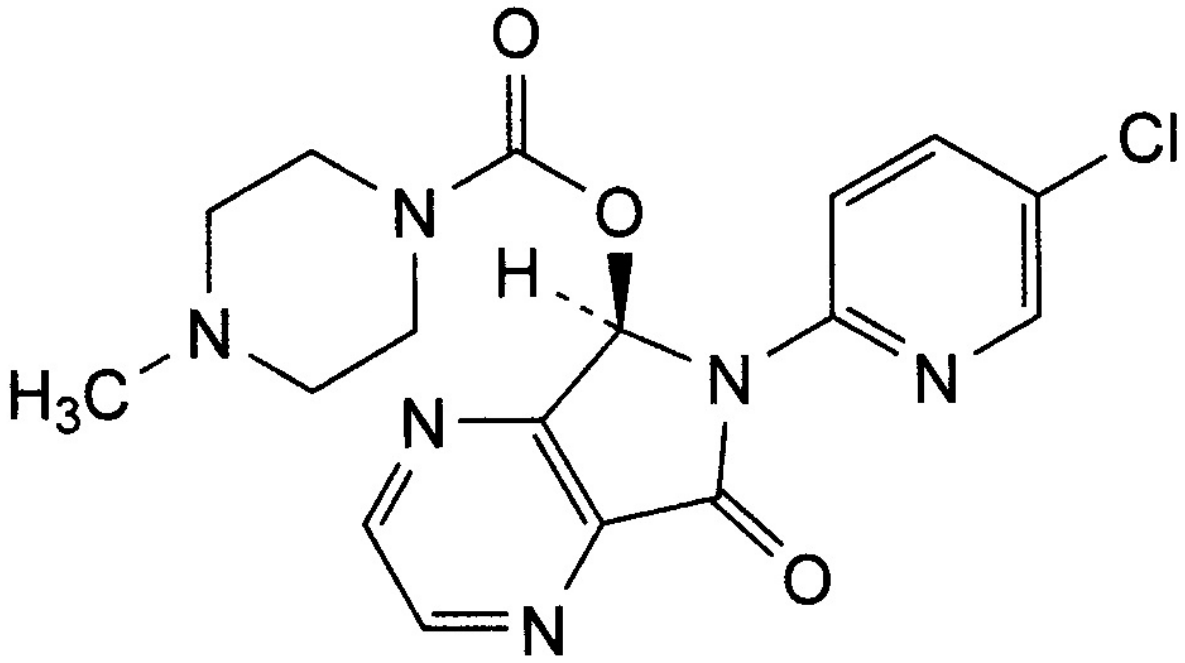

Figure 1: The chemical structure of zopiclone (3).

Preparation of anhydrous zopiclone

A dihydrate recrystallized from toluene was prepared (14). Approximately 1 g of zopiclone was added to 10 mL of toluene while stirring continuously and heating the solution to 60 ± 5°C. A few drops of cold ultrapure water were subsequently added to the solution. The beaker containing the saturated solution was covered with Parafilm®. After slow evaporation

of the toluene, crystals were obtained. The recrystallized material was removed from the solvent and dried on filter paper to ensure evaporation of surface solvent. The solid-state form was confirmed through DSC, TGA, Karl Fischer and XRPD analyses. This dihydrate solid-state form was then dehydrated by drying the recrystallization product in a laboratory oven (Binder, Tuttlingen, Germany) at 80°C for 24 h. The anhydrous form was confirmed by means of thermal analysis i.e. a melting endotherm (±150°C, a recrystallization exotherm (± 164°C), followed by a final melting endotherm (± 180°C). Furthermore it was characterized by its characteristic XRPD (Bragg) peaks, which are seen at °2θ values of 5.66, 13.58, 16.07, 16.39, 20.32, 20.52, 24.98, 25.49, 26.30 and 27.14 (15).

Preparation of amorphous zopiclone

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

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

Preparation of the amorphous solid dispersion

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

potency of the amorphous solid dispersion was 99% ± 1%. The optical rotation was also determined of zopiclone incorporated into the amorphous solid dispersion.

Differential Scanning Calorimetry (DSC)

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

Thermogravimetric analysis (TGA)

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

Infrared spectroscopy (IR spectroscopy)

IR-spectra were recorded using a Shimadzu IR Prestige-21 spectrophotometer (Kyoto, Japan) over a range of 400 - 4000 cm^{-1}. Potassium bromide (KBr) was used as a background. The diffuse reflectance method was implemented and involves grinding of approximately 1 mg of the sample with KBr and measuring its IR spectrum in a reflectance cell.

X-ray powder diffraction (XRPD)

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

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

Optical rotation

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

Hot-stage microscopy (HSM)

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

Scanning electron microscopy (SEM)

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

HPLC analysis

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

Karl Fischer (KF) titration

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

Vapor sorption analysis

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

Equilibrium solubility studies

Approximately 100 mg of crystalline zopiclone raw material, the quench cooled amorphous form and the amorphous solid dispersion were respectively weighed into test tubes (n = 7). 10 mL distilled water (25°C) was pipetted into each test tube. The test tubes were sealed with Parafilm® and tightly capped in order to prevent any leakage, followed by affixing the test tubes to a rotating axis in a water bath set at 37°C. The axis was set to rotate at 54 rpm. Withdrawals were taken on 10, 20, 30, 60, 120, 180, 240, 480, 5760 minutes for all three forms. From this data it was calculated that a period of 24 hours for both the raw material and the amorphous form was required to reach equilibrium solubility. For the solid dispersion a period of 96 hours was necessary.

Powder dissolution testing

A VanKel700 dissolution bath (Varian, Cary, USA) was used for dissolution testing. USP apparatus 2 (paddle) was set up at 37°C with a rotational speed of 100 rpm, 900 mL distilled water was added to each dissolution vessel. Approximately 200 mg of crystalline zopiclone (raw material) and 900 mg of the amorphous solid dispersion (weight equivalent to 200 mg

zopiclone) was weighed into 10 mL test tubes, to which 100 mg and 450 mg glass beads, ≤106 µm (Sigma–Aldrich, South Africa) was added respectively. 5 mL of dissolution medium (distilled water maintained at 37°C) was added to each test tube. The mixtures were agitated for a period of 120 s, using a vortex mixer. The resulting mixtures were transferred to each dissolution vessel. 5 mL of solution was withdrawn from each dissolution vessel at predetermined time intervals. The dissolution medium was not replaced after each withdrawal since a supersaturated solution is required to observe solution-mediated transformations (17). After withdrawal, the samples were filtered through a 0.45 µm PVDF filter into an HPLC vial. The filtered solutions were analyzed by HPLC.

Results

Amorphous zopiclone prepared from quench cooling of the melt

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

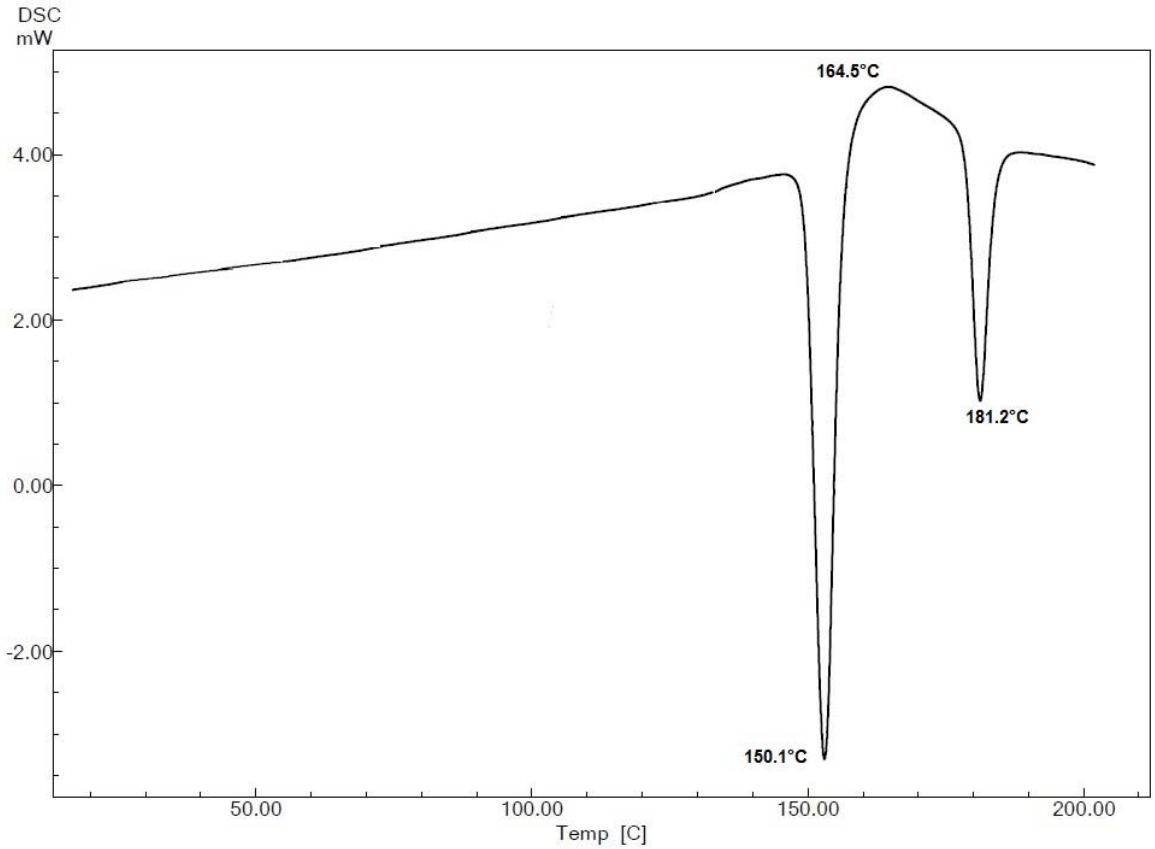

Figure 2: The DSC thermogram obtained for anhydrous zopiclone that was prepared through the dehydration of zopiclone dihydrate.

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

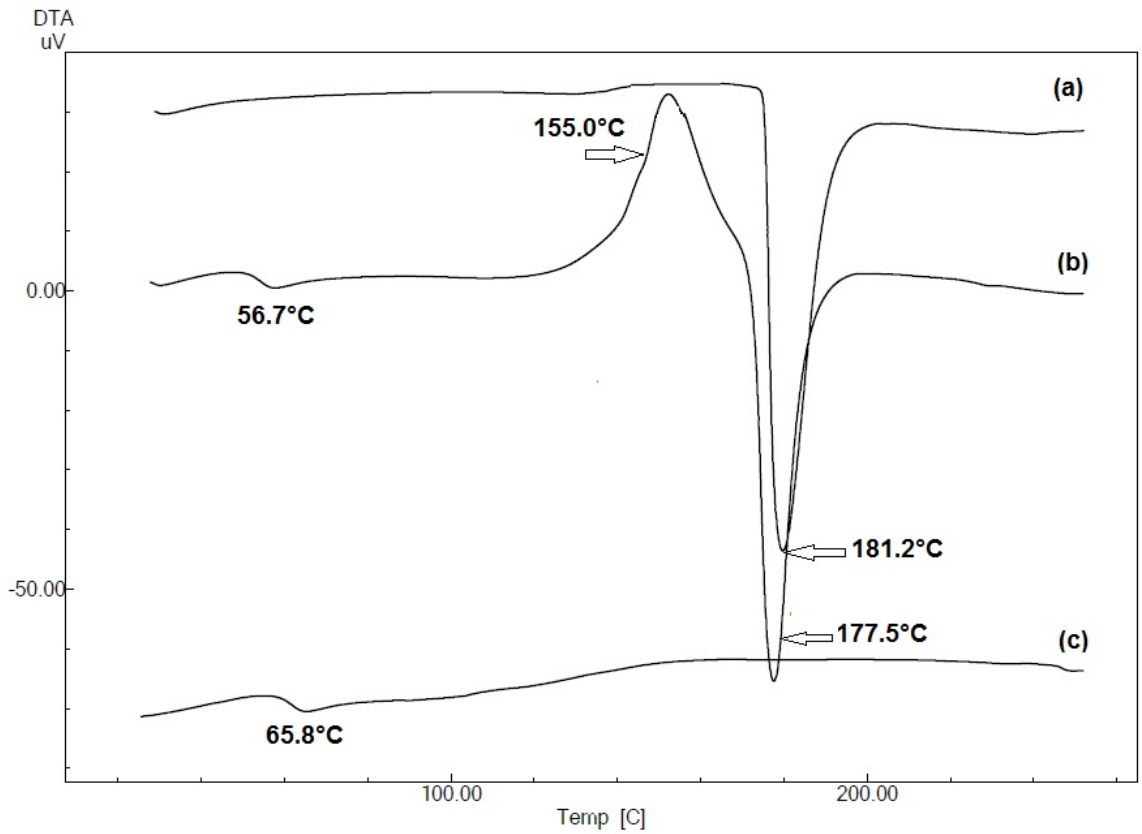

Figure 3: The DSC thermograms of (a) commercially available crystalline zopiclone, (b) amorphous zopiclone and (c) the amorphous solid dispersion.

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

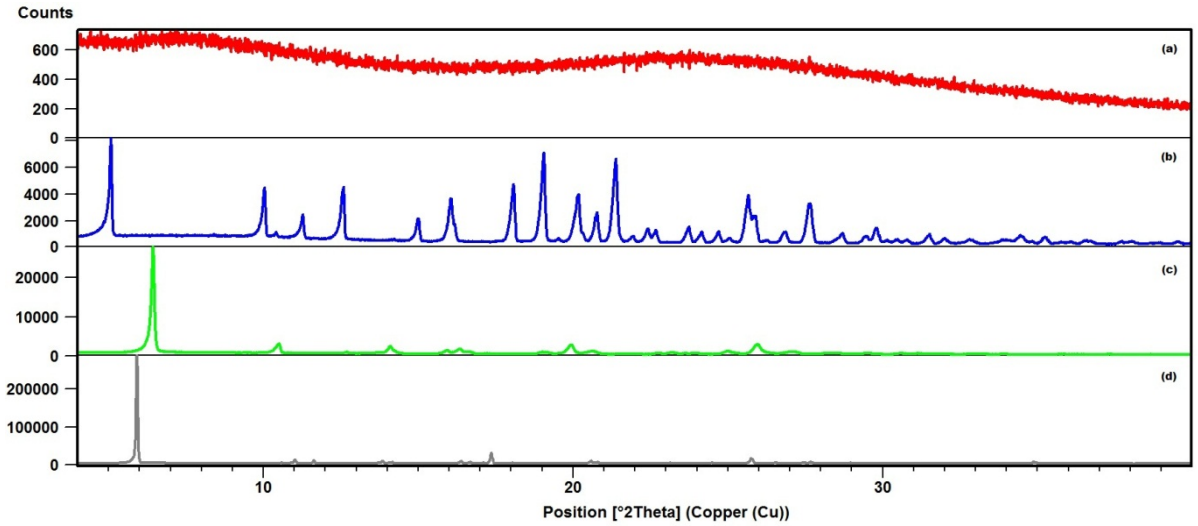

Figure 4: Overlay of the XRPD patterns of (a) amorphous zopiclone prepared through quench cooling of the melt and (b) crystalline zopiclone raw material (c) dihydrate prepared through recrystallization from toluene and (d) anhydrous zopiclone obtained from the dehydration of the dihydrate prepared through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

Physical and chemical stability of amorphous zopiclone

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

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

$$\eta = \eta_0 \exp \frac{DT_0}{T - T_0} \qquad (1)$$

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 (18-20) and D is the strength parameter. A large D value (> 30) represents a 'strong' glass forming behavior, while a low D value (<10) denotes a 'fragile' glass forming behavior. Fragility is a concept that may help explain the glass forming ability, and various other amorphous characteristics (18, 21). Amongst various methods available to determine the fragility, the dependence of T_g on the heating rate (q), in DSC measurements, was selected, due to the fact that it does

not necessitate precise thermodynamic parameters such as heat capacity (19, 20, 22). Subsequently, the fragility parameter (*m*) can be defined as:

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

and thus

$$m = \frac{\Delta H \eta^*}{(2.303\ R T_g)} \quad (3)$$

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

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

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 (ΔH^*) (27). The T_g was determined for amorphous zopiclone using DSC analysis at heating rates of 2, 4, 6, 8 and 10°C.min^{-1}. From this it was possible to calculate ΔH^* from the slope of the plot of *ln* heating rate (ϕ) vs. $1/T_g$ which subsequently enabled the calculation of *m* and *D* using Eq. (3) and (5) respectively (27).

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

The ΔH^* for amorphous zopiclone was calculated to be 556.30 kJ.mol^{-1}, the fragility parameter (*m*) 89.80 and the strength parameter (*D*) 9.15. From the calculated value of D it is clear that amorphous zopiclone, prepared through quench cooling of the melt, results in a

fragile amorphous form. With the fragility and strength parameters known T_0 can be determined by applying equation (4). The T_0 of amorphous zopiclone was determined to be 7.48°C. Considering the determined parameters it can be concluded that amorphous zopiclone prepared through quench cooling of the melt, resulted in a fragile glass with a relative low T_0. This suggests that amorphous zopiclone might be unstable and further processing techniques might induce recrystallization to the most stable form of zopiclone.

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

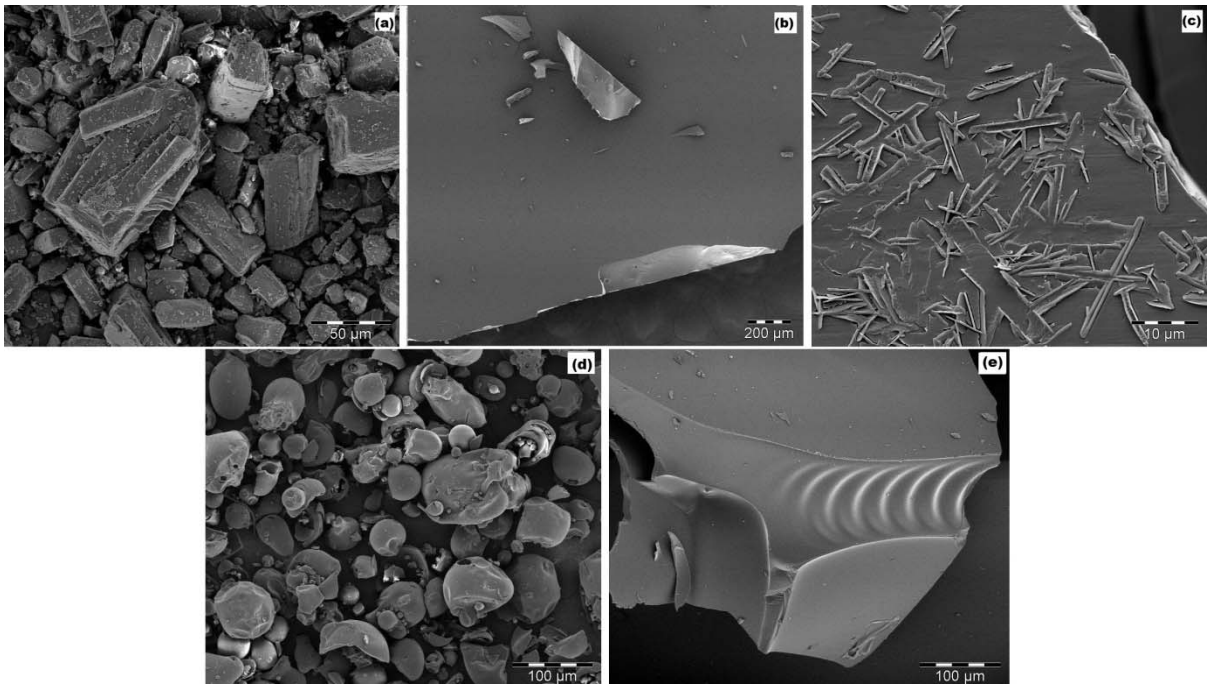

Figure 5: SEM micrographs of (a) zopiclone raw material, (b) amorphous zopiclone prepared through quench cooling of the melt, (c) surface recrystallization of amorphous zopiclone upon agitation of the sample, (d) polyvinylpyrrolidone (PVP-25) and (e) the obtained amorphous solid dispersion.

The influence of moisture on amorphous zopiclone was studied by means of moisture sorption analyses. Figure 6 depicts the moisture sorption isotherms obtained for amorphous zopiclone at ambient temperature.

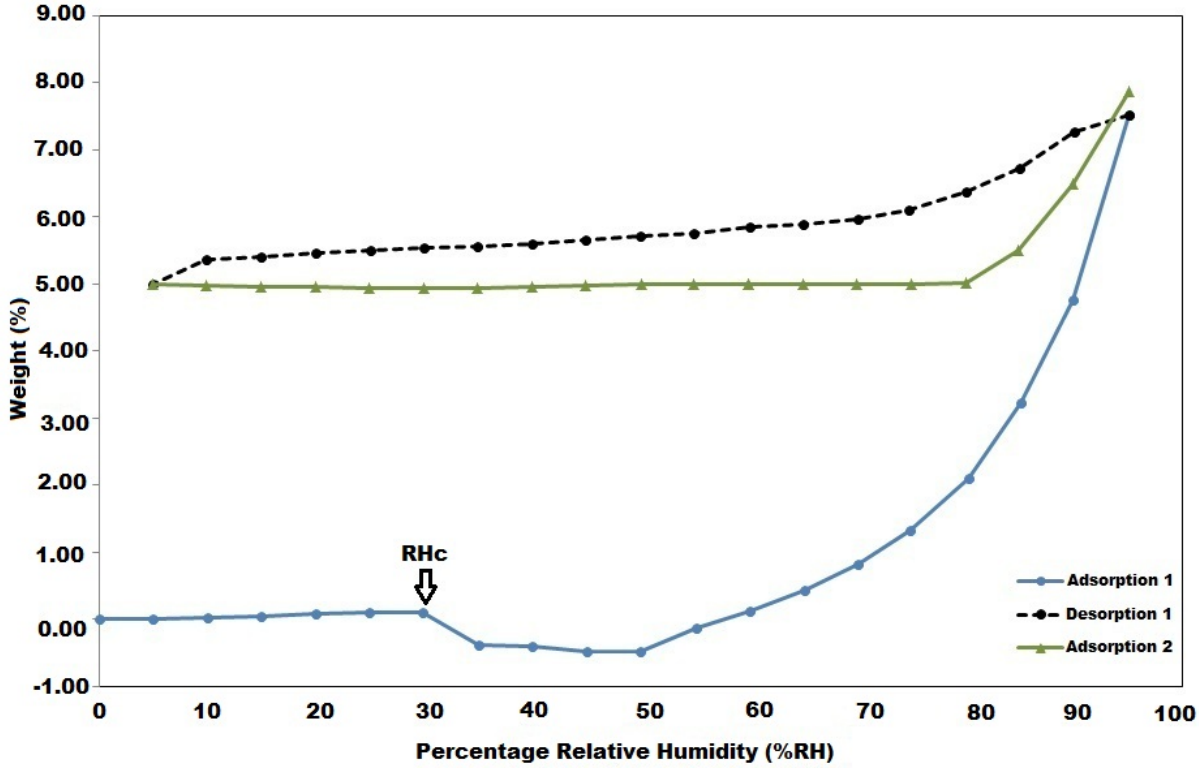

Figure 6: Moisture sorption isotherms obtained for amorphous zopiclone. The isotherms were obtained at 25°C with humidity variation of 5 - 95% RH.

The first adsorption isotherm showed 30% RH to be the humidity that would allow sufficient plasticization which would lead to the subsequent recrystallization for amorphous zopiclone. The sudden loss in sample weight can be attributed to water desorption during crystallization. Therefore the relative humidity where crystallization will occur for amorphous zopiclone was determined to be 30% RH. In terms of stability this is detrimental due to the fact that storage of amorphous zopiclone will have to be below 7.0°C and relative humidity of less than 30% RH. After the moisture sorption analysis the sample was subjected to Karl Fischer and TGA analyses. It was determined that the sample contains 8.9% water, which correlates well with the theoretically calculated water content of 8.8% for a zopiclone dihydrate. The crystallinity of the sample was furthermore confirmed by XRPD and it was evident that the XRPD pattern correlated with that obtained with the dihydrate form prepared through the recrystallization of zopiclone from toluene (Figure 7 (a) and (b)).

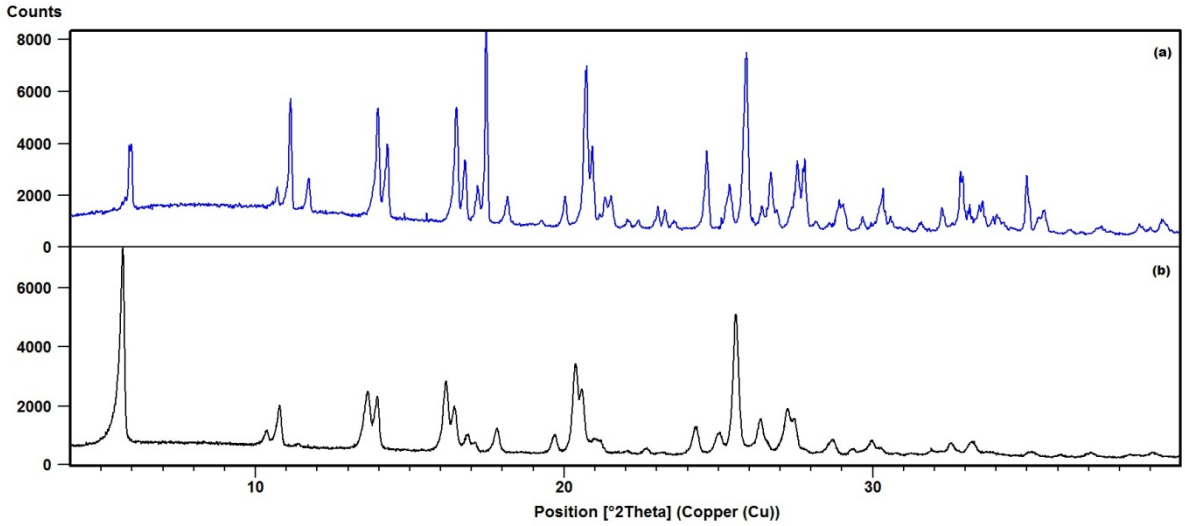

Figure 7: Overlay of the XRPD patterns obtained for (a) zopiclone dihydrate obtained by recrystallization from toluene and (b) zopiclone dihydrate obtained through the recrystallization process of amorphous zopiclone upon exposure to relative high humidity conditions.

From the determined parameters and the influence of moisture on amorphous zopiclone, it is evident that the stability is a questionable factor. The rapid recrystallization of amorphous zopiclone upon exposure to moisture also indicated that a possible experimental determination of the solubility advantage of this solid-state form will not be viable. Although it will not be possible to include amorphous zopiclone in any dosage form design processes, a stabilised amorphous form of this drug might lead to improved aqueous solubility. A thermodynamic approach, as reported by Hancock and Parks (7), was used to calculate the predicted or estimated enhancement in solubility of amorphous zopiclone. According to Hancock and Parks the solubility ratio of the two forms, σ^a/σ^c (amorphous/crystalline) at any temperature (*T*) is considered to be directly related to the difference in free energy (ΔG) between the amorphous and crystalline form (8).

$$\Delta G_T^{a,c} = -RT\ ln\frac{\sigma_T^a}{\sigma_T^c} \qquad (7)$$

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

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

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

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

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

$$\Delta S_f^c = \frac{\Delta H_f^c}{T_f^c} \quad (11)$$

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

Determination of the miscibility of zopiclone and PVP-25

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

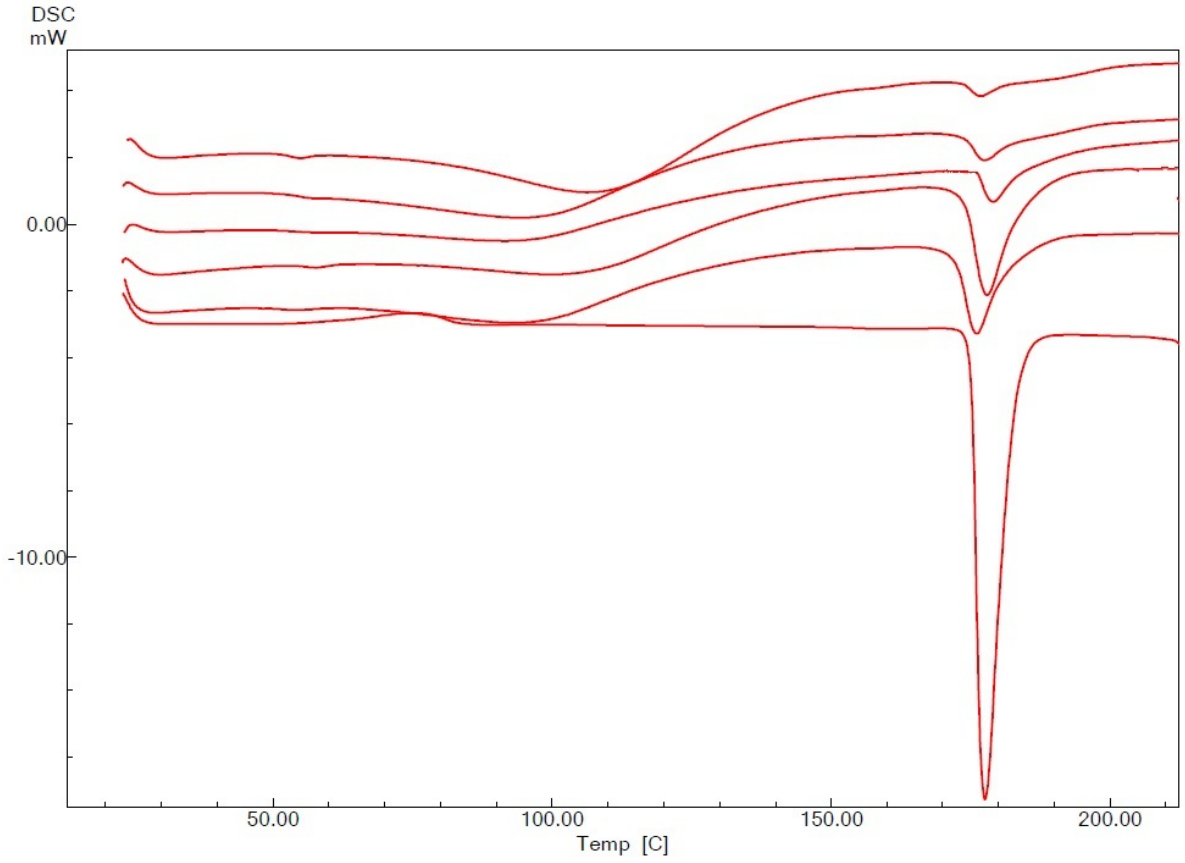

Figure 8: Overlay of the DSC thermograms obtained during the miscibility determination of crystalline zopiclone and PVP-25. Ratios are denoted as PVP:zopiclone.

The Flory-Huggins interaction parameter (χ) is a useful method to determine the free energy involved in the mixing of two components. The melting point of a pure drug occurs at a temperature at which the chemical potential of the crystalline drug is equal to the chemical potential of the molten drug (29, 30). Therefore, if the drug is miscible with a polymer, the chemical potential of the drug-polymer mixture must be less than the chemical potential of the pure amorphous drug. On the other hand, if the drug and polymer are immiscible no melting point depression is expected due to the fact that the chemical potential of the molten drug is unchanged by the presence of the polymer (29, 30). In this study the Flory-Huggins interaction parameter (χ) was determined for a zopiclone-PVP-25 mixture. Equation 12 shows the relationship between the melting temperature of the pure drug (T_M^{pure}), the depressed melting temperature of the pure drug (T_M^{mix}) and the Flory-Huggins interaction parameter (χ).

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

Where R is the gas constant, T is the absolute temperature, n_1 is the number of moles of drug, Φ_1 is the weighted volume fraction of the drug, n_2 is the number of moles of polymer

and Φ_2 is the weighted volume fraction of the polymer. For a 1:1 zopiclone: PVP-25 mixture an interaction parameter (χ) of -0.2 was calculated. A negative or close to zero interaction parameter is an indication of a miscible system (30). It can therefore be deduced that for the combination of crystalline zopiclone with PVP-25 sufficient miscibility is obtained in a weight ratio of 1:1.

Physical characterization of a zopiclone amorphous solid dispersion

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

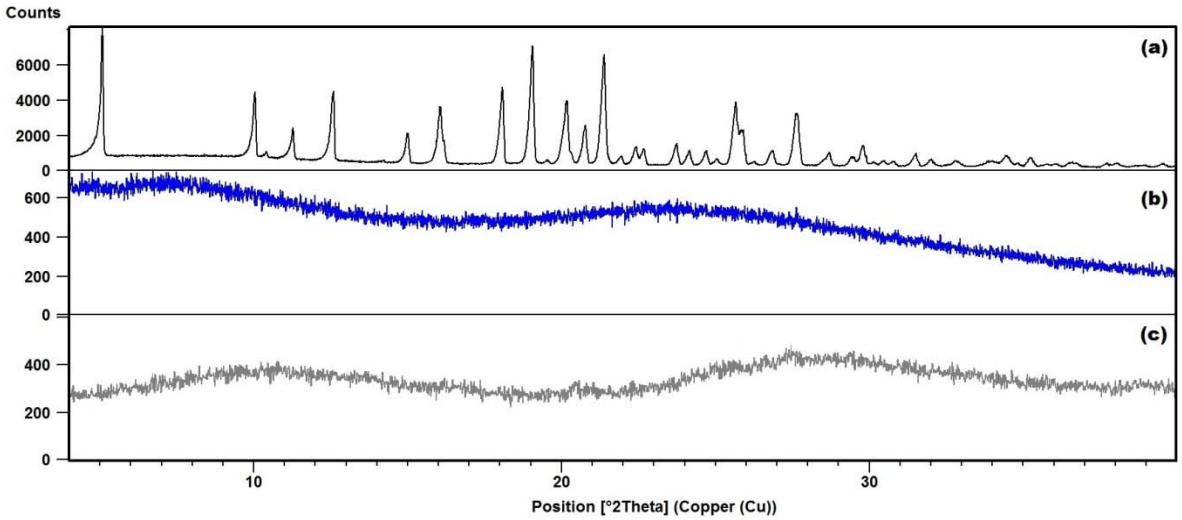

Figure 9: Overlay of diffractograms obtained for: (a) zopiclone raw material, (b) amorphous zopiclone and (c) amorphous solid dispersion.

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

The amorphous habit is confirmed by the broadening of the peaks between 4000 cm^{-1} and 3500 cm^{-1}, 3000 cm^{-1} and 2500 cm^{-1}, 2500 $cm^{-1}$and 2000 cm^{-1} (Figure 10 (b)).

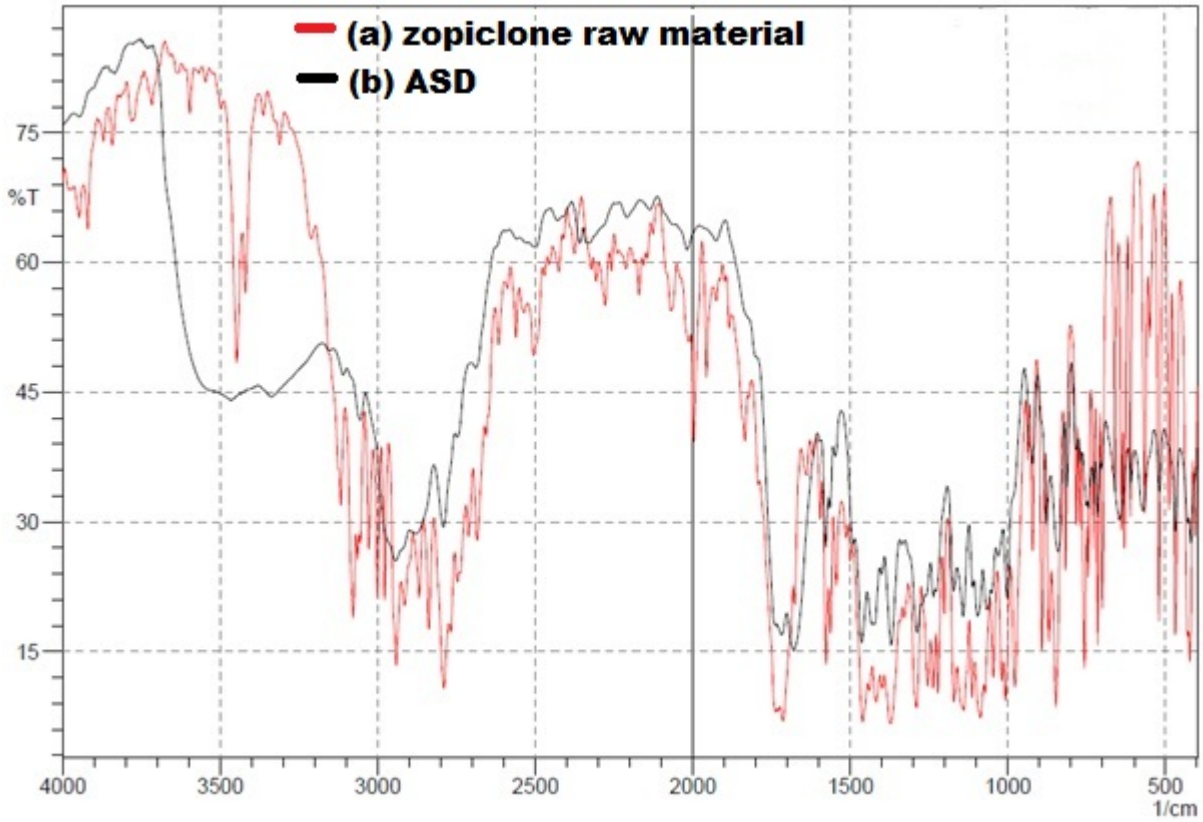

Figure 10: Overlay of the IR spectra obtained for crystalline zopiclone (a) raw material and the (b) ASD.

Borea *et al*., and Bertolasie *et al*., reported that the C=O group of the pyrrolopyrazine ring is the main receptor binding site of zopiclone on the benzodiazepine receptor complex and that the C=Cl group of the chloropyridine ring and the 4-methyl-1-piperzaine carboxylate fragment are regions responsible for an increase in the agonistic properties of zopiclone (28, 31). Table I summarizes the important functional groups and their characteristic absorption bands, all present in the ASD.

Table I: Summary of the important functional groups, their characteristic absorption bands, and the three different solid-state forms of zopiclone

Wavenumbers (cm^{-1})			
Group	**C=Cl**	**C=O**	**O-C=O**
Frequency range	700-800	1690-1760 1050-1300	1690-1760 10500-1300
Zopiclone raw material	Present	Present	Present
Amorphous solid dispersion	Present	Present	Present

The pharmaceutical significance of a zopiclone amorphous solid dispersion

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

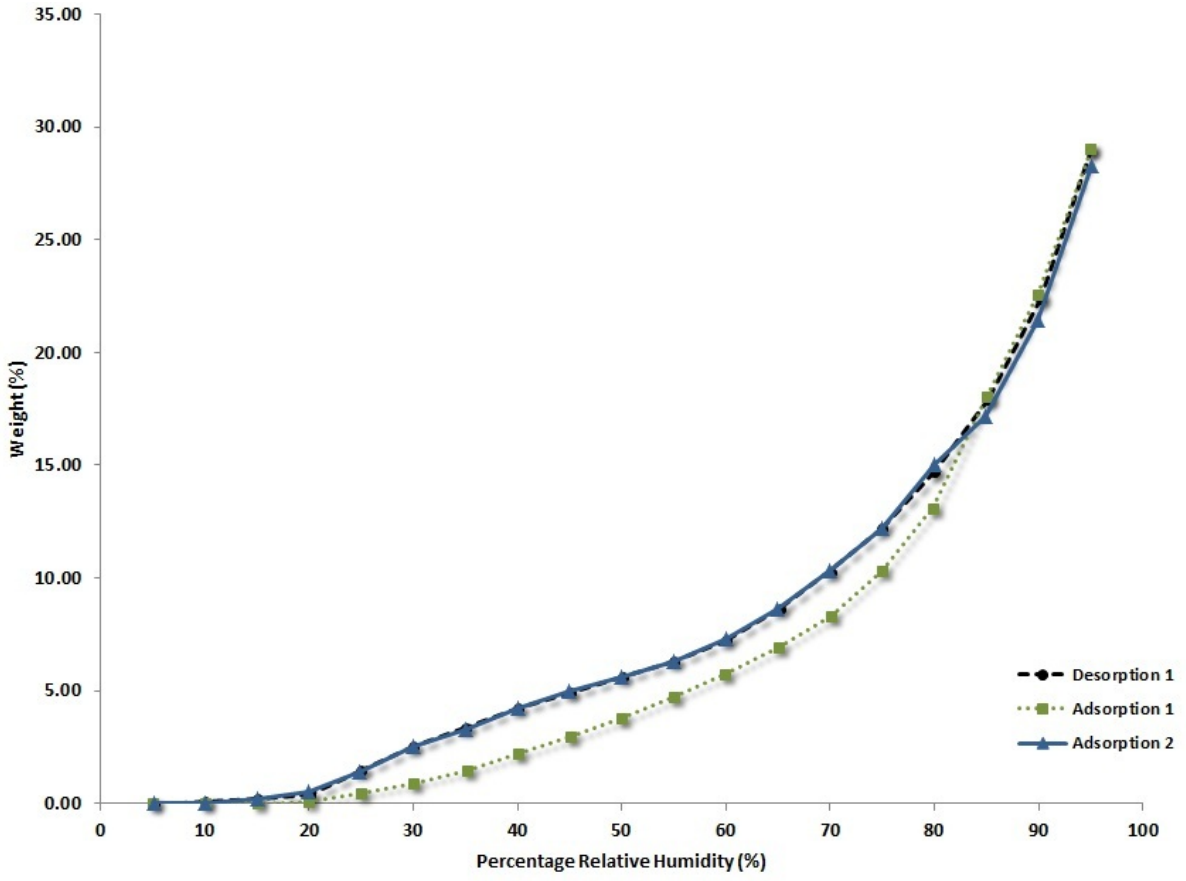

Figure 11: Vapor sorption isotherms obtained for the amorphous solid dispersion of zopiclone at ambient temperature.

XRPD experiments at ambient temperature were used to study the recrystallization tendency of the amorphous solid dispersion while exposed to a sufficient amount of water that will allow dissolution of the amorphous solid dispersion (Figure 12). From these results it is clear that although the ASD is more stable than neat amorphous zopiclone, recrystallization of zopiclone still occur through the process of solution-mediated phase transformation. Solution-mediated phase transformation is a phenomena that occurs when a metastable form of a drug (i.e. amorphous form) dissolve in a solvent phase and from that solution a stable solid-state form nucleates and grows (17, 32). Considering this, it is clear that such a transformation will typically have a detrimental effect on the dissolution behavior of a drug due to the fact that the more thermodynamically stable a solid-state form of a drug is the less soluble it becomes. Aucamp *et al*. (32), also studied the phase transformation of amorphous roxithromycin through the solution-mediated mechanism by means of XRPD analysis. During this study the detrimental effect that the recrystallization of the amorphous drug had on the dissolution rate and dissolved drug concentration was clearly demonstrated. It should however be mentioned that although recrystallization of the drug was evident from the XRPD analyses, this recrystallization process did not result in the crystal growth of a highly

crystalline hydrated form of zopiclone. Comparison of the XRPD data (Figure 12) showed that the peak intensities obtained after 210 minutes was significantly lower than that of zopiclone raw material. Therefore, it can be deduced that although recrystallization of the ASD was identified it occurs at a slow rate. However, since recrystallization of zopiclone ASD was identified with the XRPD analyses the effect of such a recrystallization on the dissolution behavior of the drug was imperative.

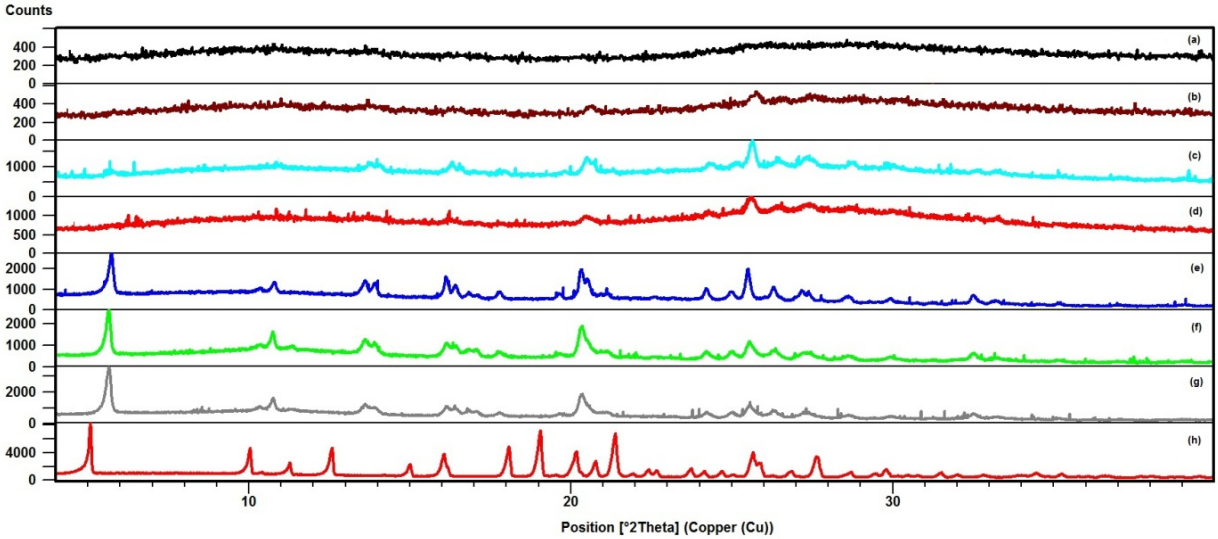

Figure 12: XRPD overlay of the amorphous solid dispersion at (a) initial, (b) 30, (c) 60, (d) 90, (e) 120, (f) 150, (g) 210 minutes and (h) reference diffractogram for crystalline zopiclone raw material.

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

The rationale for these dissolution parameters was to allow the study of the solution-mediated phase transformation of amorphous zopiclone to the stable crystalline form of zopiclone. The equilibrium solubility of crystalline zopiclone was determined to be 0.22 mg/mL, after 24 hours. The amorphous solid dispersion showed a solubility of 0.63 mg/mL after 24 hours, this is a 3-fold increase in the solubility of zopiclone. Figure 13 depicts the comparison of dissolution profiles of zopiclone amorphous solid dispersion (a) and the crystalline raw material (b) in distilled water at 37°C for a period of 24 hours. The dissolution profile of the ASD shows almost complete dissolution of zopiclone with a peak concentration of 0.56 mg/ml after 60 minutes in comparison with the lower dissolved concentration of 0.23 mg/ml obtained with crystalline zopiclone. This is a significant difference of 55.92%. The

decrease in the dissolved concentration after 60 minutes is most probably due to solution-mediated phase transformation of the amorphous drug, however it is clear that the transformation process is not a complete transformation since the dissolved concentration doesn't decrease to such an extent that it would correlate to the dissolved concentration of crystalline zopiclone. The question remains whether the resulting improved dissolution rate and higher drug solubility is truly due to the amorphous state in which the drug is captured or does the presence of the polymer play a more pronounced role?

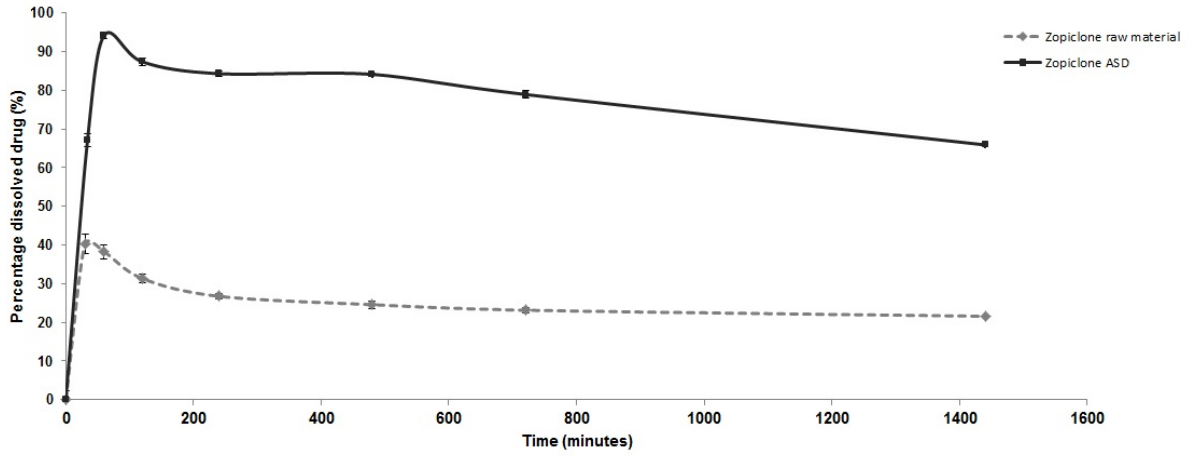

Figure 13: Comparison of the dissolution profiles of zopiclone amorphous solid dispersion and crystalline zopiclone raw material in distilled water at 37ºC.

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

4. Conclusion

During this study, an amorphous form of zopiclone was prepared by the quench cooling of the melt of anhydrous zopiclone. However, the determined fragility and strength parameters indicated that the prepared amorphous form of zopiclone is extremely fragile. It was concluded that the stability of the neat amorphous form is influenced by temperature,

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

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Disclaimer

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CHAPTER 3

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Instructions to authors (summary) – see chapter 1

My contribution to this manuscript can be outlined as follows:

The preparation and performance of the DSC, TGA, HSM and HPLC work, as well the preparation of samples for SEM, XRPD, isothermal microcalorimetry and vapor sorption analysis. I furthermore assisted with the reading and writing of the paper.

Amorphous sulfadoxine: a physical stability and crystallization kinetics study

Marique Aucamp[1*], Marnus Milne[1], Wilna Liebenberg[1]

[1]Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, Potchefstroom Campus, North-West University, 2520, Potchefstroom, South Africa

[*]Corresponding author: Marique Aucamp, Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, Potchefstroom Campus, North-West University, 2520, South Africa, Tel: +27 182994472, Fax: +27 872315432, Email: Marique.Aucamp@nwu.ac.za

Abstract

Poor aqueous solubility of drugs and the improvement thereof has always been a challenge for the pharmaceutical industry. With this, one of the focuses of the pharmaceutical research scientist involves investigating possible metastable forms of a given drug to be incorporated into solid dosage forms. The rationale being, the improved solubility offered by the metastable solid-state forms of drugs. Solubility remains a major challenge for formulation scientists, especially with antimicrobial agents where the emergence of resistance is directly dependent on the concentration and duration of the parasite exposed to the drug. Sulfadoxine-pyrimethamine combination therapies are still the recommended treatments for uncomplicated *P. falciparum* malaria. The aim of this study was to prepare an amorphous form of sulfadoxine and to investigate the stability and recrystallization behavior thereof. The amorphous form was prepared by the well-known quench cooling of the melt. The physico-chemical properties and stability of amorphous sulfadoxine were studied using hot-stage microscopy (HSM), scanning electron microscopy (SEM), x-ray powder diffractometry (XRPD), differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA) as well as microcalorimetry. The recrystallization kinetics were studied isothermally by applying the Johnson-Mehl-Avrami model and non-isothermally by applying the Kissinger model. The physical stabilization of the amorphous form was investigated using physical mixtures of amorphous sulfadoxine with polyvinylpyrrolidone-25 (PVP-25). It was proved that sulfadoxine is a good glass former with relative high physical stability, however water acts as a strong plasticizer for amorphous sulfadoxine, detrimentally affecting the stability during exposure to high moisture conditions.

Keywords: amorphous; sulfadoxine; stability, crystallization kinetics.

1. Introduction

The aqueous solubility of drugs is an age-old and everlasting challenge for the pharmaceutical industry. Usually, a poorly soluble drug leads to an array of problems - from the process of product formulation right up to and including patient treatment. As a result, the pharmaceutical industry started to consider the inclusion of amorphous solid-state forms into solid dosage forms. Current products are being revisited and possibilities that would lead to improved performance of poorly soluble drugs are being investigated extensively.

Rendering a drug amorphous is one common method used to improve the aqueous solubility of a drug (1). In turn, improved aqueous solubility could lead to improved absorption and bioavailability (2, 3, 4). The advantage of amorphous materials lies within the lack of a clearly defined molecular structure (5, 6). Due to the absence of repeating long-distance molecular order, an amorphous material displays higher molecular mobility and greater inter-molecular distances (6). They possess a higher free energy state compared to the crystalline form of the same drug, a property which can result in improved solubility and dissolution rate of the drug (6). On the other hand, the fact that it exists in a metastable state, brings forth the disadvantage that it can crystallize to a more thermodynamically stable crystalline state (7). This can occur upon exposure to increased temperature and/or moisture (8). From a manufacturing point of view this would be disadvantageous and needs to be prevented.

Sulfadoxine is a long-acting sulphonamide antibacterial agent. Sulfonamides are structural analogues and competitive antagonists of p-aminobenzoic acid. Furthermore, they are competitive inhibitors of dihydropteroate synthase, the bacterial enzyme responsible for the incorporation of p-aminobenzoic acid in the synthesis of folic acid. Artemisinin-based combination therapies (ACTs), including artesunate plus sulfadoxine-pyrimethamine, are still the recommended treatment for uncomplicated *P. falciparum* malaria today (9). In literature one can find numerous reports of poor *in vitro/in vivo* dissolution profiles of the sulfadoxine/pyrimethamine combination products (10, 11, 12, 13, 14). Although these studies report on the poor dissolution of sulfadoxine, none clarified or provided a reason for such results. Taking the aforementioned as well as the high morbidity and mortality rates due to malaria into account, (15, 16), one cannot argue that concern should be raised regarding the unsolved problem of poor solubility and dissolution of sulfadoxine.

Currently, no evidence can be found of different solid-state forms in which sulfadoxine can exist and up to this point in time no amorphous form of sulfadoxine has been reported. The objective of this study was to investigate the possibility of preparing an amorphous solid-

state form of sulfadoxine and furthermore to investigate possible stability problems associated with the amorphous form of this anti-malarial drug.

2. Materials and methods

Crystalline sulfadoxine was obtained from DB Fine Chemicals (Johannesburg, South Africa), with a potency of 99.2%. HPLC grade acetonitrile was purchased from ACE Chemicals (Johannesburg, South Africa), glacial acetic acid, triethylamine and sodium hydroxide was of analytical grade and was purchased from Merck (South Africa). The polymer (polyvinylpyrrolidone-25) was obtained from DB Fine Chemicals (Johannesburg, South Africa). Water used throughout this study was ultra-pure grade with a resistivity of 18.2 $M\Omega.cm^{-1}$.

Preparation of amorphous sulfadoxine

Amorphous sulfadoxine was prepared by heating a small portion (approximately 500 mg) of the crystalline form to approximately 198°C, subsequently quenching it to room temperature on a cool surface. The amorphous habit was confirmed by X-Ray Powder Diffraction (XRPD), Differential Scanning Calorimetry (DSC) and visually through Hot-stage microscopy (HSM). Directly after preparation the purity of the amorphous sulfadoxine sample was confirmed by means of HPLC analysis. HPLC potency analysis indicated that potency of the amorphous sulfadoxine was within the range of 99 ± 1%.

Preparation of physical mixtures

Physical mixtures of amorphous sulfadoxine combined with polyvinylpyrrolidone (PVP-25) were investigated thermally. The mixtures were prepared through mixing amorphous sulfadoxine with PVP-25 using a mortar and pestle. For a homogenous physical mixture, mixing was done for 2 minutes. The mixtures were prepared in % w/w concentration and in ratios of: 1:1, 1:2 and 1:4 (drug:polymer).

Differential Scanning Calorimetry (DSC)

A Shimadzu (Kyoto, Japan) DSC-60 instrument was used to record the DSC thermograms. Samples (3 – 5 mg) were accurately weighed and sealed in aluminum crimp cells. The samples were heated from 25 to 250°C with a heating rate of 10°C/min and a nitrogen gas purge of 35 mL/min. The onset temperatures of the thermal events are reported and the glass transition temperatures (T_g) were determined from heating scans. For non-isothermal recrystallization studies of amorphous sulfadoxine, approximately 5 mg of the amorphous form was accurately weighed and sealed into aluminum crimp cells. For the samples to which crystalline sulfadoxine was added as seeds, approximately 4 mg of neat amorphous

sulfadoxine and 1 mg of crystalline sulfadoxine were weighed into aluminum crimp cells. The same nitrogen gas purge was used as mentioned above. Heating rates of 2, 5, 7, 10, 15 and 20°C/min were used (n = 3). For the resulting Kissinger plots the peak temperatures for the recrystallization of amorphous sulfadoxine was used.

Thermogravimetric analysis (TGA)

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

Hot-stage microscopy (HSM)

A small amount of sample was placed on the center of a glass microscope slide and viewed under the microscope. The presence or absence of amorphous material was evaluated by the observance of birefringence, under cross polarized light. HSM analysis was performed with a Nikon Eclipse E4000 microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan) and a Linkam THMS600 heating stage equipped with a T95 LinkPad temperature controller (Surrey, England). During heating experiments a heating rate of 5°C/min was used.

Scanning electron microscopy (SEM)

SEM images of the purchased crystalline sulfadoxine and the quench cooled amorphous form were also obtained in order to observe possible morphological differences between the samples. For SEM analyses, samples were coated with a layer of gold/palladium using an Eiko engineering ion coater IB-2 (Eiko Engineering, Ibaraki, Japan), and were subsequently imaged using a field-emission environmental FEI Corporation, Quanta 200ESEM (Hillsboro, Oregon, USA).

X-ray powder diffraction (XRPD)

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

Isothermal microcalorimetry

A 2277 Thermal Activity Monitor (TAMIII) (TA Instruments, USA) equipped with an oil bath with a stability of ±100 µK over 24 hours was used during isothermal recrystallization kinetic studies. The temperature of the calorimeters was maintained at 35, 45, 55 or 65°C. For each analysis approximately 50 mg of amorphous sulfadoxine was accurately weighed into a 4 ml glass ampoule. For samples in which case crystalline sulfadoxine was added as seeds, approximately 2 mg of crystalline sulfadoxine was added to each glass ampoule. The ampoules were tightly sealed with an aluminum crimp cap. The samples were lowered into the equilibration position of the TAMIII, the samples were left to equilibrate for 15 minutes, subsequently the samples were lowered into the measuring position, an additional 45 minutes were allowed for the calorimetric output signal to be considered correct. The experiments were conducted until a heat flow baseline with a drift of < 100 nW was obtained for 12h. After such a baseline was obtained the samples were considered to be fully crystallized. The resulting heat flow *versus* time plots were analyzed using TAMIII Lab Assistant Software V1.3.0.153 (TA Instruments, USA). Integration of the obtained power (heat flow) – time curves, allowed the determination of the heat output of the recrystallisation process. From a heat flow *versus* heat output plot and an iterative procedure applied by the software the rate constant and enthalpy change were then calculated for the recrystallisation process.

Vapor sorption analysis

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

High-performance liquid chromatography (HPLC)

HPLC analysis was done utilizing a Shimadzu (Kyoto, Japan) UFLC chromatographic system. The system consisted of a SIL-20AC auto-sampler fitted with a sample temperature controller, a UV/VIS Photodiode Array detector (SPD-M20A) and a LC-20AD solvent delivery module. The mobile phase consisted of glacial acetic acid, triethylamine and water (10: 0.5: 800, v/v/v). This solution was diluted to 1000mL, and the pH was adjusted to 4.2, with dilute sodium hydroxide. 800mL of this solution was added to 200mL acetonitrile. The mobile phase was filtered and degassed prior to use. A Luna C18 (150 × 3.9 mm) column was used with a flow rate set to 2.0 mL/min and a wavelength of 227nm (17,18).

3. Results and discussion

Up to this point in time no comprehensive description of the solid-state characteristics of sulfadoxine is available in literature. Also no mention towards other solid-state forms in which sulfadoxine may exist can be found with literature searches. The molecular structure of sulfadoxine is illustrated in Figure 1.

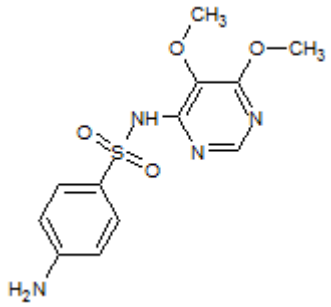

Figure 1: The chemical structure of crystalline sulfadoxine (11).

It consists of low level branched carbon skeletons, lacks molecular symmetry and consists of one benzene ring. According to a model utilized by Mahlin *et al.* (19) in a study to predict the glass forming ability of 16 novel compounds, these structural features of sulfadoxine should grant the drug a fairly high glass forming ability (19). Furthermore, sulfadoxine has four rotatable bonds along the C-S, C-NH and the two non-equivalent C-O-CH bonds. According to Mahlin *et al.* (19), this would decrease the likelihood of sulfadoxine being in the proper orientation to undergo nucleation or to be incorporated in an ordered crystalline lattice, resulting in an increased glass forming ability (19). During preliminary studies it was proved that sulfadoxine can be prepared in an amorphous solid-state form through the well-known, quench cooling of the melt, method. After the preparation of amorphous sulfadoxine the purity of the drug was confirmed through HPLC analysis. However, the fact that a drug can be classified as a good glass former does not imply that the drug will show good “glass stability”.

Glass forming ability and characterization of amorphous sulfadoxine

Figure 2 depicts the thermal analyses of both crystalline and amorphous sulfadoxine. For the commercially obtained sulfadoxine only one single melting endotherm was observed at approximately 196.76°C (ΔH_m = -122.08 J/g). The thermal analysis obtained for amorphous sulfadoxine exhibited a typical DSC thermogram of an amorphous solid-state form of a drug. The glass transition (T_g) was observed at ≅ 45.71°C (± 0.52°C), followed by a thermally induced recrystallization of sulfadoxine at 92.82°C (ΔH_c = 67.67 J/g). Subsequently, the recrystallized product melted at approximately 196.58°C. It should be noted that the scan rate used to obtain these results was 10°C/min. Therefore indicating that amorphous sulfadoxine recrystallized to the stable crystalline form upon heating above the T_g. The XRPD diffractograms of both crystalline and amorphous sulfadoxine are depicted in Figure 3. The absence of diffraction peaks obtained for amorphous sulfadoxine (Figure 3(b)) confirms the true amorphous habit thereof.

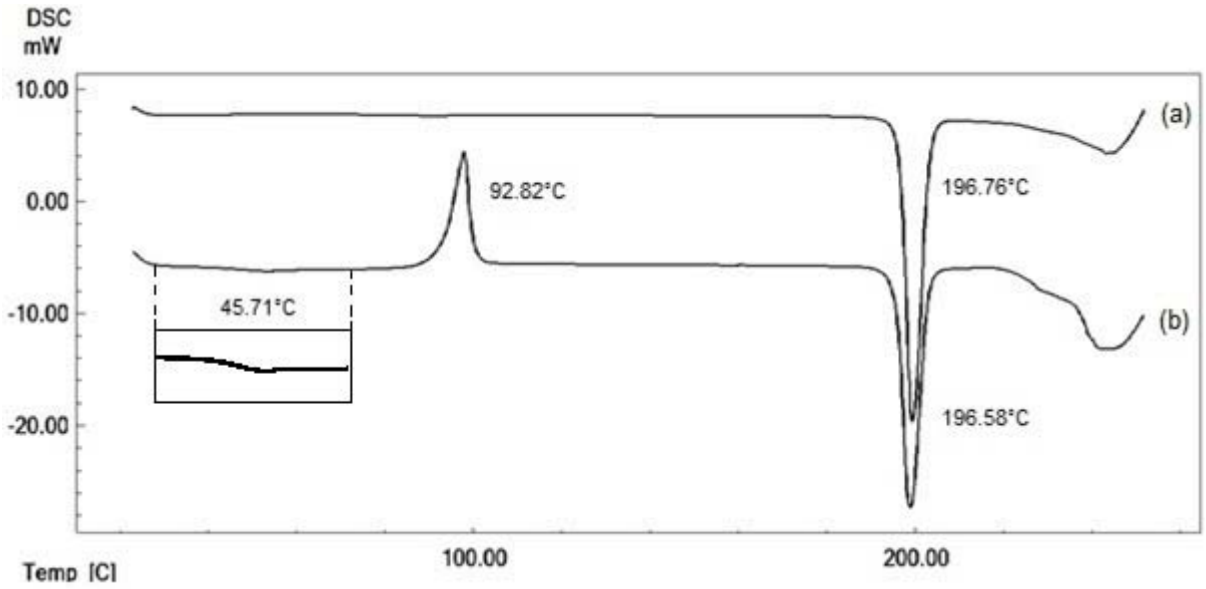

Figure 2: Overlay of DSC thermograms of (a) commercially obtained crystalline sulfadoxine and (b) amorphous sulfadoxine prepared through quench cooling of the melt, with a zoomed inset depicting the glass transition temperature (T_g).

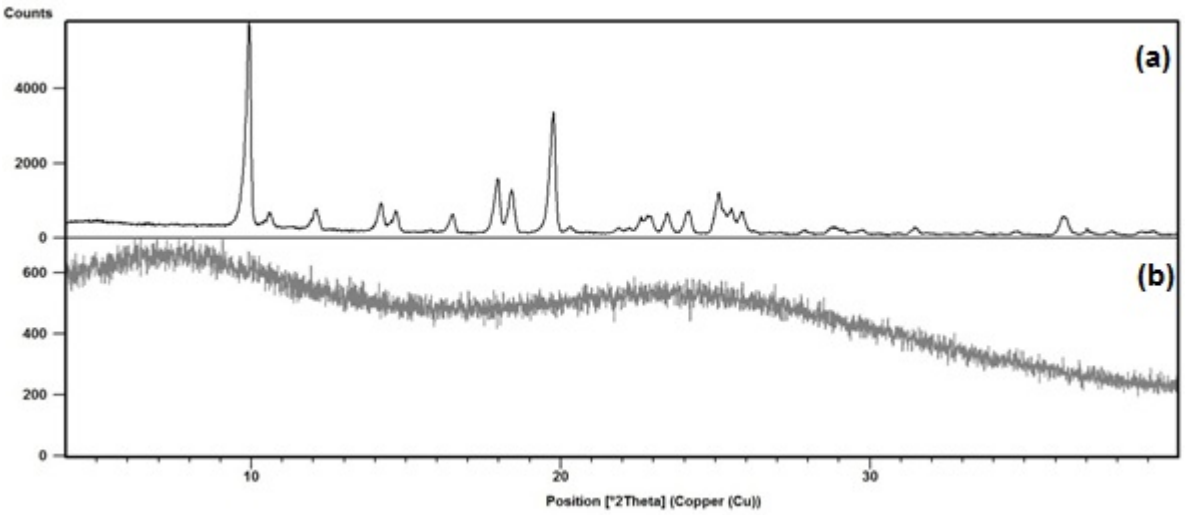

Figure 3: Overlay of XRPD patterns obtained for (a) commercially obtained crystalline sulfadoxine and (b) amorphous sulfadoxine prepared through quench cooling of the melt.

SEM images further confirmed the amorphous habit of sulfadoxine and were in good agreement with the thermal and crystallography results obtained. Figure 4(a) and (b) exhibits the plate-like like morphology of sulfadoxine. The smooth glassy surface of amorphous sulfadoxine is depicted in Figure 4(c) and (d)

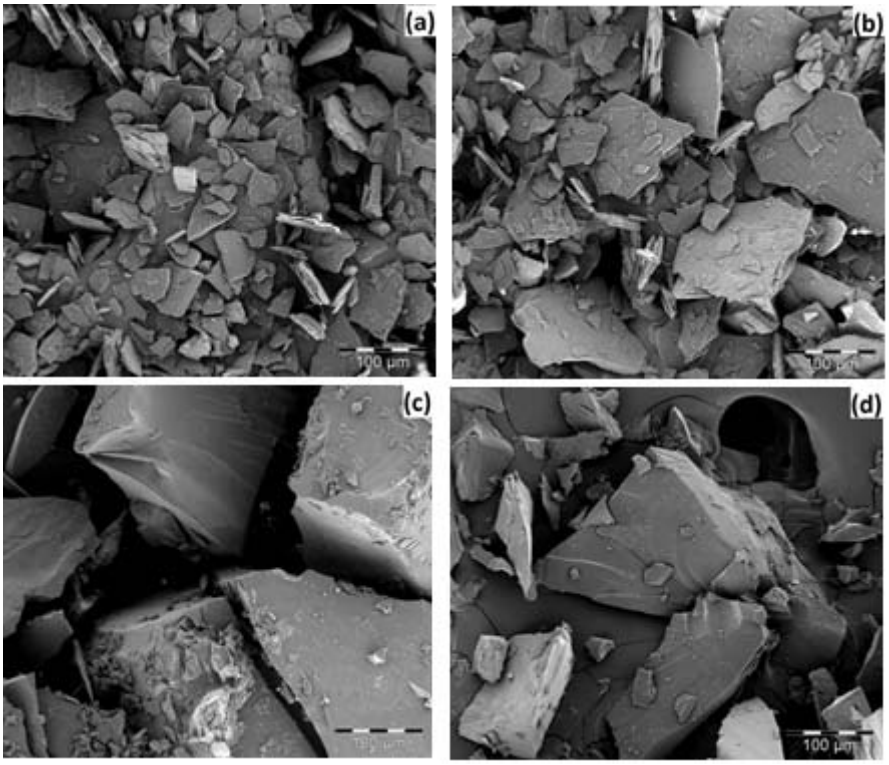

Figure 4: SEM micrographs of (a) and (b) commercially obtained sulfadoxine, (c) and (d) amorphous sulfadoxine prepared through quench cooling of the melt.

In order to accurately describe the solid-state properties of any amorphous form of a drug it is important to investigate parameters which would provide information on the physical stability thereof (20). The activation energy (ΔH^*) of the structural relaxation of amorphous sulfadoxine was determined from the glass transition temperature (T_g) at different heating rates (β) (Table I).

Table I: Glass transition temperatures (T_g) *versus* differing heating rates (β)

Heating rates (°C/min)	**T_g (°C)**
5.00	43.46
7.00	47.17
10.00	48.84
15.00	50.08
20.00	51.89

It is a well-known fact that the glass transition temperature of an amorphous solid-state form is dependent on the heating or cooling rate (β) used during thermal analyses (5, 22, 23, 24). Therefore, it should be well noted that T_g will shift when higher heating or cooling rates are being used. For this equation 1 applies:

$$\frac{d \ln|\beta|}{d1/T_g} = -\frac{\Delta H^*}{R} \qquad (1)$$

Figure 5 depicts the plot of ln(β) versus 1000/T_g. From the slope of this plot, activation energy (E_a) of 144.13 kJ.mol^{-1} was calculated. Another meaningful parameter to report in terms of an amorphous form of a drug is the fragility index. This characteristic indicates the increase in the rate of structural relaxation as a 'glassy' form of a drug approaches and passes through the glass transition region. The fragility index can be defined as the slope of a $\log_{10} \tau(T)$ versus T_g/T plot; where $T = T_g$. From the "Angell plot" for many material types it was deduced that τ (structural relaxation time) slows down to 100 s at T_g (5, 25, 26). From this, equation 2 defines the fragility index (m) as follow:

$$m = \left[\frac{d log 10\tau(T)}{d\left(\frac{Tg}{T}\right)}\right] \qquad (2)$$

The fragility of an amorphous system is a dimensionless parameter and actually describes the molecular kinetics which is dependent on the temperature of such a system (26).

Equation 2 can also be expressed in terms of temperature dependence and therefore during this study the fragility index (*m*) of amorphous sulfadoxine was calculated by applying equation 3, where the calculated apparent activation energy (E_a) was incorporated into equation 2:

$$m = \frac{1}{2.303}\left[\frac{E_a\,(T_g)}{RT_g}\right] \qquad (3)$$

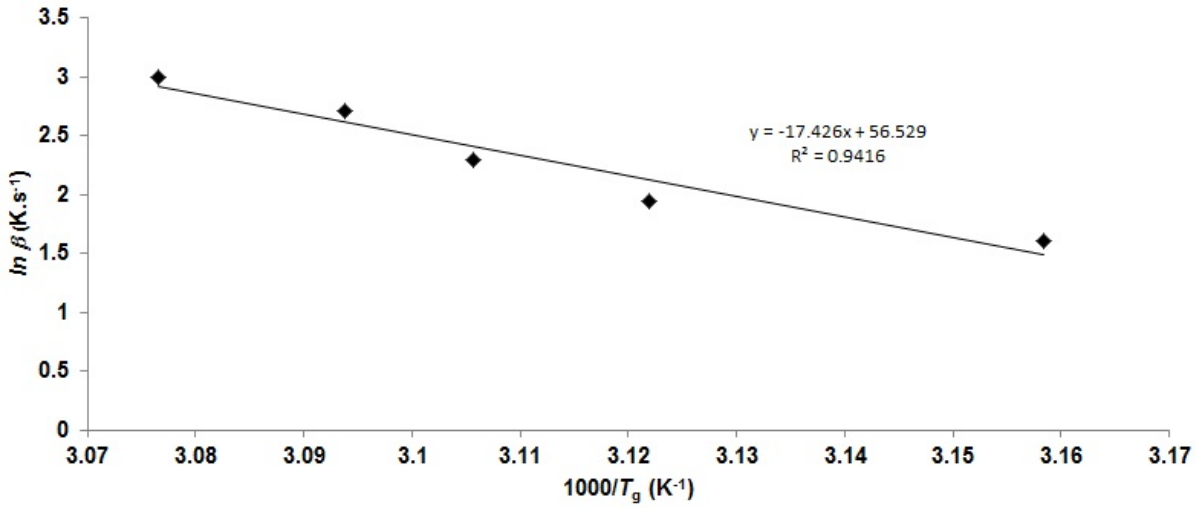

Figure 5: An Arrhenius plot for the glass transition (T_g) of amorphous sulfadoxine.

Furthermore, it was assumed that the viscosity at T_g is 10^{12}Pa s and η_0 is 10^{-5} Pa s, this allows for the calculation of the strength parameter (*D*) by applying equation 4:

$$D = \frac{666}{m-17} \qquad (4)$$

Where 17 is the order of magnitude in the viscosity change from the T_g to η_0. A parameter that closely correlates with the strength parameter is T_0 which is denoted as the temperature of zero mobility. This temperature is important in pharmaceutical amorphous solid-state forms due to the fact that it can act as a guide of storage temperature which would ensure maximum stability of the amorphous form of a drug. The correlation between *D* and T_0 can be expressed in the approximate form of equation 5 (5):

$$\frac{T_g}{T_0} = \frac{1+D}{39.1} \qquad (5)$$

In literature it is well described that strong amorphous systems will exhibit a value of $m < 40$ while a fragile system will show a value of $m > 75$ (5). In the case of the strength parameter (*D*), typical values between 3 and 7 will indicate a fragile amorphous form, while $D = 30$ to ∞ is indicative of a strong amorphous form (26). Considering this**,** it can be concluded that sulfadoxine forms a strong amorphous form (Table II).

Table II: Physico-chemical properties calculated for amorphous sulfadoxine

Physico-chemical property of amorphous sulfadoxine	Calculated values
Fragility index (*m*)	7.53
Strength parameter (*D*)	70.31
Reduced glass transition index (T_{rg})	0.68
Temperature of zero mobility (T_0) (°C)	25.06

A low fragility index is considered to be indicative of lower free energy, therefore a higher physical stability is probable for a strong amorphous system. The glass forming ability of sulfadoxine was further investigated by applying the reduced glass transition (T_{rg}) method. For this method equation 6 was used, where T_g is the glass transition temperature (K) and T_m is the melting temperature (K):

$$T_{rg} = \frac{T_g}{T_m} \qquad (6)$$

The closer the value of T_{rg} is to 1 the higher is the glass forming ability of the drug. The calculated value of 0.68 further proved the good glass forming ability of sulfadoxine. This correlating well with the structural features of sulfadoxine and the 'prediction' that sulfadoxine would be a good glass former. However a major drawback that should be kept in mind is the notably low temperature of zero mobility. From this it can be concluded that amorphous sulfadoxine will have to be stored at least below ≅ 25°C in an effort to counter a temperature dependent transformation of this metastable form to the stable crystalline form of sulfadoxine during mere storage of the bulk material.

It is a well-known fact that the amorphous solid-state form of a drug is a metastable form. As discussed previously, these metastable solid-state forms exist in a higher free energy state. Such a higher energy state results in the advantages of improved aqueous solubility and higher dissolution rates. However, the inherent instability of amorphous forms due to the meta-stability of the solid-state should always be considered as a disadvantage and should therefore be investigated extensively.

Temperature dependent recrystallization of amorphous sulfadoxine

(a) Non-isothermal recrystallization kinetics

From the thermal analysis of amorphous sulfadoxine it can be deduced that the crystallization process is a thermally activated process. The recrystallization kinetics of amorphous sulfadoxine were determined non-isothermally and isothermally. By applying Kissinger's analysis the activation energy required to obtain recrystallization of amorphous sulfadoxine was determined. It should be noted that the Kissinger model is based on first-order kinetics and therefore nucleation and crystal growth are not taken into account. This was done by measuring the crystallization temperature from various heating rates (β) (Table III).

Table III: Peak crystallization temperatures of amorphous sulfadoxine obtained during different heating rates (β)

Heating rate (°C/min)	**Peak crystallization temperatures (°C)**
5.00	94.32
7.00	95.16
10.00	98.00
15.00	100.93
20.00	102.93

From the obtained thermal analyses, the peak crystallization temperature was used (T_p). Figure 6 depicts plots of $\ln(\beta/T_p^2)$ versus $1/T_p$ obtained from DSC analyses of amorphous sulfadoxine without the presence of seeds and with crystalline sulfadoxine seeds present. The slope of the Kissinger plot is $-E_a/R$. The non-isothermal activation energy for amorphous sulfadoxine without the presence of seed crystals was determined to be 173.77 $kJ.mol^{-1}$ (± 28.70). The same thermal analysis was done on sulfadoxine amorphous solid-state form but with seed crystals present. The activation energy was determined to be 144.51 $kJ.mol^{-1}$ (± 13.08).

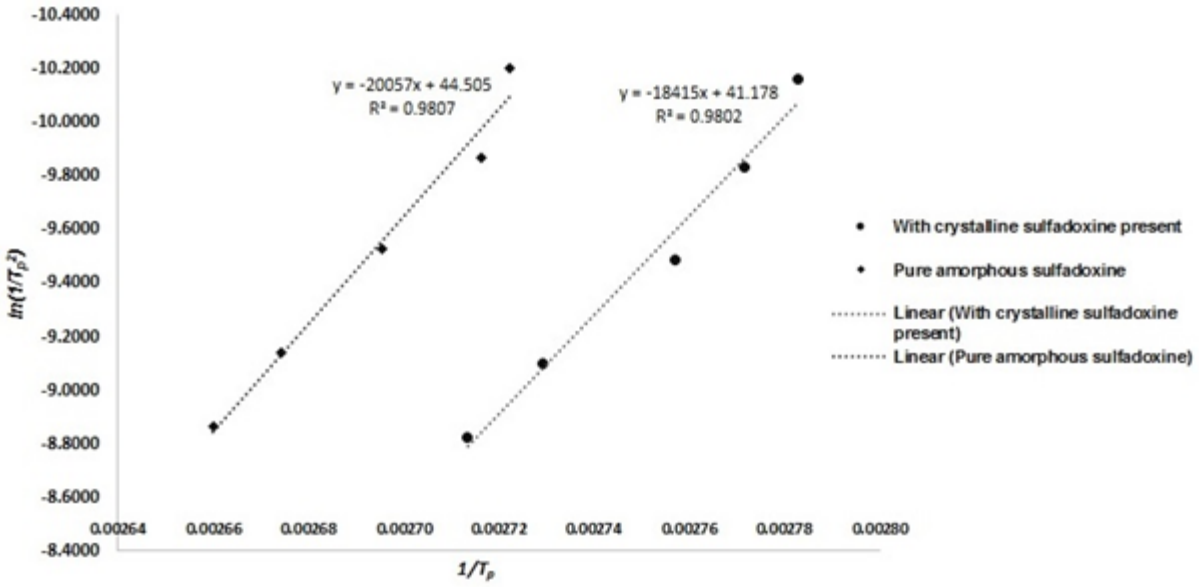

Figure 6: A Kissinger plot of the non-isothermal crystallization of amorphous sulfadoxine.

(b) Isothermal recrystallization kinetics

The recrystallization kinetics of amorphous sulfadoxine was further investigated by the application of the Johnson-Mehl-Avrami (JMA) model. This model is viewed to be a suitable model for studying the recrystallization of an amorphous form of a drug due to the fact that it takes into account that the amorphous solid-state coexists with the stable crystalline state during the phase transformation. The JMA model describes the degree of recrystallization as a function of time and can be quantified by applying equation 7:

$$\alpha = 1 - e^{-[(kt-t_0)]n} \qquad (7)$$

Where α is the fraction of crystalline drug at the temperature of storage time t, t_0 is the induction time, k is the apparent temperature dependent rate constant (time^{-1}) and n is the temperature-independent Avrami exponent (26, 27). The Avrami exponent depends on the mechanism of nucleation and growth as well as the number of dimensions (m) in which the growth of the crystals occur (28, 29). By assigning an appropriate reaction order, equation 7 can be linearized so that equation 8 is obtained:

$$[-\ln(1-\alpha)]^{1/n} = k(t - t_0) \qquad (8)$$

The various nucleation mechanisms are well described in literature (30, 31). From hot-stage microscopy (HSM) observations as well as the DSC data it was clear that the nucleation and crystal growth rate of amorphous sulfadoxine is only a function of temperature. HSM images, (Figure 7 (b)) showed that nucleation sites are formed at temperatures ≅49 - 55°C, from these nucleation sites crystals start to growth with an increase in temperature (Figure 7(c) – (d)). Considering this, three general mechanisms of nucleation would apply, namely:

(1) continuous nucleation – nuclei continue to form and grow throughout the transformation process with a first-order time dependence, (2) fixed number of nuclei – a fixed number of nucleation sites exists and growth proceeds from these preexisting sites, these nuclei will appear at one time with zero-order time dependence and (3) site-saturated nucleation, which is considered as a combination of mechanisms (1) and (2), in this scenario all nuclei is present at the onset of the isothermal process, no additional nuclei will form during the transformation but the absolute number of nuclei present is dependent on the temperature (31). Furthermore, crystal growth dimensions (*m*) is either 1 (unidimensional), 2 (bidimensional) or 3 (tridimensional). Therefore, the reaction order (Avrami exponent) (*n*), is equal to *m* + 1, if nucleation is continuous or equal to *m* if nucleation is instantaneous (mechanisms (2) or (3) (31). From HSM data (Figure 7) it was determined that amorphous sulfadoxine recrystallize in a tridimensional manner (spherulitic) and therefore the reaction order was determined to 4.

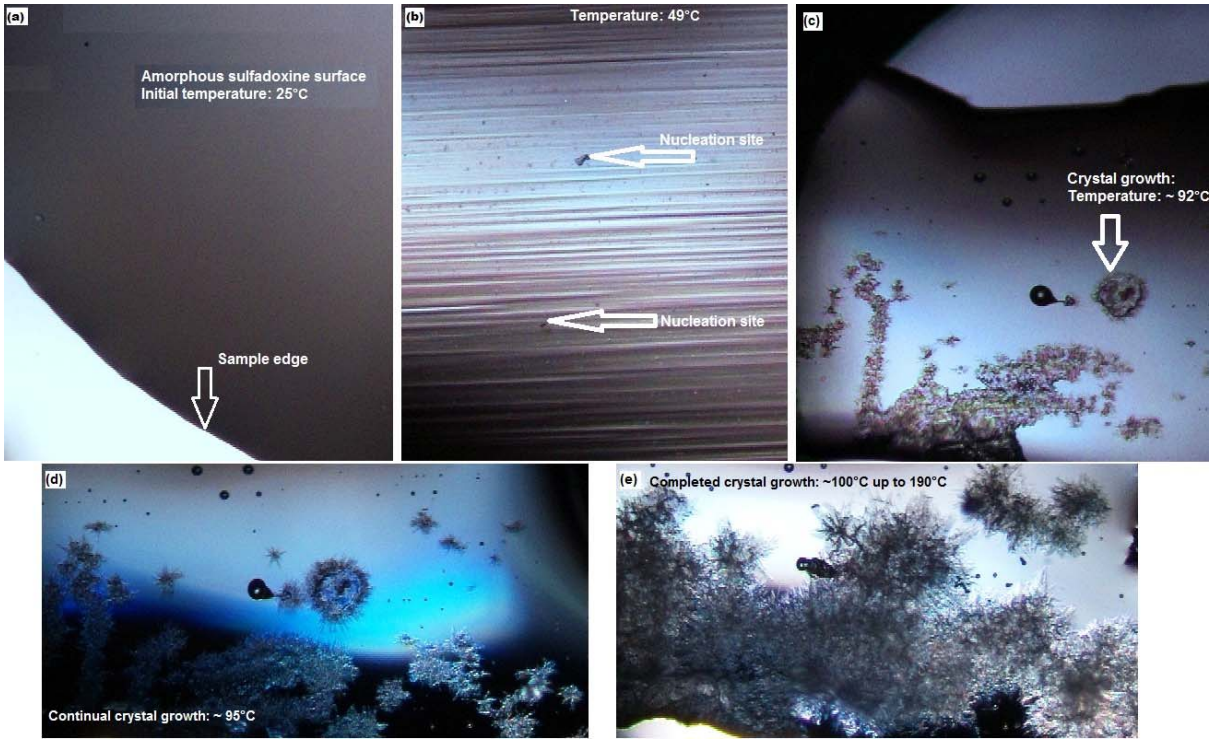

Figure 7: Photomicrographs obtained during HSM analysis of amorphous sulfadoxine, (a) sulfadoxine amorphous form at ambient temperature prior to heating, (b) sulfadoxine amorphous form during the heating process at approximately 49°C, the individual nucleation sites became visible, (c) crystal growth from the nucleation sites at a temperature of approximately 92°C, (d) continuous crystal growth can be visually observed from the nucleation sites (95°C) and (e) completed crystal growth from the nucleation sites in a tridimensional manner is visible.

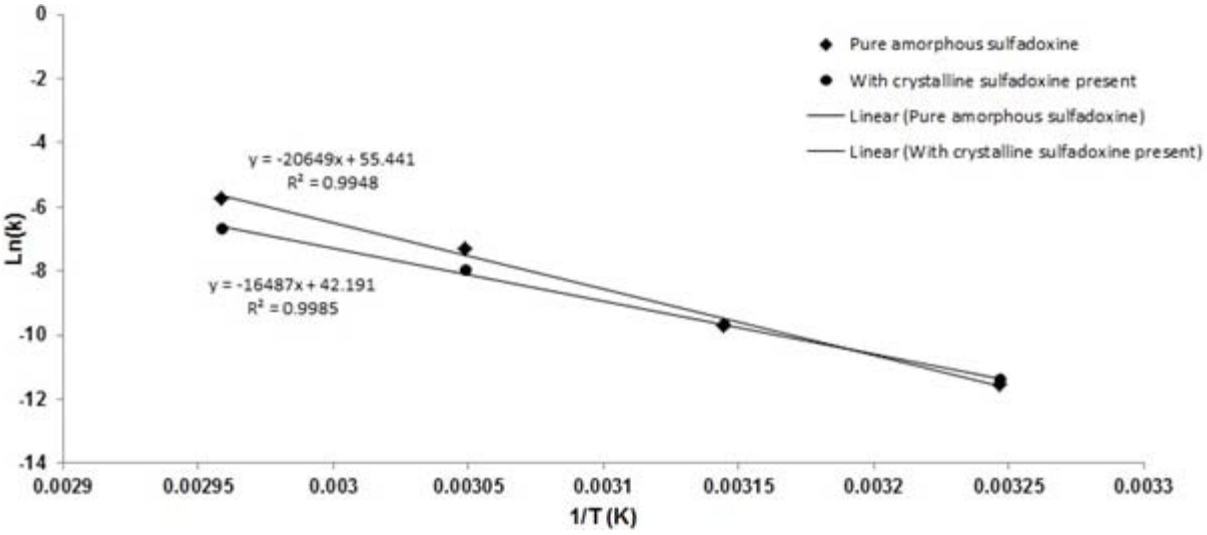

Figure 8: The Arrhenius plot of the JMA model rate constants determined through isothermal microcalorimetry.

Arrhenius plots of the crystallization rate constants (Figure 8) determined through isothermal microcalorimetry resulted in calculated activation energies of 171.68 kJ.mol^{-1} (without crystalline seeds being present) and 137.07 kJ.mol^{-1} (seeds being present). This showed to be in good correlation with the activation energies calculated through non-isothermal determinations.

Moisture induced recrystallization of sulfadoxine

As discussed previously, moisture can also affect the stability of amorphous solid-state forms of drugs. The stability of amorphous sulfadoxine during exposure to moisture was investigated by means of vapor sorption analyses. In comparison with the crystalline form of a drug an amorphous form can absorb relatively large quantities of water vapor due to greater void space or surface area. Due to the plasticizing effect of sorbed water, the glass transition temperature (T_g) can be lowered quite significantly. Furthermore, when an amorphous to crystalline phase transition occurs, the water sorption capacity of the drug will decrease significantly. This will result in an overall weight loss due to the fact that excess water becomes desorbed during the crystallization process. The specific relative humidity at which crystallization (weight loss) occurs is denoted as RH_c (32). The moisture sorption analysis of amorphous sulfadoxine showed the critical relative humidity to be 25 % RH (Figure 9). This is suspected due to the higher energy state in which amorphous sulfadoxine exists. It is clear that exposure of amorphous sulfadoxine to high moisture environments should be avoided.

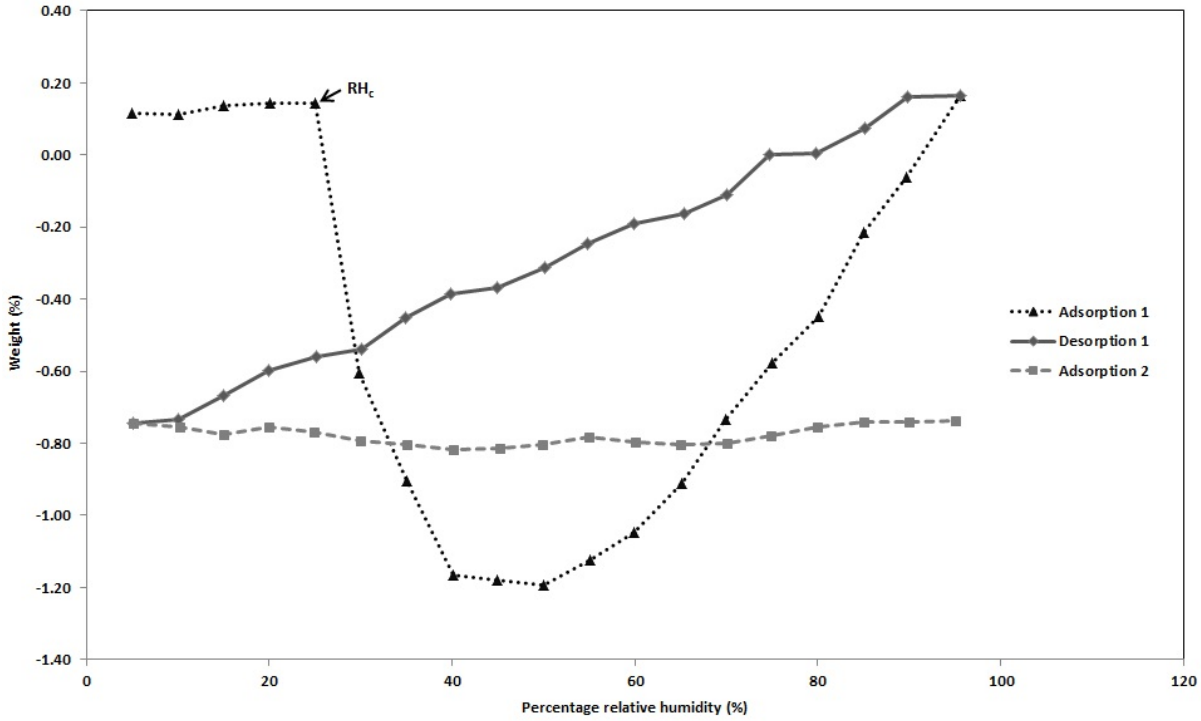

Figure 9: Moisture sorption isotherms obtained for amorphous sulfadoxine. The isotherms were obtained at 25°C with humidity variation of 5 – 95 % RH.

Due to the instability of amorphous sulfadoxine during exposure to high humidity environments it was not possible to accurately determine the solubility and dissolution behavior thereof.

Stabilization of amorphous sulfadoxine through physical mixtures

During the last decade or so, the focus moved from preparing neat amorphous forms of drugs to the preparation of amorphous solid dispersions. The advantage of this strategy is that an amorphous solid dispersion retains all the advantages of pure amorphous forms of drugs but with better physical stability. Figure 10 depicts the influence that physical mixtures of amorphous sulfadoxine with PVP-25 have on the stability of the amorphous drug.

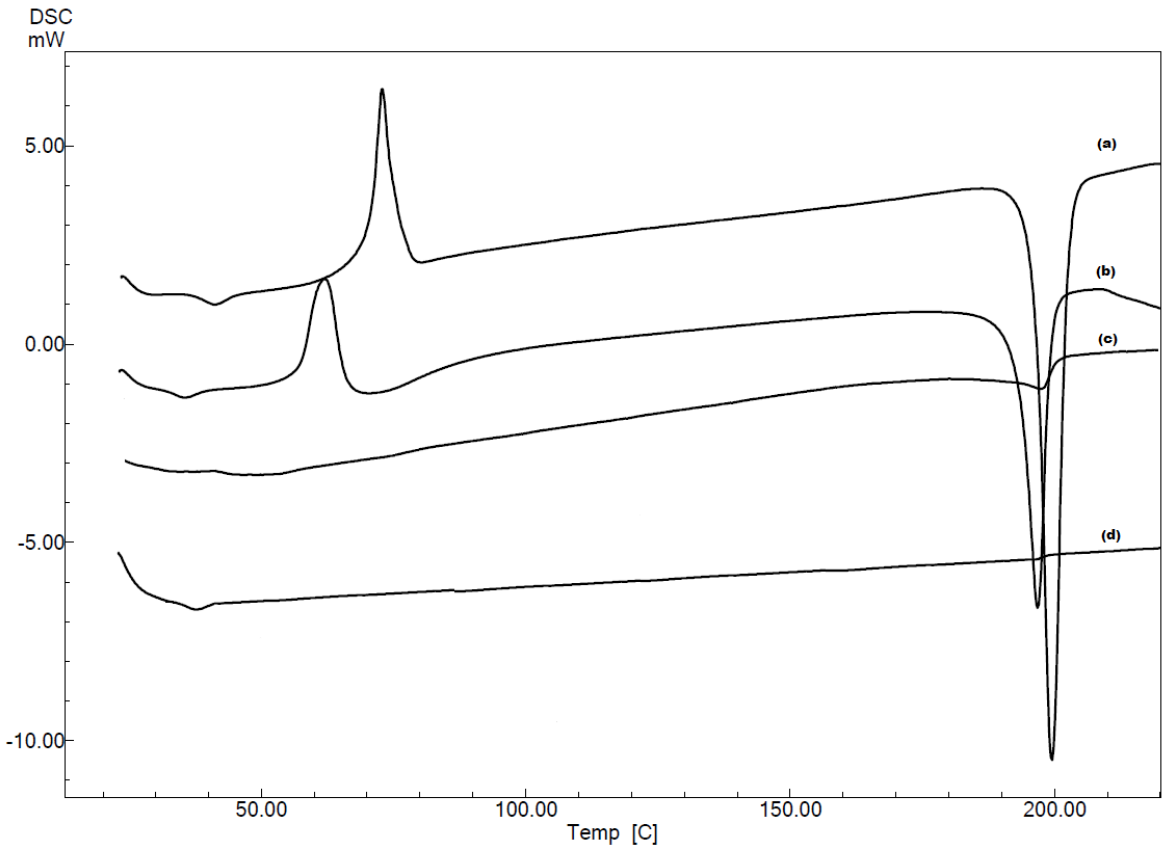

Figure 10: DSC thermograms obtained during the thermal analysis of physical mixtures of amorphous sulfadoxine with PVP-25, (a) neat amorphous sulfadoxine (b) drug:polymer (1:1), (c) drug:polymer (1:2), (d) drug:polymer (1:4).

From Figure 10 it is apparent that even a physical mixture can result in improved physical stability of amorphous sulfadoxine. A ratio of 1:4 (drug:polymer) showed that the thermally-induced recrystallization of amorphous sulfadoxine is being inhibited. The purpose of this study was not to prepare an amorphous solid dispersion of sulfadoxine, but to investigate the influence of polymer:drug physical mixtures on the stability of amorphous sulfadoxine. Future investigations into amorphous sulfadoxine prepared as an amorphous solid dispersion will be considered advantageous.

4. Conclusion

During this study, an amorphous form of sulfadoxine was successfully prepared by quench-cooling of the crystalline solid. The amorphous habit was confirmed with X-ray powder diffraction, through thermal studies and visually through HSM and SEM analyses. It was also shown that sulfadoxine has a good glass forming ability, and that the sulfadoxine glass can be classified as a strong glass. In contrast to literature's dictation that good glass forming ability and strong strength parameters infer good stability, this research heightens the fact that it is not always the case. The critical storage conditions of the amorphous solid-

state form (below 25°C and 25 % RH) needs to be strongly adhered to, in order to prevent temperature and moisture dependent transformation to the stable crystalline form. Recrystallization kinetic studies showed that the nucleation and crystal growth rate is temperature dependent and that the presence of crystal seeds significantly decreases the amount of activation energy that is necessary for the recrystallization process to start. Considering the aforementioned, pharmaceutical industries will need to ensure amorphous sulfadoxine without any crystal seeds are used during manufacturing and maintained until after patient consumption. The study further proved that a drug: polymer physical mixture of 1:4 inhibited the thermally induced recrystallization of amorphous sulfadoxine. This together with the good glass forming ability of sulfadoxine makes this long-acting sulphonamide an ideal candidate for an amorphous solid dispersion.

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CHAPTER 4

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The Editor-in-Chief

International Journal of Pharmaceutics

Hereby I would like to submit the manuscript entitled: ***Different amorphous solid-state forms of roxithromycin: a thermodynamic and morphological study*** for publication in International Journal of Pharmaceutics. With this writing I confirm that all authors read and approved this version of the manuscript. Furthermore, this manuscript has not been published previously and that it was also not submitted to another journal for simultaneous consideration.

This manuscript reports on two amorphous forms of roxithromycin, not previously reported on. One of these solid-state forms was prepared *via* a novel preparation method utilizing hot air. Furthermore, this manuscript provides a direct comparison between four amorphous solid-state form of the macrolide antibiotic, roxithromycin which greatly emphasizes the fact that different amorphous forms of a given drug are a possibility and a reality to reckon with. Currently, in literature, no evidence can be found of different morphologies of different amorphous solid-state forms of the same drug and how such differences influence the physico-chemical properties of the drug. Through this original research the term poly-amorphism or atypical poly-amorphism is elaborated on and the use of this term is encouraged.

With this it would be regarded as a great privilege if you will consider this manuscript for publication.

Kind regards

Dr. Marique Aucamp

Different amorphous solid-state forms of roxithromycin: a thermodynamic and morphological study

Marnus Milne[a], Wilna Liebenberg[a], Marique Elizabeth Aucamp[a*]

[a]Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom Campus, Potchefstroom, South Africa, 2520

[*]Corresponding author: Marique Elizabeth Aucamp, Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom Campus, Potchefstroom, South Africa, 2520, Tel: +27 182994472, Fax: +2787 231 5432, E-mail: Marique.Aucamp@nwu.ac.za

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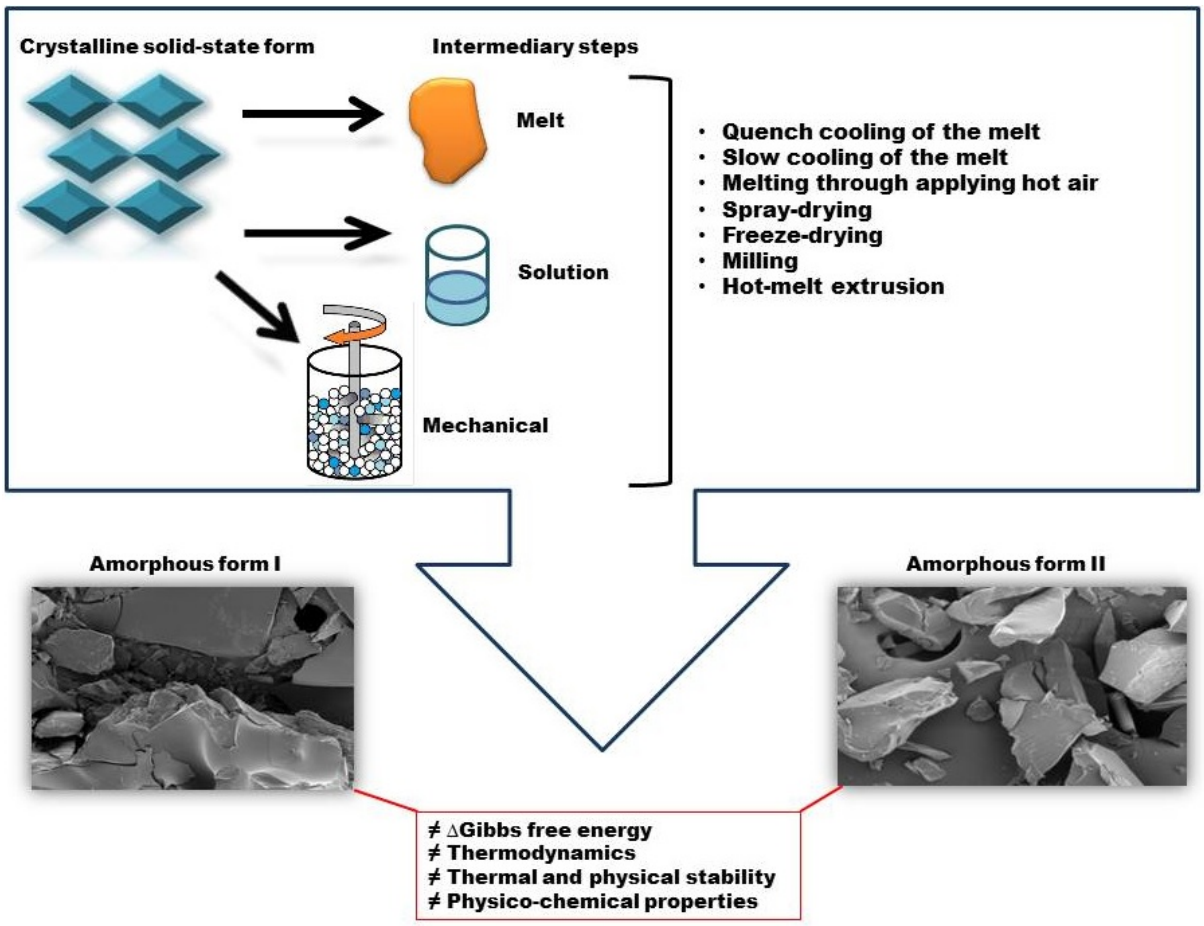

Abstract

The striking impact that different preparation methods have on the characteristics of amorphous solid-state forms has attracted considerable attention during the last two decades. The pursuit of more extensive knowledge regarding polyamorphism therefore continues. The aim of this study was firstly, to investigate the influence of different preparation techniques to obtain amorphous solid-state forms for the same active pharmaceutical ingredient, namely roxithromycin. The preparation techniques also report on a method utilizing hot air, which

although it is based on a melt intermediary step, is considered a novel preparation method. Secondly, to conduct an in-depth investigation into any physico-chemical differences between the resulting amorphous forms and thirdly, to bring our findings into context with that of previous work done, whilst simultaneously discussing a well-defined interpretation for the term polyamorphism and propose a discernment between true polyamorphism and pseudo-polyamorphism / atypical polyamorphism. The preparation techniques included melt, solution, and a combination of solution-mechanical disruption as intermediary steps. The resulting amorphous forms were investigated using differential scanning calorimetry, X-ray powder diffraction, hot-stage microscopy, scanning electron microscopy, and vapor sorption. Clear and significant thermodynamic differences were determined between the four amorphous forms. It was also deduced from this study that different preparation techniques have a mentionable impact on the morphological properties of the resulting amorphous roxithromycin powders. Thermodynamic properties as well as the physical characteristics of the amorphous forms greatly governed other physico-chemical properties *i.e.* solubility and dissolution.

Keywords: Roxithromycin, Amorphous, Preparation method, Polyamorphism, Pseudo-polyamorphism, atypical polyamorphism

Introduction

Several different preparation techniques have been identified and used in obtaining amorphous forms of drugs in current literature. Typical methods to prepare amorphous forms of a drug are based on three intermediary steps, namely melt, solution, or solid (Savolainen *et al*., 2007, Patterson *et al*., 2005). The best known method is that of quench cooling of the melt which is based on molten drug as intermediary step (Savolainen *et al*., 2007, Patterson *et al*., 2005). During the process of quench cooling of the melt, the cooling step is so rapid that the drug molecules are not able to rearrange into a crystalline lattice therefore becoming "frozen" in a disorganized state (Savolainen *et al*., 2007, Patterson *et al*., 2005). Precipitation, spray-drying and freeze-drying are considered solution-based methods, with rapid removal of the solvent thereby inhibiting the rearrangement of the drug molecules into an ordered state (Savolainen *et al*., 2007, Patterson *et al*., 2005). Milling is based on a solid-state transition as intermediary step with mechanical activation causing a direct, but time and degree of added energy dependent, disruption of the crystalline lattice (Savolainen *et al*., 2007, Patterson *et al*., 2005). Lastly, an amorphous form may be achieved through a combination of solution, mechanical activation and the melt of a drug. An example of the aforementioned would include hot melt extrusion (Savolainen *et al*., 2007, Patterson *et al*., 2005).

It follows that one cannot ignore the fact that there are indeed differences in the physico-chemical behaviour of amorphous solids formed through different preparation techniques.

Graeser *et al*., 2008 conducted a study on possible differences in physico-chemical properties and stability between amorphous forms of simvastatin prepared by cryo-milling and quench-cooling of the melt, and found that cryo-milled amorphous samples have different properties in comparison with the quench-cooled amorphous state (Graeser *et al*., 2008). Their thermodynamic parameters suggested that the cryo-milled amorphous form was less disordered compared to the quench-cooled form (Graeser *et al*., 2008). Furthermore, the cryo-milled samples had a lower stability and decreased recrystallization enthalpy (Graeser *et al*., 2008). Another study conducted by Sheth *et al.,* 2004 proved that a process of cryogrinding of piroxicam forms I (P_I) and II (P_{II}) resulted in two different amorphous forms, namely P_{AI} from form I and P_{AII} from form II. These two amorphous forms differed in terms of their recrystallization behaviour. During a study of the local structures of the amorphous phases of piroxicam the authors concluded that amorphous piroxicam forms, P_{AI} and P_{AII}, had the same local amorphous NN order but differed in their residual longer-range order - possibly explaining differences in their respective recrystallization processes (Sheth *et al*., 2004).

Savolainen *et al*., 2007 investigated the molecular level differences in the amorphous state of indomethacin prepared from both α- and γ-polymorphs using different preparative techniques - including milling, quench cooling of a melt, slow cooling of a melt and spray drying (Savolainen *et al*., 2007). Differences in the amorphous samples could be found on a molecular level between the milled samples and the quench cooling and also between the slow cooling of a melt and spray drying (Savolainen *et al*., 2007). The recrystallization temperature varied between the milled, slow cooled and quenched cooled samples (Savolainen *et al*., 2007). The recrystallization showed an onset at higher temperatures for the amorphous forms obtained from a melt intermediary step, compared to the milled and spray dried samples (Savolainen *et al*., 2007). In addition, an increase in hydrogen-bonding properties was noted with the melted samples as opposed to the milled samples (Savolainen *et al*., 2007). Patterson *et al*., 2005 investigated the influence of thermal and mechanical preparative techniques on the amorphous state of four poorly soluble compounds, and concluded that the proneness for amorphous conversion of the compounds in question, through thermal and mechanical techniques, were compound specific (Patterson *et al*., 2005). Furthermore, the amorphous test samples that were investigated were all prone to recrystallization, irrespective of whether the quench cooled or ball milled method was applied. (Patterson *et al*., 2005).

From literature overviews it is evident that differences between amorphous forms of the same drug do exist. However, most authors are either hesitant to apply the term polyamorphism to the varying amorphous forms of the same drug, or they speculate that the different amorphous forms of the given drug could be described as polyamorphism. This can be ascribed to the

current definition of polyamorphism which signifies the possible existence of two distinct amorphous states of the same material separated by a clear phase transition. It must be noted that this definition has limited application to organic compounds. Reports on polyamorphic forms which have been identified through clear phase transitions between amorphous phases were all on inorganic compounds (Hancock *et al*., 2002).

Up to this point in time no well-defined phrase or term has been established to describe the different energetic states of pharmaceutical amorphous forms of the same drug. The most appropriate term is probably that of pseudo-polyamorphism, as proposed by Shalaev and Zografi (2002) (Shalaev and Zografi, 2002). It is clear that numerous challenges still exist to fully understand the amorphous solid-state forms of pharmaceutical molecules. The lack of a clear and well-described definition to group different amorphous forms of the same drug is still evasive, and after more than three decades still challenges the pharmaceutical scientist.

Preceding studies on the investigation of possible polyamorphism emphasized and investigated differences in short range molecular order and varying molecular energetics. However, none investigated the impact that physical characteristics, i.e particle morphology could have on other physico-chemical properties of a given drug. Previous studies on amorphous roxithromycin proved that it is possible to prepare amorphous forms of this drug by means of various methods (Aucamp *et al*., 2012, Biradar *et al*., 2006). All these methods were employed in an effort to improve the aqueous solubility of this macrolide antibiotic. However, up to this point in time, no comparative study was done to investigate physical, chemical and stability differences of roxithromycin amorphous forms prepared through different methods. Considering this it was decided that amorphous roxithromycin should be investigated further in an effort to provide more insight on the term *"polyamorphism"*.

Material and methods

1.1 Materials

Crystalline roxithromycin anhydrate (Form III) was purchased from DB Fine Chemicals Pty Ltd. (Johannesburg, South Africa). Ultrapure water with a resistivity of 18.2 $M\Omega.cm^{-1}$ was used throughout this study and all other reagents used were either of chromatography or analytical grade.

1.2 Methods

1.2.1 Preparation of roxithromycin amorphous solid-state forms

The chloroform desolvated amorphous form of roxithromycin (R-CD) was prepared through a slow evaporative method followed by a desolvation process (Aucamp *et al*., 2012). 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. The desolvated amorphous roxithromycin was subsequently obtained by desolvation of the solvated solid-state form in a laboratory oven (Binder, Germany) for 24 hours at 60 ± 2°C.

The second amorphous form of roxithromycin was prepared through a quench cooling method (R-QC). Approximately 200 mg of crystalline roxithromycin was evenly distributed on the surface of a Petridish. The sample was placed in a laboratory oven (Binder, Germany) at 120 ± 5°C until a complete melt was obtained. The resulting melt was subsequently quench cooled on a cool surface (Aucamp *et al*., 2013).

A third amorphous form was prepared through slow cooling of molten roxithromycin (R-SC). Approximately 200 mg of crystalline roxithromycin was evenly distributed in a Petridish. The sample was heated to 120 ± 5°C in a vacuum oven with an applied vacuum of 1.3 kPa. After a molten product was obtained the temperature was lowered to 25 ± 5°C. The sample was left to cool down slowly.

The last amorphous form was prepared through the application of hot air (R-HA). This is a novel method allowing the sample to be heated and melted in a gentler manner. This method has the advantage of accurate temperature control with no temperature overshooting. Approximately 60 mg of crystalline roxithromycin was placed on a 0.25 mm thick stainless steel sheet. Hot air (130 ± 5°C) was directed towards the back of the stainless steel sheet. After a molten product was obtained the hot air was directed from heating underneath, towards directly on the melt to ensure complete melting of the product without any seed crystals remaining.

1.2.2 Differential Scanning Calorimetry (DSC)

A Shimadzu (Kyoto, Japan) DSC-60 instrument was used to record the DSC thermograms. Samples (3 – 5 mg) were accurately weighed and sealed in aluminum crimp cells with pierced lids. The samples were heated from 25 to 250°C with a heating rate of 10°C/min and a nitrogen gas purge of 35 mL/min. In the instances where thermal data was used to calculate activation energy (*Ea*) heating rates of 2, 4, 6, 8 and 10°C/min were employed. The onset temperatures of the thermal events are reported.

1.2.3 X-ray powder diffraction (XRPD)

Powder X-ray diffraction measurements were performed to confirm the crystalline or amorphous nature of the solid-state forms under investigation. A PANalytical (Almelo, Netherlands) Empyrean X-ray diffractometer with a PIXcel3D detector was used to record

XRPD patterns at ambient temperature. Samples were evenly distributed on a zero background sample holder. The measurement conditions for all scans were set as follows: target, Cu; voltage, 40 kV; current, 40 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; scanning speed, 2°/min (step size, 0.02°; step time, 1.0 s).

1.2.4 Hot-stage microscopy (HSM)

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

1.2.5 Scanning electron microscopy (SEM)

SEM images of the purchased crystalline roxithromycin (Form III) and the various amorphous forms were obtained in order to observe possible morphological differences between the samples. For SEM analyses, samples were coated with a layer of gold/palladium using an Eiko engineering ion coater IB-2 (Eiko Engineering, Ibaraki, Japan), and were subsequently imaged using a field-emission environmental FEI Corporation, Quanta 200 ESEM (Hillsboro, Oregon, USA).

1.2.6 Vapor sorption analysis

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

1.2.7 Equilibrium solubility determination

Approximately 100 mg of crystalline roxithromycin (Form III) was weighed into test tubes (n = 3). 10 mL distilled water (ambient temperature) was pipetted into each test tube. The test tubes were sealed with Parafilm® and tightly capped in order to prevent any leakage, followed

by affixing the test tubes to a rotating axis in a water bath set at 37°C ± 0.5 °C. The axis was set to rotate at 54 rpm. Withdrawals were taken on 720, 1440 and 2160 minutes. From this data it was calculated that a period of 24 h for roxithromycin was deemed sufficient to reach equilibrium solubility.

1.2.8 Powder dissolution testing

A VanKel700 dissolution bath (Varian, Cary, USA) was used for dissolution testing. USP apparatus 2 (paddle) was set up at 37°C ± 2°C with a rotational speed of 100 rpm, 900 mL distilled water was added to each dissolution vessel. In order to ensure particle size uniformity, all the samples were subjected to thirty seconds of light crushing with a mortar and pestle. However, care was taken not to add thermal energy, avoiding possible solid-state transformation. Approximately 500 mg of each solid-state form was respectively weighed into 10 mL test tubes, to which 250 mg glass beads, ≤106 µm (Sigma–Aldrich, South Africa) were added. 5 mL of dissolution medium (distilled water maintained at 37°C ± 2°C) was added to each test tube. The mixtures were agitated for a period of 120 s, using a vortex mixer. The resulting mixtures were transferred to each dissolution vessel. 5 mL of solution was withdrawn from each dissolution vessel at predetermined time intervals. The dissolution medium was not replaced after each withdrawal since a supersaturated solution was required to observe solution-mediated transformations (Greco and Bogner, 2012). After withdrawal, the samples were filtered through a 0.45 µm PVDF filter into an HPLC vial. The filtered solutions were analyzed by HPLC.

1.2.9 HPLC (High-performance liquid chromatograpy) analysis

HPLC analysis was done utilizing a Shimadzu (Kyoto, Japan) UFLC chromatographic system. The system consisted of a SIL-20AC auto-sampler fitted with a sample temperature controller, a UV/VIS Photodiode Array detector (SPD-M20A) and a LC-20AD solvent delivery module. The mobile phase consisted of 49.1 g/L solution of ammonium dihydrogen phosphate, adjusted to pH 5.3 with dilute sodium hydroxide. 700 mL of this buffer was mixed with 300 mL acetonitrile. The mobile phase was filtered and degassed prior to use. A Luna C18 150×3.9 mm column was used with a flow rate set to 1.5 mL/min and a wavelength of 205 nm (British Pharmacopoeia, 2015). Validation of this method provided a linear regression (r^2) of 0.9964.

Results

2.1 Investigating different preparation methods for amorphous roxithromycin

As stated previously the preparation methods of amorphous drugs can be divided into melt-, solution- or mechanically-based methods (Patterson *et al*., 2005, Graeser *et al*., 2008). Four different preparation methods were used to investigate possible differences in terms of

physical and chemical behavior as well as stability of the amorphous roxithromycin forms. This study focuses on melt- and solution-based methods and the rationale is to determine if preparation methods arising from different transitional states (melt or solution) will result in amorphous forms of roxithromycin with different physico-chemical properties. It might be considered a disadvantage that it was not possible to investigate a mechanical disruption-based method, however, no efforts of milling or other mechanical manipulation of crystalline roxithromycin rendered this drug in an amorphous state.

2.2 Amorphous habit of roxithromycin forms prepared through different preparation techniques

All the preparation methods used in this study proved to be successful in rendering roxithromycin in an amorphous state. The amorphous habit of the roxithromycin samples was confirmed by HSM and XRPD. During HSM experiments the lack of birefringence were observed for all four amorphous forms, while the crystalline nature of anhydrous roxithromycin was confirmed by the presence of birefringence. Figure 1 presents the XRPD overlay of crystalline roxithromycin, R-QC, R-CD, R-HA and R-SC and clearly indicates the crystalline habit of anhydrous roxithromycin compared to the amorphous halo which is distinctive of true amorphous solid-state forms.

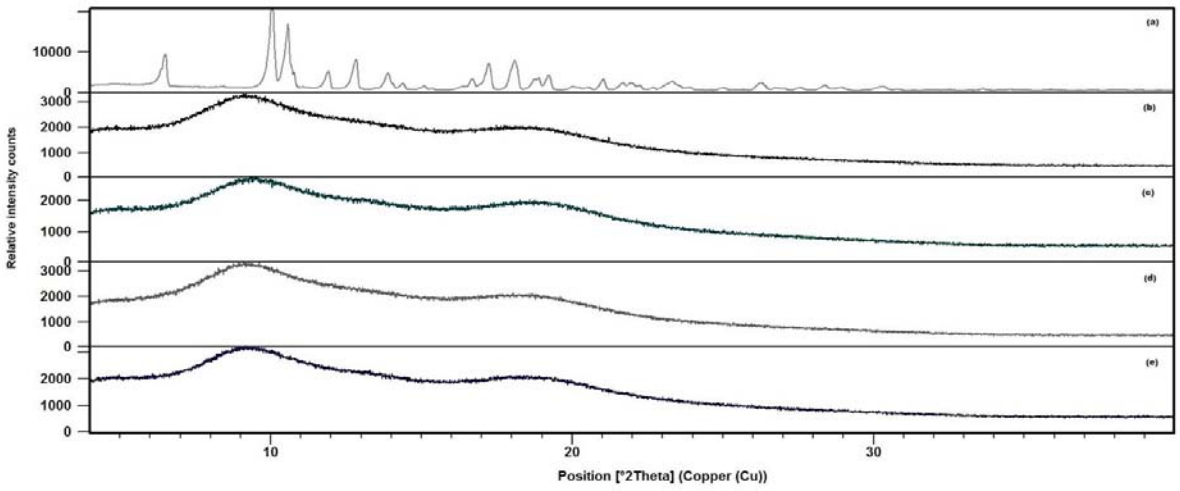

Figure 1: Overlay of the XRPD patterns of (a) crystalline roxithromycin (Form III), (b) R-QC, (c) R-CD (d) R-HA and (e) R-SC.

2.3 Thermodynamic properties of crystalline and amorphous forms of roxithromycin

Thermal analyses of crystalline roxithromycin showed a single sharp endotherm at 109.0 °C. This signified the melting endotherm and correlated well with roxithromycin (Form III) reported

in literature (Aucamp *et al*., 2013). The enthalpy (ΔH_m) and entropy of fusion (ΔSm) were obtained from the integration of the endothermic fusion event (Table 1).

Table 1: Thermodynamic properties calculated for roxithromycin (Form III) and amorphous forms prepared through different preparation techniques.

	T_m (°C)	ΔH_m (kJ/mol)	ΔS_m (J/mol/K)	$\Delta S_m/R$	T_g (°C)	T_g / T_m	ΔC_p (J.g^{-1}. (°C^{-1})
Crystalline roxithromycin (Form III)	109.0	15.7	41.1	5.1	-	-	-
R-CD	-	-	-	-	86.6	0.79	0.374
R-QC	-	-	-	-	87.2	0.80	0.405
R-SC	-	-	-	-	85.9	0.79	0.338
R-HA	-	-	-	-	88.4	0.81	0.344

2.4 Characterization of different roxithromycin solid-state forms

Other characterization properties described in this section includes the fragility (*m*) and strength indices (*D*). The fragility parameter provides insight into the amorphous materials' chemical, physical or mechanical stability with changing temperature (Shalaev and Zografi, 1996, Hancock and Zografi, 1997). It has been well described by Angell, 1997, that some materials can exhibit Arrhenius behavior over a wide range of temperatures, while others can exhibit non-Arrhenius behavior with a very distinct change in viscosity or structural relaxation time in the glass transition temperature (T_g) region (Angell, 1997). Most small organic molecules exhibit non-Arrhenius behavior (Hancock and Zografi, 1997). It is considered valuable to investigate the dynamic properties of amorphous forms of drugs, since such properties reveal information pertaining to the molecular mobility, resulting in an understanding of the physical and chemical behavior of amorphous forms.

The fragility index is a temperature dependent property of the molecular dynamics (viscosity or structural relaxation) in the glass transition temperature region of amorphous forms (Shalaev and Zografi, 1996, Hancock and Zografi, 1997, Hancock *et al*., 1998). The most common method applied for determining the fragility of an amorphous solid-state form is to fit

the molecular mobility against the temperature data obtained above the T_g to a modified Vogel-Tamman-Fulcher (VTF) equation (Eq. 1):

$$\eta = \eta_{0\exp}\left(\frac{DT_0}{T-T_0}\right) \quad (1)$$

Where: η_0 (viscosity) is taken to be 10^{-5} Pa.s (for normal liquids), T_0 is the ideal glass transition temperature (20 – 50 K) below the experimentally determined T_g and D denotes the strength parameter.

However, in the study of pharmaceutical compounds this approach is limited due to experimental difficulties in determining the temperature dependence of molecular mobility above T_g, this being due to drugs frequently being unstable at temperatures above their glass transition temperature (Hancock *et al*., 1998). An equivalent method for estimating the fragility index of an amorphous form is to apply a plot of $\log_{10}\tau(T)$ versus T_g/T at the glass transition temperature, therefore $T = T_g$. This can be expressed as Eq. 2 (Lu and Zografi, 1997):

$$m = \left[\frac{dlog_{10}\eta}{d\frac{Tg}{T}}\right]_{T=T_g} \quad (2)$$

Since the fragility index is a temperature dependent parameter, Eq. 1 can be expressed as Eq. 3 (Lu and Zografi, 1997):

$$m = \frac{\Delta H^*\ \eta}{2.303\ R\ T_g} \quad (3)$$

Considering this and the fact that the T_g is dependent on the heating or cooling rate of the amorphous form being investigated as well as the fact that the fragility index was determined by means of a conventional DSC experiment, the following equation (Eq. 4) will apply Lu and Zografi, 1997):

$$\frac{d\ln|q|}{d(\frac{1}{T_g})} = -\frac{\Delta H^*}{R} \quad (4)$$

Where ΔH^* denotes the activation energy for viscous flow and R is the universal gas constant (Lu and Zografi, 1997). By applying Eq. 4 it was possible to construct plots of *ln* heating rates (*q)* vs *1000/*T_g, the activation energy (ΔH^*) was subsequently calculated from the slope of the linear plot. An overlay of the obtained plots for R-CD, R-QC, R-SC and R-HA is provided in Figure 2.

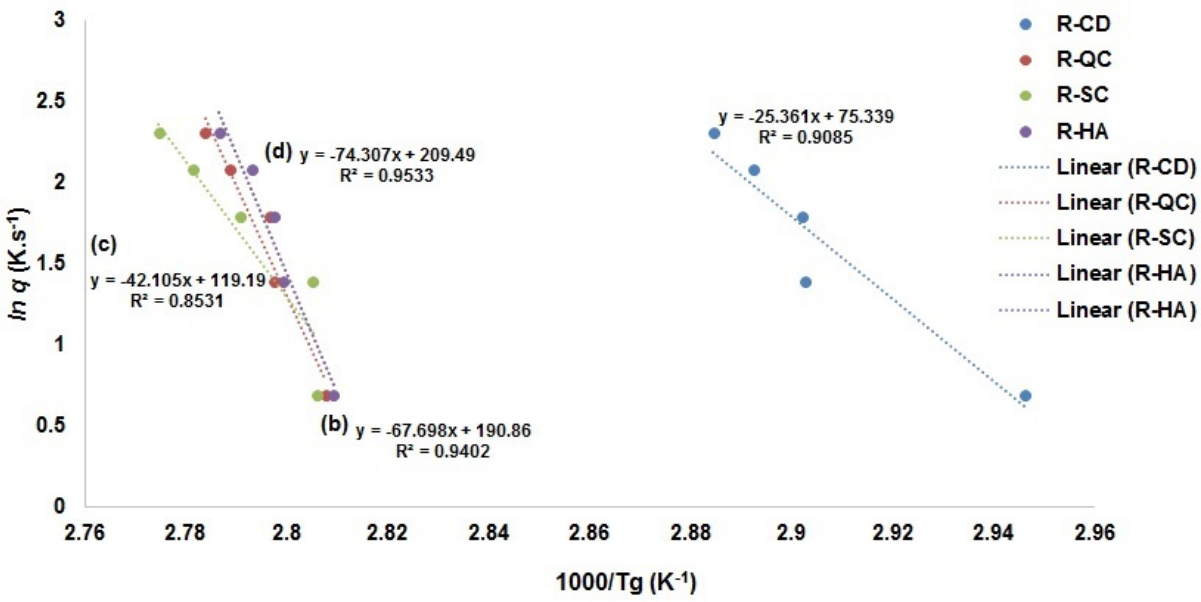

Figure 2: A plot of *ln* heating rates versus 1000/Tg for (a) R-CD, (b) R-QC, (c) R-SC and (d) R-HA.

By applying Eq. 1 the fragility index can further be related to the strength parameter (*D*) (Eq. 5) (Lu and Zografi, 1997):

$$m = \frac{D\left(\frac{Tg}{T_0}\right)}{\ln(10)\left(\frac{Tg}{T_0}-1\right)^2} \qquad (5)$$

assuming that the viscosity of the amorphous roxithromycin forms at T_g is about 10^{12} Pa.s and that η_0 equals 10^{-5} Pa.s, then the strength parameter (*D*) can be expressed as Eq.6 (Lu and Zografi, 1997):

$$D = \frac{666}{m-17} \qquad (6)$$

The strength parameter (*D*) describes the deviation from the Arrhenius behavior, where *D* = 100 indicates a true Arrhenius plot with a strong amorphous system. Therefore, any system with a *D* parameter greater than 25 is considered a strong amorphous system, while a *D* parameter less than 10 signifies a fragile system (Yu, 2001).

From the calculated data, as presented in Figure 3, it can be deduced that R-CD can be characterized as a strong amorphous form (m = 34.22, D= 38.68), while R-QC (m = 81.6, D= 10.31), R-SC (m = 50.92, D= 19.63) and R-HA (m= 89.25, D= 9.22) exhibit fragile amorphous system behaviour. It is therefore evident that these different amorphous forms vary substantially in terms of their fragility and strength parameters. Since fragility influences the

molecular dynamics of an amorphous system it is clear that definitive differences will exist between the amorphous forms of roxithromycin in terms of other physico-chemical properties.

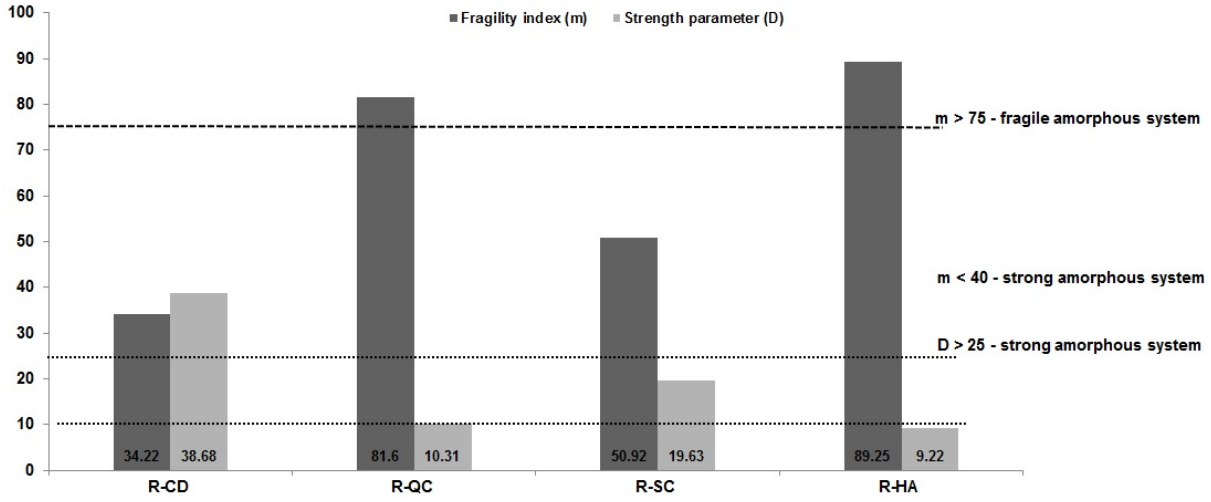

Figure 3: A plot of the calculated individual fragility indices (*m*) and strength parameters (*D*) of the different amorphous forms of roxithromycin.

2.5 Morphological differences between different amorphous forms

Visual inspection of the resulting amorphous forms of roxithromycin also indicated that the physical properties of the forms differ. Firstly, the morphology of the amorphous forms was investigated by means of scanning electron microscopy (SEM). Figure 4 depicts the SEM micrographs for each amorphous form. For both R-QC and R-CD open pores were visible. In the instance of R-CD the open pores could be ascribed to the evaporation of chloroform during the last preparation step of R-CD. In the case of R-QC it is hypothesized that the pores are due to air being trapped during the quench cooling of the molten roxithromycin. The pores could result in increased porosity of the two amorphous forms, which could subsequently influence various other physico-chemical properties, i.e. dissolution rate, moisture sorption behaviour, as well as mechanical properties of the resulting powder. For R-SC and R-HA smooth glassy-like surfaces were observed. It should be mentioned that R-HA resulted in a very brittle and delicate 'glassy' solid-state form, crumbling and cracking easily in very fine fragments. These fine fragments showed to be static, thus adhering to each other. It is hypothesized that the hot air used to prepare this amorphous form caused the surface of the resulting amorphous form to be electrostatically charged.

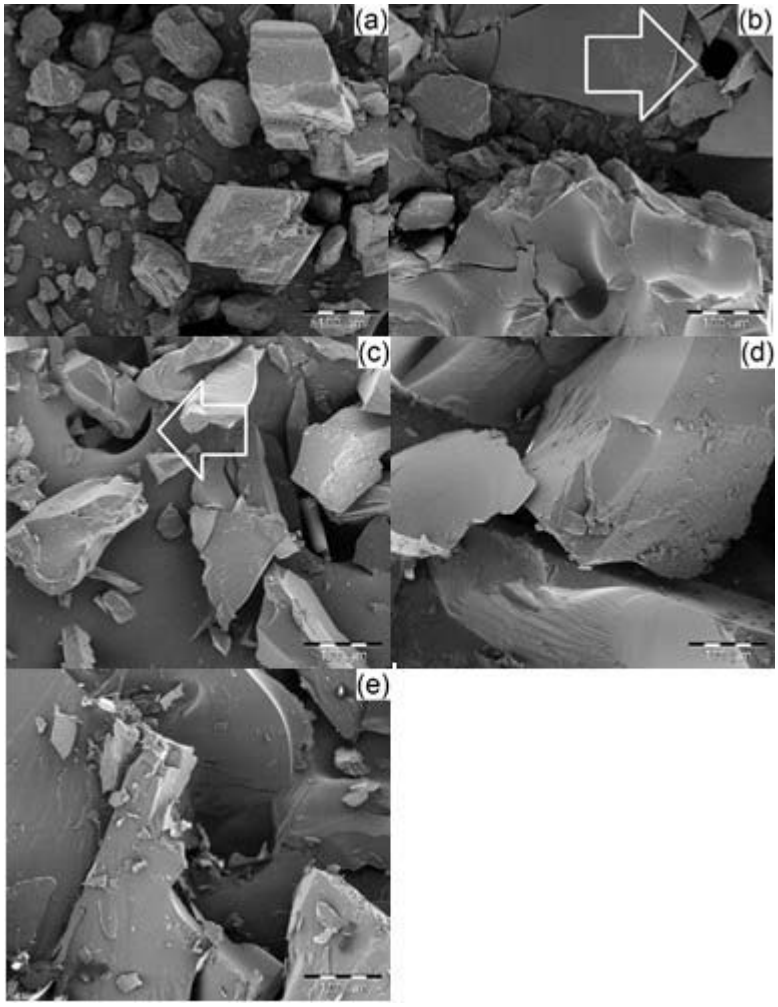

Figure 4: SEM micrographs of (a) crystalline roxithromycin (Form III), (b) R-CD, (c) R-QC (d) R-SC, and (e) R-HA.

Secondly, the ability of a solid to absorb vapor is a property that is greatly determined by surface and morphological properties. Through determining vapor sorption isotherms for a powder, a significant amount of information can be gained in terms of physico-chemical characteristics. Vapor sorption isotherms are useful to predict or explain behavior of a compound during further pharmaceutical processing steps.

A property of amorphous forms of any given compound is their enhanced ability to sorb water from the atmosphere into the bulk structure of the amorphous powder. This is in comparison with the vapor sorption of their crystalline counterparts, of which the ability to sorb water from the atmosphere is often limited to the particle surface. The sorption of water into the bulk structure of amorphous forms can lead to significant changes in chemical and physical stability and behavior of such solid-state forms (Shamblin *et al*., 1997).

It is evident from Figure 5 (crystalline roxithromycin, anhydrous) that sorption of water did not result in any hysteresis of the adsorption and desorption isotherms. The sorption isotherm of

roxithromycin Form III is considered to be a typical reversible Type I BET adsorption isotherm. A Type I isotherm is generally obtained by a microporous solid (Singh, 1982). Microporous solids can be defined as a solid having pores of internal width of less than 2 nm (Rouquerol *et al*., 1999). Furthermore, Type I isotherms represent limiting vapor uptake which is governed by the micropore volume. Therefore, once the micropores are filled with the adsorbate, little external surface is available for further adsorption to occur. It can therefore be concluded that the sorption isotherms obtained for crystalline roxithromycin is typical isotherms that would be expected from a crystalline compound that is not affected by high relative humidity conditions. It should however be mentioned that a measured adsorbed vapor percentage of 3.5% is a significant amount, but it is hypothesized that this can be ascribed to a large micropore volume. It was not possible to determine the available micropore volume for crystalline roxithromycin Form III.

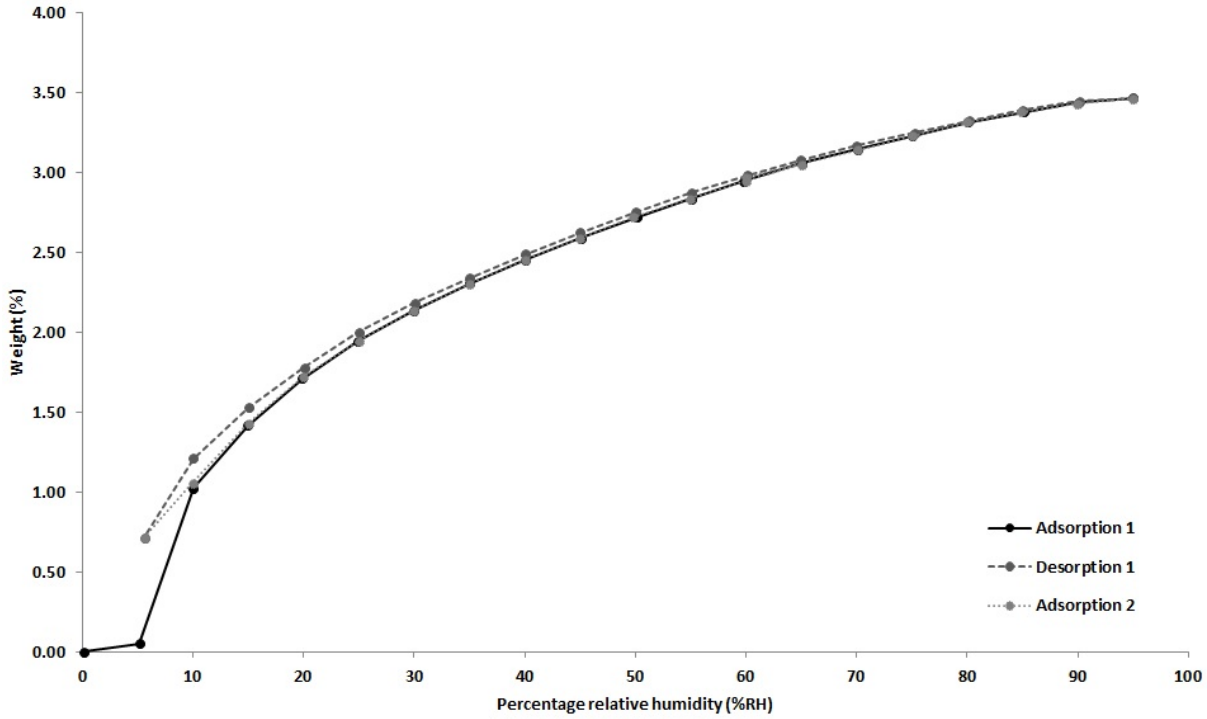

Figure 5: Vapor sorption isotherms obtained for crystalline roxithromycin (Form III) at constant ambient temperature.

Figure 6 depicts the vapor sorption isotherms obtained for amorphous form R-CD. This can be classified as a reversible Type II isotherm (Rouquerol *et al*., 1999). This type of isotherm is typically obtained by either non-porous or macroporous compounds (Rouquerol *et al*., 1999). Macroporous compounds can be defined as solids with pore width >50 nm (Rouquerol *et al*., 1999). Considering that the SEM images (Figure 4), the sorption isotherm for R-CD correlate very well with the morphology which has been visually confirmed through SEM images. Pore widths greater than 50 nm were observed for R-CD, thereby confirming the

macroporous morphology. A Type II isotherm represents unrestricted monolayer-multilayer adsorption (Rouquerol *et al*., 1999). From Figure 6 it is evident that R-CD only absorbed approximately 1.8% moisture. This being much less than that observed with roxithromycin Form III and contradicting the typical/dictated moisture sorption behavior of amorphous solid-state forms. It is hypothesized that either the adsorbed moisture remained a monolayer without the formation of multilayers, thereby limiting the amount of adsorbed moisture or that a very small amount of residual chloroform was still retained in the sample at the time of vapor sorption experiments thereby repelling water during the adsorption process. These results indicate R-CD to be a stable solid-state form, given the fact that R-CD is an amorphous form which, in theory, would be sensitive towards exposure to high relative humidity. It is a well-known fact that the presence of water can act as a plasticizer for an amorphous form, lowering the glass transition temperature and thereby altering the molecular dynamics of the amorphous form causing an increase in the molecular mobility (Zhang and Zografi, 2000). Such an increase of the molecular mobility could result in structural rearrangement of the molecule leading to the crystallization of the amorphous form. However, no recrystallization of R-CD to crystalline roxithromycin due to vapor sorption was observed (confirmed by XRPD and DSC analyses), therefore indicating that R-CD is not sensitive to high relative humidity. This is considered a significant advantage in terms of further pharmaceutical processing due to the fact that R-CD can be exposed to small quantities of moisture during processing steps without triggering recrystallization of the amorphous drug to the more stable crystalline solid-state form.

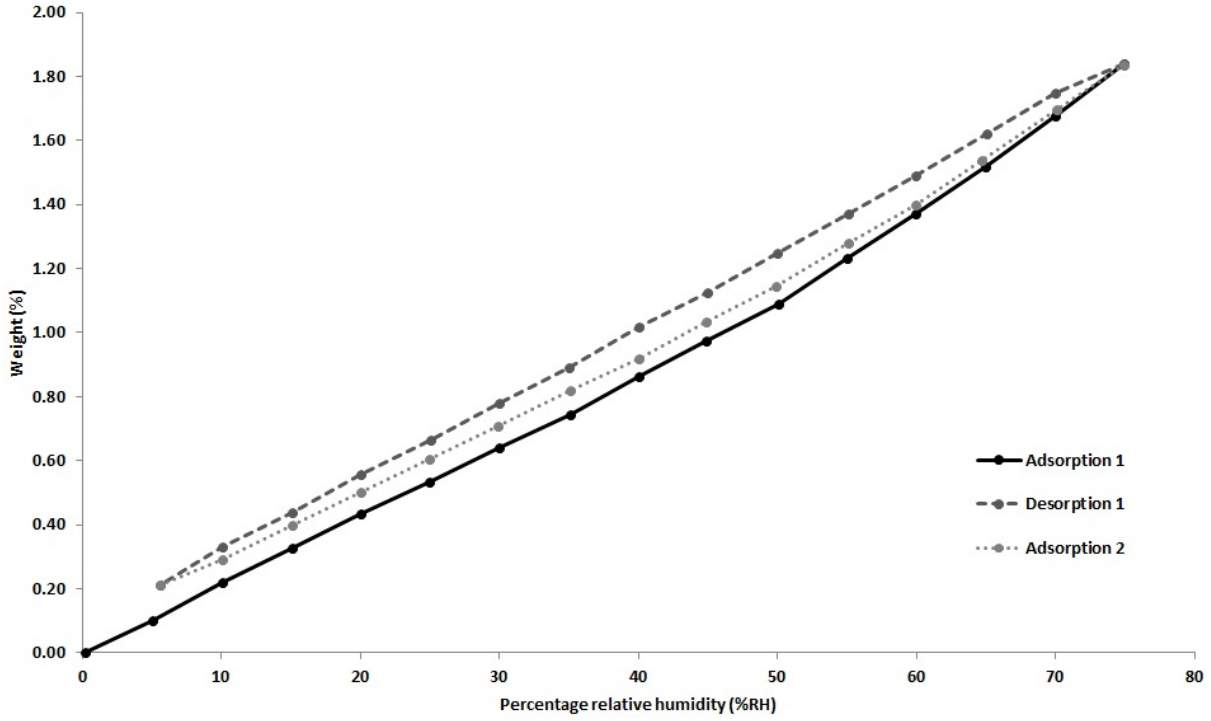

Figure 6: Vapor sorption isotherms obtained for R-CD at constant ambient temperature.

Figures 7 and 8 depict the vapor sorption isotherms obtained for R-QC and R-SC respectively. Both adsorption isotherms can be classified as Type III isotherms, which is indicative of non-porous compounds where the adsorbent-adsorbate interaction is considered to be weak (Rouquerol *et al*., 1999). For R-QC this could be considered as somewhat contradictory results due to the fact that SEM analysis showed pores to be visibly part of the morphology of R-QC, however the pores were much less in comparison with that observed for R-CD. Therefore the amount of pores observed with R-QC does not influence the morphology to such an extent that it could be classified as a micro- or macroporous powder and from vapor sorption experiments it became evident that R-QC rather constitutes a non-porous morphology.

For both R-QC and R-SC hysteresis were observed. Hysteresis is a common occurrence with (a) highly porous compounds where capillary condensation within the pores is a possibility or with (b) amorphous compounds where conformational change can occur due to water uptake, causing an increased molecular mobility (Rouquerol *et al*., 1999). In the case of R-QC and R-SC it is hypothesized that the hysteresis is ascribed to slight conformational changes due to the presence of water. These conformational changes can be possible crystallization of the amorphous state to the stable crystalline state. The conformational changes were however not complete since the two forms remained amorphous even after the sorption processes were complete (confirmed by XRPD and DSC analyses). From this it can therefore be deduced that since R-QC and R-SC are somewhat fragile amorphous systems, the presence of water initiated an increase in molecular mobility that lead to a small degree and incomplete conformational changes. However, R-QC and R-SC are still considered sufficiently stable when exposed to high relative humidities, which pose to be advantageous in terms of pharmaceutical processability.

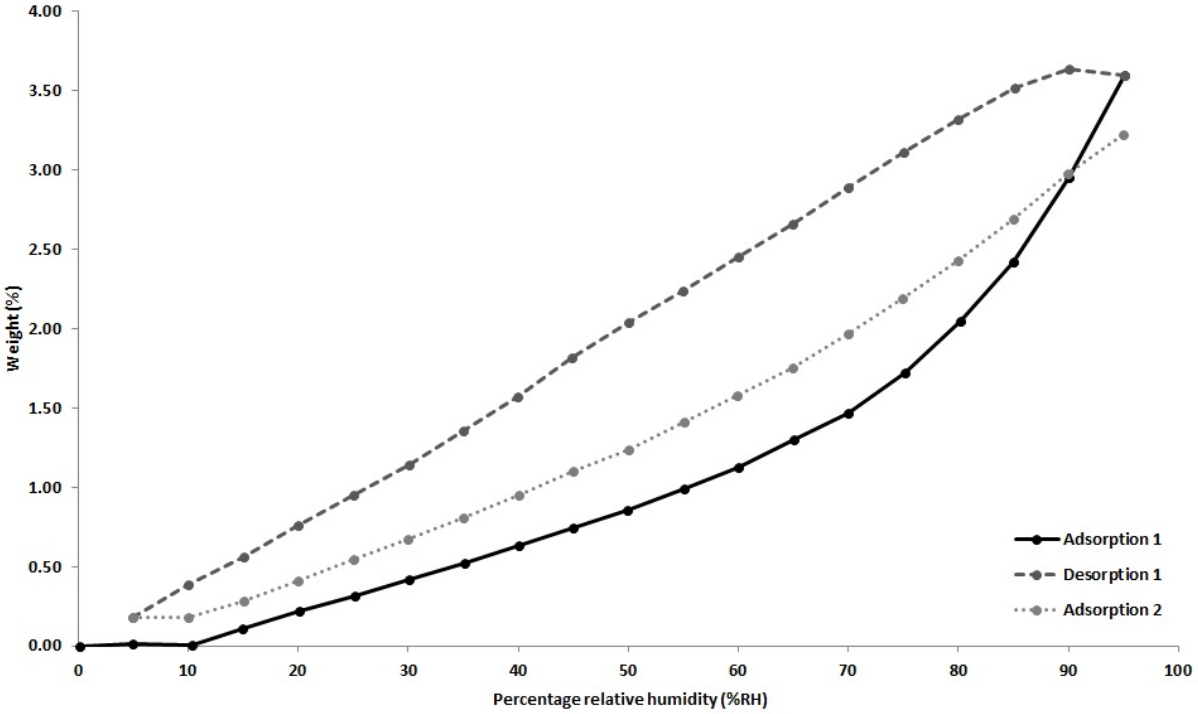

Figure 7: Vapor sorption isotherms obtained for R-QC at constant ambient temperature.

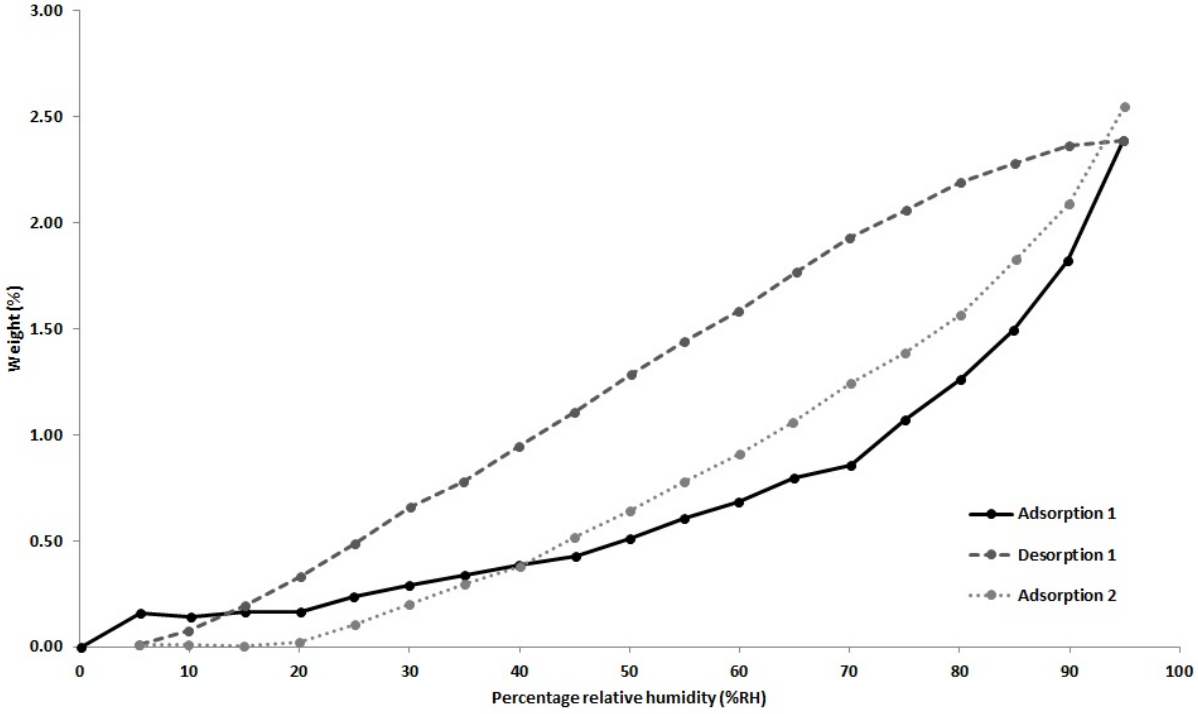

Figure 8: Vapor sorption isotherms obtained for R-SC at constant ambient temperature.

The vapor sorption isotherms obtained for R-HA (Figure 9) showed it to be a Type II isotherm, signifying it to be a non-porous compound (Rouquerol *et al.*, 1999). The observed hysteresis could be an indication of a small degree of conformational relaxation that occurred during the sorption experiments.

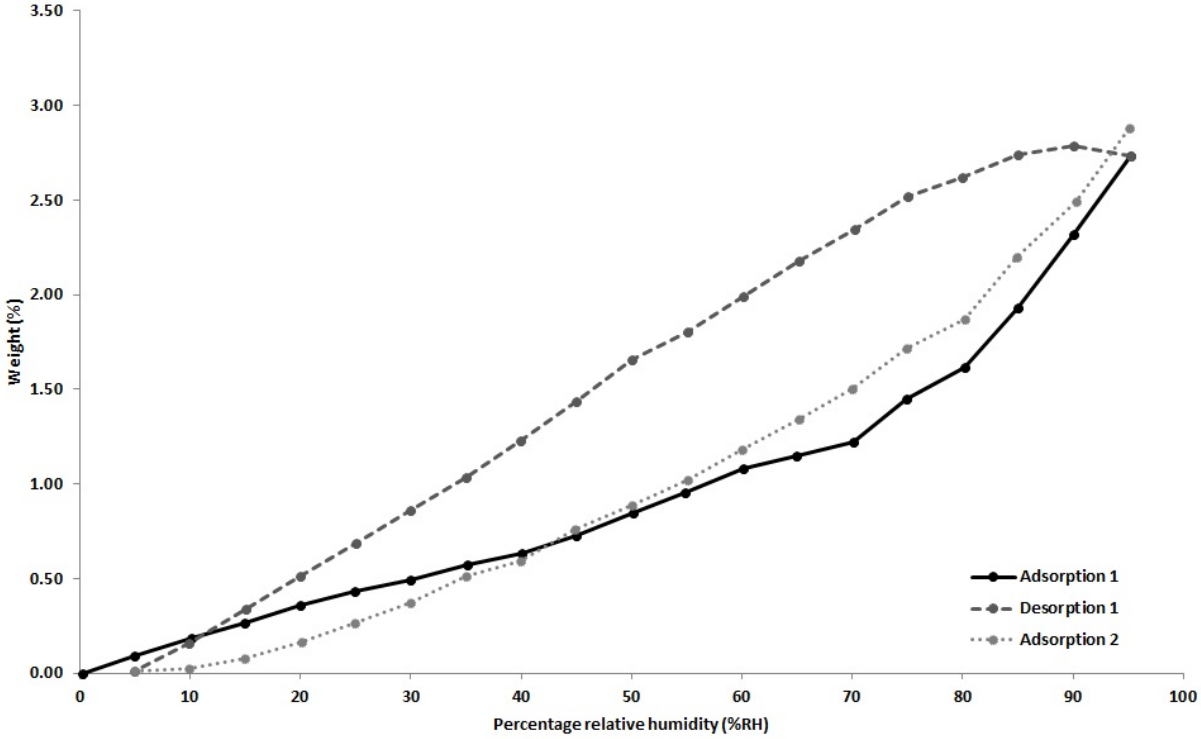

Figure 9: Vapor sorption isotherms obtained for R-HA at constant ambient temperature.

Considering the fact that there exist clear morphological differences between the different amorphous forms of roxithromycin, one cannot ignore the fact that such differences will directly impact solubility and dissolution characteristics.

2.6 Influence on solubility and dissolution properties

The equilibrium solubility of roxithromycin (Form III) was determined to be 0.034 mg/mL in distilled water at 37°C ± 0.5°C, after 24 h. The accurate determination of possible solubility enhancements of the roxithromycin amorphous solid-state forms was not possible due to the proven solution-mediated phase transformation when subjected to solubility and dissolution conditions (Aucamp *et al*., 2013). The thermodynamic approach described by Hancock and Parks, 2000, were therefore applied in order to estimate the theoretical maximum solubility improvement that would be possible with these amorphous forms (Patterson *et al*., 2005). According to the thermodynamic approach, the solubility ratio of the crystalline form to the amorphous form is considered to be directly related to the free energy difference between the two forms. Using this approach, Eq. (7) will apply:

$$\Delta G_T^{a,c} = -RTln\left(\frac{\sigma_T^a}{\sigma_T^c}\right) \qquad (7)$$

where *R* is the gas constant, $\frac{\sigma_T^a}{\sigma_T^c}$ is the solubility ratio at any given temperature of the amorphous (*a*) and crystalline (*c*), and $\Delta G_T^{a,c}$ is the free energy difference between these two

forms. The free energy difference is estimated from the entropy (*S*) and enthalpy (*H*) difference, Eq. (8):

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

and the enthalpy and entropy differences are calculated utilizing Eqs. 9, 10 and 11,

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

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

$$\Delta S_f^c = \frac{\Delta H_f^c}{T_f^c} \quad (11)$$

where T_f^c, are the melting points, ΔH_f^c and ΔS_f^c, is the enthalpy and entropy of fusion, and $C_p^a - C_p^c$, are the isobaric heat capacities (Hancock and Parks, 2000).

Table 2 summarizes the relative predicted solubility ratios for the amorphous forms of roxithromycin at 37°C. From the tabulated data it can be deduced that although there are differences in terms of the molecular dynamics as well as the morphology, a calculated/predicted thermodynamic approach results in predicted solubility ratios in close correlation with each other. Taking this aspect into account it would be useful to study the dissolution behavior of the different amorphous forms.

Table 2: Summary of the thermodynamically predicted solubility ratios for the different amorphous roxithromycin forms relative to crystalline roxithromycin.

Amorphous form	Predicted solubility ratio
R-CD	3.12
R-QC	2.88
R-SC	3.13
R-HA	3.13

From the dissolution profiles (Figure 10) it is evident that solution-mediated phase transformation of all the amorphous solid-state forms occurred during the dissolution process. This is expected due to the metastable nature of the amorphous forms. Furthermore, these results correlate very well with a previous study conducted on the quench cooled amorphous

form of roxithromycin (Aucamp *et al*., 2013). It is however interesting that all four amorphous forms did not follow the same dissolution profile.

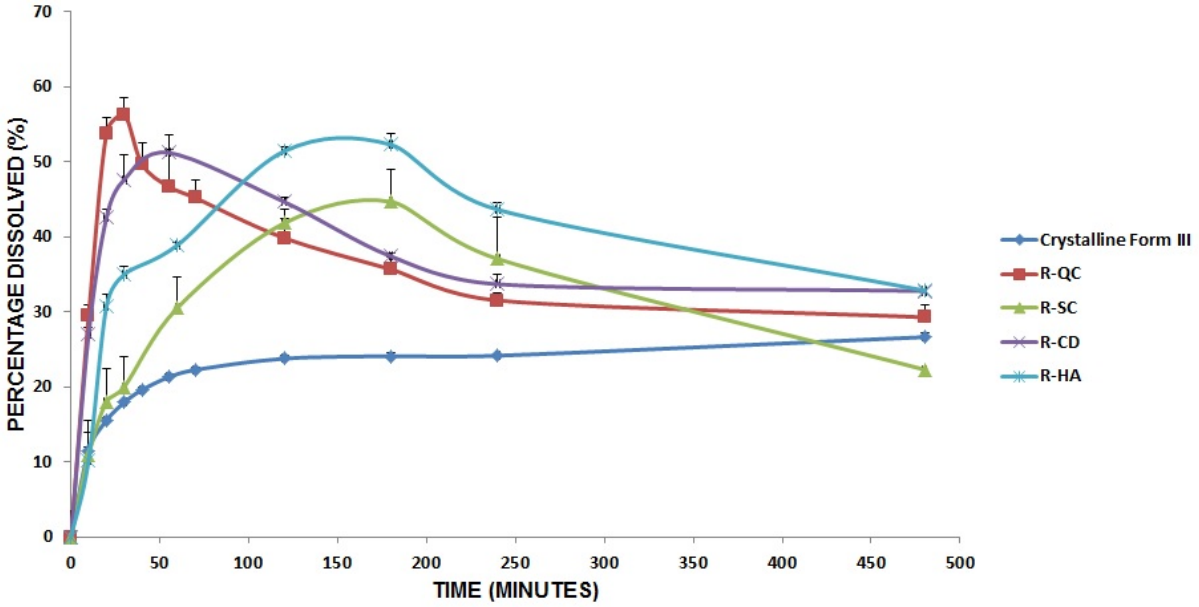

Figure 10: Overlay of the dissolution profiles of crystalline roxithromycin (Form III), R-CD, R-QC, R-SC and R-HA as obtained in distilled water at 37ºC ± 2ºC.

It should be considered that a substantial amount of factors will have an influence on the dissolution behavior of a compound and therefore it was a challenge to identify the exact cause for the differences between the dissolution profiles. Due to the significant morphological differences between the different forms, variation in dissolution profiles could be ascribed to various available surface properties. The well-known effect of surface area on the rate of dissolution as stipulated by the modified Noyes-Whitney equation (Eq. 12) was investigated in an effort to understand the dissolution behavior of the different amorphous forms:

$$\frac{dC}{dt} = \frac{AD(C_S - C)}{h} \qquad (12)$$

where *dC/dt* is the rate of dissolution, *A* is the surface area available for dissolution, *D* is the diffusion coefficient of the compound, C_S is the solubility of the compound in the dissolution medium, *C* is the concentration of drug in the medium at time *t,* and *h* is the thickness of the diffusion boundary layer adjacent to the surface of the dissolving compound (Leuner and Dressman, 2000). From this it can be seen that the rate of dissolution is directly proportional to surface area. The surface areas of all four amorphous forms as well as crystalline roxithromycin (Form III) were determined by applying the Brunauer-Emmet-Teller (BET) theory (Zhang and Zografi, 2000), denoted as Eq. 13:

$$W = \frac{W_m C_{B(\frac{p}{p_0})}}{\left[\left(1-\left(\frac{p}{p_0}\right)\right)\right]\left[\left(1-\left(\frac{p}{p_0}\right)+ C_B\left(\frac{p}{p_0}\right)\right)\right]} \qquad (13)$$

Where W is the weight of the adsorbed gas per dry weight of solid, p is the partial pressure of the gas, p_0 is the saturation pressure of the corresponding liquid at temperature, T, and W_m and C_B are constants. The specific surface areas of all solid-state forms are listed in Table 3.

Table 3: Calculated specific surface areas of crystalline roxithromycin and the different amorphous solid-state forms

Roxithromycin solid-state forms	Specific surface area (m^2/g)
Crystalline roxithromycin (Form III)	4.6
R-CD	21.4
R-QC	26.6
R-SC	22.7
R-HA	29.8

The rapid solution-mediated phase transformation of R-QC during dissolution testing could be ascribed to the higher specific surface area in relation to the other amorphous solid-state forms. It is hypothesized that the slower dissolution rate observed for R-HA is probably due to the static behaviour of the glassy powder, while in the case of R-SC it can be explained by the smooth glass-like surface morphology. Particles adhering to one another will minimize the available surface area resulting in a slower dissolution rate. The slower dissolution rate also results in a slower rate of solution-mediated phase transformation, with the transformation process only starting at about 180 minutes. This is approximately 120 minutes after the onset of the phase transformation for R-CD and R-QC. It is however very clear that accurate dissolution testing of amorphous solid-state forms remains a challenge. Particle size was not controlled in order to prevent possible solid-state transformation caused by the addition of excess energy through a milling process. Although all samples were subjected to a limited degree of milling, it was not sufficient in order to ensure an accurate particle size distribution. It is however argued that since all solid-state forms investigated were powdered for the same amount of time and to the same extent, at least some degree of comparison is possible.

Discussion

Much deliberation and different viewpoints arose from the growing interest into the investigation of different amorphous solid-state forms of the same drug. However, no literature reports exist on any first-order transitions between different amorphous states of the same drug and therefore the use of the term “polyamorphs” to describe different amorphous states

is considered debatable (Hancock *et al*., 2002, Shalaev and Zografi, 2002). Previous studies were conducted on different preparation techniques for different amorphous forms of indomethacin, glibenclamide, dipyridamole and carbamazepine and from these studies the term “pseudopolyamorphism” arose (Savolainen *et al*., 2007, Patterson *et al*., 2005). It is evident that even after a substantial amount of research has been done on the possible existence of “polyamorphs”, a proper descriptive term for more than one distinctly different amorphous form of the same drug is still evasive.

Previous work done on amorphous roxithromycin proved that this antibiotic can be prepared in an amorphous form *via* different intermediary states. These studies also showed amorphous roxithromycin to be a relative stable amorphous form with the unique thermal behaviour of not recrystallizing to the stable crystalline state once heated above T_g (Aucamp *et al*., 2012). All four preparation techniques investigated proved to render crystalline roxithromycin in true amorphous solid-state forms. Through visual observation of the different forms it was evident that some differences might exist. SEM images confirmed particle surface differences between these forms.

Thermal analyses of the four amorphous forms proved that differences do indeed exist in terms of structural dynamics. From structural relaxation data it was concluded that only R-CD forms a strong amorphous system, while R-QC, R-SC and R-HA form fragile systems with non-Arrhenius behavior. Through vapor sorption studies the powder surface differences were investigated even further. These studies proved that R-CD is a stable amorphous form, without any conformational changes occurring during exposure to high relative humidities. Although R-HA exhibited the same BET isotherm type as R-CD, the hysteresis showed that conformational changes do occur when R-HA is being subjected to standard pharmaceutical storage conditions. Both R-QC and R-SC showed typical Type III isotherms. The fact that the conformational changes were small and definitively incomplete, is considered to be an indication of stability exhibited by all four amorphous forms, considering the metastable state in which they exist.

It was however illustrated that powder dissolution testing is not the most suitable method to investigate the influence of different amorphous forms on the dissolution behavior of a drug. Reasons being, that in the case of amorphous forms one has to deal with the inevitable solution-mediated phase transformation, as well as the influences of shape, size and particle morphology during dissolution testing. Therefore, a dissolution method for the investigation of differences between various amorphous forms will include too many variables, making it an ineffective method to identify physico-chemical differences between amorphous forms of the same drug.

Based on our findings, together with that of previous work done, the following principles for different amorphous forms of the same drug can be proposed: (a) different amorphous forms of the same drug can be obtained through applying different preparative techniques, (b) amorphous forms of the same drug can differ from one another, irrespective of the intermediary step used as a preparative technique, (c) a specific preparation method will always provide a specific amorphous state, (d) different amorphous forms differ in terms of thermodynamic properties, which will influence all other physico-chemical properties of the solid-state forms and (e) different amorphous forms vary in terms of morphological properties, influencing the powder properties and subsequent drug performance during pharmaceutical processing. It is understandable that the term polyamorphism cannot be applied to different amorphous forms obtained through different preparation techniques that are not distinguished through clear first-order phase transitions. Through reviewing this study and many others it is suggested that the term pseudo-polyamorphism or atypical polyamorphism should be used to describe amorphous forms of the same drug that was obtained through different preparation methods. These pseudo-polyamorphous forms differ in terms of molecular dynamics and physico-chemical properties, and do not convert from one amorphous state to another via a first order phase transition.

Conclusion

It was demonstrated that different preparation techniques such as melt, solution, and a combination of a solution intermediary step followed by mechanical disruption could be used to obtain amorphous forms of roxithromycin. Caution should be used when characterizing different amorphous forms of a single compound, since each technique used to prepare these forms influences the thermodynamic properties as well as the morphology of the specific amorphous forms obtained. More importantly, pharmaceutical industries can take advantage of the physico-chemical and thermodynamic differences resulting from various preparative techniques to meet their specific product development needs.

Acknowledgements

The authors are very thankful for the financial support received from the National Research Foundation (NRF) of South Africa (Grant nr: TTK13020718661) as well as the Centre of Excellence for Pharmaceutical Sciences (Pharmacen) at the North-West University, Potchefstroom, South Africa. Without their contribution, this study would not have been possible.

Disclaimer

Any opinion, findings and conclusions, or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regard thereto.

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CHAPTER 5

This chapter was submitted to *Die Pharmazie as a short communication.*

Marnus Milne, Wilna Liebenberg, Marique Aucamp. 2015. A non-isothermal dehydration study of zopiclone dihydrate

Current impact factor: 1.052

Instructions to authors (summary)

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The journal Die Pharmazie publishes reviews, experimental studies, letters to the editor, as well as book reviews. The following fields of pharmacy are covered: Pharmaceutical and medicinal chemistry, pharmaceutical analysis and drug control, pharmaceutical technology, biopharmacy (biopharmaceutics, pharmacokinetics, biotransformation), experimental and clinical pharmacology, pharmaceutical biology (pharmacognosy), history of pharmacy. Articles are published in English (preferred) or German and are classified as:

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Letter to the editor

13 November 2015

To the editor of Die Pharmazie journal

It would be considered a privilege if you would consider our short communication for publication in Die Pharmazie.

The solid-state forms in which a drug can exist have a significant impact on product processing as well as the behaviour and performance of the drug after patient administration. Zopiclone can exist in more than one solid-state form, one of which is a dihydrate. It is imperative to investigate processes that would cause solid-state transformations of a given drug. This short communication reports on the dehydration of this dihydrate with emphasis on the kinetics involved in this dehydration process.

Thanking you in advance and kind regards

Dr. Marique Aucamp

A non-isothermal dehydration study of zopiclone dihydrate

Authors: M. Milne, M.E. Aucamp* and W. Liebenberg

Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa

*Corresponding author: Marique Aucamp, Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa. Tel.: +27182994472; Fax: +27875432. Email address: Marique.Aucamp@nwu.ac.za

Abstract

Previous studies have shown that zopiclone, in the solid-state, can either exist as true polymorphs, a dihydrate or an anhydrate. In order to gain better insight into the behaviour of pharmaceutical hydrates throughout the manufacturing and storage processes, it is important to characterise the effects of water loss on crystal hydrates. The dehydration behaviour of zopiclone Form B has never been reported on and was therefore investigated by non-isothermal kinetic studies. From the dehydration kinetics as well as hot-stage microscopy (HSM) it was determined that the dehydration of zopiclone Form B requires little activation energy and that the dehydration occurs without complete disruption of the molecular framework.

Keywords: zopiclone, dehydration kinetics, activation energy, anhydrate

Introduction

It is widely known that approximately one third of pharmaceutical substances are capable of forming hydrates (Vippagunta *et al.,* 2001). A hydrate is defined as a solid adduct which contains both the principal compound, in this case the drug, as well as water. Different hydrates of the same drug are a common occurrence. The inclusion of water molecules into a crystal lattice will unequivocally change the shape, symmetry, dimensions and capacity of the unit cell. Subsequently, causing that each hydrate of a given drug will exhibit different physico-chemical properties (Khankari & Grant, 1994). Previous studies conducted on different solid-state forms of zopiclone showed that this hypnotic drug can exist as true polymorphs, an anhydrate or a dihydrate (Form B). Form B has a lower melting point, indicating it to be a thermodynamically less stable form in comparison with the described anhydrate (Terblanche, 1999, Giovannini *et al*., 2001, Milne, 2015). Solid-state form transformations due to hydration or dehydration during processing or once formulated into a final product should always be kept in mind. Therefore, it is considered imperative to investigate the dehydration behaviour of a drug, since dehydration can lead to significant

changes in terms of drug powder properties, solubility and dissolution rate, to name but just a few.

Investigations, results and discussion

Zopiclone Form B was recrystallised from toluene. From TGA data it was observed that Form B starts to dehydrate at approximately 56.8°C (heating rate of 10°C/min). During the whole dehydration process Form B loses 8.8% weight, which confirms a stoichiometric 1:2 (zopiclone: water) ratio. In this study the dehydration kinetics of Form B was investigated through the application of two well-known model free methods, namely; the Kissinger and Ozawa-Flynn-Wall methods. Most existing reaction models that have been developed are based on equation (1) (Khawam & Flanagan, 2006):

$$\frac{d\alpha}{dt} = k(1-\alpha)^n \qquad (1)$$

Where in this case, α, is the degree of dehydration, t is time and n is the reaction order. By applying the Kissinger method, equation (2) can be applied to kinetic data:

$$\ln\left(\frac{q}{T^2}\right) = \ln\left(\frac{AR}{E_a}\right) - \frac{E_a}{RT} \qquad (2)$$

Where q is the heating rate, T is the peak temperature of dehydration, E_a is the activation energy and A is the pre-exponential factor. From the dehydration data obtained by applying different heating rates a Kissinger plot of $ln\ (q/T^2)$ against $1000/T$ was plotted. The E_a was calculated to 86.46 ± 1.5 kJ.mol^{-1}. The second model free approach used was that of Ozawa-Flynn-Wall (OFW). This method is an integral method that determines $\frac{-E_a}{R}$ from the slope of a plot of log (q) against $\frac{1}{T}$. This model can be expressed as:

$$\log(q) = \log\left(\frac{AE_a}{RG_\alpha}\right) - 2.315 - 0.4567\frac{E_a}{RT} \qquad (3)$$

Under the isoconversion assumption, G_α reaches a given value, therefore becoming a constant. Therefore, this allows equation 3 to be written as:

$$\log(q) \cong -0.4567\frac{E_a}{RT} \qquad (4)$$

Through applying the linear plot of $log\ (q)$ against $\frac{1}{T}$ and equation 4, the E_a for dehydration of zopiclone Form B was calculated to be 71.83 ± 1.0 kJ.mol^{-1}. The two methods correlated well with one another with a calculated $p > 0.005$. The poor linearity obtained for the 9.0% dehydration can be attributed to the fact that dehydration reached completeness, since an 8.8% weight loss was obtained for Form B.

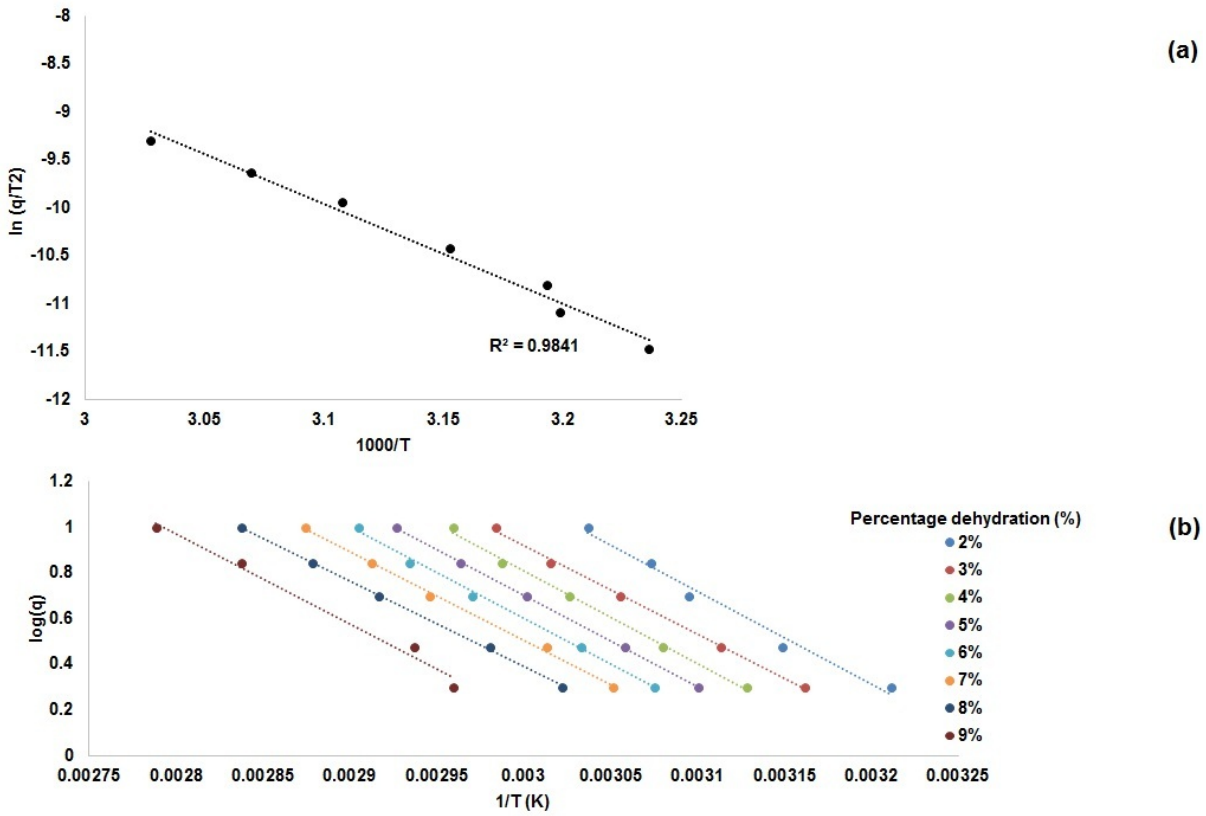

Figure 1: (a) A Kissinger plot and (b) OFW method plot, both obtained from dehydration of zopiclone Form B at different heating rates (*q*).

The thermal stability of Form B can further be evaluated by using equation 5:

$$\Delta T = T_{on} - T_b \qquad (5)$$

Where (ΔT) represents the relative stability of the hydrate, (T_{on}) the onset temperature of dehydration, and (T_b) the solvent boiling point. The hydrate will be considered "stable" should the obtained value be positive and unstable / reactive should the ratio be negative (Caira, 2004). The calculated value of the ratio was -43.19°C, therefore suggesting that a complete disruption of the host framework, in order for the water molecules to be released, is not necessary. HSM micrographs (Figure 2 (a)) shows that dehydration starts at one edge of the crystals, moving in one direction, Figure 2(b) shows the resulting anhydrate, clearly indicating the intactness of the crystal.

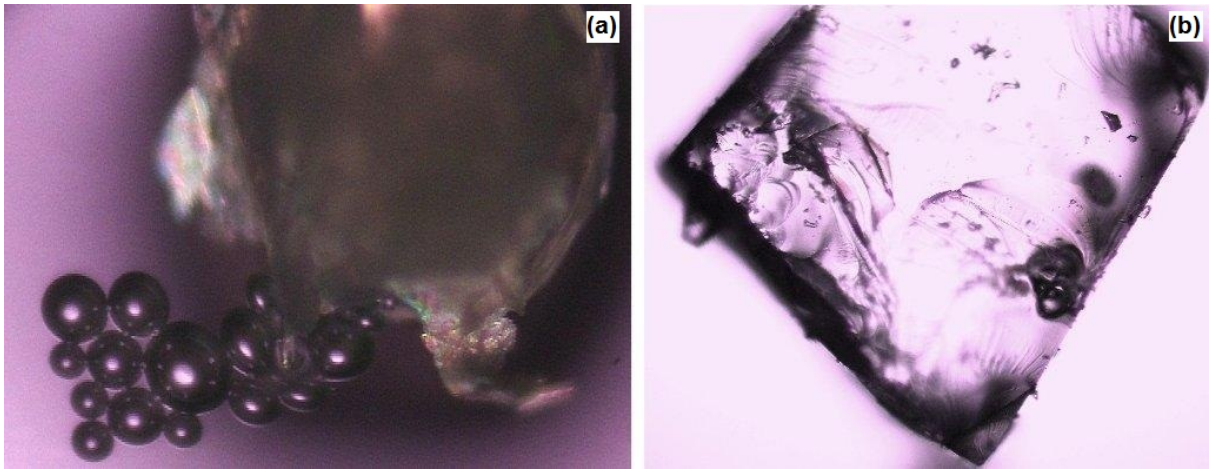

Figure 2: HSM micrographs of form B crystal (a) and the obtained anhydrate (b).

Experimental

Crystalline anhydrous zopiclone was purchased from DB Fine Chemicals Pty Ltd. (Johannesburg, South Africa). Toluene used in this study was of analytical grade and ultrapure water with a resistivity of 18.2 MΩ.cm^{-1} was used. For the recrystallisation of Form B approximately 1 g of zopiclone anhydrate was dissolved in 100 mL toluene, stirring continuously and heating the solution to 60 ± 5°C. An aliquot of cold ultrapure water was added drop wise to the solution. The solution was left for slow evaporation to occur. Dehydration studies were done using a Shimadzu (Kyoto, Japan) DTA-60 instrument. Samples were accurately weighed (4 mg ± 0.5 mg) into aluminium crucibles. Each sample was allowed to equilibrate to 25°C for 5 minutes. Heating rates of 1, 1, 5, 2, 3, 5, 7 and 10 °C/min were used and each sample was heated to 200 °C (n = 2). The dehydration kinetics was determined by applying two model free methods, namely, the Kissinger and OFW method. HSM was performed with a Nikon Eclipse E4000 microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan) and a Linkam THMS600 heating stage equipped with a T95 LinkPad temperature controller (Surrey, England).

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Concluding remarks

The unique properties brought about by the amorphous solid-state, can be ascribed to both molecular level structure and macroscopic properties. The susceptibility for recrystallisation of the metastable amorphous form to the stable crystalline form can be influenced by several factors. Furthermore, each amorphous form of a given drug is unique in terms of physico-chemical and stability properties. What makes amorphous forms of drugs even more interesting and to some extent challenging to work with is the unpredictability of these metastable forms. Better understanding of each amorphous solid-state form of any given drug can be brought about by studying not only the physical and chemical behaviour but also the thermodynamic properties and how this influences the molecular dynamics. Knowledge gained through this can result in the development of newer or more advanced methods for not only stabilising metastable forms but also to further enhance the performance and behaviour of these solid-state forms.

This study is comprised of four individual studies. The research conducted on zopiclone involved the preparation of an intermediary solid-state form, i.e. a dihydrate with a lower melting point in comparison with the commercial available anhydrate form. This intermediary form facilitated easier melting and quenching of molten zopiclone to obtain amorphous zopiclone. The neat amorphous solid-state form prepared through quench cooling of the melt resulted in a relative unstable amorphous form which recrystallised to the stable crystalline form when exposed to increasing temperatures, relative high humidity or sample agitation. From this study an amorphous solid dispersion of zopiclone was prepared, rendering the amorphous form stable with improved aqueous solubility and a higher dissolution rate. Currently, in literature this is the first reported amorphous solid dispersion of zopiclone.

Forthcoming from the preparation method of amorphous zopiclone it became evident that a recrystallisation process of crystalline zopiclone from toluene resulted in a dihydrate (Form B). This form has previously been described in literature. However, an interesting observation made was that dehydration of this form starts at a significantly low temperature (≅ 57°C). Furthermore, from thermal studies it became evident that the dehydration of this solid-state form does not require much added energy (i.e. a significant increase in temperature). From a short non-isothermal investigation it was determined that zopiclone Form B, easily dehydrates in a temperature region of ≅ 57 – 98°C. Due to this characteristic, zopiclone Form B can be considered a metastable form which easily will convert to an anhydrate.

The second study reported on the physical stability and crystallisation kinetics of amorphous sulfadoxine. Currently, in literature this is the first reported and investigated amorphous form of sulfadoxine. The amorphous sulfadoxine was successfully prepared by quench-cooling of the crystalline solid. Even though sulfadoxine has a good glass forming ability, and the quench cooled method led to the formation of a strong glass, sulfadoxine still exhibited strong recrystallisation tendencies. This recrystallisation tendency was especially driven by high temperatures and moisture. Given the fact that physical mixtures of this amorphous drug with a polymer resulted in inhibition of the temperature dependent crystallisation of the metastable form to the more stable crystalline form, proved that amorphous sulfadoxine can be considered a viable option to improve its poor aqueous solubility. Further studies on amorphous sulfadoxine in combination with different types and ratios of polymers will form a sound basis for possible inclusion of amorphous sulfadoxine into solid dosage forms.

The third study reported on the thermodynamic and morphological properties of different amorphous solid-state forms of roxithromycin. This study was conducted in an attempt to further investigate the phenomenon of polyamorphism within organic pharmaceutical compounds. Four different methods with differing intermediary steps, namely, melt, solution, and a combination of solution followed by mechanical disruption, were employed to successfully obtain amorphous roxithromycin. All these preparation methods resulted in dissimilar thermodynamic as well as morphological properties. Form this and an extensive literature study it was concluded that the term polyamorphism cannot be applied to describe differing amorphous solid-state forms of drugs when the various amorphous forms do not transform from one to the other through a first order transition. First order transition from one amorphous form to another is mainly reported to occur in inorganic compounds and none has thus far been reported in organic compounds. It is therefore proposed that the term pseudo-polyamorphism or atypical polyamorphism would be a more suitable term to describe the clear differences between varying amorphous forms of the same organic compound.

This study emphasised the previously made statement that each amorphous form of a given drug is unique in terms of physico-chemical and stability properties. During this study zopiclone proofed to follow all rules currently established in this field. Low glass forming ability results in low stability, but which can be overcome by formulation adjustments. Sulfadoxine deviates from these rules, and shows that good glass forming ability does not always infer good stability. Roxithromycin is an ideal case where more than one preparation method can be utilised to obtain an amorphous form. More interesting is that these different routes all results in stable

definitively different amorphous forms. With this in mind, one can safely admit that there is still much to explore and learn from this interesting research area. The most valuable fact learned from this study on different amorphous forms of three distinctly different drugs is that each amorphous compound should be treated and evaluated individually. Also knowing that for each amorphous solid-state form, the physico-chemical and stability properties thereof remain unpredictable.