

Investigation of the physico-chemical properties of amorphous solid-state forms of azithromycin

A Joubert
11781963

Thesis submitted for the degree *Doctor Philosophiae* in
Pharmaceutics at the Potchefstroom Campus of the North-West
University

Promoter: Dr M Aucamp
Co-Supervisor: Dr N Stieger
Assistant Supervisor: Prof W Liebenberg

October 2016



Table of contents

Table of contents	i
Acknowledgements	x
Abstract.....	xi
Uittreksel.....	xiii

Chapter 1

Solid-state properties of active pharmaceutical ingredients

1.1 Introduction.....	1
1.2 Physical states of active pharmaceutical ingredients	2
1.2.1 The gas/vapour and liquid phases	3
1.2.2 Solid-state pharmaceutical chemistry.....	3
1.3 The crystalline phase	4
1.4 Polymorphism.....	6
1.5 Solvates.....	7
1.5.1 Stoichiometric solvates	8
1.5.2 Non-stoichiometric solvates.....	8
1.6 Hydrates	9
1.7 Salts	10

1.8	Amorphous, non-crystalline, or glassy phase.....	11
1.8.1	Preparation and preparation techniques of amorphous forms.....	13
1.8.2	Advantages of amorphous forms	15
1.8.3	Disadvantages of amorphous forms.....	16
1.9	Methods to improve solubility.....	17
1.10	Phase transformations.....	18
1.11	Conclusion.....	20
1.12	Reference list.....	21

Figures & Tables

Figure 1.1:	Different physical states of an active pharmaceutical ingredient and the effects of temperature changes on these states (Ymén, 2011).....	2
Figure 1.2:	Illustration of the different solid-state forms of APIs (Hilfiker <i>et al.</i> , 2006)	4
Figure 1.3:	Indication of the axes and angles of a cell unit in crystals (Ymén, 2011)	5
Table 1.1:	Different axes and angle combinations of a cell unit in crystals (Ymén, 2011)	6
Figure 1.4:	Images to illustrate the different principles of crystalline solids in relation to solvents (Griesser, 2006).....	8
Figure 1.5:	Solvate classification, described in conjunction with different types of binary systems (Griesser, 2006).....	9
Figure 1.6:	Graphic representation of the crystallisation process, or the glass transition of a compound (Craig <i>et al.</i> , 1999).....	12
Figure 1.7:	Graphic representation of the formation of a glassy phase during which the crystallisation process is temperature dependent (Craig <i>et al.</i> , 1999)	13

Chapter 2

Azithromycin: a macrolide (azolide) antibiotic

2.1	Introduction.....	25
2.2	Macrolide classification.....	26
2.2.1	Early development of macrolides.....	26
2.2.2	The macrolide structure.....	27
2.2.3	Physical and chemical properties of azithromycin.....	28
2.2.3.1	<i>Definition and appearance</i>	29
2.2.3.2	<i>pH</i>	29
2.2.3.3	<i>Water content</i>	29
2.2.3.4	<i>Loss on drying</i>	29
2.2.3.5	<i>Solubility and solubility characteristics</i>	30
2.2.3.6	<i>Melting point</i>	31
2.2.3.7	<i>Acid stability</i>	31
2.2.3.8	<i>Dissociation constant</i>	32
2.3	Pharmaceutical uses and advantages of azithromycin.....	32
2.4	Solid-state forms of azithromycin.....	35
2.5	Conclusion.....	36
2.6	Reference list.....	38

Figures & Tables

Figure 2.1: The chemical structure of azithromycin anhydrous and x H ₂ O, representing a monohydrate or dihydrate, according to the United States Pharmacopeial Convention (USPC, 2015a).....	27
--	----

Chapter 3

Materials and methods

3.1	Introduction.....	43
3.2	Materials.....	43
3.3	Methods used for the preparation of amorphous azithromycin.....	43
3.4	Characterisation methods.....	45
3.4.1	Differential scanning calorimetry.....	45
3.4.2	Thermogravimetric analysis.....	46
3.4.3	Scanning electron microscopy.....	47
3.4.4	Fourier-transform infrared spectroscopy.....	48
3.4.5	X-ray powder diffraction.....	48
3.4.6	Vapour sorption studies.....	50
3.4.7	Heat of solution determinations (solution calorimetry).....	52
3.4.8	Solubility studies.....	53
3.4.9	Powder dissolution studies.....	53
3.4.10	High performance liquid chromatography analysis.....	53
3.5	Conclusion.....	54
3.6	Reference list.....	55

Figures & Tables

Figure 3.1: Thermogram illustrating DSC thermal behaviour of an amorphous form (Saunders & Gabbott, 2011).....	46
Figure 3.2: Water sorption isotherm of a typical solid (Heng & Williams, 2011).....	51
Figure 3.3: Isotherm classification types (Reutzel-Edens & Newman, 2006).....	51

Chapter 4

Different amorphous solid-state forms of azithromycin

4.1	Introduction.....	58
4.2	Preparation methods for different azithromycin amorphous forms	58
4.3	Physico-chemical characterisation of the obtained azithromycin amorphous solid-state forms.....	61
4.3.1	Determination of the thermodynamics of the azithromycin amorphous forms.....	61
4.3.2	The amorphous habit of the differently prepared azithromycin amorphous forms.....	69
4.3.3	The stability of the five azithromycin amorphous forms	75
4.4	Conclusion and summary	84
4.5	Reference list.....	87

Figures & Tables

Table 4.1:	A summary of all the preparative techniques and the corresponding codes used for the prepared amorphous forms	59
Figure 4.1:	Overlay of the XRPD diffractograms obtained for A-TB (slow evaporation from a solution of azithromycin in tert-butyl alcohol) and A-DH (commercially obtained azithromycin dihydrate).....	60
Table 4.2:	Thermodynamic properties calculated for crystalline azithromycin dihydrate and five differently prepared amorphous forms of azithromycin.....	61
Figure 4.2:	An overlay of DSC thermograms obtained for (a) A-QC, (b) A-SC, (c) A-HA, (d) A-RV, (e) A-SD and (f) A-DH. With the red marks indicating the onset and end temperatures of either the glass transition temperatures (T _g) or the melting of A-DH.	62

Figure 4.3: Arrhenius plots for the glass transition temperature (T_g) affected by varying heating rates (q). Intermediary state: melt with (a) A-QC, (b) A-SC and (c) A-HA.....	65
Figure 4.4: Arrhenius plots for the glass transition temperature (T_g) affected by varying heating rates (q). Intermediary state: solution with (a) A-RV and (b) A-SD.....	66
Table 4.3: The calculated fragility index (m), strength parameter (D), zero mobility temperature (T_0) and reduced glass transition ratio (T_{rg}).....	68
Figure 4.5: XRPD diffractogram of crystalline A-DH.	70
Figure 4.6: An overlay of XRPD diffractograms obtained for A-QC, A-SC, A-HA, A-RV and A-SD immediately after preparation of the amorphous forms.....	71
Table 4.4: FTIR peak listings reported for azithromycin dihydrate.	72
Figure 4.7: FTIR spectra for (a) azithromycin dihydrate and (b) amorphous azithromycin as reported by Jasanada <i>et al.</i> (2001).	72
Figure 4.8: FTIR spectrum obtained for crystalline A-DH.	73
Figure 4.9: An overlay of the FTIR spectra obtained for A-QC, A-SC, A-HA, A-RV, A-SD and A-DH.....	74
Table 4.5: FTIR peak listings for the different amorphous azithromycin preparations (A-QC, A-SC, A-HA, A-RV and A-SD).....	74
Figure 4.10: Vapour sorption isotherm obtained for A-QC. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.....	76
Figure 4.11: Vapour sorption isotherm obtained for A-SC. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.....	76
Figure 4.12: Vapour sorption isotherm obtained for A-HA. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.....	77
Figure 4.13: Vapour sorption isotherm obtained for A-RV. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.....	77
Figure 4.14: Vapour sorption isotherm obtained for A-SD. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.....	78
Figure 4.15: SEM micrographs obtained for A-QC.	79

Figure 4.16: SEM micrographs obtained for A-SC.....	79
Figure 4.17: SEM micrographs obtained for A-HA.....	80
Figure 4.18: SEM micrographs obtained for A-RV.....	80
Figure 4.19: SEM micrographs obtained for A-SD.....	81
Figure 4.20: A typical dissolution profile obtained during the study of solution-mediated phase transformation of an API (Aucamp <i>et al.</i> , 2015).....	82
Figure 4.21: An overlay of the dissolution profiles obtained for A-QC, A-SC, A-HA, A-RV, A-SD and A-DH in distilled water at 37°C.....	83
Figure 4.22: The calculated surface area and maximum percentage absorbed moisture for all azithromycin amorphous solid-state forms investigated for SMPT.....	84

Chapter 5

Instructions for Authors for Drug Development and Industrial Pharmacy

5.1 About the journal.....	89
5.1.1 Aims and scope	89
5.1.2 Editor-in-chief.....	90
5.2 Manuscript submission	90
5.3 Rapid publication	90
5.4 Manuscript preparation	91
5.4.1 File preparation and types.....	91
5.4.2 Title page	91
5.4.3 Abstract.....	91
5.4.4 Main text.....	92
5.4.4.1 Original articles	92

5.4.4.2 Reviews	92
5.4.5 Acknowledgments and declaration of interest sections.....	93
5.4.5.1 Acknowledgments section	93
5.4.5.2 Declaration of interest section.....	93
5.4.6 References	94
5.4.7 Tables.....	94
5.4.8 Illustrations	94
5.4.9 Notes on style	95
5.4.9.1 General Style.....	95
5.4.9.2 Abbreviations and nomenclature.....	97
5.4.9.3 Footnotes.....	97
5.4.10 Editorial policies	97
5.4.10.1 Authorship.....	97
5.4.10.2 Redundant publication and plagiarism	98
5.4.10.3 Peer review	98
5.4.10.4 Ethics and consent	99
5.4.10.5 Copyright and permissions	99
5.4.10.6 Declaration of interest.....	100
Letter to the editor.....	101
The stability of amorphous azithromycin: a thermodynamic and kinetic perspective	102

Chapter 6

Concluding remarks

Concluding remarks 126

Acknowledgements

The author of this thesis would like to take this opportunity to show his gratitude and appreciation by acknowledging and giving a tremendous heart-warming word of thanks to the following people and institutions:

The author is 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 (Pharmacem) at the North-West University, Potchefstroom, South Africa. Without their contribution, this study would not have been possible.

A special thanks to the Research Institute for Industrial Pharmacy (RIIP)[®] incorporating CENQAM[®] and in particular thanks to Dr. E. Swanepoel for granting me the opportunity to enroll for this *Doctor Philosophiae* degree while working full time and for the granting and understanding in approving of study leave requests etc.

Also very special thanks to Prof. W. Liebenberg, Dr. M. Aucamp, Dr. N. Stieger and Mr. N. Barnard who helped with the performing of the different analyses and instrument setups. I would like to thank Prof. W. Liebenberg, Dr. M. Aucamp and Dr. N. Stieger for their time, inputs, guidance and uplifting words during the tuff and exhausting times.

Thanks to Dr. L. Tiedt for the SEM analysis on the different prepared samples.

A very special thanks to my family that supported me through this journey and all their uplifting words that kept me going. And then finally to my beautiful and dearest wife Sanri Joubert that stood by me during this time through all the low's and all the high's. Thank you for the support and understanding and giving me this opportunity to do my *Doctor Philosophiae*.degree. I know I had to say No many times to spend time with you. I appreciate you more than you will ever know. Love you.

Abstract

Azithromycin is chemically modified from the macrolide, erythromycin and thereby shows improved efficacy and has more advantages above other macrolides. Azithromycin is currently the most prescribed and used macrolide antibiotic worldwide with much less frequent oral administration required. The biggest disadvantage of azithromycin is its poor water solubility. Poor water solubility of an active pharmaceutical ingredient (API) is seen as a critical factor, which can have a detrimental effect on not only the bioavailability of the API, but also the effective treatment of patients. A complete physico-chemical characterisation is extremely important for an API as it may exist in different solid-state forms which can display different physico-chemical and thermodynamic properties. Stability, solubility, dissolution rate, bioavailability, particle morphology, powder flow, powder colour and tableting behaviour are all properties that can be influenced by these differences. The better the dissolution rate of an API, the better is the absorption from the gastrointestinal tract (GIT), leading to improved bioavailability of the API. Preparing an amorphous solid-state form of an API is an effective and easy way to improve the aqueous solubility. However, the inherent instability of these solid-state forms is usually detrimental.

Preparation methods such as quench cooling of the melt, slow cooling of the melt, hot-air melting, ambient solvent evaporation, rapid solvent evaporation and spray-drying, all with different intermediary states (melt or solution), were identified and selected for the preparation of amorphous azithromycin. The possibility of solution-mediated, solvent-mediated and solid-solid phase transformations of amorphous azithromycin was also investigated. The principal goal was to investigate the impact and to illustrate the effect of the different preparation methods on the physico-chemical characteristics of each obtained amorphous solid-state form of azithromycin. XRPD and FTIR positively characterised the amorphous habit of the different preparation techniques in this study showing that each mentioned preparation method can indeed create amorphous forms of azithromycin. The thermodynamic properties of all the preparation techniques reflected a fragility index (m) lower than 75 and a high strength parameter (D), meaning that a lower free energy is present that will lead to a higher physical stability. Two amorphous forms prepared from a solution intermediary state showed significantly higher temperatures of zero mobility making them more physically stable during ambient storage conditions. The amorphous form prepared from a spray-drying technique showed the lowest activation energy for structural relaxation, thereby contradicting the temperature of zero mobility finding of this amorphous form. The stability of amorphous forms can also be influenced by means of solid-solid phase transformation, solvent-mediated phase transformation and solution-mediated phase

transformation (SMPT). Vapour sorption experiments proved that the different amorphous forms are not influenced by solvent-mediated phase transformations. Dissolution was used to investigate the possibility of SMPT of the amorphous forms. The rates of SMPT differed and in some instances the transformation was not visible from dissolution data, proving that the rate of SMPT was too rapid. Through investigation of the recrystallisation behaviour of amorphous quench cooled azithromycin, it became evident that the recrystallisation process followed a first-order reaction rate. An 8-fold solubility enhancement in comparison with the solubility of crystalline azithromycin was determined by applying a Nogami plot.

This study proved that it is possible to prepare different amorphous forms of the same API and that these different amorphous forms differ substantially in terms of particle morphology, physical stability and ultimately in terms of dissolution rates.

Keywords: azithromycin; poor water solubility; stability; fragility index; solution-mediated phase transformation (SMPT); Nogami

Uittreksel

Asitromisien, 'n makrolied (asaliëd) antibiotikum wat chemies gemodifiseer is vanaf eritromisien, toon verbeterde effektiwiteit sowel as ander voordele bo ander makroliede. Met asitromisien is minder dosisse op 'n dag nodig en tans is dit die antibiotikum wat wêreldwyd die meeste voorgeskryf word. Die grootste nadeel van asitromisien is sy swak wateroplosbaarheid. Swak wateroplosbaarheid van 'n geneesmiddel word beskou as 'n kritiese faktor wat 'n nadelige uitwerking kan hê, nie net op die biobeskikbaarheid van die geneesmiddel nie, maar ook die doeltreffende behandeling van pasiënte. Omdat die moontlikheid bestaan dat geneesmiddels in meer as een vaste-toestand vorm kan bestaan, is volledige fisies-chemiese karakterisering uiters belangrik. Verskillende vaste-toestand vorme kan verskillende fisies-chemiese en termodinamiese eienskappe toon. Stabiliteit, oplosbaarheid, dissolusietempo, biobeskikbaarheid, poeiermorfologie, poeiervloei eienskappe, geneesmiddel kleur en tableteringsgedrag is almal eienskappe wat beïnvloed kan word deur hierdie verskille. Hoe hoër die dissolusietempo van 'n geneesmiddel, hoe beter kan die geneesmiddel opname vanuit die spysverteringskanaal wees, wat dan kan lei tot moontlike verbeterde biobeskikbaarheid van die geneesmiddel. Voorbereiding van 'n amorge vaste-toestand vorm van 'n geneesmiddel is 'n doeltreffende en maklike manier om die wateroplosbaarheid te verbeter, maar die inherente onstabiliteit van hierdie vaste-toestand vorme is gewoonlik nadelig.

Vorbereidingsmetodes soos vinnige afkoeling van die gesmelte geneesmiddel, stadige afkoeling van die gesmelte geneesmiddel, smelting verkry deur die toepassing van warm lug verhitting, oplosmiddel verdamping by kamertemperatuur, vinnige oplosmiddel verdamping en sproeidroging, almal met verskillende tussenganger fases (gesmelte geneesmiddel of geneesmiddel in oplossing), is geïdentifiseer en gekies vir die voorbereiding van amorge asitromisien. Die moontlikheid van oplossing-bemiddelde, oplosmiddel-bemiddelde en vastevorm-vastevorm fase oorgange van amorge asitromisien is ook ondersoek. Die doel was om die impak te ondersoek en om die effek te illustreer van die verskillende bereidingsmetodes op die fisies-chemiese eienskappe van elke amorge vaste-toestand vorm van asitromisien. In die studie is tegnieke soos XRPD en FTIR gebruik om die amorfisiteit van amorge asitromisien, soos verkry vanuit verskillende voorbereidingstegnieke, te bevestig. Die termodinamiese eienskappe van al die voorbereidingstegnieke weerspieël 'n breekbaarheidsindeks (m) laer as 75 en 'n hoë sterkte parameter (D), wat beteken dat 'n laer vrye energie teenwoordig is wat sal lei tot 'n moontlike hoër fisiese stabiliteit. Twee amorge vorme wat voorberei was deur 'n oplossing tussenganger fase, het aansienlik hoër

temperature van nul mobiliteit (T_0) gelewer, wat hulle fisies meer stabiel maak tydens berging by kamertemperatuur. Die amorfte vorm wat voorberei was deur sproeidroging het die laagste aktiveringsenergie vir strukturele ontspanning gewys en sodoende die bevinding van die temperatuur van nul mobiliteit van hierdie amorfte vorm weerspreek. Vogabsorpsie studies het bewys dat die verskillende amorfte vorme nie beïnvloed word deur oplosmiddel-bemiddelde fase oorgange nie. Dissolusiestudies was gebruik om die moontlikheid van oplossing-bemiddelde fase oorgang (OBFO) van die amorfte vorme te ondersoek. Die tempo van OBFO het verskil en in sommige gevalle was die fase oorgang van die amorfte vorm na die meer stabiele kristallyne vorm, nie sigbaar nie, wat bewys dat die tempo van OBFO te vinnig was. Deur die ondersoek van die rekristallasie gedrag van amorfte asitromisien, berei deur middel van vinnige afkoeling van die gesmelte geneesmiddel, het dit duidelik geword dat die rekristallasie 'n eerste-orde reaksie is. 'n 8-Voudige oplosbaarheidsverbetering in vergelyking met die oplosbaarheid van kristallyne asitromisien is bereken deur die toepassing van 'n Nogami plot.

Hierdie studie het bewys dat dit moontlik is om verskillende amorfte vorme van dieselfde geneesmiddel te berei en dat hierdie verskillende amorfte vorme wesenlik kan verskil in terme van poeiermorfologie, fisiese stabiliteit en uiteindelik in terme van dissolusietempo.

Sleutelwoorde: asitromisien; swak water oplosbaar; stabiliteit; breekbaarheidsindeks; oplossing-bemiddelde fase oorgang (OBFO); Nogami

Chapter 1

Solid-state properties of active pharmaceutical ingredients

1.1 Introduction

Different solid-state forms of organic and inorganic compounds had been recorded, as early as in the eighteenth century. The earliest known reports that describe the occurrence of more than one crystal form for a single compound, are those by two independent researchers, Davey and Klaproth. Both in 1798, Davey reported that a diamond was a form of carbon, while Klaproth discovered that aragonite and calcite had the same chemical compositions. In the early nineteenth century, Mitscherlich received credit for discovering that certain crystals of arsenate and phosphate, respectively, had shown different solid-state properties. Berzelius, who was Mitscherlich's mentor, also discovered two polymorphic forms that were initially named, isomorphism and dimorphism. Frankenheim, in 1839, was the front runner in investigating the existence of polymorphism in potassium nitrate. He investigated phase changes, resulting from the scratching of a compound, or through physical contact of one polymorph with another. He also established many of the principles of polymorphism that are still recognised and applied today. These principles were mostly founded during his intense studying of mercuric iodide (Bernstein, 2002).

Mallard researched the field of geometrical crystallography in the 1870s and identified the structural basis of polymorphism. He recognised that crystals consisted of tiny, so called crystallites. These crystallites could pack and arrange themselves in different ways to form different crystal forms. He noticed that such different arrangements of the crystallites had produced different physical properties and that the most densely packed ones were the more ideal forms. Mallard wrote: "It has been known for a long time that when the same substance displays two fundamentally incompatible forms, often belonging to two different chemical systems, these two forms are always only slightly different and the symmetry of the less symmetrical is very similar to that of the other". Two different types of polymorphism, monotropic and enantiotropic, were characterised by Lehmann in 1891. The research of Ostwald in 1897, was responsible for significant developments in the science of polymorphism. He focused on the relative stability of different polymorphs, as well as on that of the less stable (metastable) forms. He discovered that in a particular solvent, the more

stable polymorphic forms had demonstrated lower equilibrium solubility than the unstable forms (Bernstein, 2002).

Different solid-state forms of active pharmaceutical ingredients (APIs) play an important role in commercial and industrial applications. This is because their structural differences result in a variation of physico-chemical properties. This chapter elaborates more on the different solid-state forms in which APIs can exist and on the influence that these different forms may have on the suitable design of a drug.

1.2 Physical states of active pharmaceutical ingredients

Pure APIs can be prepared to exist in different physical states, or phases, when certain processing parameters are changed. There are three physical states in which an API can be visible, or expressed, i.e. the gas/vapour, liquid and solid phases. The solid phase may consist of a crystalline phase (polymorph 1, 2, 3, etc.), or of an amorphous/glass phase. These phases and the effects that temperature changes may have on a particular phase, are illustrated in Figure 1.1 (Ymén, 2011).

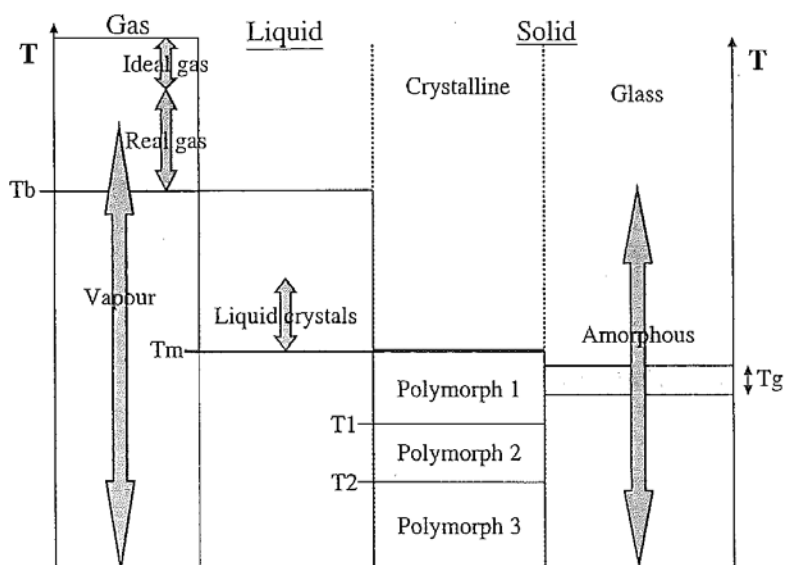


Figure 1.1: Different physical states of an active pharmaceutical ingredient and the effects of temperature changes on these states (Ymén, 2011).

1.2.1 The gas/vapour and liquid phases

A gas consists of molecules that are moving freely and independently from each other in the vessel or container, in which it is contained. These molecules can collide with each other from time to time, which causes gas pressure. An increase in molecule collisions will increase the gas pressure. The equation used to describe this phase is:

$$pV = nRT \quad (1)$$

where p is the pressure, V is the volume, n is the number of moles, T is the temperature and R is the constant for gas.

An ideal gas would consist of point-like, small molecules, with insignificant interactions. Most gas molecules may undergo lesser collisions, which make these gasses less than ideal. Deviation from this ideal gas behaviour is attributable to the facts that gas molecules possess volume themselves as well as intermolecular interactions and attractive forces. A gas is closer to being ideal, when it is at high temperatures and if a gas is cooled, then deviation from ideal gas behaviour should increase as temperature approaches the condensation point. A specific conformation may be dominating at this near condensation point. Different conformations are possible in a single gas, and does a rise in temperature cause the molecules to vibrate and rotate even more. Even when various conformations are present in a gas at a time, only one gas phase can exist, which will be isotropic (same physical properties). The density and viscosity of the molecules (relative to solid and liquid states of matter) will be low, whereas their compressibility will be high. The rotations and vibrations of the molecules will reduce gradually, as the temperature drops. The condensation point temperature will cause the colliding molecules to aggregate, to form a liquid. This temperature is dependent upon the strength and number of the bonds among the molecules and also upon the sizes of the molecules. Larger molecules will have more and stronger intermolecular bonds and higher boiling points, than those of smaller molecules. A liquid vapour is formed when the liquid reaches an equilibrium with its gas phase (Ymén, 2011).

1.2.2 Solid-state pharmaceutical chemistry

The definition of a solid-state, according to the Oxford Dictionary (2015), is the state, or phase of matter, during which the materials are neither fluid, nor liquid and during which they can maintain their boundaries without any outside support. The molecules, or atoms are unable to move freely, because of their fixed positions with respect to each other (Oxford Dictionaries, 2015). APIs may consist of organic molecules only, or they may contain

organic solvent molecules (solvates), water (hydrates), organic or inorganic anions, organic cations, metal cations (metal salts), or neutral organic molecules (co-crystals) (Ymén, 2011). The diversity that is being attained by an API in its solid-state relies upon the range of order, the balance between entropy and enthalpy to define the free energy region, the molecular assemblies and the non-covalent interactions (Rodríguez-Spong *et al.*, 2004).

It is well known that APIs can exist in different solid-state forms. These different forms can be crystalline, or amorphous, or a combination of both. In the following sections, the different solid-state forms, as depicted in Figure 1.2, are discussed. To fully understand solid-state chemistry of APIs, however, the principles of the crystalline state should be discussed first and foremost.

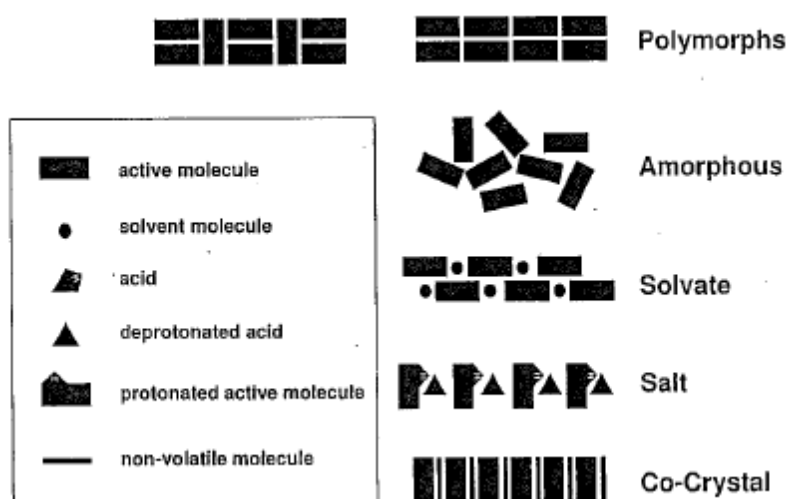


Figure 1.2: Illustration of the different solid-state forms of APIs (Hilfiker *et al.*, 2006).

1.3 The crystalline phase

Molecular movements decrease when a liquid is cooled. The liquid properties and cooling rate will determine whether crystallisation, or glass formation of the molecules would occur. Crystallisation would normally occur at, or below the melting or freezing point of small molecules, at low cooling rates. The intermolecular bonds that form are strong enough to stop translational motion, but not rotational or vibrational motion. Larger molecules would have, just as in the case of the boiling point, a higher freezing or melting point, than those of smaller molecules. Crystallisation may be recorded as an exothermic event, as the entropy decreases and enthalpy is released (Ymén, 2011).

The number of molecules per unit and the intermolecular distances make the crystalline phase similar to the liquid phase, as the compressibility and density, for example, are similar for those two phases. Long range molecular order and a lack of translational motion in the crystals are two of the biggest differences that exist between crystals and liquids. Although there may be exceptions, each molecule in a crystal is fixed in a variety of symmetric positions and conformations. The molecules are usually bound together by weak hydrogen, or van der Waal's forces (Ymén, 2011).

The crystal structure is described as a brick structure that is three-dimensional. Crystal structures consist of identical cell units. Every cell has three axes (a, b and c), with three angles (α , β and γ). Seven possible crystal systems can be derived from the different combinations of these axes and angles. Figure 1.3 illustrates the cell units' axes and angles, whereas Table 1.1 summarises the different axes and angle combinations that are possible (Ymén, 2011). These cell units contain more than one molecule and when they have the same conformation, they have symmetric positions. An asymmetric unit is known as the smallest part of the unit. The fracture and habit of crystals are related to the crystal structure. The directions of strong intermolecular bonds have shorter axes, whereas the directions of weak bonds have long ones. The shorter axes will have a higher crystal growth rate, such as needle shaped crystals. Very thin plate shaped crystals, however, have long axes of 90° to the plane of the plate (Ymén, 2011).

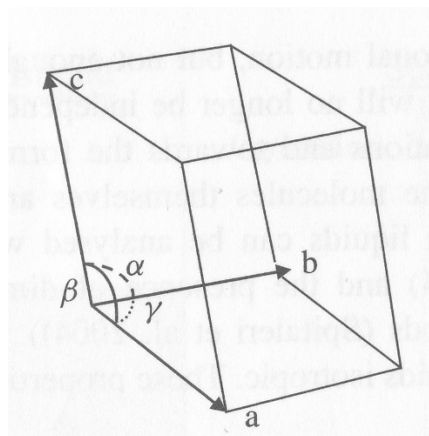


Figure 1.3: Indication of the axes and angles of a cell unit in crystals (Ymén, 2011).

Table 1.1: Different axes and angle combinations of a cell unit in crystals (Ymén, 2011)

Crystal system	a	b	c	α	β	γ
Triclinic	a	b	c	$\neq 90^*$	$\neq 90^*$	$\neq 90^*$
Monoclinic	a	b	c	90	$\neq 90$	90
Orthorhombic	a	b	c	90	90	90
Tetragonal	a	b	a	90	90	90
Trigonal	a	a	a	$\neq 90^*$	$\neq 90^*$	$\neq 90^*$
Hexagonal	a	b	a	90	120	90
Cubic	a	a	a	90	90	90

*In the trigonal system all three angles are the same but in the triclinic they are all different.

Crystals are likely to fracture at the weak bonds, when pressure, or forces are administered on them. Graphite and talcum are examples of such crystals. It is very important to manage the crystal size and habit, as these would influence processes, like granulation, tableting, filtration and flow properties. The chosen solvent and the super-saturation level for crystallisation are the most common methods of affecting the crystal habit and growth. In accordance with recommended practice within the pharmaceutical industry, the purer the API that is being crystallised, the better, since impurities, or foreign molecules may also affect the crystal size and habit (Ymén, 2011).

1.4 Polymorphism

Polymorphism is a term that originated from the Greek words, *poly* (many/much) and *morphe* (form) (Bernstein, 2002; Hilfiker, 2006). Crystal polymorphism is a phenomenon that is related to the solid-state of an API. It is the ability of a compound, molecule, or element in the solid-state to exist in different crystalline forms. It hence is the ability, or possibility of molecules to crystallise into several different crystal structures, or crystal arrangements, having the same chemical composition, atom types and covalent bonding sequence (Bernstein, 2002; BP, 2015; Haleblan & McCrone, 1969; Hilfiker *et al.*, 2006; Vipagunta *et al.*, 2001; Ymén, 2011). Lehmann characterised two different types of polymorphism. Monotropic polymorphism involves two forms, where the one form undergoes an irreversible phase change into the second form. Enantiotropic polymorphism occurs where both forms can show reversible phase transitions (form 1 changes into form 2, while form 2 changes back into form 1) (Bernstein, 2002; Ymén, 2011).

1.5 Solvates

Solvates can be defined as molecules of a solvent that are embedded in, or incorporated into the host lattice (Grant & Lohani, 2006). Solvation is defined as the formation of crystals when an API is mixed with a solvent. When used as a solvent, water is classified as part of a sub-group of the solvates, i.e. hydrates (Ymén, 2011) (paragraph 1.6). APIs are exposed to solvents in most of the pharmaceutical production and manufacturing stages and at times even to solvent vapours. Many prominent procedures being used in the pharmaceutical and chemical industries are solvent based, such as crystallisation, precipitation and re-crystallisation, which imply that a desired product is produced by separating, or purifying it from a suitable solvent mixture, or solvent. Wet granulation, lyophilisation, co-acervation and spray drying are other solvent based procedures that are also used. The aim of these production processes is to create single component, solid crystals that are free, or almost free of impurities. Once a solvent is added to an API, the solvent can become entrapped within the crystalline solid. The term, residual solvents, is used when a solvent cannot be completely removed from a crystalline solid with the available drying techniques. This can cause significant problems and huge setbacks during manufacturing and production (Griesser, 2006).

It is well documented that crystallisation from different solvents can yield different crystalline, solid-state forms. It is still not fully understood how this kinetic phenomenon occurs. Different activation energies for primary nucleation may be provided by different solvents. Alternatively, different solubilities can lead to different interactions, such as solute-solute and solvent-solute interactions (Ymén, 2011).

Molecules are attached to each other by means of intermolecular interactions, or bonds, of which the most common ones are hydrogen, van der Waal's and dipole-dipole bonding. In some instances, the solvent molecules may be so tightly bound that extreme conditions are necessary to remove, or to desolvate the crystalline solid. The solvent may play such an inseparable part of the crystalline solid, that when the solvent is removed, it may lead to a collapse in the crystal lattice. Contrary, when solvent molecules are loosely bound, desolvation would not cause the crystal lattice to collapse. The morphology of the crystalline solids are the determining factor of the affinity that molecules have for each other, which determines the amount of solvent being adsorbed on the surface of the crystals. The solvent becomes entrapped within the growing crystal, which is referred to as liquid inclusion (Bernstein, 2002; Griesser, 2006). Figure 1.4 illustrates the different principles of crystalline solids in relation to solvents.

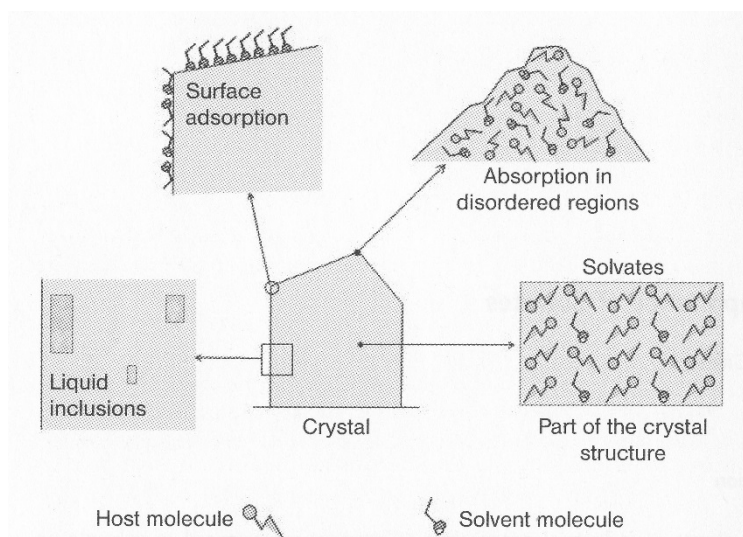


Figure 1.4: Images to illustrate the different principles of crystalline solids in relation to solvents (Griesser, 2006).

A crystalline solvate can be described as a solvent that is coordinated in a solid, or a solvent that is accommodated by the crystal structure. Solvates can be classified into two groups, i.e. stoichiometric and non-stoichiometric solvates (Griesser, 2006).

1.5.1 Stoichiometric solvates

Stoichiometric solvates are known as molecular compounds. The binary phase diagram, as adapted from Griesser (2006) (Figure 1.5), shows the classification of solvates in relation to typical types of binary systems. The solvate is an individual phase, while the binary phase diagram illustrates an eutectic/peritectic system with parent components (API and solvent) (Griesser, 2006). Stoichiometric solvates hence have a fixed ratio of API to solvent. The desolvation of a stoichiometric solvate always results in a different crystal structure, or in a disordered (amorphous) state (Griesser, 2006).

1.5.2 Non-stoichiometric solvates

Griesser (2006) describes a non-stoichiometric solvate as a type of inclusion compound. These solvates can also be described as interstitial co-crystals, or interstitial solid solutions. The crystal structure can only form in the presence of this solvent. These solvents are usually found in structural channels, or voids and fill the spaces within these channels. The structures that form are usually irregularly shaped crystals that can't pack close to each

other. The crystal structures of the solvates remain intact/fixed, whereas the solvent can attract a range of molar compound ratios. The amount of solvent in the structure will depend upon the temperature and upon the partial pressure that the solvent generates in the environment of the solid. Dipeptide structures are good examples of such solvates and consist of channels, called nano-tubes that host different solvent molecules, as well as water molecules (Griesser, 2006).

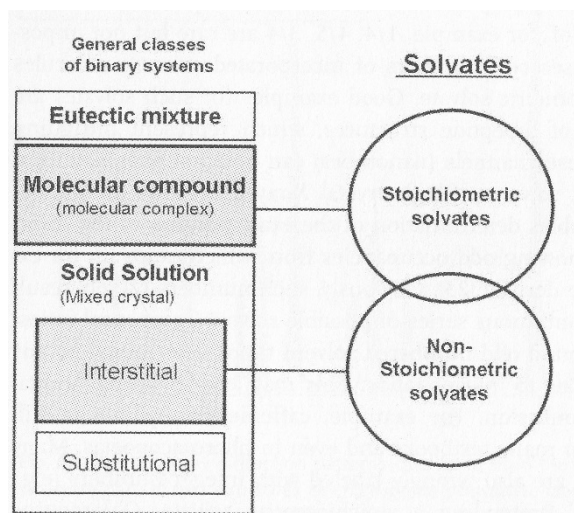


Figure 1.5: Solvate classification, described in conjunction with different types of binary systems (Griesser, 2006).

1.6 Hydrates

Water is the most common solvent present in APIs. APIs may come into contact with water during processes, such as during aqueous film coating, crystallisation, wet granulation, spray drying and lyophilisation. APIs can also be exposed to water during storage in a humid atmosphere, or to materials in a dosage form that contain water and that are capable of transferring it to other ingredients. The water molecules can be adsorbed to the surface of the solid, or can be absorbed into the bulk solid structure. The adsorption of water to the surface of a solid is dependent upon the specific surface area, whereas its absorption into the bulk solid structure is independent of the specific surface area (Khankari & Grant, 1995).

Hydrates can either be characterised as stoichiometric, or non-stoichiometric, depending upon the nature of the bonding of the water molecules, as well as the crystal lattice arrangement (Jeffrey, 1969). A hydrate is a solid that contains both the parent compound (the anhydrate of an API) and water. A hydrate is formed when the solvent is water, or when water is present in the organic solvent during crystallisation. The water molecules occupy definite positions within the crystal lattice, usually through the formation of co-ordinate

covalent bonds, or hydrogen bonds with the API molecules. When the water molecules are incorporated into the crystal lattice, it produces a new cell unit, which is different from that of the anhydrate. The hydrate may therefore have different physical properties, compared to the anhydrate.

This incorporation of the water molecules changes the symmetry, shape, dimensions and capacity of the cell unit. It will also alter the crystal behaviour through the following:

- The interaction of the electron vibrations with light quanta, changes the refractive index.
- The movement of the electrons in an electric field changes the electrical conductivity.
- The interaction of the molecular motions with heat quanta, changes the thermal conductivity.

Changes in the bonds between the host molecules themselves and the additional bonds that form between the water molecules and the host molecules, will alter the co-operation between the molecules within the crystal lattice. The solubility and physico-chemical stability of the solid will be modified, when there is a change in its thermodynamic activity, due to hydration. If there is any change in solubility, there will be a change in the dissolution rate. The changes in stability and dissolution rate will modify the bio-availability and performance of the product. Solubility depends upon pressure, temperature and the nature of the solid form (hydrate or anhydrate) and it is proportional to the thermodynamic activity of the solid form. The solubility behaviour of an anhydrate shows that it is always more soluble in water than a hydrated form of the same API. Molecular bonding between water and the API (forming the hydrate) causes the available free energy of the API to decrease, whereby the water solubility of the API also decreases (Khankari & Grant, 1995). Azithromycin can exist as either an anhydrate, monohydrate, or dihydrate (Jasanada *et al*, 2001; Li & Trask, 2005; USPC, 2015).

1.7 Salts

If a pharmaceutical molecule is basic or acidic, a salt can be created by adding a suitable base, or acid. These salts can be crystalline. Salts have a higher water solubility and bio-availability that makes them a popular choice in pharmaceutical formulations (Hilfiker *et al.*, 2006). Salts are developed from many pharmaceutical molecules. Hydrochloric-, sulphuric- and fumaric acids are some of the popular acids being used. Salt formation takes place, when a molecule consists of basic and acidic groups, as is the case with most

pharmaceutical molecules. An additional step in the synthesis will cause a salt to form from the neutral molecule, which will have a higher molecular weight than the neutral molecule. The creation of a salt will have to be justified, if the advantages are comparable to those of the neutral molecules. Most drug compounds are weak electrolytes, capable of forming salts. This widening of the selection basis may lead to the creation of new chemical entities. Each possible salt of a drug compound can be characterised by its individual physico-chemical properties profile. These properties may have a bigger range than that of a limited set of polymorphs of the neutral molecules. Manufacturers change the state of aggregation of their compounds in an attempt to prepare salts that are easier to handle. The solid magnesium and sodium salts of valproic acid liquid, for instance, are much more preferable to produce a solid dosage form. Salt formation during product development precedes the morphic state studies (Stahl & Sutter, 2006).

1.8 Amorphous, non-crystalline, or glassy phase

All crystalline solids contain some areas of low crystallinity, or regions of disorder. If the bulk of the material consists of such disordered regions, it is known as an amorphous form (Saunders & Gabbot, 2011). Amorphous forms of an API can be distinguished from the crystalline form(s) of the same API, by their lack of macroscopic and microscopic properties (fracture mechanism, particle shape and birefringence). Amorphous solids all lack long range molecular order, but they do possess some degree of the short range molecular order that is present in crystals, whereas they have no crystal lattice arrangement. The x-ray powder diffractogram (XRPD) of an amorphous solid exhibits a broad “halo” pattern, with no noticeable diffraction pattern (paragraph 4.3.2) (Craig *et al.*, 1999; Saunders & Gabbot, 2011; Ymén, 2011).

When a complex and a highly molecular liquid are rapidly cooled to avoid crystallisation, the onset of crystallisation may result in over-crystallisation to form a super-cooled liquid that may reach a glassy state (Adrjanowicz *et al.*, 2012; Ymén, 2011). Such liquids have a high viscosity and a continual cooling will increase the viscosity to such an extent that the molecular movement will almost completely stop. Amorphous materials are sometimes referred to as a glass phase. The application of such technical detail is required when you wish to distinguish between the three types of amorphous liquids, i.e. an “over-cooled liquid” (glass), a normal and a super-cooled liquid. The physical properties of a super-cooled liquid undergo the same gradual changes when it is cooled down from the melting point (T_m) (Craig *et al.*, 1999), until glass formation, as when it is melted above the melting point (T_m). These changes come to a halt when glass formation is reached (the molecules are so

closely packed together that these gradual changes stop) and the molecules cannot reach their equilibrium positions (Adrjanowicz *et al.*, 2012; Ymén, 2011). A new form of material will form when the cooling process is continued. Specific heat C_p and specific volume V_{sp} are the most common physical properties that will change during glass formation. Transition temperature T_g of solid-state during heating is the point where the API changes from the solid-state to a rubber-like (almost liquid) state. This is not a true transition phase. The lower the cooling rate of the liquid, the lower the C_p , V_{sp} , and T_g , due to the fact that molecules will pack more efficiently at slower rates (Ymén, 2011).

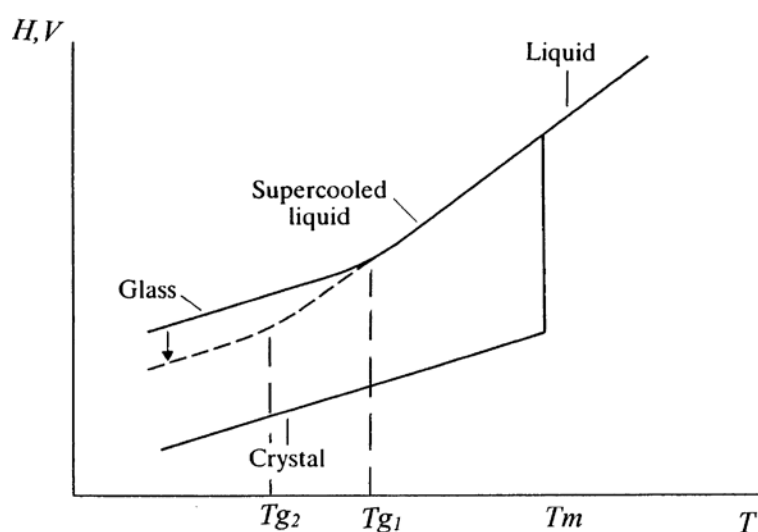


Figure 1.6: Graphic representation of the crystallisation process, or the glass transition of a compound (Craig *et al.*, 1999).

Figure 1.6 illustrates the essential differences between the formation of amorphous and crystalline forms (Craig *et al.*, 1999). When the temperature is lowered from the liquid phase to the melting point (T_m), transition into the crystalline form occurs (if super-cooling does not occur). When the phase is below T_m , a thermodynamically stable phase is reached. The exothermic crystallisation process will lead to a sudden contraction of the phase. This is caused by a decrease in free volume. Free volume is the difference between the actual volume and the total volume that is being displaced by the constituent molecules. Because of this, both specific volume (V) and enthalpy (H) decrease at T_m . When a glassy phase is formed, the cooling process is too rapid for the crystallisation process to occur. This could either be caused by the molecular shape and size that don't favour the crystallisation process, or by the use of a rapid cooling rate. Since there is no lack of coherence in the volume and enthalpy upon cooling of the material below T_m , it leads to the formation of a

super-cooled liquid. Upon further cooling, a point is reached where the material freezes into a glassy phase. The bonds between the molecules in essence remain the same as in the liquid. However, the rotational and translational motions of these molecules are drastically reduced, with vibrational motions taking place below T_g . The dashed line in Figure 1.6 that partly corresponds to the solid line, represents a system being cooled at a slower rate. The log dashed lines represent the T_g for a fast cooled (T_{g1}) and a slow cooled (T_{g2}) phase (Craig *et al.*, 1999).

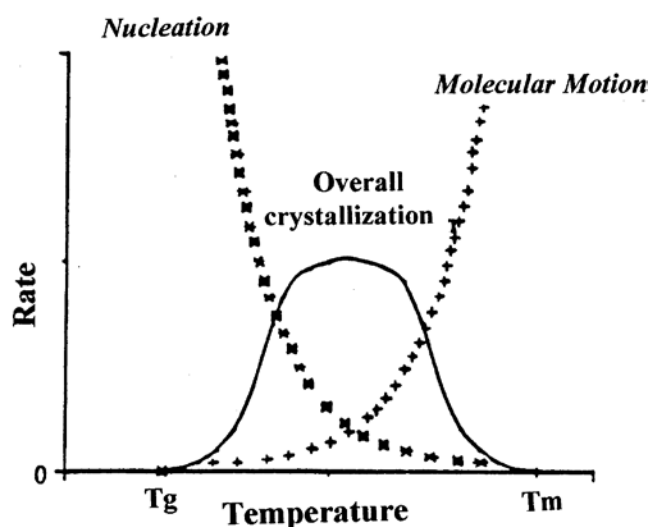


Figure 1.7: Graphic representation of the formation of a glassy phase during which the crystallisation process is temperature dependent (Craig *et al.*, 1999).

Figure 1.7 illustrates that when the temperature decreases, the nucleation rate may be expected to increase (Craig *et al.*, 1999). As the temperature, however, decreases, the molecular mobility also decreases, especially below T_g . The molecular diffusion becomes slower and a reduction in the crystallisation rate takes place. The maximum crystallisation rate will occur between T_g and T_m . The devitrification risk is lowered if a sample is stored below T_g , due to the molecules having lower mobility, which causes crystal growth to halt or decrease. However, storage below T_g is not a guarantee for the physical stability of an amorphous solid-state form (Craig *et al.*, 1999).

1.8.1 Preparation and preparation techniques of amorphous forms

The preparation of amorphous solids from some materials is fairly easy (good glass formers), but may be very difficult for others (poor glass formers), based upon their

thermodynamic and kinetic properties. As mentioned, when considering the thermodynamic properties of a material, an amorphous solid can form from the more stable crystalline counterpart, possibly because the molecules have a high internal degree of freedom and hence a disorderly arrangement. The kinetic properties of an amorphous form have a slow crystallisation rate that allows for the solid-state to transform into a glass state, while no crystallisation occurs. The conformational flexibility and configurational equilibria of molecules are the general causes of the reduced crystallisation tendencies of APIs. These conformationally flexible molecules can exist as multiple conformers in a crystallising medium, while these molecules must select the “right” ones from the “wrong” during the crystallisation process. This difficult process will not occur in rigid molecule structures. The conformers in crystals correspond to high energy and low concentration conformers in solution, which will amplify the effect. The average molecule undergoes a significant conformational change during the crystallisation process. Good examples of these crystallisation tendencies are illustrated by the two stereoisomers, mannitol (easy glass former) and sorbitol (poor glass former).

Amorphous forms, and in particular poor glass formers, can be induced deliberately, or accidentally, by preventing crystallisation through processes, like grinding, milling, cryo-milling, compression, (mechanical stress), wet granulation (chemical stress), conventional drying, heating, cooling, or a combination of these (melt extrusion) (Morris *et al.*, 2001; Murphy *et al.*, 2002; Graeser *et al.*, 2008; Yu, 2001). The most frequently used techniques for the formation of amorphous forms would be by means of freeze drying and spray drying, and precipitation through the addition of anti-solvents (Morris *et al.*, 2001; Graeser *et al.*, 2008). The rapid freezing in a freeze drying process will favour the formation of the amorphous form (Yu, 2001).

Amorphous forms may also be prepared through the rapid cooling of the melted API, or through the precipitation of the API from a suitable solvent system (Craig *et al.*, 1999; Graeser *et al.*, 2008). The cooling rate and liquid properties will affect glass formation (Ymén, 2011). Quench cooling (melted sample is exposed to a cold surface) is the most easiest, inexpensive and commonly used preparation technique (Graeser *et al.*, 2008; Yu, 2001). Another way of preparing an amorphous form is through dehydration/desolvation. The dehydration of a crystalline hydrate is probably the gentlest way to create an amorphous form (Yu, 2001). The sample is exposed to heat (below melting point) to remove the water/solvent (Vippagunta *et al.*, 2001). Another route towards the amorphous state is the introduction of impurities. The impurity effect may cause a poor glass former to exist in the amorphous state in a multi-component formulation (Yu, 2001). Many techniques or procedures to prepare amorphous forms from the crystalline counterparts are reflected by

the manifold published patents (Jasanada *et al.*, 2001; Li & Trask, 2005; Odendaal *et al.*, 2013). The amorphous preparation techniques that were used during this thesis are discussed in Chapter 3.

1.8.2 Advantages of amorphous forms

Glasses, or amorphous forms, have a dual nature. They have the mechanical properties of solids, whilst having the disorderly molecular characteristics of liquids, which make them desirable compounds for inclusion into pharmaceutical products (Adrjanowicz *et al.*, 2012). Amorphous APIs have a higher kinetic solubility and dissolution rate, than their crystalline counterparts (Savolainen *et al.*, 2009). This higher solubility could potentially lead to a higher bio-availability (Graeser *et al.*, 2008). A perfect amorphous form can be characterised as a solid-state form, having exceptional stability and solubility characteristics. It is, however, difficult to find such combinations of desirable properties.

The different amorphisation routes possess different enthalpies, structures and physical stabilities that will lead to different dissolution and solubility rates (Adrjanowicz *et al.*, 2012).

Because amorphous forms have a random orientation of molecules, it will lead to an excess of free energy, entropy and enthalpy (Babu & Nangia, 2011; Graeser *et al.*, 2008). These thermodynamic characteristics of amorphous forms will account for better solubility and bio-availability characteristics, when compared to their crystalline counterparts (Aucamp *et al.*, 2015b; Byrn *et al.*, 1994). Amorphous forms are therefore more soluble, because an amorphous solid requires less energy to transfer one molecule into the solvent solution (Aucamp *et al.*, 2015b), with a faster ability to dissolve (Yu *et al.*, 1998).

If APIs are poorly soluble in water, it will reflect in their lower bio-availability profiles. This will influence the dosage form regimen and it may require higher, or more frequently administered quantities that may increase potential adverse side effects. A possible solution may be to shift the focus towards amorphous forms that possibly have better solubilities and hence better bio-availability profiles. Several studies have shown that amorphous forms of macrolide antibiotics have demonstrated significant improvements in terms of their aqueous solubilities. Solubility studies by Adrjanowicz *et al.* (2012) showed that amorphous antibiotics (azithromycin, clarithromycin and roxithromycin) had been significantly more soluble than their poorly soluble crystalline counterparts. Comparative dissolution studies were performed on the amorphous and crystalline forms of 9,3"-diacetylmidecamycin (a macrolide antibiotic derived from midecamycin) and their dissolution rates were compared. The amorphous forms clearly showed higher dissolution rates than their counterparts (Sato *et al.*, 1981).

In another solubility study by Aucamp *et al.* (2015b), an increase in the water solubility of an amorphous azithromycin preparation was noted, when compared to the crystalline azithromycin dihydrate. This study also demonstrated an improvement in the permeability of the preparation through the intestinal tissue (Aucamp *et al.*, 2015b).

1.8.3 Disadvantages of amorphous forms

Amorphous forms are thermodynamically unstable, or less stable (metastable) than their counterpart crystalline forms. The amorphous form tends to transform into its more thermodynamically stable crystalline form, through nucleation and crystal growth. Such a transformation can occur either during normal ambient storage, or during pharmaceutical processing, or even during patient consumption (Adrjanowicz *et al.*, 2012; Byrn *et al.*, 1994; Craig *et al.*, 1999; Saunders & Gabbot, 2011; Strachan *et al.*, 2005; Yu *et al.*, 1998). Amorphous forms are more prone to moisture uptake (hygroscopic), due to the larger voids in between the molecules and may this also be regarded as a disadvantage (Byrn *et al.*, 1994; Yu *et al.*, 1998).

Amorphous forms may undergo solution-mediated phase transformation into less soluble crystalline forms during dissolution, or even during exposure to sufficient solvent, which would allow the formation of a saturated solution (Greco & Bogner, 2011). The possible conversion of the amorphous form into its stable crystalline form during dissolution, will result in the dissolution rate gradually changing towards that of the crystalline form (Savolainen *et al.*, 2009).

In terms of product development, it may be quite a challenge to include amorphous solid-state forms into suitable dosage forms. As mentioned, amorphous forms exist in a higher free energy state, which would make these forms more 'reactive' to stimuli that would facilitate the crystallisation of an amorphous form into a more thermodynamically stable solid-state form. Considering this, amorphous forms must be used with caution during product formulation. It is important to limit the exposure of the amorphous form to a variety of pharmaceutical processing steps and also to continuously monitor the solid-state form. Crystallisation of an amorphous form may be triggered by temperature fluctuations, milling, grinding, or physical agitation and exposure to solvents. In light thereof, it is evident that processes, such as granulation, milling, particle size reduction and coating, among others, can be somewhat challenging, when the solid-state form of the drug is an amorphous form.

It is clear that the disadvantages being presented by amorphous solid-state forms could significantly negatively impact the development of solid dosage forms that contain amorphous forms. However, if sufficient information is available on the physical, chemical

and thermodynamic stability of the specific amorphous drug that is incorporated, the formulation of an amorphous form into a suitable dosage form is indeed achievable.

1.9 Methods to improve solubility

One of the biggest challenges in the pharmaceutical industry is the improvement of the solubility and dissolution properties of poorly soluble APIs. To increase the dissolution rate and solubility of poorly soluble APIs, different techniques can be applied, of which one method is to reduce their particle sizes (high pressure homogenisation). A reduction of the particle size into the nano-meter range will increase the surface area of the API, which will in turn increase its dissolution rate (Craig, 2002; Zhang *et al.*, 2007). These smaller particles will furthermore adhere better to the gastro-intestinal (GI) tract wall and result in a longer contact time, improve wettability, require lower dosages to obtain the same clinical effect and hence a reduction in the possible loss of a drug through diarrhoea, that would result from high dosages. All of these factors will improve the drug's bio-availability and affordability (Zhang *et al.*, 2007). Other methods, like complexation and the use of hydrophilic carriers, can also possibly improve the wettability and dissolution rate of an API.

The solubility of an API can also be improved by employing methods, like the formation of a co-crystal, salt, or an amorphous solid. These methods may lead to phase changes that can occur through several mechanisms (Greco & Bogner, 2011). Other methods that can be employed are the formulation of solid dispersions, micro-emulsions (cinnamon oil) (Nirmala *et al.*, 2013; Yu, 2001) and nano-suspensions (Zhang *et al.*, 2007). Solid dispersions can be defined as the dispersion of one or more API in an inert matrix. The APIs in solid dispersions may exist in finely solubilised, crystalline, or amorphous states. The API can be molecularly dispersed in crystalline particles or amorphous particles, or clusters (Adeli & Mortazavi, 2014; Yu, 2001). A solid dispersion is a process that is used to enhance the solubility and hence bio-availability of an API (Yu, 2001).

Several studies are described in the literature regarding the strategies that were applied to improve the solubility and dissolution rates of azithromycin. In one such study, urea was used as a water soluble carrier (Arora *et al.*, 2010). In another study by Adeli (2014), a solid dispersion of azithromycin was prepared with polyethylene glycol (PEG 6000), sorbitol, poloxamer 188, or sodium lauryl sulphate (SLS). These mixtures were exposed to a solvent-anti-solvent (SAS) super-critical fluid CO₂ (SCF CO₂) process and the dissolution data did indicate that an enhanced azithromycin dissolution rate could be achieved (Adeli, 2014). The solubility of azithromycin in SCF CO₂ and its correlation outcomes, using semi-empirical models, were also studied and the correlation results did compare well with the experimental

results (Asiabi *et al.*, 2015). Mixed non-ionic surfactants and mixed oils (micro-emulsions) that were of the alcohol free, U-type, including mixtures of water, propylene, sucrose laurate, ethoxylated mono-diglyceride, isopropylmyristate and peppermint oil, were used in a study by Fanun to investigate their abilities to solubilise azithromycin. The integration of a poorly soluble API into a delivery system, such as a micro-emulsion, may offer protection against degradation, both *in vitro* and *in vivo*, it can control the release of the API and the target area can be reached. The U-type, newly formulated micro-emulsions showed an increase in the solubilisation of azithromycin (Fanun, 2012).

1.10 Phase transformations

Phase transformations can significantly impact the performance of an API, either during pre-formulation, product formulation, or patient treatment. A major concern is the occurrence of a phase transformation, in the absence of any knowledge, or even just an awareness thereof. A phase transformation can be described as any transition/reaction from one solid-state form of an API into another, resulting in the same chemical composition, but a different molecular arrangement (Aucamp *et al.*, 2015a). A pharmaceutical hydrate can be dehydrated, or hydrated to a higher hydration level, while a pharmaceutical solvate can be desolvated, whereas a crystalline form can change into an amorphous form, or *vice versa*.

The crystalline form of an API may be thermodynamically stable, or unstable. When it is stable, it has a certain interval of pressure (p) and temperature (T). The unstable form is lacking such an interval. When any combination of p and T is encountered, the form will be unstable. A metastable form is a stable form, which is encountered outside of its stability interval. The presence of activation energy (E_a) is the reason for a metastable form to exist. The E_a prevents the metastable form from transforming into the more stable form. The kinetic properties of a metastable form will prevail above its thermodynamic properties. When the metastable form of an API has to be avoided, or when it must be transformed into the more thermodynamically stable form, the focus must shift towards its thermodynamic properties. The completion of a transformation from one form into another (the time it takes for all of the molecules to transform) will depend upon the magnitude of the E_a . The Boltzmann factor $e^{-E_a/RT}$ represents the fraction of molecules with minimum energy at temperature (T) for reaction (phase transformation) at a certain energy level. The higher the E_a for a phase transformation, the less energy the molecules have to complete the transformation and the longer it will take for all of the molecules to transform. This means a slower transformation rate. E_a is not a constant and it may be increased when a phase transformation occurs too fast, so that it becomes super-heated, or super-cooled. This can

be observed when a solution is evaporated too rapidly, so that it causes an increase in the E_a that will result in the crowding of the molecules. This crowding of molecules makes it more difficult for them to complete the conformation and to find their place in the crystal lattice. This could typically lead to the formation of a glass (Ymén, 2011).

An API can crystallise from a solution, or from a melt. The difference in the Gibbs energy between the saturated solution and the crystals $G_{(sol)} - G_{(crystal)}$ is responsible for the phase transformation and it is the driving force thereof. If the solution is exactly saturated, the difference in the Gibbs energy will be zero. When the solution becomes over-saturated, or super-saturated the $G_{(sol)}$ will increase to $G_{(super-saturated)}$. Cooling, evaporation, or the addition of a solvent will create a super-saturated environment. The API will be less soluble in this environment. The crystallisation of an API from a super-saturated solution is initiated through crystal growth and nucleation. Primary nucleation can be heterogeneous, or homogeneous. Heterogeneous nucleation occurs on foreign surfaces, while homogeneous nucleation will occur in the absence of any foreign surfaces. Foreign surfaces include different types of equipment and solid impurities, for example. Secondary nucleation occurs when the already formed crystals disintegrate to induce new nucleation (Ymén, 2011).

Different types of transformation can occur in a given API. Zhang *et al.* (2004) categorised the possible transformations as solution-mediated, solvent-mediated, solid-solid and solid-melt-solid transformations.

Solution-mediated transformation occurs when a solvent is introduced to the solid-state in liquid or vapour form, to then act as a mediator to induce transformation. A typical example of solution-mediated transformation is recrystallisation that may result in the formation of polymorphs, solvates and hydrates. This type of transformation is also possible during dissolution, when the metastable form transforms into the more stable form. A super-saturated solution during dissolution, followed by nucleation of a less soluble phase and the growth of that phase, are the three steps that can occur during such solution-mediated phase transformation process (Greco & Bogner, 2011; Zhang *et al.*, 2004).

Contrary, solvent-mediated transformation occurs when the solvent mediates the transformation through an interaction between the undissolved solid-state and the solvent that is introduced in liquid, or vapour form. This can be observed when an anhydrous form converts into a hydrate or solvate form, during the crystallisation of an amorphous form into the hydrate or solvate form, and where there is a solvent exchange that causes a change in the structure of the API (Aucamp *et al.*, 2015a). Vapour sorption on the surface of the starting material can also induce transformation into a solvate, hydrate, or a more stable form.

Solid-solid transformations can also occur without an intermediate solution or melt phase, where the one form can change into another.

Solid-melt-solid transformation occurs when the solid form is induced by the heating and melting thereof, followed by cooling of the solid. The result can be a crystalline, or an amorphous form (Aucamp *et al.*, 2015a).

1.11 Conclusion

It is of great importance during the drug development process to do a complete physico-chemical characterisation of pharmaceutical solids. It is well known that pharmaceutical solids can exist in different forms, such as crystalline polymorphs, solvates, hydrates, co-crystals, salts and even amorphous forms.

Crystalline polymorphs have the same chemical composition, but different crystal structures. These differences cause polymorphs to have different solubilities, melting points and densities. Solvate or hydrate forms contain solvent, or water molecules within the crystal structure. Amorphous forms have no long range molecular order, i.e. they are non-crystalline (Byrn *et al.*, 1994). The different forms can display different chemical and physical properties, such as differences in stability, dissolution, bio-availability, morphology, powder flow, colour and tablet behaviour (Holzgrabe *et al.*, 1999).

Previous studies on the amorphous solid-state form of azithromycin proved that the dissolution rate of this antibiotic can be increased when rendered in an amorphous state (Aucamp *et al.*, 2015b), as its aqueous solubility is improved (Adrjanowicz *et al.*, 2012). In theory, generally an amorphous form represents the most energetic solid-state of an API. This could mean that amorphous forms may have an advantage with regards to their bio-availability and solubility properties and may the preparation of amorphous forms hence improve the solubility and dissolution rates of APIs. With the higher free energy, however, some degree of stability is sacrificed (Aucamp *et al.*, 2015b; Hancock & Parks, 2000). Usually, the most thermodynamically stable form is chosen for the development of final pharmaceutical products (Craig *et al.*, 1999; O'Neil & Edwards, 2011). However, metastable forms have in recent years attracted the attention of formulators, due to their enhanced dissolution and bio-availability profiles. As a result, the amorphous forms of azithromycin were investigated during this study, as discussed in the next chapters.

1.12 Reference list

Adeli, E. 2014. A comparative evaluation between utilizing SAS supercritical fluid technique and solvent evaporation method in preparation of azithromycin solid dispersions for dissolution rate enhancement. *The journal of supercritical fluids*, 87:9-21.

Adeli, E. & Mortazavi, S.A. 2014. Design, formulation and evaluation of azithromycin binary solid dispersion using Kolliphor® series for the solubility and *in vitro* dissolution rate enhancement. *Journal of pharmaceutical investigation*, 44:119-131.

Adrjanowicz, K., Zakowiecki, D., Kaminski, K., Hawelek, L., Grzybowska, K., Tarnacka, M., Paluch, M. & Cal, K. 2012. Molecular dynamics in supercooled liquid and glassy states of antibiotics: azithromycin, clarithromycin and roxithromycin studied by dielectric spectroscopy. advantages given by the amorphous state. *Molecular pharmaceutics*, 9:1748-1763.

Arora, S.C., Sharma, P.K., Irchhaiya, R., Khatkar, A., Singh, N. & Gagoria, J. 2010. Development, characterization and solubility study of solid dispersions of azithromycin dihydrate by solvent evaporation method. *Journal of advanced pharmaceutical technology & research*, 1:221-228. <http://www.japtr.org> Date of access: 22 Oct. 2015.

Asiabi, H., Yamini, Y., Latifeh, F. & Vatanara, A. 2015. Solubilities of four macrolide antibiotics in supercritical carbon dioxide and their correlations using semi-empirical models. *The journal of supercritical fluids*, 104:62-69.

Aucamp, M.E., Liebenberg, W. & Stieger, N. 2015a. Solvent-interactive transformations of pharmaceutical compounds. (*In* Mastai, Y. ed. Crystallization. InTech:Rijeka. p. 2-26.)

Aucamp, M., Odendaal, R., Liebenberg, W. & Hamman, J. 2015b. Amorphous azithromycin with improved aqueous solubility and intestinal membrane permeability. *Drug development and industrial pharmacy*, 41:1100-1108.

Babu, N.J. & Nangia, A. 2011. Solubility advantage of amorphous drugs and pharmaceutical cocrystals. *Crystal growth & design*, 11:2662-2679.

Bernstein, J. 2002. Polymorphism in molecular crystals. Clarendon, Oxford: Oxford University Press.

British Pharmacopoeia. 2015. Appendix IF.

Polymorphism, <https://www.pharmacopoeia.com/bp-2016/appendices/appendix-01/appendix-01-f/appendix-i-f--polymorphism.html?published-date=2015-08-03#p2p06455>

Date of access: 07 Nov. 2015.

Byrn, S.R., Pfeiffer, R.R., Stephenson, G., Grant, D.J.W. & Gleason, W.B. 1994. Solid-state pharmaceutical chemistry. *Chemistry of materials*, 6:1148-1158.

Craig, D.Q.M. 2002. The mechanisms of drug release from solid dispersions in water-soluble polymers. *International journal of pharmaceutics*, 231:131-144.

Craig, D.Q.M., Royall, P.G., Kett, V.L. & Hopton, M.L. 1999. The relevance of the amorphous state to pharmaceutical dosage forms: glassy drugs and freeze dried systems. *International journal of pharmaceutics*, 179:179-207.

Fanun, M. 2012. Solubilization of azithromycin in microemulsions based on mixed nonionic surfactants and mixed oils. *Journal of dispersion science and technology*, 33:185-190.

Graeser, K.A., Strachan, C.J., Patterson, J.E., Gordon, K.C. & Rades, T. 2008. Physicochemical properties and stability of two differently prepared amorphous forms of simvastatin. *Crystal growth and design*, 8:128-135.

Grant, D.J.W. & Lohani, S. 2006. Thermodynamics of polymorphs. (In Hilfiker, R. ed. *Polymorphism in the pharmaceutical industry*. Weinheim, Germany: Wiley. p. 21-42).

Greco, K. & Bogner, R. 2011. Solution-mediated phase transformation: significance during dissolution and implications for bioavailability. *Journal of pharmaceutical sciences*, 101:2996-3018.

Griesser, U.J. 2006. The importance of solvates. (In Hilfiker, R. ed. *Polymorphism in the pharmaceutical industry*. Weinheim, Germany: Wiley. p. 211-234).

Haleblian, J. & McCrone, W. 1969. Pharmaceutical applications of polymorphism. *Journal of pharmaceutical sciences*, 58:911-929.

Hancock, B.C. & Parks, M. 2000. What is the true solubility advantage for amorphous pharmaceuticals? *Pharmaceutical research*, 17:397-404.

Hilfiker, R. 2006. *Polymorphism: in the pharmaceutical industry*. Weinheim, Germany: Wiley.

Hilfiker, R., Blatter, F. & Von Raumer, M. 2006. Relevance of solid-state properties for pharmaceutical products. (In Hilfiker, R. ed. *Polymorphism in the pharmaceutical industry*. Weinheim. Germany: Wiley. p. 1-20).

Holzgrabe, U., Wawer, I. & Diehl, B. 1999. *NMR spectroscopy in drug development and analysis*. Weinheim, Germany:Wiley.

Jasanada, B., Santos, M., Isidro, L.G. & Felix, F.M. 2001. Preparation of non-crystalline and crystalline dihydrate forms of azithromycin. (Patent: EPO 1 103558 A2).

Jeffrey, G.A. 1969. Water structure in organic hydrates. *Accounts of chemical research*, 2:344-352.

Khankari, R.K. & Grant, D.J.W. 1995. Pharmaceutical hydrates. *Thermochimica Acta*, 248:61-79.

Li, Z.J. & Trask, A.V. 2005. Crystal forms of azithromycin. (Patent: US 6977243B2).

Morris, K.R., Griesser, U.J., Eckhardt, C.J. & Stowell, J.G. 2001. Theoretical approaches to physical transformations of active pharmaceutical ingredients during manufacturing processes. *Advanced drug delivery reviews*, 48:91-114.

Murphy, D., Rodríguez-Cintrón, F., Langevin, B., Kelly, R.C. & Rodríguez-Hornedo, N. 2002. Solution-mediated phase transformation of anhydrous to dihydrate carbamazepine and the effect of lattice disorder. *International journal of pharmaceutics*, 246:121-134.

Nirmala, M.J., Viswanadha, S., Mukherjee, A. & Chandrasekaran, N. 2013. Improved physico-chemical aspects of azithromycin through novel microemulsion system. *International journal of pharmacy and pharmaceutical sciences*, 5:700-702.

Odendaal, R.W., Liebenberg, W. & Aucamp, M. 2013. Composition comprising an amorphous non-crystalline glass form of azithromycin. (Patent: US 2013/0059805A1).

O'Neil, A. & Edwards, H. 2011. Spectroscopic characterization. (In Storey, R.A. & Ymén, I. ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 71-134).

Oxford Dictionaries. Language matters. 2015. Solid-state. <http://www.oxforddictionaries.com/definition/english/solid-state#solid-state5> Date of access: 07 Nov. 2015.

Rodríguez-Spong, B., Price, C.P., Jayasankar, A., Matzger, A.J. & Rodríguez-Hornedo, N. 2004. General principles of pharmaceutical solid polymorphism: a supramolecular perspective. *Advanced drug delivery reviews*, 56:241-274.

Saunders, M. & Gabbot, P. 2011. Thermal analysis-conventional techniques. (In Storey, R.A. & Ymén, I. ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 135-186).

Sato, T., Okada, A., Sekiguchi, K. & Tsuda, Y., 1981. Difference in physico-pharmaceutical properties between crystalline and noncrystalline 9,3"-diacetylmidcamycin. *Chemical and pharmaceutical bulletin*, 29:2675-2682.

Savolainen, M., Kogermann, K., Heinz, A., Aaltonen, J., Peltonen, L., Strachan, C. & Yliruusi, J. 2009. Better understanding of dissolution behaviour of amorphous drugs by *in situ* solid-state analysis using Raman spectroscopy. *European journal of pharmaceutics and biopharmaceutics*, 71:71-79.

- Stahl, P.H. & Sutter, B. 2006. Salt selection. (*In* Hilfiker, R. *ed.* Polymorphism in the pharmaceutical industry. Weinheim, Germany: Wiley. p. 309-332).
- Strachan, C.J., Rades, T. & Lee, C.J. 2005. Determination of the optical second harmonic response of pharmaceutical solid–solid mixtures. *Optics and lasers in engineering*, 43:209-220.
- United States Pharmacopeial Convention. 2015. Monographs: azithromycin. http://www.uspnf.com/uspnf/pub/data/v38330/usp38_nf33s0_m6740.xml Date of access: 11 Jul. 2015.
- Vippagunta, S.R., Brittain, H.G. & Grant, D.J.W. 2001. Crystalline solids. *Advanced drug delivery reviews*, 48:3-26.
- Ymén, I. 2011. Introduction to the solid state-physical properties and processes. (*In* Storey, R.A. & Ymén, I. *ed.* Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 1-34).
- Yu, L., Reutzel, S.M. & Stephenson, G.A. 1998. Physical characterization of polymorphic drugs: an integrated characterization strategy. *Pharmaceutical science & technology today*, 1:118-127.
- Yu, L. 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Advanced drug delivery reviews*, 48:27-42.
- Zhang, D., Tan, T., Gao, L., Zhao, W. & Wang, P. 2007. Preparation of azithromycin nanosuspensions by high pressure homogenization and its physicochemical characteristics studies. *Drug development and industrial pharmacy*, 33:569-575.
- Zhang, G.G.Z., Law, D., Schmitt, E.A. & Qiu, Y. 2004. Phase transformation considerations during process development and manufacture of solid dosage forms. *Advanced drug delivery reviews*, 56:371-390.

Chapter 2

Azithromycin: a macrolide (azolide) antibiotic

2.1 Introduction

The word, antibiotic, was first used by Selman Waksman in 1941 and at the time, it described any small molecule that had been produced from microbes to inhibit the growth of other microbes (Clardy *et al.*, 2009). Today, antibiotics are biosynthesised through protein synthesis (Khosla, 2010). Antibiotics have hence been around for more than a hundred years and are being used in the treatment of different infections in both animals and humans. Currently, it is impossible to treat bacterial infections without antibiotics and anti-bacterial agents (Adrjanowicz *et al.*, 2012).

One of the first antibiotics that had been documented was penicillin, as observed and identified by Alexander Fleming in 1928 (as quoted in Sköld, 2010). Since then, scientists were prompted to discover new types of antibiotics. Erythromycin, a first generation macrolide, was identified in the 1950's, whereas the second generation macrolides (azithromycin, roxithromycin, dirithromycin, flurithromycin and clarithromycin) were synthesised in the 1970's and 1980's through a semi-synthetic approach (Adrjanowicz *et al.*, 2012). Azithromycin is classified as a macrolide antibiotic and falls in the same group as erythromycin, roxithromycin and clarithromycin (Rossiter, 2010). Azithromycin currently is the world's most widely consumed antibiotic, thereby making it one of the best-selling antibiotics (Nirmala *et al.*, 2013) on the market (Čulić *et al.*, 2001; Wipo, 2013). This is understandable, as it has such a broad activity spectrum against both gram-positive and gram-negative bacteria. Azithromycin is mainly used for upper and lower respiratory infections, but also for some specific sexually transmitted diseases, for skin and tissue infections (Abu-Gharbieh *et al.*, 2004; Amsden, 1996; Čulić *et al.*, 2001; Hoepelman & Scheider, 1995), and even for the treatment of trachoma (chronic inflammatory eye disease). This macrolide antibiotic may in future also be used in malaria prophylaxis and treatment (Sood, 1999). Azithromycin is furthermore also considered a good choice for the treatment of opportunistic infections in acquired immunodeficiency syndrome (AIDS) patients (Abu-Gharbieh *et al.*, 2004; Amsden, 1996; Hoepelman & Scheider, 1995).

Several solid-state forms in which azithromycin can exist, have been reported, including anhydrate, monohydrate and dihydrate crystalline forms, as well as several amorphous forms (Gandhi *et al.*, 2002; Li & Trask, 2005; Jasanada *et al.*, 2001; Odendaal *et al.*, 2013).

2.2 Macrolide classification

2.2.1 Early development of macrolides

In 1957, R.B Woodward originally proposed the term, macrolide, as an acronym for macrolactone glycoside that belongs to structures, consisting of a macrolactone, or macrocyclic lactone, and to which one or more de-oxy-sugar residues, or amino-sugars, are attached (Čulić *et al.*, 2001; Katz & Mankin, 2009; Retsema & Wu, 2001). Following the discovery of penicillin, the pharmaceutical industry strived towards discovering new and improved antibiotics, of which erythromycin were one of them. Erythromycin, also a macrolide antibiotic (Rossiter, 2010), was the first natural macrolide antibiotic being discovered and it is also known as the first generation macrolide. It was discovered in the early 1950's and it has been officially clinically used since 1952, as an oral out-patient drug (Čulić *et al.*, 2001; Miroshnyk *et al.*, 2008; Weisblum, 1998). It was discovered in the Philippines from purified soil samples that contained *Streptomyces erythreus* (Sood, 1999). Continuous soil screening had occurred during the 1950's in search for new natural macrolide products. Only a few were isolated and developed, such as spiramycin in Europe, carbomycin in the United States of America, and josamycin and midecamycin in Japan. Tylosin was also developed, but mainly for veterinary use (Čulić *et al.*, 2001; Katz & Mankin, 2009).

Rapid elimination, poor bio-availability and acid instability were some of the reasons why these safe and effective macrolides had to be administered three to four times a day. During the 1960's, scientists had started to improve the pharmacokinetic properties that would allow for the less frequent administration of these antibiotics. The starting point for chemical derivitisation was to employ the natural products themselves (Katz & Mankin, 2009). The recognition of *Campylobacter*, *Mycoplasma*, *Legionella* and *Chlamydia* pathogens during the 1980-1990's, further rekindled the interest in macrolides and a new group (second generation) of semi-synthetic erythromycin derivatives, such as azithromycin, clarithromycin and roxithromycin was developed (Katz & Mankin, 2009; Miroshnyk *et al.*, 2008).

Azithromycin was discovered in the 1980's by a group of Croatian scientists at Pliva and it had been marketed for a few years in central and Eastern Europe under the name, Sumamed® (Xu *et al.*, 2012). Neither spectrum, nor potency improvements were displayed by these second generation macrolides, but they did have longer half-lives (*in vivo*), which reduced oral administration to once, or twice a day. Third generation macrolides, namely ketolides, were developed in the late 1980's, mainly to address and to overcome the rise of bacterial macrolide resistance at the time that caused respiratory tract infections (Čulić *et al.*, 2001; Katz & Mankin, 2009). Azithromycin received clinical approval in 1992 from the United

States (US) Food and Drug Administration (FDA), since then, Pfizer has marketed it under the brand name, Zithromax[®] (Kremer, 2002; Xu *et al.*, 2012).

2.2.2 The macrolide structure

Erythromycin is produced by bacteria, i.e. *Saccharopolyspora erythraea*, also called *Streptomyces* or *Streptomycetes* species, and it was originally isolated from these pathogen species (Čulić *et al.*, 2001; Kanfer *et al.*, 1998; Katz & Mankin, 2009; Retsema & Wu, 2001). Pikromycin was the first macrolide that was discovered through soil screening. Methymycin and narbomycin were also discovered, but they were never developed as drugs. Bacteria that are members of the *Actinomycetales* and mucelia forming, gram-positive bacteria order, are responsible for the production of many macrolides as natural products. *Saccharopolyspora*, *Micromonospora*, *Streptomyces* and *Actinoplanes* are common genera that can produce macrolides. Antibiotics, including the macrolides, that are produced by the *Micromonospora* species, end with the spelling, '-micin' (e.g. rosamicin), while compounds that are produced by the *Streptomyces* species, end with '-mycin' (e.g. azithromycin) (Kanfer *et al.*, 1998; Katz & Mankin, 2009).

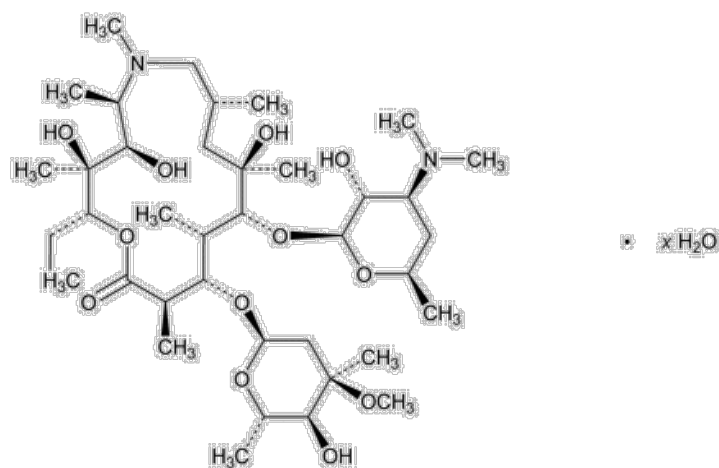


Figure 2.1: The chemical structure of azithromycin anhydrous and x H₂O, representing a monohydrate or dihydrate, according to the United States Pharmacopeial Convention (USPC, 2015a).

The term, macrolide, is derived from its structure that consists of a macrocyclic lactone nucleus ring, to which one, or multiple amino-sugars are attached, or linked by means of glycosidic bonds. Macrolides can hence be classified on the basis of the number of carbon atoms in the macrocyclic ring. The most commonly used and the most important macrolides consist of a 14-16 carbon atom macrocyclic ring and are also called 14-16-membered ring

compounds. These 14-16-membered macrolides can be sub-divided into natural products and semi-synthetic derivatives. The semi-synthetic derivatives can, depending on the type of chemical modification to the core erythromycin A structure, be further divided into three sub-groups.

- **14-membered macrolides:** Erythromycin A, B, C, D, E and F, clarithromycin, roxithromycin, oleandomycin, dirithromycin and flurithromycin are examples of this first group. They only consist of carbon and oxygen atoms, with two monosaccharide entities namely, D-desosamine and L-cladinose (Katz & Mankin, 2009; Miroshnyk *et al.*, 2008; Retsema & Wu, 2001).
- **15-membered macrolides:** Azithromycin (Figure 2.1) (an azolide) is the only 15-membered macrolide available that uniquely contains carbon, oxygen and nitrogen atoms (Adeli & Mortazavi, 2014; Hoepelman & Scheider, 1995; Kremer, 2002). Azithromycin is produced from erythromycin through a Beckmann re-arrangement that includes a methyl-substituted azolide nitrogen atom at position C9 (Katz & Mankin, 2009; Miroshnyk *et al.*, 2008; Retsema & Wu, 2001). It hence is a semi-synthetic derivative and a structural analogue of erythromycin (Nirmala *et al.*, 2013). According to Čulić *et al.* (2001), biotechnology permits the modification and interchanging of specific parts of various biosynthetic gene clusters, like polyketide synthase, to produce new macrolide structures. This recombinant biosynthesis is the process of artificial natural product formation. Azithromycin is also known as an azolide, due to its nitrogen modification. D-desosamine and L-cladinose sugars are also present, similarly as in erythromycin. The azolide nitrogen causes a dibasic chemical behaviour, whereas almost all other macrolides have a monobasic chemical behaviour.
- **16-membered macrolides:** Spiramycin, tylosin, mirosamicin, kitasamycin, rokitamycin, rosaramicin and josamycin belong to this group, which in addition contain oxygen and carbon atoms (Amsden, 1996; Amsden, 2001; Hoepelman & Scheider, 1995; Kanfer *et al.*, 1998; Katz & Mankin, 2009; Miroshnyk *et al.*, 2008; Retsema & Wu, 2001; Weisblum, 1998).

2.2.3 Physical and chemical properties of azithromycin

In this section, the known physical and chemical properties, as reported by the British Pharmacopoeia and other literature sources, are discussed.

2.2.3.1 Definition and appearance

According to the pharmacopoeia (BP, 2016; USPC, 2015a), the purity of a reference, or bulk material, should contain the equivalent of not less than 945 µg and not more than 1030 µg of azithromycin per mg. This is calculated on the anhydrous base. The anhydrate, monohydrate and dihydrate solid-state forms of azithromycin are reported to be crystalline. Azithromycin is a white, or almost white powder (BP, 2016; O'Neil, 2006; USPC, 2015b). It is derived from a fermentation product as a semi-synthetic product (BP, 2016).

2.2.3.2 pH

A 0.2% azithromycin solution, consisting of a 1:1 methanol:water ratio has a pH of 9.0 to 11.0 (BP, 2016; USPC, 2015a; USPC, 2015b).

2.2.3.3 Water content

Azithromycin can be available with zero, one or two molecules of water. Karl Fischer titration showed that anhydrous azithromycin will not contain more than 2.0% of water. According to the pharmacopoeia (BP, 2016; USPC, 2015a), the label claim for azithromycin dihydrate's water content must be between 4.0% and 5.0%. Azithromycin monohydrate may contain 1.8% to 4.0% of water, with the exception that it may contain 4.0% to 6.5%, when the specifications for the loss on drying test are met. However, it is quite possible that the identification and characterisation of the hydration level of azithromycin may be inaccurate. Theoretically, azithromycin monohydrate should contain 2.4% of molecular water and azithromycin dihydrate, 4.8%. If azithromycin monohydrate is characterised to contain 4.0% of water, one must always keep in mind that a transformation into azithromycin dihydrate is almost inevitable, due to the fact that only a small amount of molecular water is necessary to possibly trigger a phase transformation. Broad water content specifications, such as those for azithromycin, could lead to significant discrepancies and challenges during product processing steps, as well as during compliance testing with regards to other specifications, such as solubility and dissolution.

2.2.3.4 Loss on drying

The percentage of volatile substances is determined by means of thermogravimetric analysis. The total percentage loss that is allowed for azithromycin monohydrate is 4.0% to 6.5%. The analysis is conducted between 25°C and 150°C. Azithromycin monohydrate

must not lose more than 4.5% of its weight, between 25°C and 70°C. It must also not lose more than 1.8% to 2.6% of its weight between the two inflection points of 70°C and 130°C (USPC, 2015a). Again, these hydration level specifications for azithromycin could be problematic, especially if one considers the fact that a theoretical moisture loss of 2.4% is indicative of an azithromycin monohydrate. If azithromycin monohydrate loses more than 4.5% of its weight between 25°C and 70°C, it is more likely to be characterised as a dihydrate than a monohydrate. The specifications for loss on drying and water content (paragraph 2.2.3.3) show how critical extensive characterisation, as well as the use of multiple analytical techniques is during the physico-chemical characterisation of pharmaceutical raw materials.

2.2.3.5 Solubility and solubility characteristics

Azithromycin is slightly soluble to practically insoluble in water. Azithromycin is freely soluble in anhydrous ethanol and methylene chloride (BP, 2016; USPC, 2015b).

Solubility is dependent upon pressure, the nature of the solid form (anhydrate or hydrate) and temperature. Intermolecular interactions will be influenced by the presence of water molecules, which will affect solubility of the API. The solubility behaviour rule for an anhydrous form of a substance is that it is always better soluble in water than the hydrated forms of the same substance. If this is the case, then azithromycin anhydrous would be more soluble in water, than azithromycin monohydrate and dihydrate. Azithromycin monohydrate would also be more soluble in water than its dihydrate. The reason for the differences in solubility is attributable to the intimate interaction that has already taken place between the hydrate and the water. Because the available free energy of a hydrated form will be less for further interaction with water, its solubility will decrease. Anhydrous forms will hence have more free energy than their corresponding hydrates and be more soluble in water (Khankari & Grant, 1995).

Drug absorption (extent and rate) from the gastrointestinal (GI) tract is affected by several physiological factors (absorption mechanisms, small intestine transit time, GI blood flow, gastric emptying, GI pH level and colonic transit time), dosage form related (tablet, gel, solution, capsule, emulsion and suspension) and physico-chemical factors (solid-state form, solubility, particle size, stability, hydrogen bonding, polar-non-polar surface area, lipophilic action and diffusivity (Dahan *et al.*, 2009).

Azithromycin has a lipophilic nature, with a poor solubility profile in aqueous environments and a poor bio-availability of about 37% after oral administration, due to its poor solubility (Kremer, 2002; Nirmala *et al.*, 2013, Kanatani & Guglielmo, 1994; Sood, 1999; Aucamp *et*

et al., 2015). Azithromycin is classified as a BCS class II drug (poorly soluble, highly permeable), which can be attributed to its poor solubility in aqueous environments (Amidon *et al.*, 1995; WHO, 2006; Zhang *et al.*, 2007). It is well known that poor solubility can lead to poor dissolution rates, which will have a direct impact on the bio-availability of a drug and result in a decrease in treatment efficacy (Yu, 2001).

The bio-availability of azithromycin can be decreased by as much as 50%, when taken with food and it must therefore be taken a few hours before, or after meals (Kanatani & Guglielmo, 1994).

2.2.3.6 Melting point

The melting points of azithromycin anhydrous, monohydrate and the dihydrate differ. Anhydrous azithromycin melts at about 113°C to 115°C and the dihydrate at about 126°C (Galichet, 2004; O'Neil, 2006; USPC, 2015b). The melting of azithromycin dihydrate was also recorded at 134°C to 141°C and that of the monohydrate at about 139°C to 156°C (Gandhi *et al.*, 2002). These substantially different outcomes probably are as a result of different heating rates and temperature programs being used, when generating the melting data of the different hydrated forms of azithromycin.

2.2.3.7 Acid stability

Azithromycin is more acid stable than erythromycin (Gandhi *et al.*, 2002; Kremer, 2002). The older macrolides only contain carbon and oxygen structure rings. Since azithromycin is chemically modified, with the insertion of nitrogen into the structure ring to increase in its basicity, it is less acid labile. The chemical modification (dibasic nature), or structural change of azithromycin hence decreases its degradation in an acid medium and its acid stability and oral bio-availability is better than those of erythromycin. As a result of this modification, the decomposition of azithromycin primarily occurs *via* the acid-catalysed hydrolysis of the ether bond into the neutral cladinose sugar (Fiese & Steffen, 1990). It has been demonstrated that 10% of azithromycin degradation occurs within 20 minutes in a pH 2 acidic environment, compared to the 3.5 seconds within which erythromycin degrades to the same extent. It degrades six times slower than erythromycin at this pH, which explains its better tolerability in an acid environment (Hoepelman & Scheider, 1995; Kanatani & Guglielmo, 1994; Retsema & Wu, 2001; Sood, 1999). Although azithromycin shows higher acid stability, it is not completely acid stable. Extended exposure to stomach acid would

ultimately lead to the degradation of azithromycin into des-cladinose-azithromycin (Curatolo *et al.*, 2011).

2.2.3.8 Dissociation constant

One of the most important parameters that describe an active pharmaceutical ingredient's (API's) physico-chemical properties is its acid dissociation constant, expressed as its pKa value. Most APIs have acidic and basic functionalities, with dissociation constants and pH that control their ionisation states. The dissociation constant characterises the acid-base equilibrium of the respective ionisation states in a solution, which is indicative of the deprotonation (dissociation) potentials at a given pH. The ionisation state can consist of cationic (positive), neutral, or anionic (negative) charges. A charge difference is present in either the acidic, or basic forms of an API and at least one of them is ionised. The ionisation state of an API has certain properties that may influence its ultraviolet (UV) absorption, volatility, water solubility, reactivity with chemical oxidants, its affinity for an association with proteins, membrane permeability and therapeutic activity. The neutral form is more lipophilic and shows higher membrane permeability, while the ionised forms normally are more water soluble (Babić *et al.*, 2007; Nowak *et al.*, 2015). According to Galichet (2004), the dissociation constant of azithromycin is 8.1 and 8.8. Azithromycin anhydrous showed a pKa value of 8.74 (Prankerd, 2007). The pKa of azithromycin was also recorded by Zur *et al.* (2014), with the two nitrogen groups undergoing dissociation at 8.8 and 8.9 (mean of 8.85). Azithromycin is protonated at physiological pH and less than 4% will be in its neutral, non-ionised form (Zur *et al.*, 2014). Computational methods can also be used to calculate the pKa value of an API. Babic *et al.* (2007) made use of SPARC and calculated the pKa of azithromycin as being 7.34. SPARC is an on-line calculator that calculates pKa values solely from an API's chemical structure, and from its micro- and macroscopic pKa values (Babic, 2007; Bakheit *et al.*, 2014).

2.3 Pharmaceutical uses and advantages of azithromycin

As stated previously, azithromycin is one of the best-selling antibiotics in the pharmaceutical industry, due to the fact that it is currently the most widely prescribed antibiotic in the world (Adeli & Mortazavi, 2014; Nirmala *et al.*, 2013). Azithromycin is bacteriostatic (suppresses the multiplication of bacteria) and bactericidal (eliminates or kills bacteria). Azithromycin has a very broad spectrum of activity and it is very effective against both gram-positive and gram-negative aerobes, atypical, anaerobic pathogens and other pathogens (Abu-Gharbieh *et al.*, 2004; Amsden, 2001; Kremer, 2002; Retsema & Wu, 2001).

The pathogens can be classified as follows:

- **Gram-positive aerobes:** *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Streptococcus aureus*, *Streptococci groups C, F and G* and *Streptococci viridans*.
- **Gram-negative aerobes:** *Haemophilus influenzae*, *Haemophilus ducreyi*, *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, *Bordetella pertussis*, *Listeria monocytogenes* and *Legionella pneumophila*.
- **Atypical pathogens:** *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Treponema pallidum* and *Mycoplasma pneumoniae*.
- **Anaerobes:** *Peptostreptococcus species*, *Prevotella bivia*, *Escherichia coli*, *Salmonella species*, *Yersinia enterocolitica* and *Shigella species*.
- **Other pathogens:** *Ureaplasma urealyticum* and *Campylobacter jejuni*.

The majority of azithromycin prescriptions are for upper and lower respiratory tract infections, including otitis media, acute bronchitis, acute exacerbations of chronic bronchitis, acute bacterial exacerbations of chronic obstructive pulmonary disease (COPD), laryngitis, sinusitis, tonsillitis, pharyngitis and community acquired pneumonia.

Secondly, disease causing pathogens, like *Legionella pneumophila* (Legionnaire's disease), *Neisseria gonorrhoeae* (gonorrhoea), *Borrelia burgdorferi* (Lyme disease), *Helicobacter pylori* (peptic ulcer), *Mycobacterium tuberculosis*, *Chlamydia trachomatis* (urethritis and cervicitis), *Haemophilus ducreyi* (Chancroid) (Sood, 1999; Weisblum, 1998) and *Mycobacterium avium* complex (in AIDS patients) (AIDS infonet; Katz & Mankin, 2009; Sood, 1999; Rubinstein, 2001) have also been successfully treated with azithromycin. Azithromycin can further be used to treat *Streptococcus pyogenes* and *Propionibacterium acnes*, which are responsible for soft tissue and skin infections (Abu-Gharbieh *et al.*, 2004; Amsden, 1996; Hoepelman & Scheider, 1995). A study has shown that long term treatment with azithromycin has improved the lung function in children, suffering from cystic fibrosis (Katz & Mankin, 2009; Kremer, 2002; Retsema & Wu, 2001; Rubinstein, 2001; Sood, 1999). It has also been proven useful in the treatment of *Rickettsia* (Kanfer *et al.*, 1998). Effectiveness has furthermore been demonstrated by intravenous azithromycin in the treatment of pelvic inflammatory disease, in combination with other antibiotics (Kremer, 2002). Azithromycin is the most effective of all macrolides against the parasite, *Toxoplasma gondii*. Trachoma (chronic inflammatory eye disease), caused by *Chlamydia trachomatis*, was treated in a clinical trial in Gambia with a single dose, which was as effective as 6 months of treatment with doxycycline.

A malaria prophylaxis study, using azithromycin, was conducted on Indonesians in Irian Jaya. It showed a protective efficacy of 71.6% against *Plasmodium falciparum* and 98.9% against *Plasmodium vivax*, compared to the placebo group. Another trial among children (aged 5 to 14 years) was conducted, during which azithromycin (20 mg/kg, once a week for 3 weeks) was given as a prophylactic regimen. Although new infections did continue to develop during treatment, only 35% of the children taking the azithromycin prophylactic treatment were infected, compared to the 58% of reported infections among the control group. Azithromycin may hence have a future in malaria treatment and prophylaxis (Sood, 1999; Bygott & Fry, 2008).

One of its major advantages is that azithromycin has a higher acid stability, than the first generation macrolides (Blandizzi *et al.*, 1998; Katz & Mankin, 2009; Kremer, 2002). Azithromycin is 300 times more acid stable, compared to the older macrolides. A study was conducted by treating patients with azithromycin, suffering from gastric *Helicobacter pylori* (*H. pylori*). The outcomes showed that the azithromycin concentration had been significantly higher in the gastric mucosa than in the plasma, indicating that the drug had been largely retained in the target tissue, resulting in a fairly good eradication of *H. pylori*. The high concentration in the gastric mucosa may, however, have also resulted from the co-administration of omeprazole during this study (Blandizzi *et al.*, 1998).

Azithromycin also shows a longer elimination time after administration (average half-life of azithromycin is 68 hours), which leads away from the frequent administration of the first generation antibiotics of three to four times a day, to only once or twice daily (Amsden, 1996; Katz & Mankin, 2009; Kremer, 2002). It also has more favourable pharmacokinetic parameters, than other macrolides. Azithromycin shows a two to eight-fold increase in spectrum activity against *H. influenza*, compared to erythromycin (Amsden, 2001; Retsema & Wu, 2001). Azithromycin has an excellent safety record and is the drug of choice for the treatment of gram-negative bacteria (Katz & Mankin, 2009).

Azithromycin, clarithromycin and telithromycin have better gastrointestinal tolerability, than erythromycin. Although GI intolerance remains the primary adverse side effect of these three agents, the occurrence thereof has been significantly reduced compared to that of erythromycin (Nirmala *et al.*, 2013; Zuckerman, 2004). As mentioned, azithromycin has a dibasic chemical behaviour, compared to the monobasic chemical behaviours of the other macrolides. These differences in behaviours are of great importance with regards to their cellular dynamics. Azithromycin displays high tissue concentrations, prolonged retention and extensive cellular uptake, long after administration of the final dose of treatment. The peak concentration in granulocytes during treatment was recorded as about 80 mg/L and even after 7 days of completion of treatment, the concentration was measured at 32 mg/L

and higher. The concentrations of azithromycin in the lymphocytes/monocytes were even higher. The 14- and 16-membered macrolides were found to have shorter half-lives and showed moderate cellular and tissue penetration (Amsden, 2001; Katz & Mankin, 2009; Kremer, 2002; Van Bambeke & Tulkens, 2001). Another study revealed that azithromycin showed high concentrations in the epithelial lung fluid, often exceeding the minimal inhibitory concentration (MIC_{90}), by killing 90% of respiratory pathogens. This could be the reason for its extreme effectiveness against respiratory tract infections (Čulić *et al.*, 2001). Another significant advantage of the macrolides is that they are also used as an alternative in patients that are allergic to penicillin treatments (Kanfer *et al.*, 1998; Retsema & Wu, 2001). Azithromycin is safe for administration to pregnant women and is classified as a pregnancy category B drug, with an improved drug interaction profile (Amsden, 1996; Rossiter, 2010; Williams, 2001). In conclusion, azithromycin has less adverse effects, than other macrolides (Kremer, 2002; Hoepelman & Scheider, 1995). The three major side effects of azithromycin are its impact on gastrointestinal motility (prokinetic action that occurs during motilin receptor stimulation), its potential drug metabolism inhibiting effect (cytochrome P450 iso-enzymes inhibition) and its pro-arrhythmic effect, due to the prolongation of the QT interval of the electro-cardiogram (blockade of the HERG K^+ channels) (Abu-Gharbieh *et al.*, 2004).

2.4 Solid-state forms of azithromycin

It is well known that APIs can exist in multiple solid-state forms. The different solid-state forms will differ in terms of their physical and chemical properties. This gives scientists the opportunity to choose the optimal solid-state form that would enhance product manufacturing and also ensure effective patient treatment. It is essential to know and understand the different solid-state forms of APIs during the formulation and manufacturing of new dosage forms, since critical properties, like solubility and particle morphology vary among the different forms (Hancock & Parks, 2000).

As already mentioned, azithromycin can exist in different crystalline solid-state forms, i.e. as anhydrides, monohydrates, or dihydrates. All three these forms have well defined molecular arrangements, resulting in their crystalline solid-state forms (Jasanada *et al.*, 2001; Li & Trask, 2005; USPC, 2015a). The dihydrated form is obtained when azithromycin is crystallised in any mixture of acetone and water; or in tert-butanol and water; or in tert-butanol, petroleum ether and water, whilst an ethanol and water mixture will produce the monohydrate (Gandhi *et al.*, 2002; Jasanada *et al.*, 2001; Li & Trask, 2005; Timoumi *et al.*, 2014). Drying of the hydrates will lead to an anhydrous form. Harsh dehydration processes lead to the formation of amorphous forms (Timoumi *et al.*, 2014). During the literature

search of this study, several patents and scientific reports regarding the existence of amorphous azithromycin were found. According to Aucamp *et al.* (2015), azithromycin dihydrate can be heated in an oven, until a molten product is obtained. The subsequent molten sample can then be quench cooled on a cold surface to produce the amorphous form. Amorphous azithromycin can also be prepared by melting the crystalline solid-state form under vacuum, followed by a slow cooling process (Li & Trask, 2005). Another amorphous azithromycin preparation method involved the spray drying of a 2% w/v solution in acetone (Lyon & Shalaev, 2007). Amorphous azithromycin dihydrate can also be prepared by dissolving it in tert-butanol, followed by filtering and solidifying it in a cooling bath, or it can be dissolved in ethanol, filtered and evaporated to dryness by means of quick evaporation (Jasanada *et al.*, 2001).

Upon review of all of the above solid-state forms of azithromycin, it has become clear that pharmaceutical processing steps can have distinct and significant influences on the phase transformation of one azithromycin solid-state form into another. The objective of this study therefore was to investigate the possible transformations that may occur in amorphous azithromycin (paragraph 1.10).

2.5 Conclusion

Azithromycin is a derivative that is chemically modified from the 14-membered macrolide, erythromycin. This 15-membered macrolide, possesses increased efficacy. The numerous advantages of azithromycin make it the most prescribed and used macrolide antibiotic, worldwide. Despite of all its advantages, however, its biggest disadvantage is its poor water solubility, with a low bio-availability of only 37%, following oral administration. In this chapter, many of the explored techniques to improve the poor solubility of this antimicrobial agent, were discussed. This study focused on the preparation of amorphous solid-state forms of azithromycin, as another possible methods of improving its solubility. To date, limited studies are available regarding the differences in physico-chemical properties of different amorphous forms of the same API. An investigation into the different amorphous forms of azithromycin and a comparison of the advantages and disadvantages of each amorphous form should hence add much value to existing knowledge on amorphs.

Previous studies on the amorphous forms of another macrolide, roxithromycin, proved that solution-mediated, solvent-mediated and solid-melt-solid phase transformations were important factors to consider during processing, formulation and product testing procedures. The possibility of solution-mediated, solvent-mediated and solid-melt-solid phase

transformations of amorphous azithromycin were also investigated during this study, as discussed in further chapters.

2.6 Reference list

- Abu-Gharbieh, E., Vasina, V., Poluzzi, E. & De Ponti, F. 2004. Antibacterial macrolides: a drug class with a complex pharmacological profile. *Pharmacological research*, 50:211-222.
- Adeli, E. & Mortazavi, S.A. 2014. Design, formulation and evaluation of azithromycin binary solid dispersions using Kolliphor® series for the solubility and *in vitro* dissolution rate enhancement. *Journal of pharmaceutical investigation*, 44:119-131.
- Adrjanowicz, K., Zakowiecki, D., Kaminski, K., Hawelek, L., Grzybowska, K., Tarnacka, M., Paluch, M. & Cal, K. 2012. Molecular dynamics in supercooled liquid and glassy states of antibiotics: azithromycin, clarithromycin and roxithromycin studied by dielectric spectroscopy. advantages given by the amorphous state. *Molecular pharmaceutics*, 9:1748-1763.
- Aids Infonet. 2014. *Mycobacterium avium* complex (MAC). Fact sheet no. 514. <http://www.aidsinfonet.org> Date of access: 13 Jul. 2015.
- Amidon, G.L., Lennernäs, H., Shah, V.P. & Crison, J.R. 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharmaceutical research*, 12:413-420.
- Amsden, G.W. 1996. Erythromycin, clarithromycin and azithromycin: are the differences real? *Clinical therapeutics*, 18:56-72.
- Amsden, G.W. 2001. Advanced-generation macrolides: tissue-directed antibiotics. *International journal of antimicrobial agents*, 18:S11-S15.
- Aucamp, M., Odendaal, R., Liebenberg, W. & Hamman, J. 2015. Amorphous azithromycin with improved aqueous solubility and intestinal membrane permeability. *Drug development and industrial pharmacy*, 41:1100-1108.
- Babić, S., Horvat, A.J.M., Pavlović, D.M. & Kaštelan-Macan, M. 2007. Determination of pKa values of active pharmaceutical ingredients. *Trends in analytical chemistry*, 26:1043-1061.
- Bakheit, A.H.H., Al-Hadiya, B.M.H. & Abd-Elgalil, A.A. 2014. Azithromycin. (In Brittain, H.G. ed. Profiles of drug substances, excipients, and related methodology. Vol. 39. Amsterdam: Elsevier Inc. p. 1-40).
- Blandizzi, C., Malizia, T., Gherardi, G., Costa, F., Marchi, S., Marveggio, C., Natale, G., Senesi, S., Bellini, M., Maltinti, G., Campa, M. & Del Tacca, M. 1998. Gastric mucosal distribution and clinical efficacy of azithromycin in patients with *Helicobacter pylori* related gastritis. *Journal of antimicrobial chemotherapy*, 42:75-82.
- British Pharmacopoeia. 2016. Monographs: azithromycin. <https://www.pharmacopoeia.com/bp->

2016/monographs/azithromycin.html?published-date=2015-08-03&text=azithromycin Date of access: 09 Feb. 2016.

Bygott, J. & Fry, G. 2008. Azithromycin. (In Schlagenhauf-Lawlor, P. ed. Travelers' malaria. 2nd ed. Hamilton: BC Decker. p. 131-133). https://books.google.co.za/books?hl=en&lr=&id=54Dza0UH yngC&oi=fnd&pg=PA1&dq=%22travelers'+malaria+schlagenhauf-lawlor&ots=uH_bJpEi6F&sig=-nWwQpCcURPsWhFx E6N7RTqMhHg#v=onepage&q=%22travelers'%20malaria%20schlag enhauf-lawlor&f=false Date of access: 01 Oct. 2015.

Clardy, J., Fischbach, M. & Currie, C. 2009. The natural history of antibiotics. National institute of health. *Current biology*, 19:R437-R441. <http://www.sciencedirect.com/science/article/pii/S096098220900918X> Date of access: 01 Oct. 2015.

Čulić, O., Eraković, V. & Parnham, M.J. 2001. Anti-inflammatory effects of macrolide antibiotics. *European journal of pharmacology*, 429:209-229.

Curatolo, W., Liu, P., Johnson, B.A., Hausberger, A., Quan, E., Vendola, T., Vatsaraj, N., Foulds, G., Vincent, J. & Chandra, R. 2011. Effects of food on a gastrically degraded drug: azithromycin fast-dissolving gelatin capsules and HPMC capsules. *Pharmaceutical research*, 28:1531-1539.

Dahan, A., Miller, J.M. & Amidon, G.L. 2009. Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs. *Journal of the American Association of Pharmaceutical Scientists*, 11:740-746.

Fiese, E.F. & Steffen, S.H. 1990. Comparison of the acid stability of azithromycin and erythromycin A. *Journal of antimicrobial chemotherapy*, 25:39-47.

Galichet, L.Y. 2004. Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material. 3rd ed. London: Pharmaceutical Press.

Gandhi, R., Pillai, O., Thilagavathi, R., Gopalakrishnan, B., Lal Kaul, C. & Panchagnula, R. 2002. Characterization of azithromycin hydrates. *European journal of pharmaceutical sciences*, 16:175-184.

Hancock, B.C. & Parks, M. 2000. What is the true solubility advantage for amorphous pharmaceuticals? *Pharmaceutical research*, 17:397-404.

Hoepelman, I.M. & Schneider, M.M.E. 1995. Azithromycin: the first of the tissue-selective azalides. *International journal of antimicrobial agents*, 5:145-167.

- Jasanada, B., Santos, M., Isidro, L.G. & Felix, F.M. 2001. Preparation of non-crystalline and crystalline dihydrate forms of azithromycin. (Patent: EPO 1 103558 A2).
- Kanatani, M.S. & Guglielmo, B.J. 1994. The new macrolides azithromycin and clarithromycin. *Western journal of medicine*, 160:31-37.
- Kanfer, I., Skinner, M.F. & Walker, R.B. 1998. Analysis of macrolide antibiotics. *Journal of chromatography A*, 812:255-286.
- Katz, L. & Mankin, A.S. 2009. Macrolides. (In Schaechter, M. ed. *Encyclopedia of microbiology*. 3rd ed. Oxford: Academic Press. https://books.google.co.za/books?hl=en&lr=&id=rLhdW5YzuO4C&oi=fnd&pg=PP2&dq=KATZ+Macrolides.+Encyclopedia+of+microbiology,&ots=p_gcpp4bQF&sig=PvPR-hxpn4mQJyMD_7Kv9i_6KSo#v=snippet&q=macrolides&f=false Date of access: 02 Oct. 2015.
- Khankari, R.K. & Grant, D.J.W. 1995. Pharmaceutical hydrates. *Thermochimica Acta*, 248:61-79.
- Khosla, C. 2010. Thematic minireview series on antibacterial natural products: new tricks for old dogs. *The journal of biological chemistry*, 285:1-2.
- Kremer, C.J. 2002. Azithromycin: a new macrolide. *Primary care update for obstetrics/gynecology*, 9:174-175.
- Li, Z.J. & Trask, A.V. 2005. Crystal forms of azithromycin. (Patent: US 6977243B2).
- Lyon, D.K. & Shalaev, E. 2007. Process for forming amorphous azithromycin particles. (Patent: WO 2007/007148A1).
- Miroshnyk, I., Mirza, S., Zorky, P.M., Heinämäki, J., Yli-Kauhaluoma, J. & Yliruusi, J. 2008. A new insight into solid-state conformation of macrolide antibiotics. *Bioorganic & medicinal chemistry*, 16:232-239.
- Nirmala, M.J., Viswanadha, S., Mukherjee, A. & Chandrasekaran, N. 2013. Improved physico-chemical aspects of azithromycin through novel microemulsion system. *International journal of pharmacy and pharmaceutical sciences*, 5:700-702.
- Nowak, P., Olechowska, P., Mitoraj, M., Woźniakiewicz, M. & Kościelniak, P. 2015. Determination of acid dissociation constants of warfarin and hydroxywarfarins by capillary electrophoresis. *Journal of pharmaceutical and biomedical analysis*, 112:89-97.
- Odendaal, R.W., Liebenberg, W. & Aucamp, M. 2013. Composition comprising an amorphous non-crystalline glass form of azithromycin. (Patent: US 2013/0059805 A1).

- O'Neil, M.J., ed. 2006. The Merck index. An encyclopedia of chemicals, drugs and biologicals. 14th ed. Whitehouse Station, N.J.: Merck.
- Pranker, R.J. 2007. Critical compilation of pKa values for pharmaceutical substances. (*In* Brittain, H.G. ed. Profiles of drug substances, excipients, and related methodology. Vol. 33. Amsterdam: Elsevier Inc.).
- Retsema, J. & Fu, W. 2001. Macrolides: structures and microbial targets. *International journal of antimicrobial agents*, 18:S3-S10.
- Rubinstein, E. 2001. Comparative safety of the different macrolides. *International journal of antimicrobial agents*, 18:S71-S76.
- Rossiter, D., ed. 2010. South African medicines formulary. 9th ed. Cape Town: Health and Medical Publishing Group.
- Sköld, O. 2010. Antibiotics and antibiotic resistance. Hoboken, N.J.: Wiley. <https://books.google.co.za/books?id=qq83AT5aQ1wC&pg=PT5&dq=SK%C3%B6LD,+O.++2010.++Antibiotics+and+antibiotic+resistance&hl=en&sa=X&ved=0CBMQ6AEwAGoVChMlrJa0pITnyAlVwrcUCh1sOwm6#v=onepage&q=SK%C3%B6LD%2C%20O.%20%202010.%20%20Antibiotics%20and%20antibiotic%20resistance&f=false> Date of access: 02 Oct. 2015.
- Sood, S.K. 1999. Macrolides: clarithromycin and azithromycin. *Seminars in pediatric infectious diseases*, 10:23-30.
- Timoumi, S., Mangin, D., Peczalski, R., Zagrouba, F. & Andrieu, J. 2014. Stability and thermophysical properties of azithromycin dihydrate. *Arabian journal of chemistry*, 7:189-195.
- United States Pharmacopeial Convention (USPC). 2015a. Monographs: azithromycin. http://www.uspnf.com/uspnf/pub/data/v38330/usp38_nf33s0_m6740.xml Date of access: 11 Jul. 2015.
- United States Pharmacopeial Convention (USPC). 2015b. Safety data sheet: Azithromycin. <http://www.usp.org/pdf/EN/referenceStandards/msds/1046056.pdf> Date of access: 11 Jul. 2015.
- Van Bambeke, F. & Tulkens, P.M. 2001. Macrolides: pharmacokinetics and pharmacodynamics. *International journal of antimicrobial agents*, 18:S17-S23.
- Weisblum, B. 1998. Macrolide resistance. *Drug resistance updates*, 1:29-41.
- Williams, J.D. 2001. Evaluation of the safety of macrolides. *International journal of antimicrobial agents*, 18:S77-S81.

Wipo. 2013. The world intellectual property organization. Azithromycin: a world best-selling antibiotic-Pliva. <http://www.wipo.int/ipadvantage/en/details.jsp?id=906> Date of access: 05 Jul. 2015.

World Health Organization. 2006. WHO expert committee on specifications for pharmaceutical preparations. Annex 8: Proposal to waive *in vivo* bioequivalence requirements for WHO model list of essential medicines immediate-release, solid oral dosage forms. Geneva. WHO technical report series, (937):391-425.

Xu, Z., Flavin, M.T. & Eiznhamer, D.A. 2012. Macrolides and ketolides. (*In* Dougherty, T.J. & Pucci, M.J. *ed.* Antibiotic discovery and development. New York: Springer. p. 181-228).

Yu, L. 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Advanced drug delivery reviews*, 48:27-42.

Zhang, D., Tan, T., Gao, L., Zhao, W. & Wang, P. 2007. Preparation of azithromycin nanosuspensions by high pressure homogenization and its physicochemical characteristics studies. *Drug development and industrial pharmacy*, 33:569-575.

Zuckerman, J.M. 2004. Macrolides and ketolides: azithromycin, clarithromycin, telithromycin. *Infectious disease clinics of North America*, 18:621-649.

Zur, G., Soback, S., Weiss, Y., Perry, E., Lavy, E. & Britzi, M. 2014. Azithromycin pharmacokinetics in the serum and its distribution to the skin in healthy dogs and dogs with pyoderma. *The veterinary journal*, 200:122-126.

Chapter 3

Materials and methods

3.1 Introduction

It is of great importance to explore and make use of multiple techniques during the physico-chemical characterisation of an active pharmaceutical ingredient (API) and during solid-state chemistry. The use of multiple complementary techniques is essential in performing a complete and conclusive physico-chemical study. The different characterisation techniques that were used during this study included both chemical and thermal methods and did the research focus on the preparation and characterisation of six different amorphous solid-state forms of azithromycin.

3.2 Materials

Azithromycin dihydrate was purchased from DB Fine Chemicals (Pty) Ltd (Johannesburg, South Africa), having a potency of 96.5% (on the dried base). Potassium dihydrogen orthophosphate (batch number [B/N]: 1028963) and sodium hydroxide (B/N: 1032685) were obtained from Saarchem (Pty) Ltd (Krugersdorp, South Africa). Ethanol (analytical grade) and acetonitrile (chromatography grade) were purchased from ACE Chemicals (B/N: 30590 and D3I062233I, respectively), tert-butanol (B/N: STBD6053V) was purchased from Sigma Aldrich (Johannesburg, South Africa). Ultra-pure water with a resistivity of $\leq 18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$, produced with a Rephile (Shanghai City, China) Direct-Pure UP Ultrapure & RO Lab Water System, was used throughout the study.

3.3 Methods used for the preparation of amorphous azithromycin

A variety of methods exist for the preparation of an amorphous solid-state form of a single API. Preparation techniques include, but are not limited to melt cooling, solution based (precipitation, spray drying or freeze drying) and mechanically based methods (milling) (Graeser *et al.*, 2008).

The following methods were selected to prepare amorphous solid-state forms of azithromycin during this study:

Method 1: Amorphous azithromycin was prepared according to the quench cooling of the melt method (Aucamp *et al.*, 2015). This method involved the melting of azithromycin dihydrate (crystalline solid-state form) in a Petri dish at approximately 140°C in a laboratory oven (Binder, Tuttlingen, Germany). Upon complete melting of the crystalline material, it was removed from the oven and subsequently quench cooled on a cold surface.

Method 2: This process is considered a slow cooling method. Crystalline azithromycin dihydrate was heated in a Petri dish in a vacuum oven (Vismara, Lodigiano, Italy) at 110°C to 120°C (150 mmHg). After the sample had completely melted, the oven was left to cool to ambient temperature and the sample left under vacuum for 12 hours. The amorphous solid-state form was collected and stored with desiccant in a vacuum desiccator, until further physico-chemical characterisation (Li & Trask, 2005).

Method 3: This amorphous form was prepared by applying hot air. This preparation method is new and innovative, with the first report on it by Milne *et al.* (2016). It is a delicate heating and melting method. An advantage of this method is that it is suitable to melt APIs, which show degradation close to the melting temperature. The temperature can be controlled accurately and the stream of hot air can be applied directly and closely to the sample. The biggest disadvantage for this method is that the cooled product becomes very static and difficult to use during laboratory analyses.

Approximately 60 mg of crystalline azithromycin dihydrate was placed on a 0.25 mm thick stainless steel sheet. Hot air (140 ± 5°C) was directed towards the back of the stainless steel sheet. After a molten product was obtained, the hot air was directed from the top, directly on the melt, to ensure complete melting of the product, without any seed crystals remaining.

Method 4: This method involved dissolving 5 g of crystalline azithromycin dihydrate in 25 mL of ethanol. The solution was filtered and the solvent evaporated at room temperature at 150 mmHg. The solid obtained was subsequently dried at 80 mmHg and 60°C (Jasanada *et al.*, 2001).

Method 5: This method involved the spray drying of azithromycin from a 2% (w/v) solution in acetone. The spray dryer used was a Büchi B-90 Nano Spray-Dryer (Flawil, Switzerland), equipped with a B-295 Inert Loop and supplied with an inert atmosphere in the form of nitrogen gas. Spray drying parameters were as follows: 7 µm spray nozzle; inlet temperature = 92°C; pump speed = 3; gas flow = 120 l/min; pressure = 36 mbar. The

resulting amorphous azithromycin was collected and stored with desiccant in a vacuum desiccator at ambient temperature, until further testing.

Method 6: Amorphous azithromycin was prepared by dissolving 5 g of crystalline azithromycin dihydrate in 25 mL of tert-butyl alcohol (TBA) at 30°C. The solution was filtered and solidified in a cooling bath. The obtained solid was dried in a vacuum oven at 80 mmHg and 40°C (Jasanada *et al.*, 2001).

3.4 Characterisation methods

3.4.1 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is the most widely used thermal analysis technique for both the identification and characterisation of solid-state forms. Thermal analysis is the term used for describing all of the analytical techniques that measure the physical and chemical properties of a sample as a function of temperature and/or time. It provides quantitative information about the relative stability of polymorphic modifications, the monotropic, or enantiotropic nature of the transitions and the energies involved between phase changes. DSC is based upon the principle that a change in a sample's physical state is accompanied by the absorption, or liberation of heat. DSC measures the energy changes that may occur during the heating of a sample, the cooling of a sample, or when the sample is held isothermally. It also measures the exact temperature at which such changes take place. The DSC technique hence determines the enthalpy that accommodates any changes, by recording the difference in the heat flow between an inert reference and the sample being analysed (Bernstein, 2002; Craig, 2006; Saunders & Gabbott, 2011).

Figure 3.1 illustrates a typical DSC trace, or thermogram of an amorphous form, where the glass transition can be observed as a step in the base line and can it sometimes be followed by re-crystallisation and subsequent melting. The glass transition (T_g) is a step/shift in the baseline when the heat flow is increased. A complete description of the T_g is offered in section 1.8. The small peak at the top of the step represents a molecular relaxation. Thereafter, cold crystallisation takes place, which is indicated by a broad exothermic peak and finally, an endotherm when the crystals melt. Some degree of broadening of peaks may surface during any phase change during a DSC measurement. In theory, a pure single crystal should result in a narrow peak at a slow heating rate, but peak broadening occurs as a direct result of the thermal gradients that occur across the sample. Thermal gradients are caused by the time it takes for energy to transfer throughout the complete sample (Saunders & Gabbott, 2011).

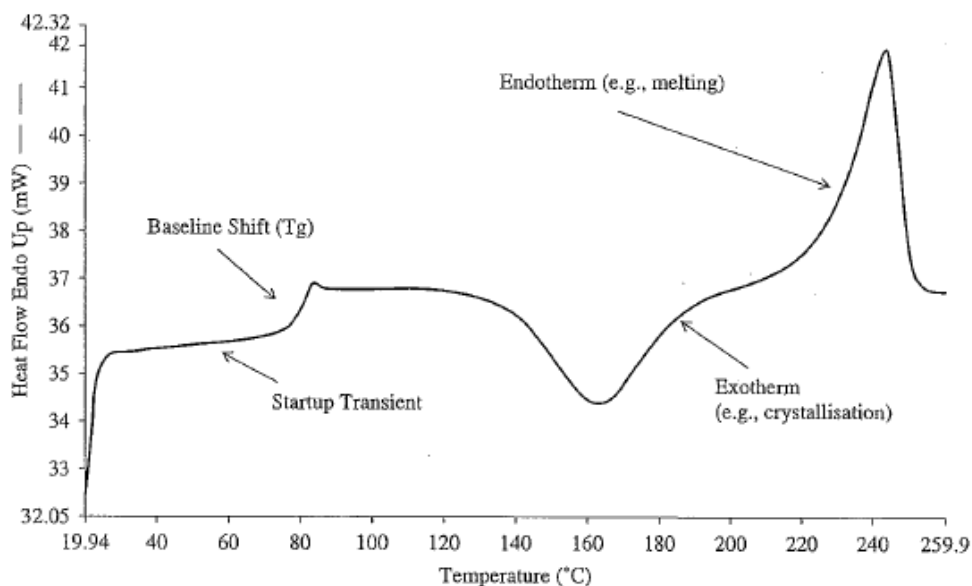


Figure 3.1: Thermogram illustrating DSC thermal behaviour of an amorphous form (Saunders & Gabbott, 2011).

DSC analysis was used to determine the thermodynamic properties of the prepared amorphous forms of azithromycin. A reference pan was used to measure and compare the difference in heat flux to that of the sample. In this study, a DSC-60 Shimadzu instrument (Kyoto, Japan) was used to record the DSC thermograms. Samples, weighing approximately 3 - 5 mg were placed in aluminium crimp cells and heated to 250°C at a heating rate of 10°C/min and a nitrogen gas purge of 35 ml/min. In those instances where the structural relaxation of the different amorphous forms were determined, varying heating rates (i.e. 2, 5, 10, 15 and 20°C/min) were applied. The onset temperatures of thermal events were used throughout this investigation of the different azithromycin amorphous forms.

3.4.2 Thermogravimetric analysis

Thermogravimetric analysis (TGA) involves the measurement of the weight of mass change that occurs as a function of temperature and/or time. It offers information regarding the presence of volatile substances, such as solvents and water, which form the bases of solvates and hydrates. TGA measurements also allow for the determination of residual solvents, and of the oxidative and thermal stabilities of a material. It is also possible to determine the decomposition, degradation and sublimation of a compound. TGA is usually

performed in conjunction with DSC analyses, as the loss of solvates, or hydrates can be mistaken for melting point peaks, whereas the TGA can distinguish between these traces (Bernstein, 2002; Craig, 2006; Saunders & Gabbott, 2011).

TGA measurements were conducted on a Shimadzu DTG-60 instrument (Kyoto, Japan) to determine the percentage weight loss (%) of samples during heating. Samples (3 – 5 mg) were accurately weighed into open aluminium crucibles. The samples were heated from 25°C to 250°C at a heating rate of 10°C/min and a nitrogen gas purge of 35 mL/min. This method was useful in determining the weight changes relative to the temperatures, and the outcomes were indicative thereof whether the investigated samples contained any solvent. This was especially useful in those cases where solvent evaporation was used as a preparation method of the amorphous forms of azithromycin.

3.4.3 Scanning electron microscopy

Scanning electron microscopy (SEM) is a powerful characterisation technique, as it offers higher magnification and resolution than optical microscopy. It can be very useful in understanding the differences in the properties of polymorphs and in assisting with their characterisation. The investigation of the properties of surfaces can be conducted by means of SEM (Bernstein, 2002). It produces images that help to observe topographical features, like cracks, crystal faces and surface roughness. SEM generates signals with signal processors that produce a wide variety of image types that are received from the electron interactions with the sample. A scanning electron beam is emitted from a cathode source, which strikes a sample, and are x-rays and electrons released, which then penetrate (a few micrometres) into the surface and are they scattered within a volume (interaction volume). The signals that are released from the samples during this bombardment of electrons and x-rays contain topographical and chemical information that are recorded by detectors (Nichols *et al.*, 2011).

An FEI Quanta 200 FEG SEM, equipped with an X-Max 20 EDS system (FEI, USA) was used to obtain micrographs of the various solid-state forms. In preparation, samples were adhered to a small piece of carbon tape, mounted onto a metal stub and coated with a gold-palladium film (Eiko Engineering Ion Coater IB-2, USA). According to Byrn *et al.* (1999), SEM provides details about the morphology, crystal habit and other details that are encompassed by the sub-optical to macromolecular size range.

3.4.4 Fourier-transform infrared spectroscopy

Infrared spectroscopy is a standard and useful technique for the analysis and/or characterisation of solids, requiring only a small sample amount for analysis. Fourier-transform infrared spectroscopy (FTIR) is based upon the vibrational modes (bound atoms) measurement and is the absorption range usually between 400 cm^{-1} and 4000 cm^{-1} . Since FTIR is mainly a technique for investigating molecular properties and not so much solid-state properties of an API, it can be used complementary to the characterisation of crystalline and non-crystalline forms. FTIR is extremely sensitive to the conformation of a solid and its structure and is it therefore ideal for comparing the conformations and structures of a compound in different solid-state forms (Byrn *et al.*, 1994). This method monitors the vibrational characteristics of bound atoms as changes in the position and width of infrared absorption bands are attributable to the variations in molecular conformation and hence bonding environment among the different solid-state forms.

In summary, FTIR provides a “fingerprint” of the solid-state form being investigated (Yu *et al.*, 1998), as it is sensitive to short range molecular order, when studying molecular vibrations. Therefore, when characterising a substance in its solid form, this technique is complementary to diffraction studies, which focus on long range order instead. FTIR helps with the identification of functional groups within a molecule and structural changes within a solid form.

FTIR spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer (Shimadzu, Kyoto, Japan) over a range of $400 - 4000\text{ cm}^{-1}$. Potassium bromide (KBr) (B/N: B0224007814) was used as a reference. The diffuse reflectance method was implemented, which involved the grinding of the sample with KBr and measuring its IR absorbance spectrum in a reflectance cell.

3.4.5 X-ray powder diffraction

X-ray powder diffraction (XRPD) is one of the most important techniques for the identification and characterisation of crystalline and amorphous forms (also described as the gold standard). XRPD offers knowledge about the three-dimensional structures of solids, i.e. their structural conformation, and about the hydrogen bonding and crystal packing of a crystal structure. XRPD further helps to understand the physical properties of the solid-state of an API (Bernstein, 2002; Gilmore, 2011). XRPD is useful for both qualitative (fingerprint analysis and phase identification) and quantitative phase analysis. The quantification of powdered mixtures can be determined by using diffraction data by means of the Rietveld

method. The ability of this method deals with a wide range of sample related effects through the use of the diffraction patterns as a whole (Scarlett & Madsen, 2006). Materials with unknown or partially known crystal structure can possibly, be quantitatively analysed by XRD without having to perform a crystal structure analysis beforehand. This can be done through the PONKCS method (Bergold *et al.*, 2013).

The scattering of the X-rays is as a result of their interaction with the electrons in an atom and does this interference occur between X-rays that scatter from different parts of the atom. The intensity of the scattering can be described by an atomic scattering factor (f_{atom}). This factor is a function of the number of electrons around the central nucleus. The scattering power of an individual atom decreases with an increase in the scattering angle 2θ (Gilmore, 2011). The X-ray diffraction pattern of a solid is generated when the Bragg condition is satisfied, which can be described as ($n\lambda = 2d \sin \theta$). The wavelength of the X-ray radiation = λ , and the particular spacing between individual parallel planes = d . When the angle θ between the set of planes and the incident radiation result in a constructive interference, the Bragg condition is satisfied. The XRPD pattern is expressed as the diffraction intensity as a function of 2θ . This can be considered the fingerprint of a solid. The d spacing values indicate the dimensions of the cell unit (Chapter 1), whereas the intensities stem from the contents of the cell unit and the way that the molecules and atoms are arranged therein (Bernstein, 2002).

Although amorphous forms lack the long range molecular order characteristic to crystals, they do have a degree of short range molecular order (over a few Ångstroms) (Ymén, 2011; Saunders & Gabbott, 2011). XRPD analysis hence illustrates that crystals don't have sharp peaks, nor do they generate Bragg diffraction peaks in the X-ray beam (Gilmore, 2011; Ymén, 2011). When a non-crystalline or amorphous form is examined using XRPD, they exhibit a broad "halo" effect, with no noticeable diffraction pattern (Murthy & Minor, 1990; Greco & Bogner, 2011).

The positions of the peaks on the XRPD pattern map the periodic spacing of the atoms in the solid-state (Lu & Rohani, 2009). Analysis of the XRPD pattern can help classify the sample as being either crystalline, semi-crystalline, or amorphous. X-ray powder diffraction patterns were obtained, using a PANalytical Empyrean diffractometer (PANalytical, Almelo, Netherlands). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/min (step size, 0.025°; step time, 1.0 sec).

3.4.6 Vapour sorption studies

Vapour sorption, or water sorption has become a routine check for evaluating the hygroscopicity at the onset of pharmaceutical development to help with the characterisation of solid-state materials. Typically, water vapour is used, but the use of volatile solvents, like ethanol and hexane is not uncommon (Heng & Williams, 2011; Reutzel-Edens & Newman, 2006). Hygroscopicity in pharmaceutical language is the term used to describe the moisture uptake of materials. Water vapour and the environment can have a major impact on the physical and chemical processes in a compound. Water molecules may attach to the surface of a solid, upon exposure to water vapour. This could occur through ion-dipole, or through specific hydrogen bonding interactions with functional groups on the surface of the solid, as well as through van der Waal's forces. Water interacts with solids through sorption at the surface of the solid, deliquescence, capillary condensation and incorporation into the lattice. Water sorption on the surfaces of solids can occur in the form of clusters, multi-molecular layers, monolayers, or individual molecules that will eventually result in condensed water. Various physical changes can occur during water sorption in amorphous solids. The water can dissolve in the solid, because of the disordered state of the system water acts as plasticiser) and can this significantly lower the T_g of the material. A lower T_g can result in greater molecular mobility and in the crystallisation of the amorphous material (Reutzel-Edens & Newman, 2006).

Atmospheric humidity may influence moisture content within a solid. This relationship between the equilibrium moisture content of a solid upon exposure to a controlled humidity atmosphere at constant temperature is quantifiable with a water sorption isotherm. An isotherm describes the amount (mass), or equilibrium water content of the sorbed vapour (per unit mass), plotted as a function of the concentration of the vapour that is present (percentage relative humidity) at a constant temperature. If water is used, it refers to the percentage relative humidity (% RH) of the water activity (a_w), with maximum values of 100% and 1.0, respectively. Figure 3.2 illustrates a typical water sorption isotherm of a solid. There is a reflection of sorption and desorption curves and the differences between the two curves are referred to as, hysteresis, which may be caused by the swelling of the solid, adsorbate dissolution and pore filling (Heng & Williams, 2011). Sorption-desorption isotherms are obtained gravimetrically by measuring the mass change of a sample with changes in the % RH. The different isotherm types are illustrated in Figure 3.3 (Reutzel-Edens & Newman, 2006).

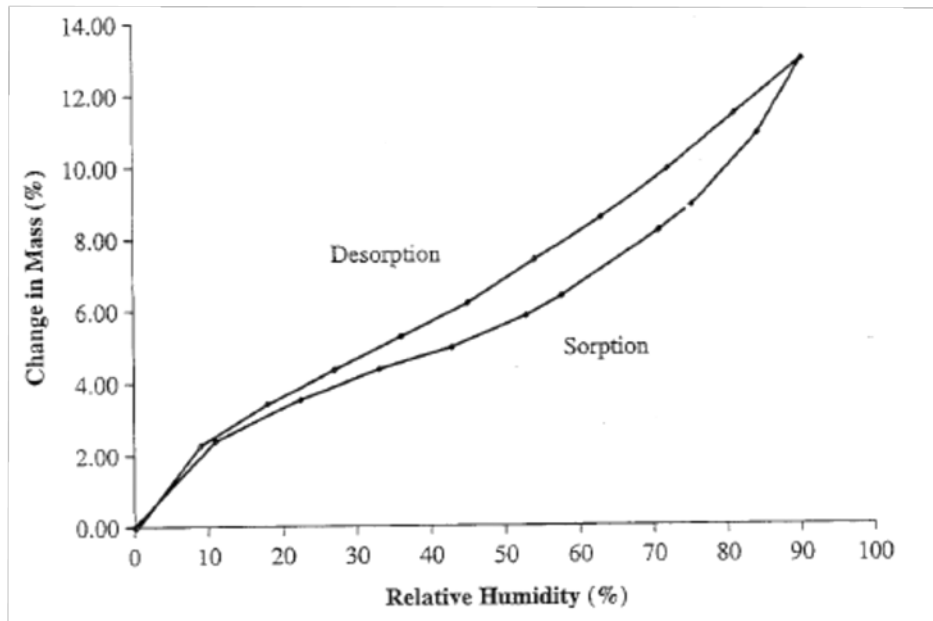


Figure 3.2: Water sorption isotherm of a typical solid (Heng & Williams, 2011).

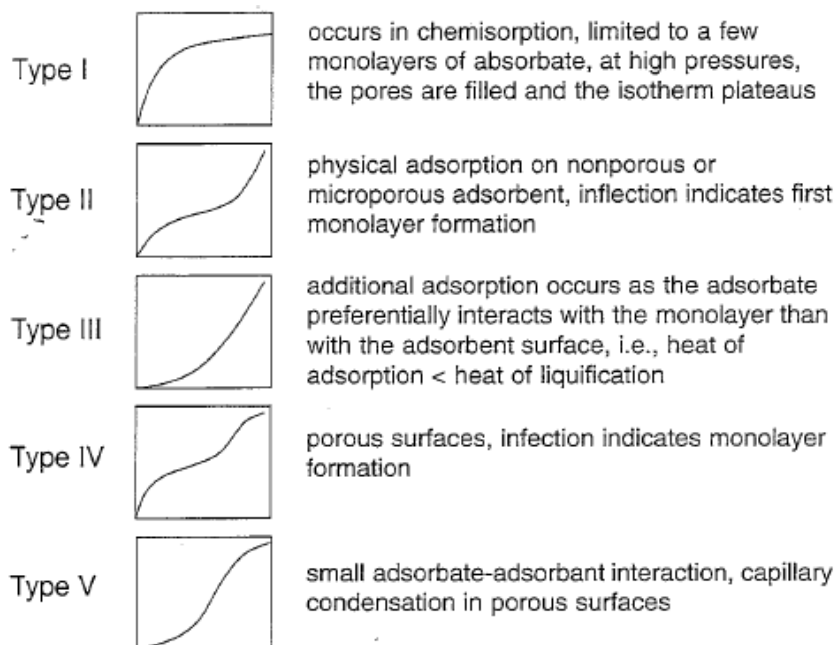


Figure 3.3: Isotherm classification types (Reutzler-Edens & Newman, 2006).

The moisture sorption analyses were performed by utilising a VTI-SA vapour sorption analyser (TA Instruments, USA). The micro-balance was calibrated prior to each vapour sorption run, by using a 100 mg standard weight. The micro-balance was set to zero, prior

to weighing the sample into a quartz sample container. The sample was carefully transferred into the sample holder and was care taken to evenly distribute the sample. The percentage relative humidity and temperature settings were programmed by using the TA Instruments Isotherm software. The initial RH range was programmed to increase from 5% to 95% RH, followed by a decrease from 95% to 5% RH. The final absorption phase was also programmed to increase from 5% to 95% RH. A drying phase of 40°C, with a weight loss criterion of not more than 0.01% in 2 minutes, was set to run prior to the percentage relative humidity increase. Care was taken that the temperature of the drying phase was not set too close to the glass transition (T_g) temperature of amorphous azithromycin. The temperature was set at a constant 25°C throughout the % RH increase. The program criteria were set to a 0.0001% weight change, or a 2 minute stability of weight gained or lost, before the program would continue to the next set parameter.

3.4.7 Heat of solution determinations (solution calorimetry)

Solution calorimetry is an effective method for determining the heat of solution under constant atmospheric pressure, of any API. The measured heat of solution of an API is actually a measurement of three steps, i.e (1) the endothermic breaking of bonds within the API and (2) within the used solvent, followed by (3) the formation of interactions between the dissolved API and the solvent (USPC, 2015a). The heats of solution were obtained by utilising a 2277 Thermal Activity Monitor (TAMIII) instrument (TA Instruments, USA), equipped with an oil bath, having a stability of $\pm 0.0001^\circ\text{C}$ over a period of 24 hours. The solution calorimeter consisted of a thin-walled 25 ml Pyrex glass reaction vessel, equipped with a thermistor for monitoring the temperature. The vessel was attached to the cylindrical calorimeter, which contained a Wheatstone bridge (resistance measuring device). The software used was the SolCal programme.

An amount of the substance was weighed into a 1 mL crushing ampoule, which was closed with a wax cover that fitted tightly into the ampoule opening. The ampoule was subsequently attached to the gold stirrer. 20 ml of ethanol was pipetted into the 25 ml Pyrex glass reaction vessel. The reaction vessel was fixed securely to the cylindrical calorimeter backbone. The apparatus was then lowered into the calorimeter. The gold stirrer was set to a stirring speed of 300 rpm. The experimental setup included two calibration runs, before the breaking of the ampoule and two thereafter, to generate a measurement baseline. The heat of solution results were calculated, using the SolCal software.

3.4.8 Solubility studies

The concept of solubility is based upon the state of equilibrium between the solute (API) and the solution. The solid-state and the free energy associated with different lattice energies, largely determine the solubility characteristics of an API (Grant & Higuchi, 1990). Excess amounts of crystalline and amorphous solid-state forms were weighed and transferred into amber test tubes, fitted with screw caps. Water was added to each of the test tubes and placed in a water bath at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$, fixed to a rotating axis (54 rpm) for a period of 24 hours. The subsequent solutions were each filtered through a $0.45\ \mu\text{m}$ polyvinylidene fluoride (PVDF) filter and diluted, after which HPLC analyses were performed on the filtrates, according to the specifications of the HPLC method (section 3.4.10).

3.4.9 Powder dissolution studies

A VanKel700 (Varian, USA) dissolution bath was used for dissolution testing. USP (2015b) apparatus 2 (paddle) was used at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$, with a rotational speed of 100 rpm. 900 ml of distilled water (dissolution medium) was added to each dissolution vessel. For solution-mediated phase transformation kinetic studies, the dissolution bath temperatures were set at 25°C , 30°C and 35°C . Approximately 600 mg of powder each was weighed into three 10 ml test tubes, to which 300 mg of glass beads ($\leq 106\ \mu\text{m}$) (Sigma-Aldrich, South Africa) each was added. 5 ml of the dissolution medium was added to each test tube. The mixtures were agitated for a period of 120 seconds, using a vortex mixer. The resultant mixtures were transferred into 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 super-saturated solution was required to observe solution-mediated transformations. After withdrawal, the samples were each filtered through a $0.45\ \mu\text{m}$ PVDF filter into an HPLC vial for analysis on HPLC.

3.4.10 High performance liquid chromatography analysis

For the high performance liquid chromatography (HPLC) analysis of azithromycin a Phenomenex[®] Luna C₁₈ ($5\ \mu\text{m}$) 150 mm x 4.6 mm reverse phase column was used as stationary phase. A mobile phase, consisting of 70 parts of 0.06 M phosphate buffer at pH 6.0 (pH adjusted with 1.0 M sodium hydroxide solution) and 30 parts of acetonitrile was used at a flow rate of 1.0 ml/min. All samples were analysed, using an isocratic elution method at

a wavelength of 210 nm. The injection volume of each sample was 15 μ l (Odendaal *et al.*, 2012).

3.5 Conclusion

This chapter described the variety of solid-state characterisation techniques and methods that were used during this study. In the next chapters the following objectives that were investigated will be discussed:

- To prepare different amorphous forms as describe in literature / patents and to fully characterise the forms to establish differences in terms of stability and other physico-chemical properties.
- To study the solution-mediated transformation of different amorphous azithromycin solid-state forms during dissolution studies.
- To compare the dissolution profiles of the different amorphous forms with that of the commercially available crystalline form.
- To at the conclusion of this study, be able to draw a comparison between the amorphous solid-state forms and the crystalline form of azithromycin regarding stability and solubility.

3.6 Reference list

- Aucamp, M., Odendaal, R., Liebenberg, W. & Hamman, J. 2015. Amorphous azithromycin with improved aqueous solubility and intestinal membrane permeability. *Drug development and industrial pharmacy*, 41:1100-1108.
- Bergold, S.T., Goetz-Neunhoeffler, F. & Neubauer, J. 2013. Quantitative analysis of C–S–H in hydrating alite pastes by in-situ XRD. *Cement and concrete research*, 53:119–126.
- Bernstein, J. 2002. Polymorphism in molecular crystals. Clarendon, Oxford: Oxford University press.
- Byrn, S.R., Pfeiffer, R.R., Stephenson, G., Grant, D.J.W. & Gleason, W.B. 1994. Solid-state pharmaceutical chemistry. *Chemistry of materials*, 6:1148-1158.
- Byrn, S.R., Pfeiffer, R.R. & Stowell, J.G. 1999. Solid-state chemistry of drugs. 2nd ed. West Lafayette, Indiana: SSCI Inc.
- Craig, D.Q.M. 2006. Characterization of polymorphic systems using thermal analysis. (In Hilfiker, R. ed. Polymorphism in the pharmaceutical industry. Weinheim, Germany: Wiley-VCH. p. 43-79).
- Gilmore, C.J. 2011. X-ray diffraction. (In Storey, R.A. & Ymén, I. ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 35-70).
- Graeser, K.A., Strachan, C.J., Patterson, J.E., Gordon, K.C. & Rades, T. 2008. Physicochemical properties and stability of two differently prepared amorphous forms of simvastatin. *Crystal growth and design*, 8:128-135.
- Grant, D.J.W. & Higuchi, T. 1990. Solubility behaviour of organic compounds. New York: Wiley.
- Greco, K. & Bogner, R. 2011. Solution-mediated phase transformation of haloperidol mesylate in the presence of sodium lauryl sulfate. *Journal of the American Association of Pharmaceutical Scientists, PharmSciTech*, 12:909-916.
- Heng, J.Y.Y. & Williams, D.R. 2011. Vapour sorption and surface analysis. (In Storey, R.A. & Ymén, I. ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 245-285).
- Jasanada, B., Santos, M., Isidro, L.G. & Felix, F.M. 2001. Preparation of non-crystalline and crystalline dihydrate forms of azithromycin. (Patent: EPO 1 103558 A2).
- Li, Z.J. & Trask, A.V. 2005. Crystal forms of azithromycin. (Patent: US 6977243B2).

- Lu, J. & Rohani, A. 2009. Polymorphism and the crystallization of active pharmaceutical ingredients (APIs). *Current medicinal chemistry*, 16:884-905.
- Milne, M., Liebenberg, W. & Aucamp, M.E. 2016. Different amorphous solid-state forms of roxithromycin: a thermodynamic and morphological study. *International journal of pharmaceutics*, 498:304-315.
- Murthy, N.S. & Minor, H. 1990. General procedure for evaluating amorphous scattering and crystallinity from X-ray diffraction scans of semicrystalline polymers. *Polymer: The international journal for the science and technology of polymers*, 31:996-1002.
- Nichols, G., Luk, S. & Roberts, C. 2011. Microscopy. (In Storey, R.A. & Ymén, I. ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 287-355).
- Odendaal, R.W., Liebenberg, W. & Aucamp, M.E. 2012. A novel reversed-phase LC method for quantitative detection of azithromycin in bulk drug and tablet formulations in various aqueous media. *Pharmazie*, 67:984-986.
- Reutzel-Edens, S.M. & Newman, A.W. 2006. Physical characterization of hygroscopicity in pharmaceutical solids. (In Hilfiker, R. ed. Polymorphism in the pharmaceutical industry. Weinheim, Germany: Wiley-VCH. p. 235-258).
- Saunders, M. & Gabbott, P. 2011. Thermal analysis: conventional techniques. (In Storey, R.A. & Ymén, I. ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 135-186).
- Scarlett, N.V.Y. & Madsen, I.C. 2006. Quantification of phases with partial or no known crystal structures. *Powder diffraction*, 21:278-284.
- United States Pharmacopeial Convention. 2015a. General chapters: <696> Characterization of crystalline solids by microcalorimetry and solution calorimetry. <http://www.uspnf.com/uspnf/pub/index?usp=38&nf=33&s=2&officialOn=December 1, 2015> Date of access: 08 Apr. 2016.
- United States Pharmacopeial Convention. 2015b. General chapters: <1092> The dissolution procedure: development and validation. <http://www.uspnf.com/uspnf/pub/index?usp=38&nf=33&s=2&officialOn=December 1, 2015> Date of access: 08 Apr. 2016.
- Ymén, I. 2011. Introduction to the solid state: physical properties and processes. (In Storey, R.A. & Ymén, I. ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 1-34).

Yu, L., Reutzel, S.M. & Stephenson, G.A. 1998. Physical characterization of polymorphic drugs: an integrated characterization strategy. *Pharmaceutical science & technology today*, 1:118-127.

Chapter 4

Different amorphous solid-state forms of azithromycin

4.1 Introduction

The aspect of being able to prepare more than one amorphous form of any given API remains a highly debatable topic. Currently, a relative small number of literature and research reports can be found on this topic. However, it is clear and makes sense that different preparative techniques can possibly result in physical, chemical and/or thermodynamic differences and thereby different amorphous forms of an API. Therefore, during the first part of this study a series of preparation methods for the manufacturing of several azithromycin amorphous forms have been investigated. The primary goal of this section of the study was to investigate the impact and to illustrate the effect of the different preparation methods on the physico-chemical characteristics of each amorphous solid-state form. Preparation methods such as quench cooling of the melt, slow cooling of the melt, hot-air melting, ambient solvent evaporation, rapid solvent evaporation and spray-drying were used. See Chapter 3 for a more in depth description of the preparation methods.

4.2 Preparation methods for different azithromycin amorphous forms

It is a well-known fact that a variety of preparation methods can be used to obtain an amorphous form of an API. In some cases only a single, specific method will be suitable to prepare an amorphous solid-state form, while in other instances several methods will render a crystalline API into an amorphous state or different amorphous forms. The question remains whether these different preparation methods will lead to the same amorphous form with essentially the same physico-chemical properties or will different amorphous forms be the result.

Several amorphous preparation methods have been reported in the literature. When one look into the different preparative techniques, it is clear that these methods are based on different intermediary states. The intermediary states include: melt, solution or solid. With fast or slow cooling of a molten API the intermediary state is a molten state. If the cooling of molten API allows that the molecules cannot rearrange themselves into an ordered crystalline lattice, the molecules therefore become “frozen” in a disorganised state, thereby resulting in amorphous forms. The quench cooling of the melt is the most commonly or

frequently used method for an amorphous preparation. Liquid nitrogen can also be used to quench cool a melted solid. In the case of a solution state being the intermediary state, either fast or slow precipitation of the API can prevent the formation of crystalline solid-state forms. The solid intermediary state includes mechanical disruption of the crystalline structure through grinding, milling (Patterson *et al.*, 2005; Savolainen *et al.*, 2009), crushing and/or compression of the crystalline API. However, these amorphous forms may revert back to the crystalline form more easily than those amorphous forms obtained by melt or solution-based intermediary stages (Graeser *et al.*, 2008). There can also be combinations of the intermediary stages (solution and mechanical) where for instance, the product from an evaporative step is grinded or milled (Graeser *et al.*, 2008).

In this study a set of different preparation techniques that have different intermediary states were identified and selected in an endeavour to prepare different amorphous solid-state forms of azithromycin. This will shed some light on the effect of different preparation methods / intermediary steps on the obtained physical, chemical and stability properties of amorphous azithromycin. It should however be mentioned that it was not possible to investigate the solid intermediary state due to the fact that grinding or milling did not render crystalline azithromycin into an amorphous state.

Table 4.1: A summary of all the preparative techniques and the corresponding codes used for the prepared amorphous forms

Method	Preparative technique	Intermediary state	Code
1	Quench cooling of the melt	Melt	A-QC
2	Slow cooling of the melt	Melt	A-SC
3	Hot-air melting	Melt	A-HA
4	Rapid solvent evaporation	Solution	A-RV
5	Spray drying	Solution	A-SD
6*	Ambient solvent evaporation	Solution	A-TB

The abbreviations A-QC, A-SC, A-HA, A-RV and A-SD for each azithromycin amorphous form will be used hereafter. Azithromycin dihydrate will be known as A-DH.

*It should be mentioned that although Jasanada *et al.* (2001) indicated that A-TB would also render crystalline azithromycin into an amorphous form, it was not possible to prepare an amorphous solid-state form through the proposed method. Several attempts were made to prepare an amorphous form through slow evaporation of tert-butyl alcohol, however each attempt resulted in a crystalline form similar to A-DH, as showed in Figure 4.1 (from XRPD analyses). The XRPD pattern for A-TB (Figure 4.1) exhibit sharp characteristic peaks which show that the azithromycin amorphous preparation method (A-TB) rather yields the thermodynamically more stable A-DH. Thereby, indicating that the method taken from the work of Jasanada *et al.* (2001) was not transferable or repeatable. It might also be a possibility that the preparation method of A-TB might have produced an amorphous form, but that this amorphous form was so unstable that crystallisation back to the more thermodynamic stable A-DH occurred rapidly. Therefore, for the purpose of this study A-TB was not included into any further investigations.

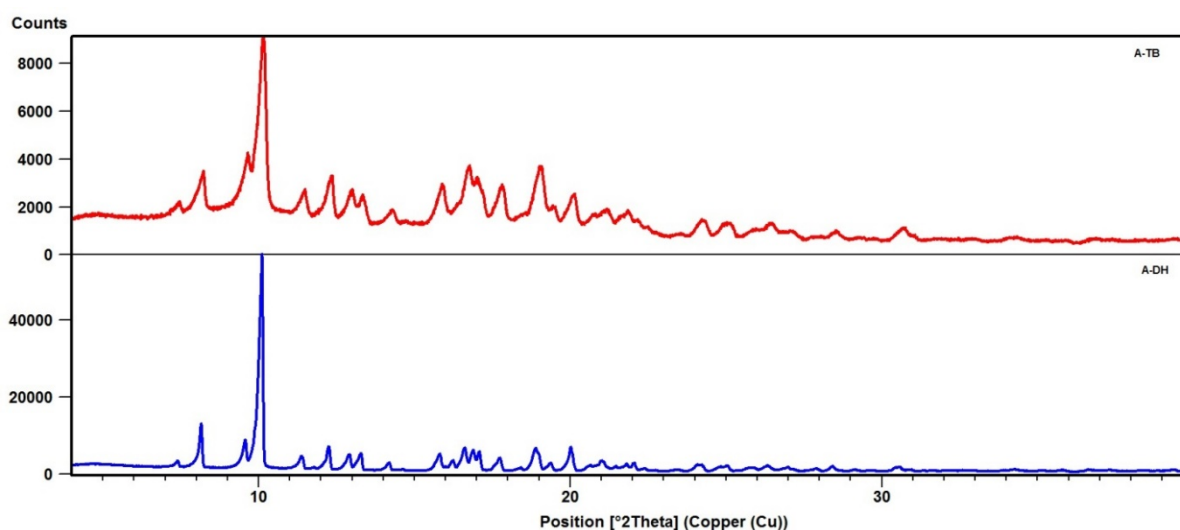


Figure 4.1: Overlay of the XRPD diffractograms obtained for A-TB (slow evaporation from a solution of azithromycin in tert-butyl alcohol) and A-DH (commercially obtained azithromycin dihydrate).

4.3 Physico-chemical characterisation of the obtained azithromycin amorphous solid-state forms

There exist a multitude of techniques that can be applied for the screening, identification and complete characterisation of crystalline and amorphous forms. Techniques like differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), Fourier-transform infrared spectrophotometry (FTIR), X-ray powder diffraction (XRPD), solubility studies, powder dissolution studies, scanning electron microscopy (SEM) as well as vapour sorption experiments were used in this study (BP, 2015; Ymén, 2011).

4.3.1 Determination of the thermodynamics of the azithromycin amorphous forms

All the prepared amorphous solid-state forms were thermally investigated through DSC and TGA analyses. The results obtained for crystalline azithromycin dihydrate and the amorphous forms were compared with each other. The corresponding glass transition (T_g) and melting temperatures are outlined in Table 4.2.

Table 4.2: Thermodynamic properties calculated for crystalline azithromycin dihydrate and five differently prepared amorphous forms of azithromycin.

Solid-state form	T_m (°C)	ΔH_m (kJ/mol)	ΔS_m (J/mol/K)	T_g (°C)	Percentage moisture loss (%)
A-DH	110.6	54.1	141.0	-	7.2
A-QC	-	-	-	103.6	0.5
A-SC	-	-	-	106.5	0.1
A-HA	-	-	-	106.0	1.0
A-RV	-	-	-	103.0	2.5
A-SD	-	-	-	103.1	0.8

The thermogram obtained for A-DH compares well with that reported in literature. According to Jasanada *et al.* (2001) A-DH should show a single endotherm that may vary from 115 - 135°C. Figure 4.2 illustrates an endotherm with an onset temperature of about 111°C for A-DH, this is considered to be in correlation with that reported by Jasanada *et al.* (2001) and Aucamp *et al.* (2015), due to the fact that the heating rate will affect the onset temperature of the thermal event in question. During heating of all the amorphous forms, no thermally induced recrystallisation was observed. This was considered to be advantageous since it implies that all five amorphous forms will remain stable during further processing techniques which would require the addition of heat, i.e. drying or even compression which could generate significantly high temperatures.

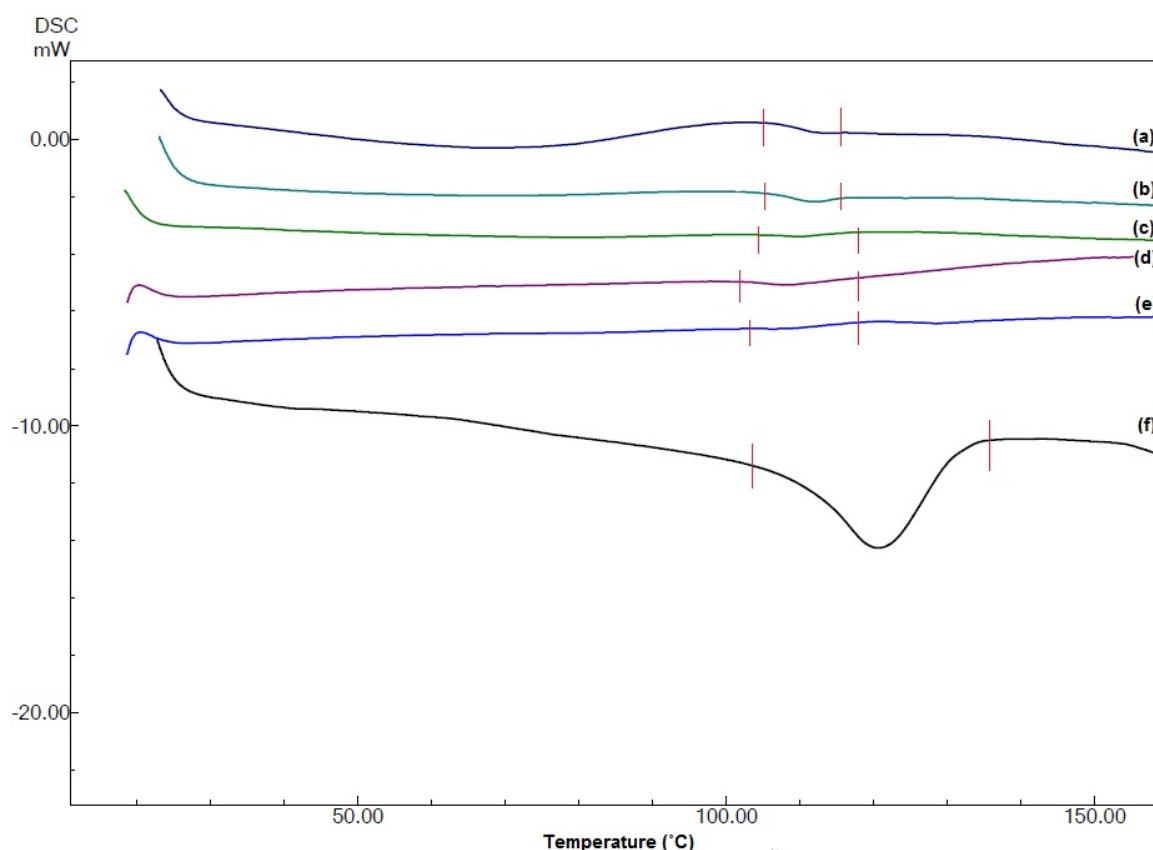


Figure 4.2: An overlay of DSC thermograms obtained for (a) A-QC, (b) A-SC, (c) A-HA, (d) A-RV, (e) A-SD and (f) A-DH. With the red marks indicating the onset and end temperatures of either the glass transition temperatures (T_g) or the melting of A-DH.

The activation energy (ΔH^\ddagger) of the structural relaxation of all the prepared azithromycin amorphous forms were determined from the glass transition temperature (T_g) at different

heating rates (q). The location (in the sense of temperature) of the glass transition region depends on the cooling or heating rate (q) as this sets the time scale (Δt) for the relaxation process at each temperature. An increase in (q) shifts the transition region upwards in temperature (Moynihan, 1993). 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 (q) used during thermal analyses (Yu, 2001; Ramos *et al.*, 2003; Aucamp *et al.*, 2012). For this equation 1 applies:

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

If the location of the glass transition region is denoted by a glass transition temperature (T_g) it may be shown that the dependence of (T_g) on cooling rate (q) is given by equation 1. Whereas (ΔH) is the activation enthalpy for the structural relaxation time for small departures from equilibrium and R is the ideal gas constant (Moynihan, 1993). Figures 4.3 and 4.4 depicts the plots of $\ln(q)$ versus $1000/T_g$. Using these plots it was possible to calculate the activation energy (E_a) for the structural relaxation of each amorphous form. These results are presented in Table 4.3. The aspect of fragility is also considered to be a valuable parameter to investigate in terms of amorphous solid-state forms of APIs. The fragility index is indicative of the rate increase of the structural relaxation of an amorphous form as it approaches and passes through the glass transition temperature 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 (Yu, 2001; Angell, 1997; Lu & Zografis, 1997). From this, equation 2 defines the fragility index (m) as follow:

$$m = \left[\frac{d \log_{10} \tau(T)}{d \left(\frac{T_g}{T} \right)} \right] \quad (2)$$

The fragility of an amorphous system is a dimensionless parameter and actually describes the molecular kinetics which is temperature dependent (Lu & Zografis, 1997). Therefore, equation 2 can also be expressed in terms of temperature dependence (equation 3), where the calculated apparent activation energy (E_a) was incorporated into equation 2:

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

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:

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

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 . This temperature can be described as the temperature of zero mobility and is important in pharmaceutical amorphous solid-state forms due to the fact that it can act as a guide of a suitable storage temperature which would ensure maximum stability of the amorphous form of an API. The correlation between D and T_0 can be expressed in the approximate form of equation 5 (Yu, 2001):

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

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 $m > 75$ (Yu, 2001). 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 (Lu & Zografi, 1997). The glass forming ability of azithromycin 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):

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

The closer the value of T_{rg} is to 1 the higher is the glass forming ability of the API. All calculated T_{rg} -values was found to be very close to 1, thereby indicating that for each method used the glass forming ability was significantly high (Table 4.3).

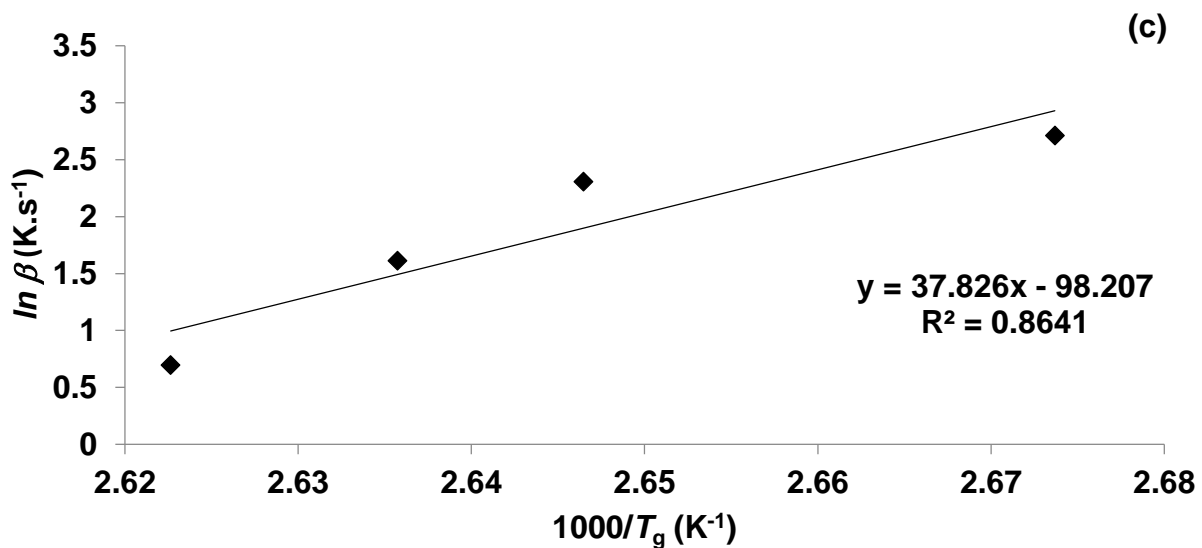
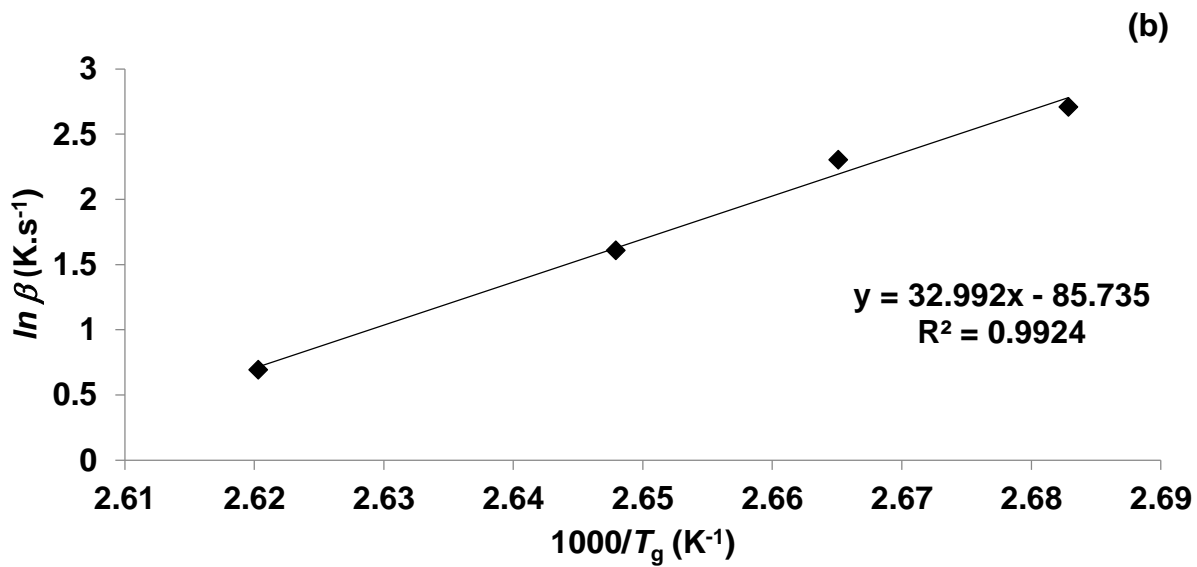
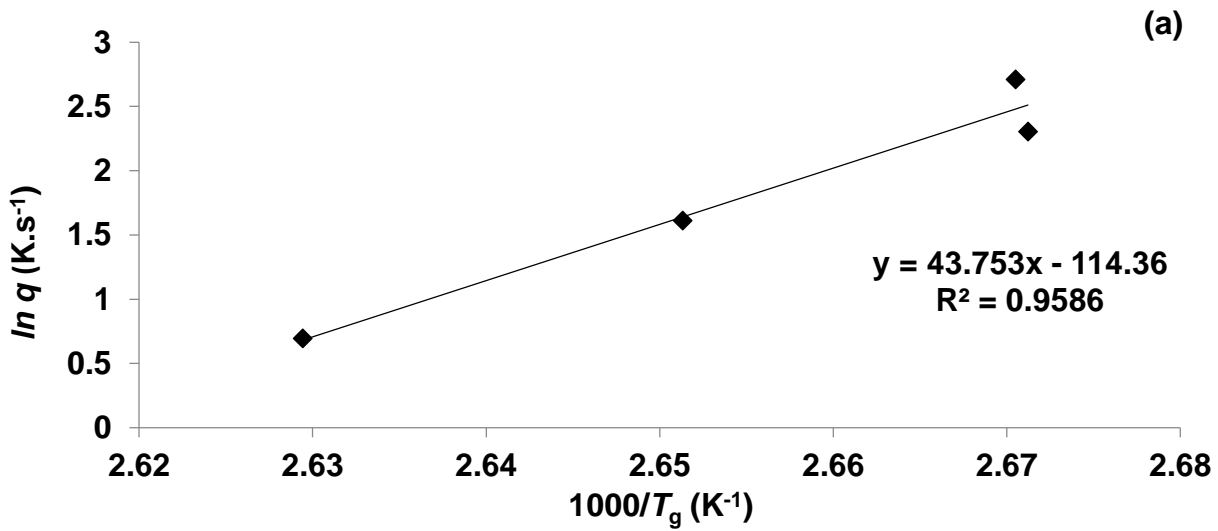


Figure 4.3: Arrhenius plots for the glass transition temperature (T_g) affected by varying heating rates (q). Intermediary state: melt with (a) A-QC, (b) A-SC and (c) A-HA.

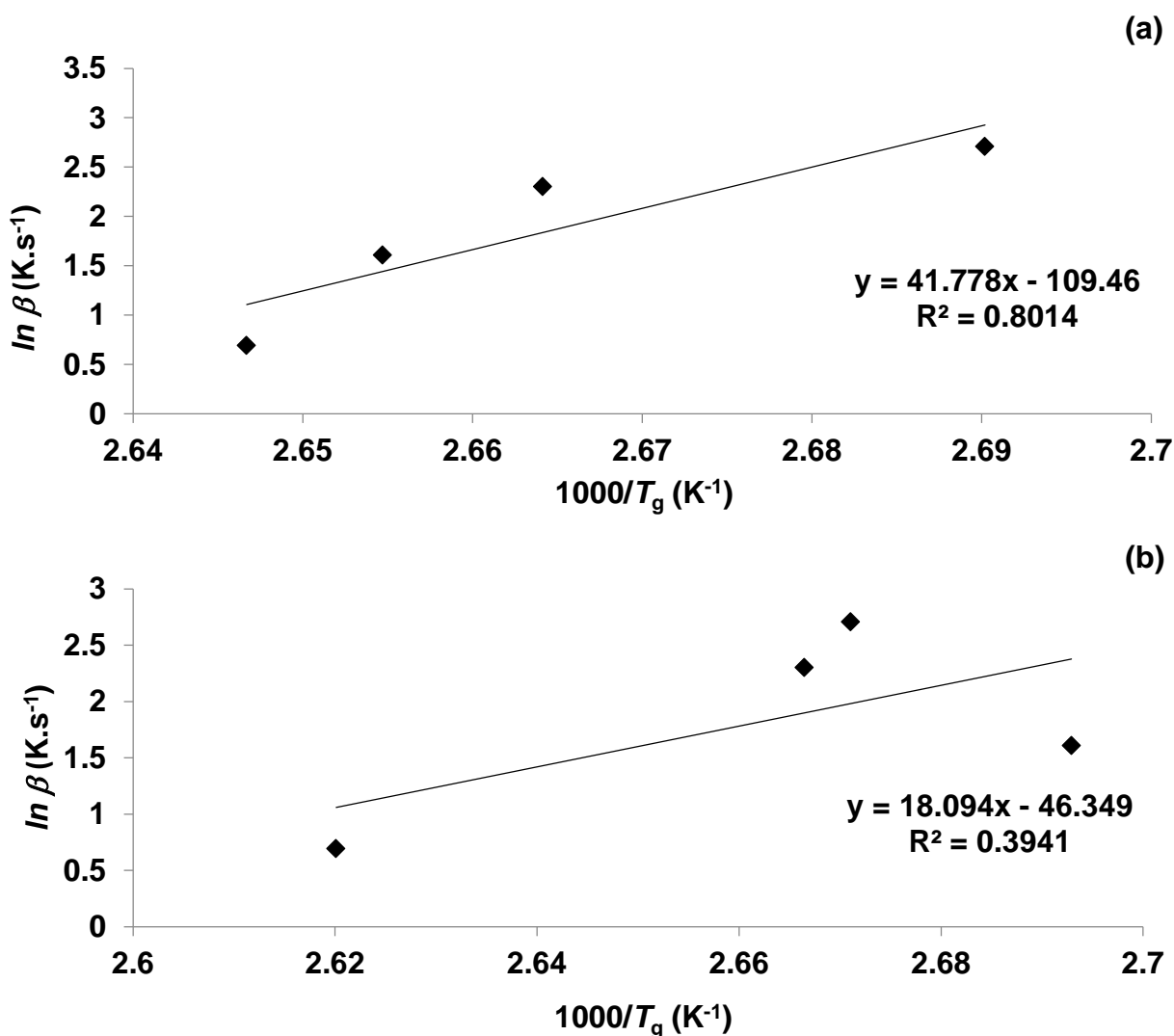


Figure 4.4: Arrhenius plots for the glass transition temperature (T_g) affected by varying heating rates (q). Intermediary state: solution with (a) A-RV and (b) A-SD.

The calculated thermodynamic data for all five amorphous forms of azithromycin is presented in Table 4.3. None of the amorphous forms of azithromycin showed fragility indices (m) higher than 75, therefore these amorphous forms can all be characterised as non-fragile systems. All calculated strength parameters (D) also showed the amorphous forms to be strong amorphous systems. A low fragility index and high strength parameter is considered to be indicative of lower free energy, therefore higher physical stability is a probability for amorphous forms characterised as exhibiting 'strong' thermodynamic behaviour.

From a thermodynamic perspective A-SD showed to be the amorphous form with the least physical stability, due to significantly lower activation energy for structural relaxation, a lower fragility index and strength parameter. On the other hand both A-RV and A-SD exhibit significantly higher temperatures of zero mobility, which is an advantage in terms of stability of these amorphous forms during ambient storage. All the amorphous forms showed reduced glass transition ratios (T_{rg}) very close to 1.0, thereby indicating that the glass formation ability were very good with regards to each preparation method applied.

Considering the presented data in Table 4.3 it is clear that from a thermodynamic perspective the amorphous forms do differ from one another. An interesting observation is that both amorphous forms prepared from a solution intermediate state showed the highest T_0 temperatures. However further investigation into the physico-chemical characteristics of these forms will probably provide even more evidence of differences between the five amorphous forms. It would be worthwhile to determine whether the intermediary states used to prepare these amorphous forms, plays a determinant role in the physico-chemical differences of the various amorphous forms.

Table 4.3: The calculated fragility index (m), strength parameter (D), zero mobility temperature (T_0) and reduced glass transition ratio (T_{rg})

Amorphous form	Activation energy for structural relaxation (E_a) (kJ.mol⁻¹)	Fragility index (m)	Strength parameter (D)	Temperature of zero mobility (T_0) (°C)	Reduced glass transition ratio (T_{rg})
A-QC	363.76	15.51	447.10	9.04	0.94
A-SC	264.47	13.81	208.94	19.47	0.96
A-HA	314.52	16.43	1161.23	3.52	0.96
A-RV	347.36	18.14	583.39	56.04	0.93
A-SD	150.40	7.85	72.83	53.96	0.93

4.3.2 The amorphous habit of the differently prepared azithromycin amorphous forms

The amorphous habit of all the prepared amorphous forms of azithromycin was confirmed through XRPD analyses. Figures 4.5 and 4.6 depict all XRPD diffractograms obtained during this study. The XRPD pattern obtained for crystalline A-DH (Figure 4.5) correlated well with that reported in current literature (Aucamp *et al.*, 2015), with characteristic Bragg peaks detected at 8.17, 9.59, 9.87 and 10.13 °2 θ . From the overlay of the XRPD diffractograms obtained with all five amorphous solid-state forms, it is clear and evident that there is no indication of sharp or characteristic peaks in all the diffractograms (Gilmore, 2011, Blanco *et al.*, 2004 and Li & Trask, 2005). The diffractograms only show a broad or halo pattern with no diffraction. By looking at Figure 4.6 it shows that the different azithromycin amorphous preparation methods (A-QC, A-SC, A-HA, A-RV and A-SD) used in this study can indeed create an amorphous form of A-DH and all forms can be considered amorphous.

Another technique which is very often used to identify and characterise the amorphous nature of a compound is that of FTIR. The absorbance peaks detected through FTIR analyses will show characteristic broadening. This can be ascribed to the randomly orientated molecules and lack of a crystalline lattice. Jasanada *et al.* (2001) identified the peak listings or distinctive markers for the characterisation of A-DH. The characteristic peaks were detected at wavenumbers (cm⁻¹) 3560, 3496, 1740, 1470, 1380, 1344, 1282, 1268, 1251 and 1093. This can also be seen in Table 4.4 and Figure 4.7(a) and Figure 4.8. Table 4.4 is a comparison of A-DH between the different literature findings. The 2 sharp peaks at about 3560 and 3496 cm⁻¹ indicates the free hydrogen bonded hydroxyl, hydroxyl and intramolecular hydrogen bonded hydroxyl functional groups (Aucamp *et al.*, 2015). The peaks of interest between 3000 to 3600 cm⁻¹ represents the water molecules present within the molecular structure of azithromycin dihydrate (Odendaal *et al.*, 2013).

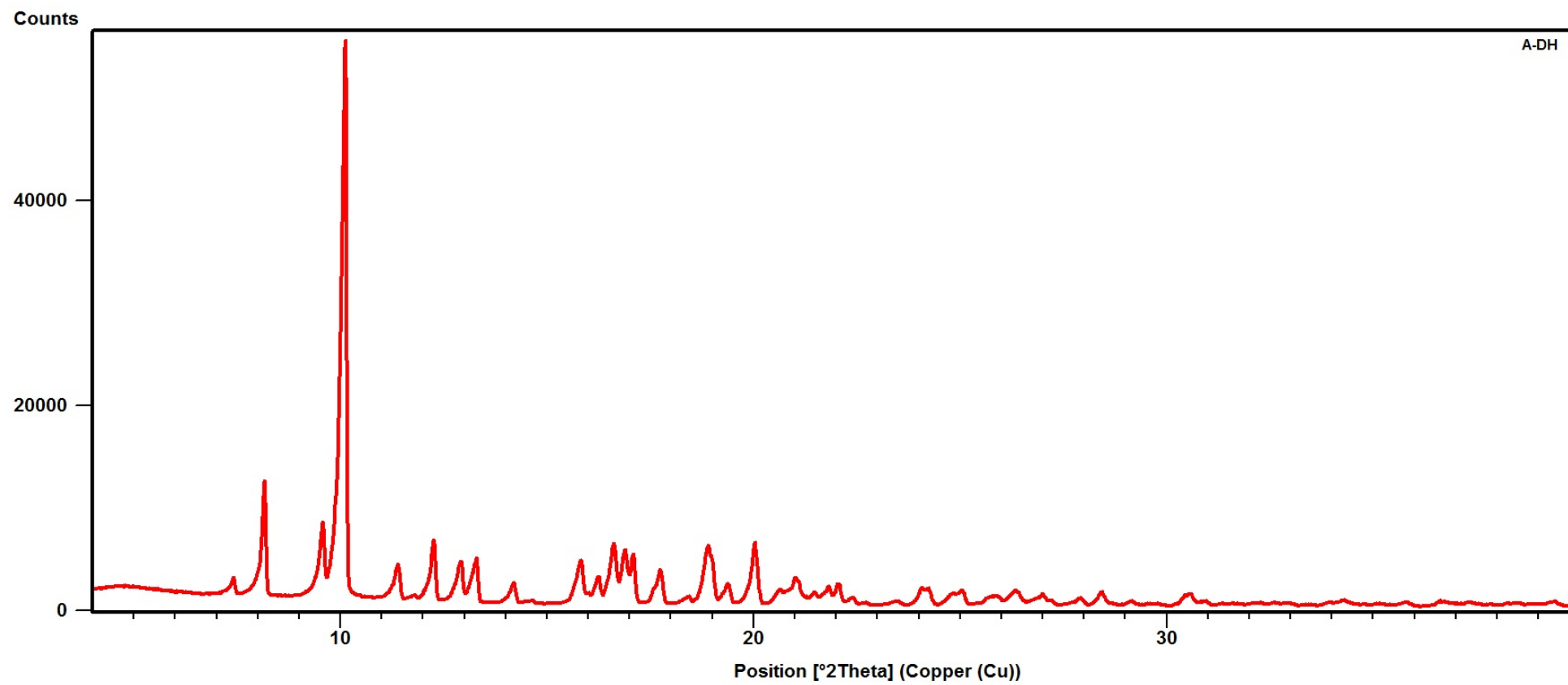


Figure 4.5: XRPD diffractogram of crystalline A-DH.

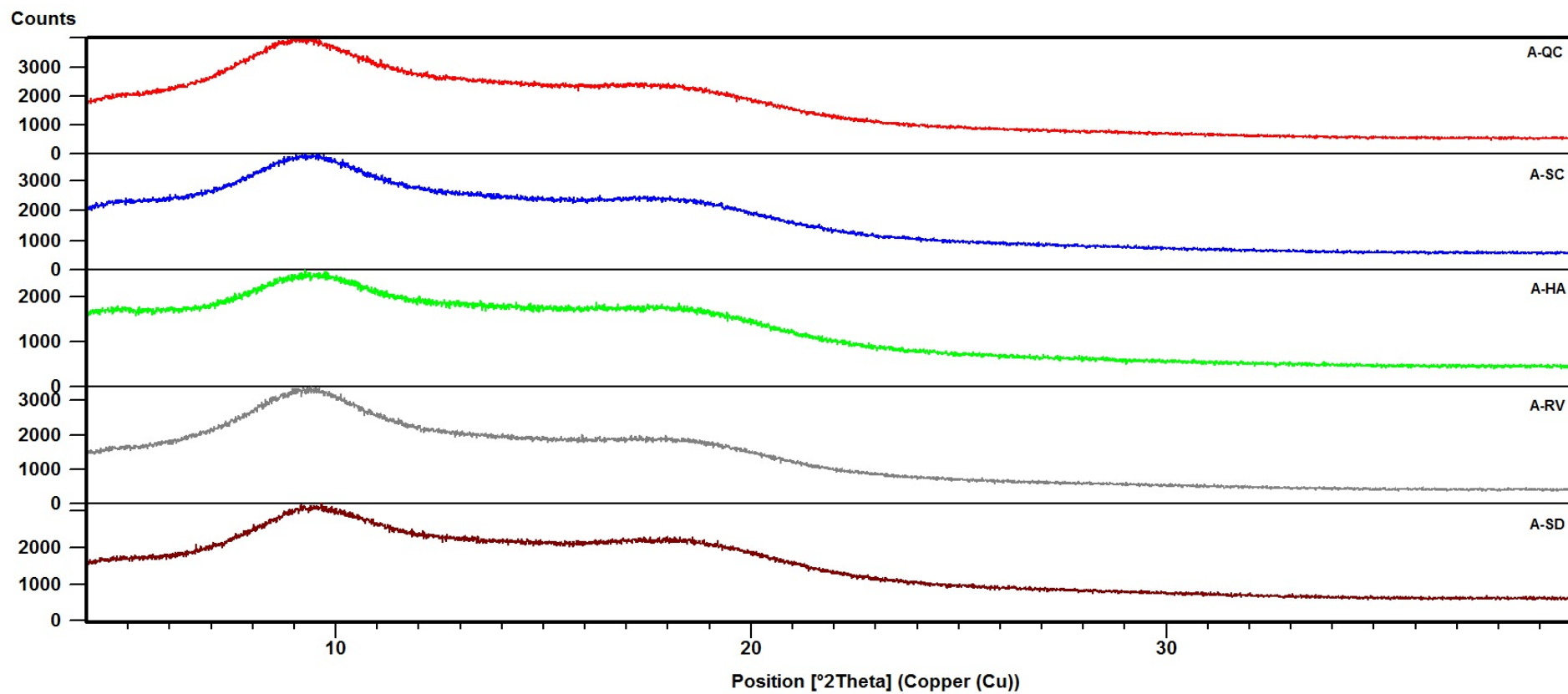


Figure 4.6: An overlay of XRPD diffractograms obtained for A-QC, A-SC, A-HA, A-RV and A-SD immediately after preparation of the amorphous forms.

Table 4.4: FTIR peak listings reported for azithromycin dihydrate.

Number	Wavenumber (cm ⁻¹)	
	Jasanada <i>et al.</i> , 2001, Odendaal <i>et al.</i> , 2013, Aucamp, 2015	A-DH
1	3560	3567
2	3496	3496
3	1740	1720
4	1470	1469
5	1380	1379
6	1344	1343
7	1282	1282
8	1268	1269
9	1251	1250
10	1093	1095

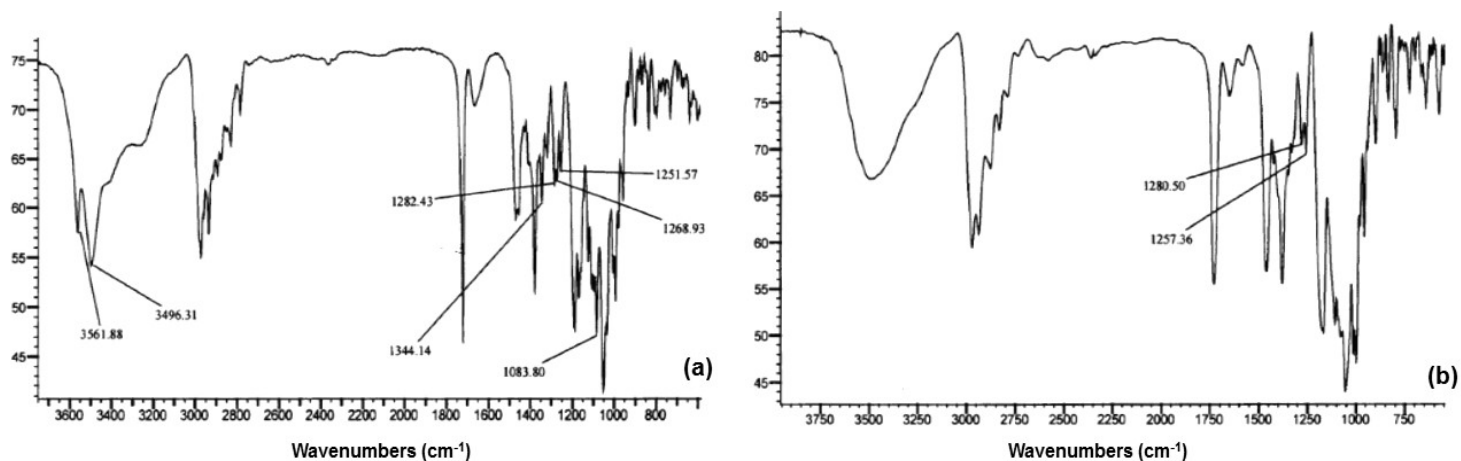


Figure 4.7: FTIR spectra for (a) azithromycin dihydrate and (b) amorphous azithromycin as reported by Jasanada *et al.* (2001). Spectra adopted from Jasanada *et al.* (2001).

The prepared amorphous forms of azithromycin were also analysed by means of FTIR and the distinctive markers or peaks were slightly different than that of A-DH. The obtained results are tabulated in Table 4.5 and depicted in Figure 4.9. The amorphous forms showed broad peaks at 3500 cm^{-1} (hydroxyl group stretch) (Aucamp *et al.*, 2015) and no peak at 3251 cm^{-1} which means that there are no hydrogen bonded hydroxyl and intramolecular hydrogen bonded hydroxyl functional groups (Odendaal *et al.*, 2013). As stated previously, the broadening of the peaks can be attributed to the random arrangement of the azithromycin molecules as it exists in the amorphous form (Aucamp *et al.*, 2015).

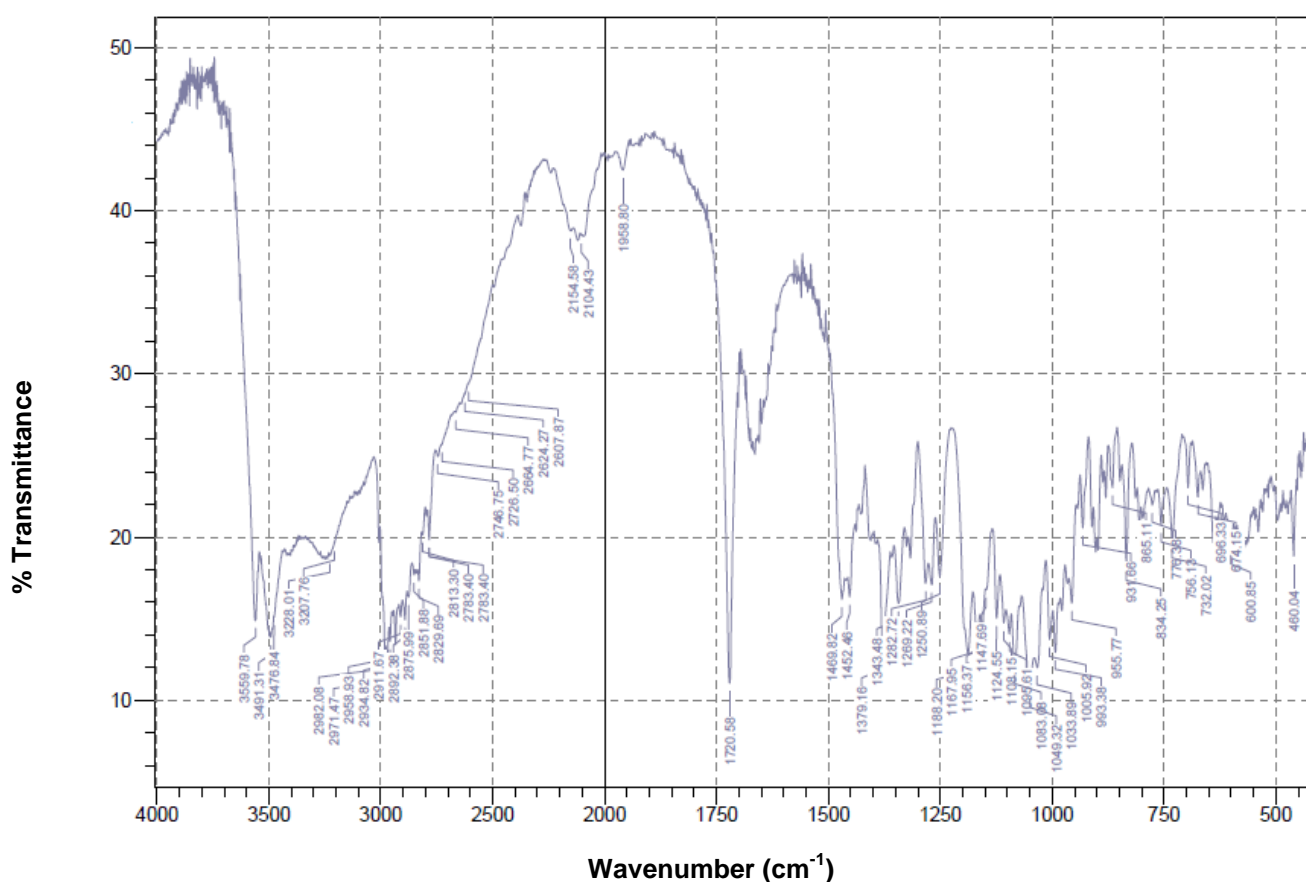


Figure 4.8: FTIR spectrum obtained for crystalline A-DH.

Jasanada *et al.* (2001) also reported that the amorphous form prepared by their research group does not present peaks at 1344 and 1083 cm^{-1} . As seen from Figure 4.7(a) and Figure 4.8, A-DH presented 3 sharp peaks at 1282 , 1269 and 1251 cm^{-1} whereas the amorphous forms only showed 2 sharp peaks at 1280 and 1257 cm^{-1} (Jasanda *et al.*, 2001; Figure 4.7(b)).

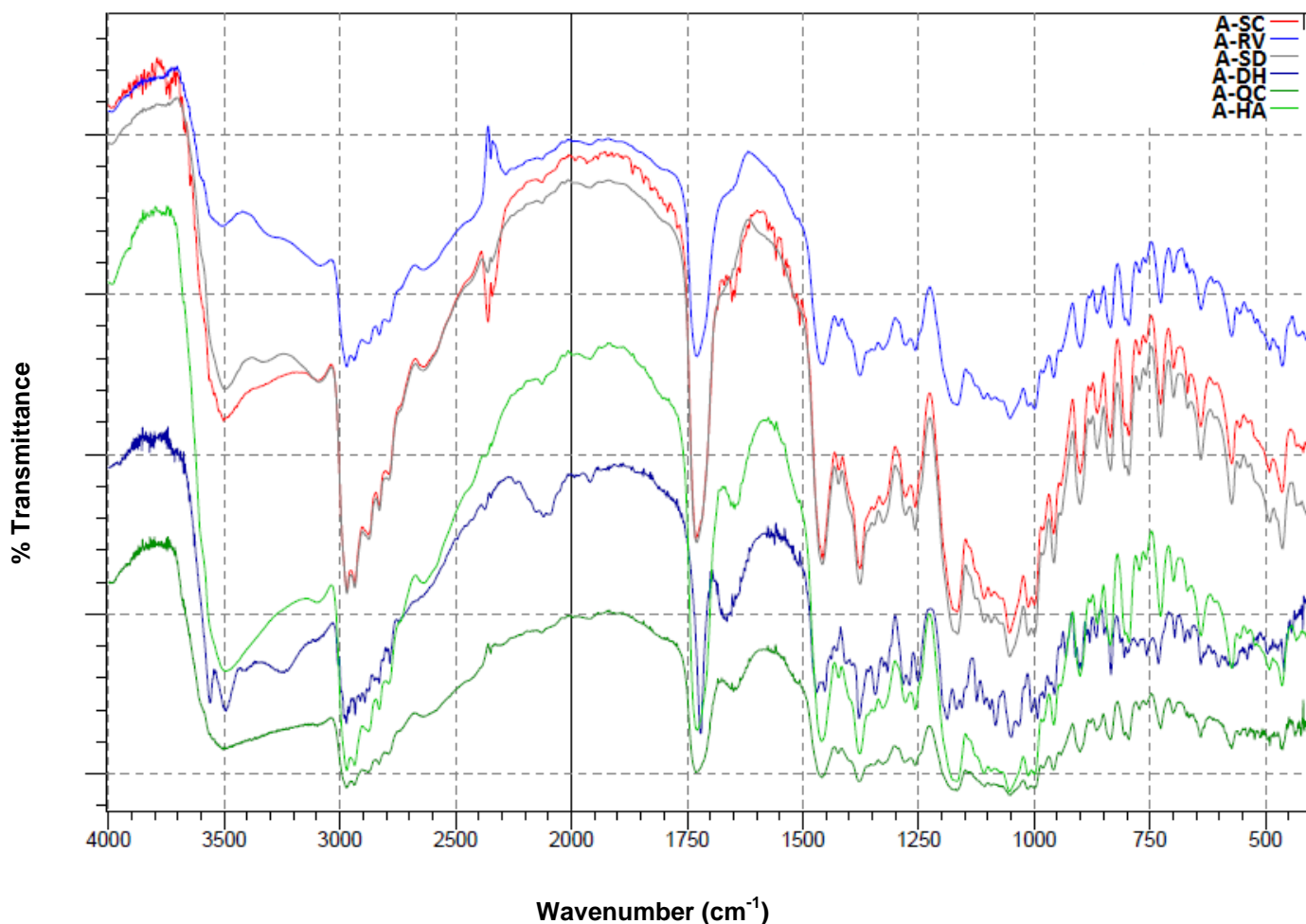


Figure 4.9: An overlay of the FTIR spectra obtained for A-QC, A-SC, A-HA, A-RV, A-SD and A-DH.

Table 4.5: FTIR peak listings for the different amorphous azithromycin preparations (A-QC, A-SC, A-HA, A-RV and A-SD).

	Wavenumber (cm ⁻¹)				
Number	A-QC	A-SC	A-HA	A-RV	A-SD
1	3500	3503	3495	3501	3497
2	3074	3096	3092	3084	3092
3	1730	1730	1728	1728	1728
4	1280	1280	1279	1279	1279
5	1257	1257	1258	1258	1258

4.3.3 The stability of the five azithromycin amorphous forms

(a) Solid-solid phase transformation

From the thermodynamic data presented in section 4.3.1 (Table 4.3) it became apparent that all five amorphous forms possess good physical stability. However, it is always best practice to investigate the stability of amorphous forms in-depth, rather than to just consider the amorphous forms to be stable based on calculated thermodynamic properties. The transformation of an amorphous state to a more thermodynamically stable form can occur due to several triggers or conditions to which the amorphous forms are being exposed. One such trigger is that of physical agitation. It might be the case that mere scraping, milling or grinding could lead to the transformation of the amorphous form to a more stable crystalline form. During this study it was found that physical agitation did not affect the amorphous habit of any of the five amorphous forms. The samples were scraped from surfaces, powdered and milled without recrystallisation occurring. This can be clearly seen from Figure 4.6 since all the amorphous forms were physically handled, scraped from preparation surfaces and milled prior to analyses. However, the possible formation of microcrystals or seeds which could be undetectable by XRPD cannot be excluded.

(b) Solvent-mediated phase transformation

Another typical method that could trigger the crystallisation of an amorphous form to a crystalline form is that of solvent-mediated phase transformation. The possibility of this transformation was investigated by means of vapour sorption experiments. A significant difference between a crystalline and amorphous form of the same API is that the amorphous form possess the ability to absorb a significant large quantity of moisture due to greater void spaces. Due to the plasticising effect of sorbed water, the T_g can be lowered quite significantly, which in turn could lead to crystallisation of the amorphous form. If crystallisation of the amorphous form occurs by means of exposure to water vapour, the water sorption capacity of the API will decrease significantly, due to the re-ordering of the API molecules into a well-organised crystal lattice. This process will result in an overall weight loss during vapour sorption experiments due to the fact that water molecules become expelled from the newly ordered crystalline lattice (Burnett, 2004).

Figures 4.10 – 4.14 show the vapour sorption isotherms obtained with each amorphous form. None of the vapour sorption isotherms showed any rapid and/or distinct weight loss, which is an indication that no recrystallisation of the solid forms occurred.

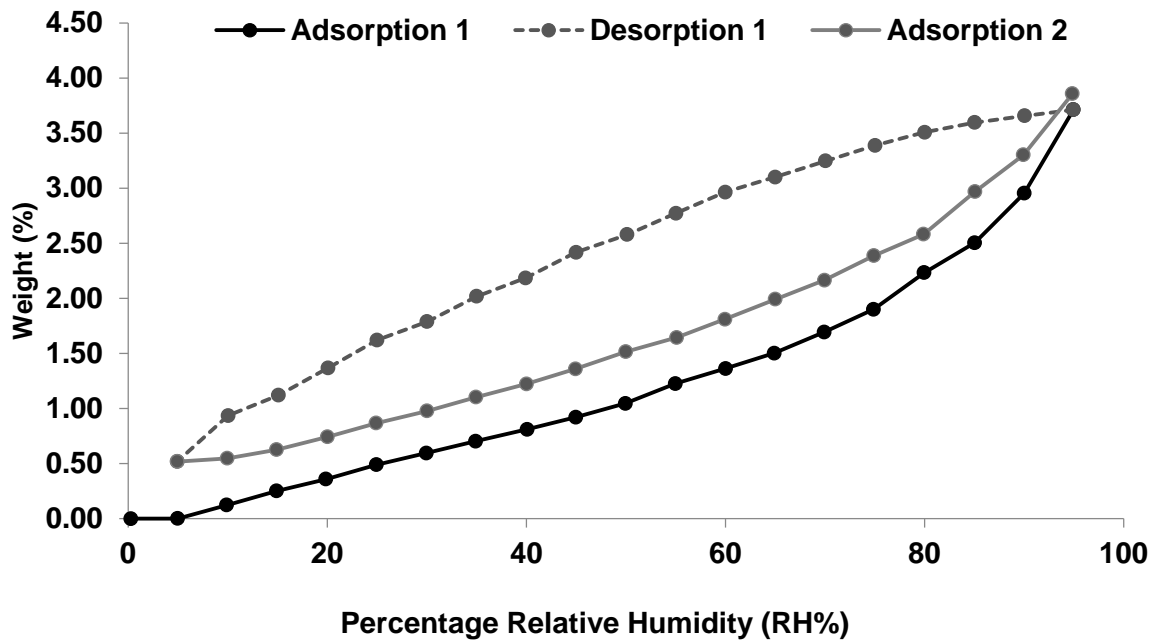


Figure 4.10: Vapour sorption isotherm obtained for A-QC. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.

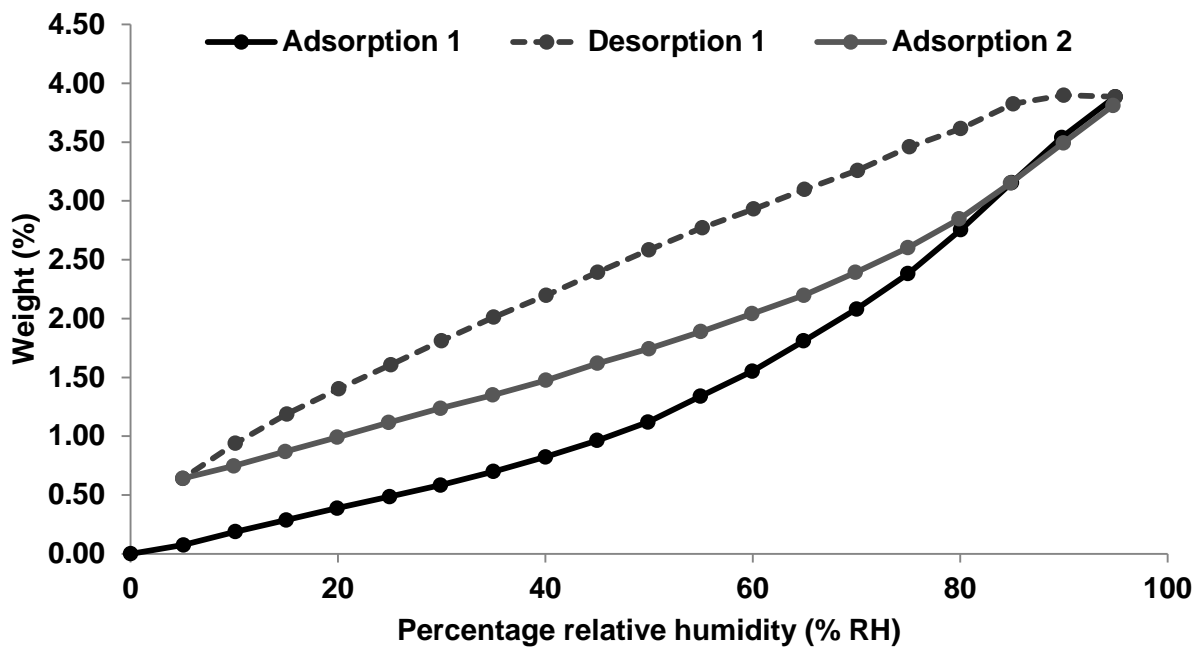


Figure 4.11: Vapour sorption isotherm obtained for A-SC. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.

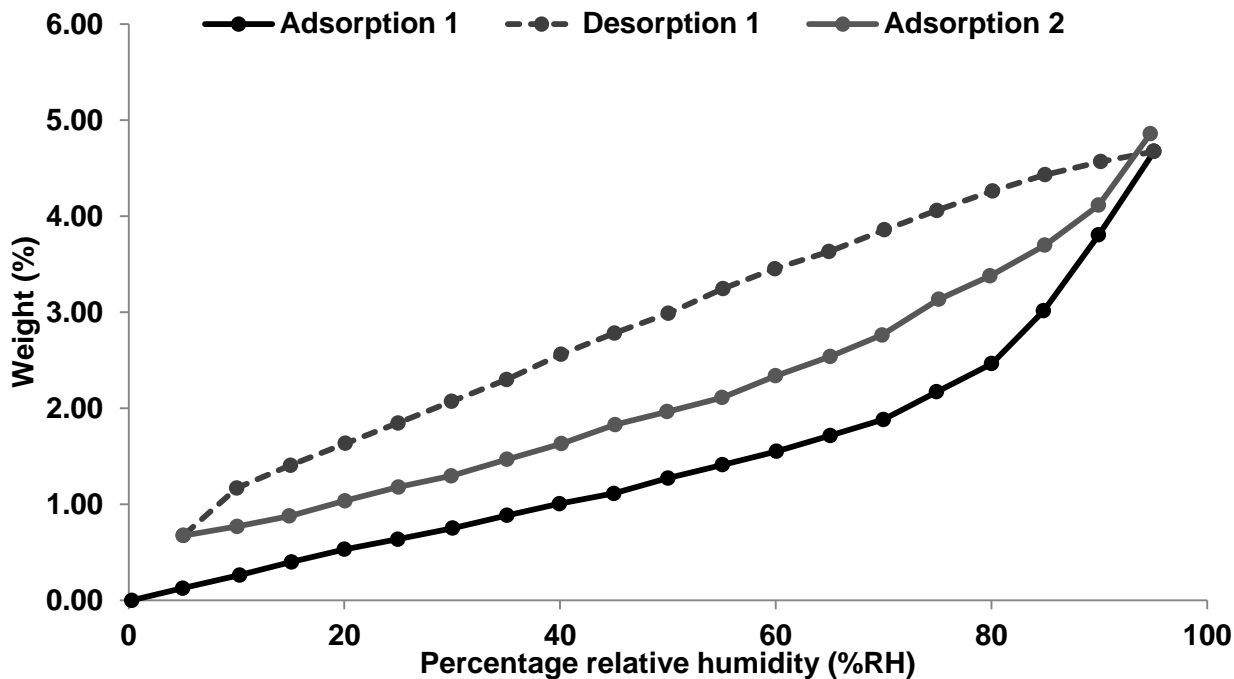


Figure 4.12: Vapour sorption isotherm obtained for A-HA. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.

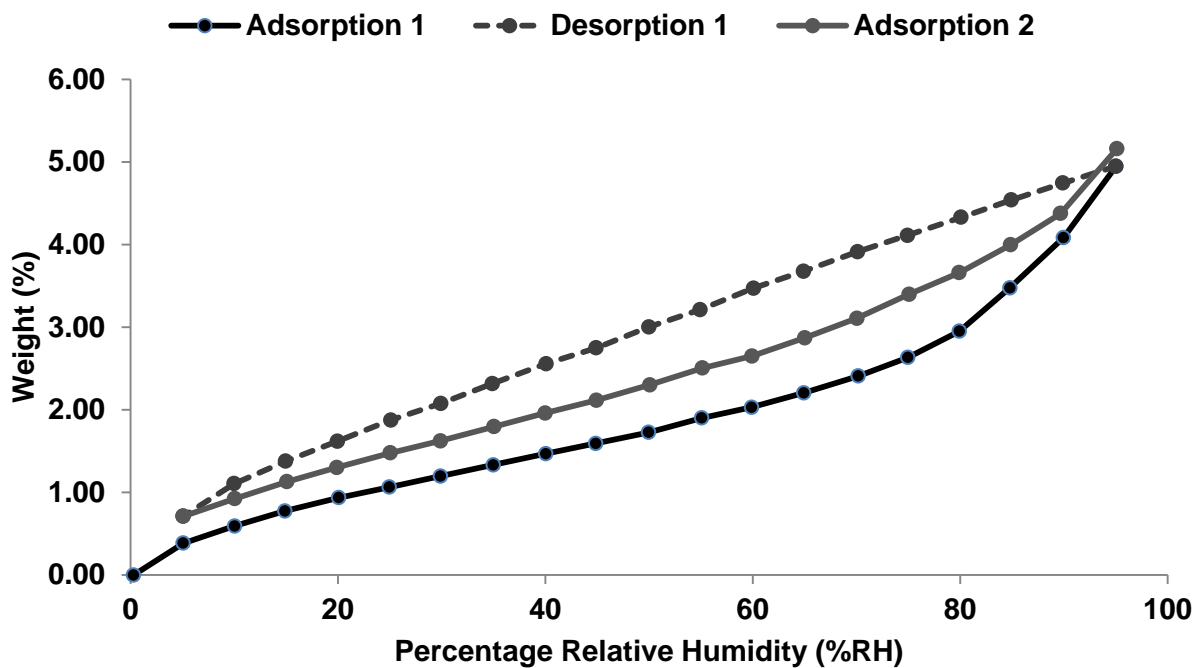


Figure 4.13: Vapour sorption isotherm obtained for A-RV. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.

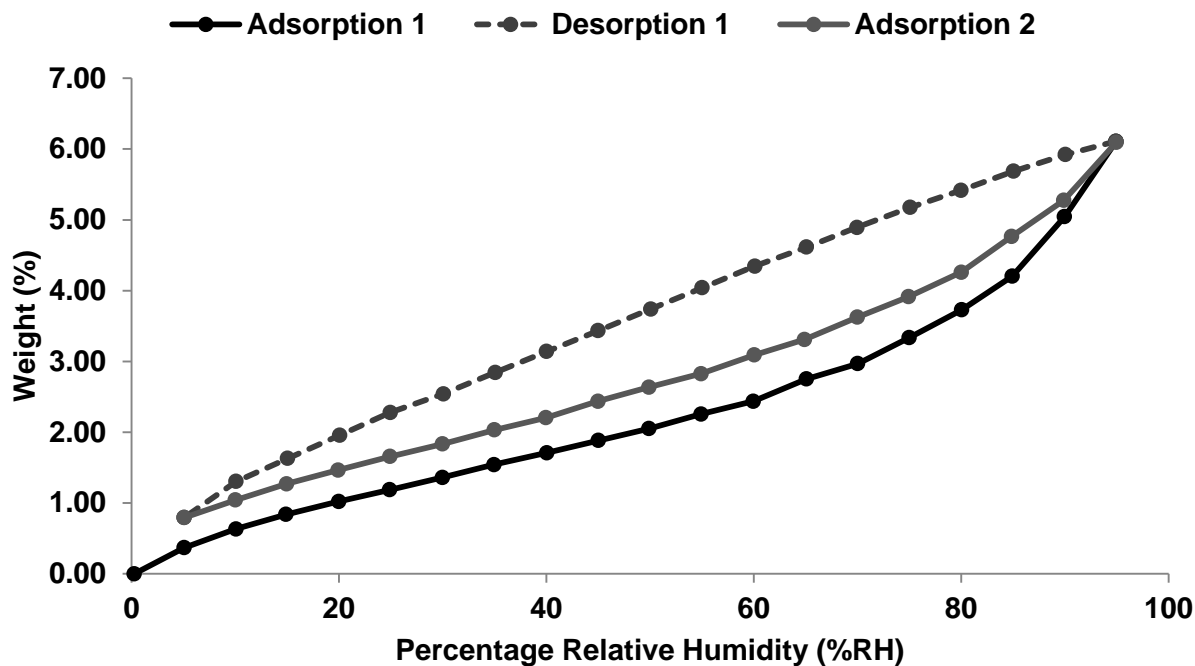


Figure 4.14: Vapour sorption isotherm obtained for A-SD. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.

Some critical information pertaining to the morphology of powder particles can be obtained from investigating vapour sorption isotherms of a compound. Although this does not relate directly to solvent-mediated phase transformation investigations, it is imperative to discuss in order to explain further stability results obtained. Both absorption steps followed the same isotherm pattern for all five amorphous forms but some degree of hysteresis was visible in all instances. The hysteresis can be identified by the deviation in the isotherm pattern between the initial absorption and subsequent desorption step. 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). However, the degree of molecular relaxation was too little to induce any recrystallisation of the amorphous forms back to azithromycin dihydrate.

Interestingly, A-QC, A-SC and A-HA, all showed typical Type III sorption isotherms. This isotherm type is indicative of non-porous compounds where the adsorbent-adsorbate interaction is considered to be weak (Rouquerol *et al.*, 1999). The obtained isotherm type is in good correlation with the SEM micrographs obtained for all three amorphous forms (Figures 4.15 – 4.17).

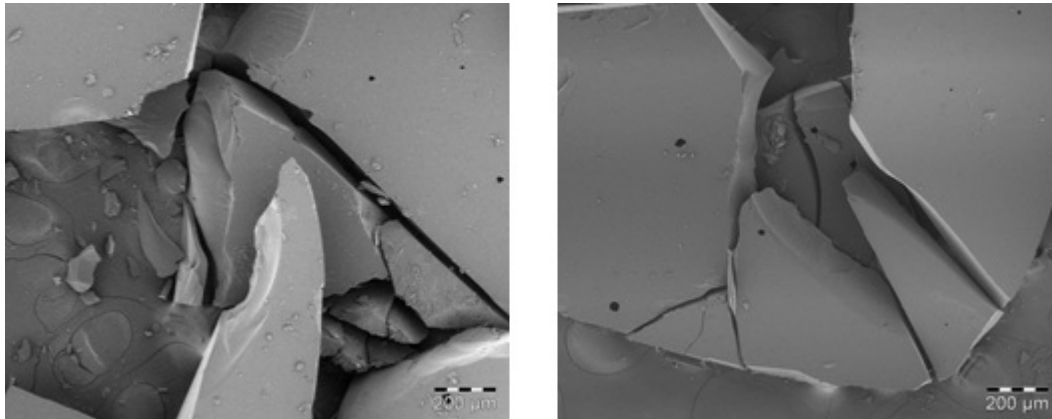


Figure 4.15: SEM micrographs obtained for A-QC.

The SEM micrographs clearly indicate the smooth 'glassy'-like surface of the particles, with almost no pores visible. It is also quite interesting that all three methods which involved melt as an intermediary step resulted in Type III sorption isotherms.

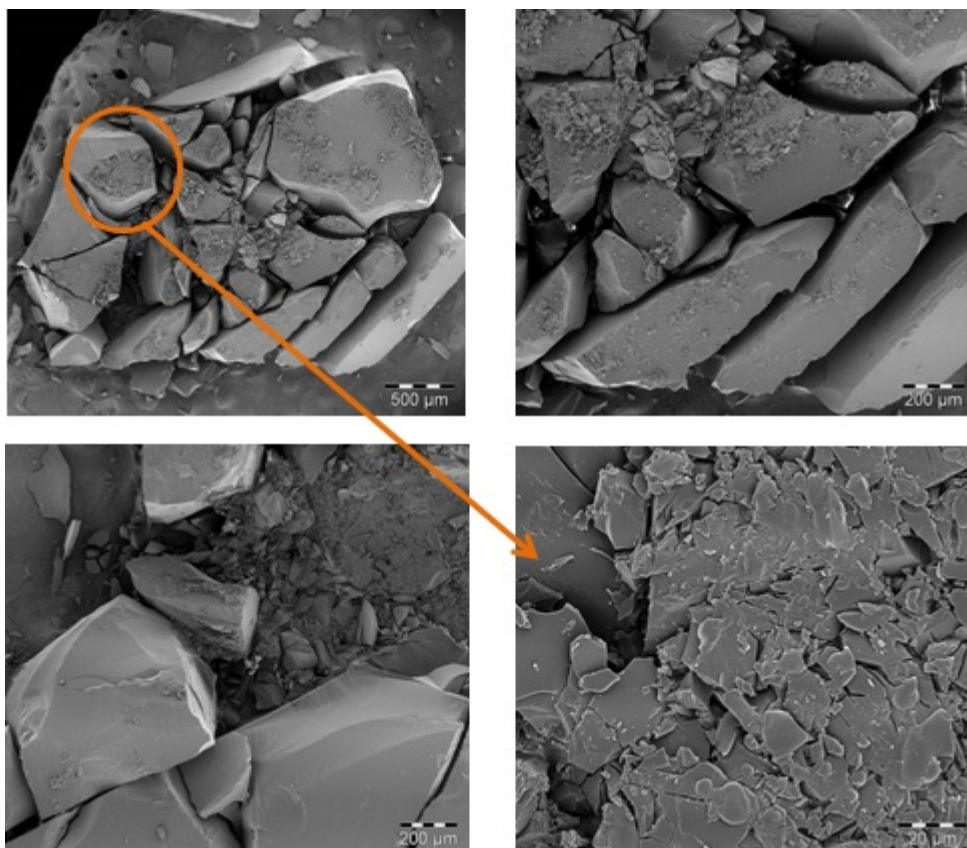


Figure 4.16: SEM micrographs obtained for A-SC.

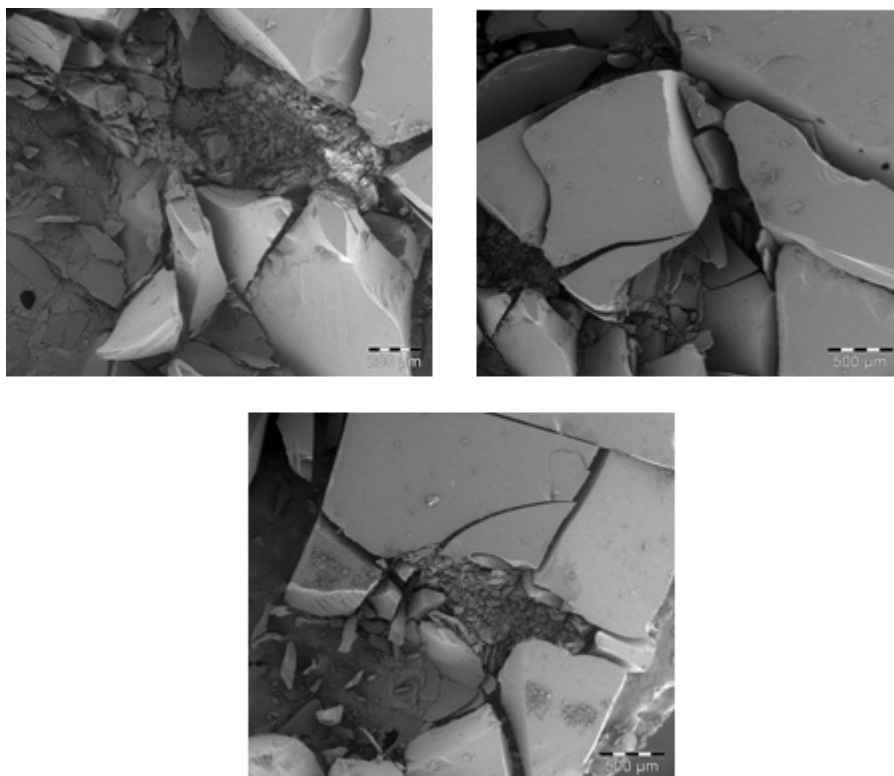


Figure 4.17: SEM micrographs obtained for A-HA.

Both A-RV and A-SD showed typical Type II vapour sorption isotherms. Type II isotherms represent unrestricted monolayer-multilayer adsorption (Rouquerol *et al.*, 1999). This can also be explained in correlation with the obtained SEM results. Figures 4.18 and 4.19 depict the SEM micrographs of A-RV and A-SD respectively. From the SEM images the flake-like morphology of A-RV is visible. This type of morphology could allow the absorption of more water vapour than in comparison with the more rigid, smooth surfaces of A-QC, A-SC and A-HA.

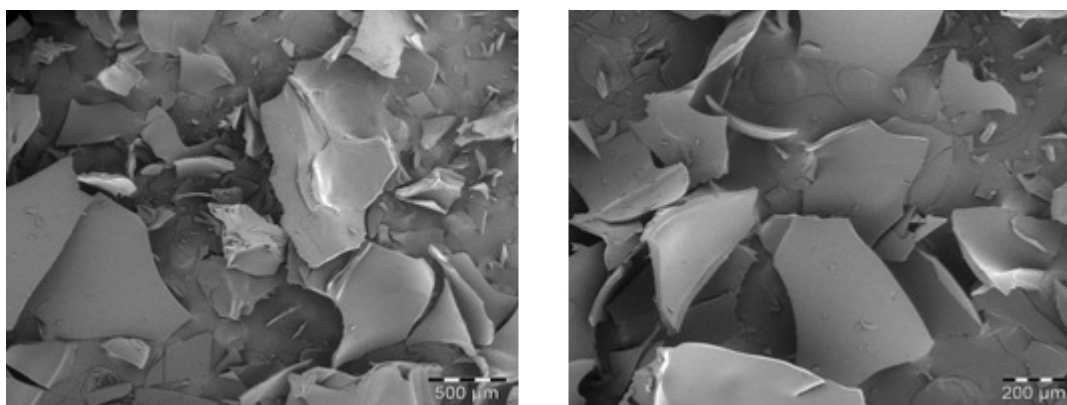


Figure 4.18: SEM micrographs obtained for A-RV.

On the other hand the raisin-like morphology of the amorphous form obtained from the spray-drying process is clearly visible from Figure 4.19. The round particle morphology with lots of folds/creases will definitely provide a larger area for monolayer-multilayer moisture sorption to occur. This also correlates with the amount of moisture that was absorbed by all 5 amorphous forms. Both A-QC and A-SC absorbed a maximum moisture percentage of 3.8%, A-HA absorbed a maximum moisture percentage of 4.9%, in contrast with A-RV and A-SD which absorbed 5.5 and 6.1%, respectively. It was interesting to see that both A-QC and A-SC show 3.8% moisture absorption as literature usually indicates that it is a general trend for amorphous materials to be more hygroscopic with greater moisture sorption capabilities. A more intensive study may be conducted in future to investigate this phenomenon.

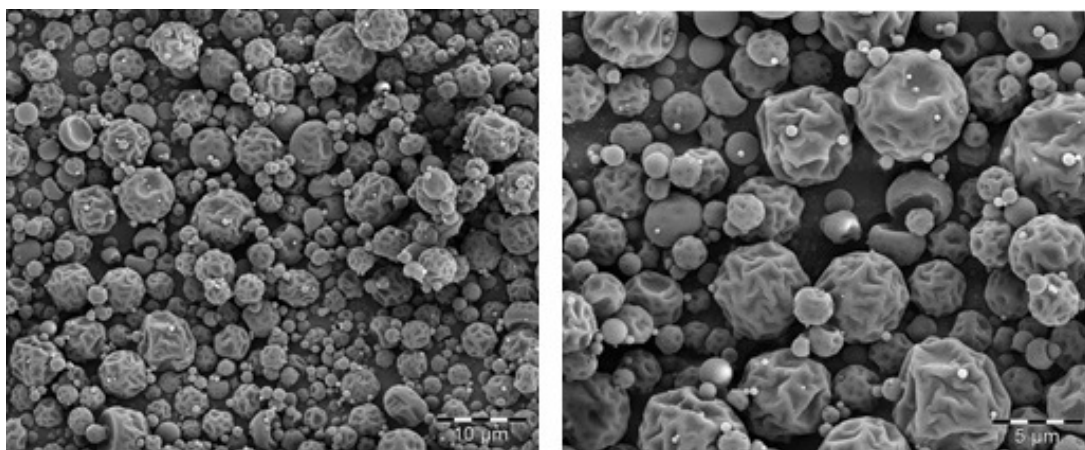


Figure 4.19: SEM micrographs obtained for A-SD.

(c) Solution-mediated phase transformations (SMPT)

Another problem usually associated with the stability of amorphous solid-state forms is the process of solution-mediated phase transformations (SMPT). This type of solid-state transformation is more commonplace than probably realised by pharmaceutical scientists. It is usually quite a challenge to detect and investigate SMPT of APIs, the reason being that limited techniques exist which allow on-line or in-line analysis of this transformation process. The equipment currently available is also quite expensive and specialised, therefore meaning that it won't be part of the general equipment setup of a normal pharmaceutical laboratory. The reason for the above mentioned drawbacks lies within the complexity of analysing solid-state characteristics of the API when it is actually part of a solution or suspension.

However, an easy method to use to allow the identification of SMPT is that of dissolution testing. When a super-saturated solution of the API is obtained it will lead to the nucleation and crystal growth of a more stable solid-state form of the API. Since a more stable solid-state form possess less Gibbs free energy it is less soluble and therefore a decrease in the dissolved API concentration will be observable. Figure 4.20 depicts a typical dissolution profile of an amorphous or anhydrous form of an API transforming to a more stable crystalline solid-state form *via* SMPT (Forms I and II transforming to Form III).

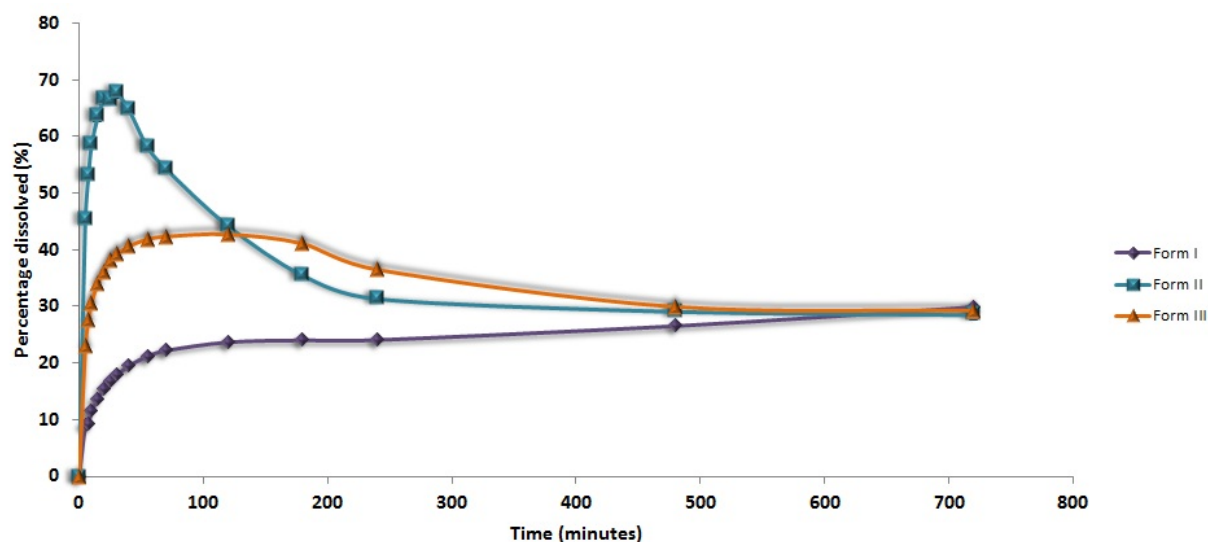


Figure 4.20: A typical dissolution profile obtained during the study of solution-mediated phase transformation of an API (Aucamp *et al.*, 2015).

Subsequently, the stability of the amorphous azithromycin forms was further investigated by means of dissolution testing. Figure 4.21 depicts an overlay of the dissolution profiles obtained for each amorphous form. The dissolution profile of A-DH is also included in the combined graph so as to show how the dissolution behaviour of the five amorphous forms compares with that of A-DH. From Figure 4.21 it is evident that the dissolution profiles of the different amorphous forms differ significantly. The rates of SMPT differ and in some instances the transformation is not visible by a significant decrease in the dissolved API concentration.

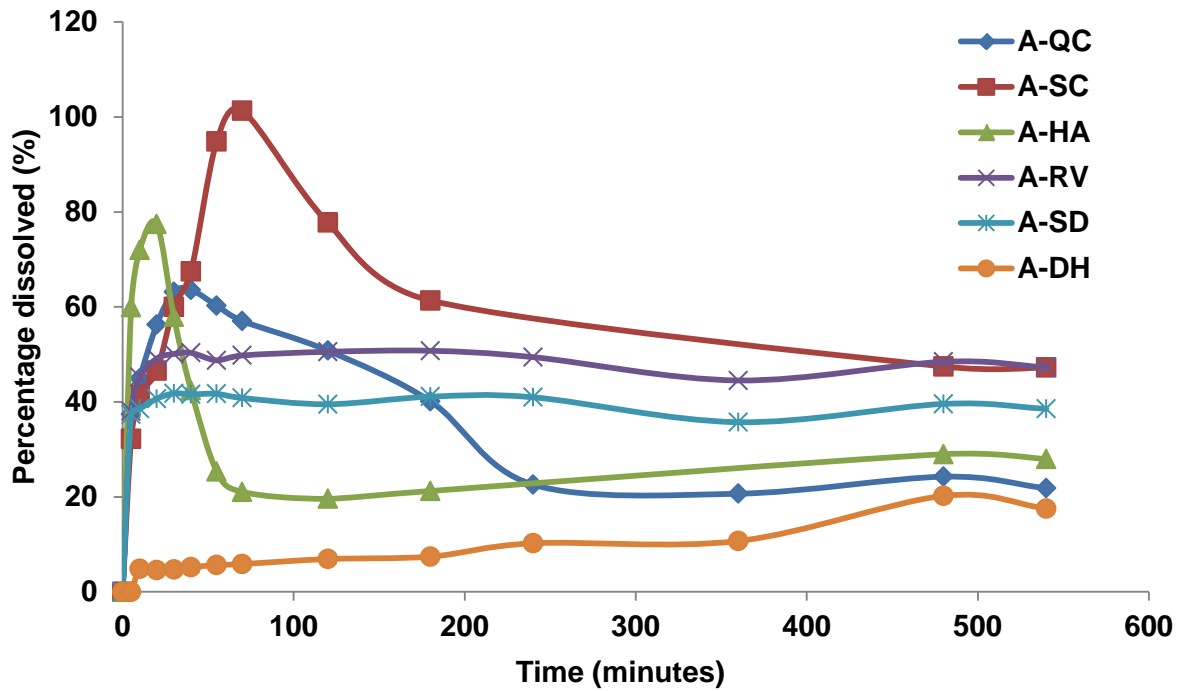


Figure 4.21: An overlay of the dissolution profiles obtained for A-QC, A-SC, A-HA, A-RV, A-SD and A-DH in distilled water at 37°C.

It is possible to discuss and explain these differences in relation to the particle morphology as well as the surface area of each amorphous form. The surface areas of all five amorphous forms as well as that of A-DH were determined by applying the Brunauer-Emmet-Teller (BET) theory, denoted as equation 7:

$$W = \frac{W_m C_B \left(\frac{p}{p_0}\right)}{\left[\left(1 - \left(\frac{p}{p_0}\right)\right)\right] \left[\left(1 - \left(\frac{p}{p_0}\right)\right) + C_B \left(\frac{p}{p_0}\right)\right]} \quad (7)$$

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 area for A-QC was calculated to be 1.59 m²/g. In the instance of A-QC the SMPT process takes a longer time to reach completion, as can be deduced from the steady decrease of the slope of the percentage dissolved API curve. The SMPT process takes about 450 minutes to reach completion. In comparison the specific surface area for A-SC, A-HA, A-RV and A-SD were calculated to be 4.61, 42.51, 43.57 and 52.73 m²/g, respectively (Figure 4.22). The SMPT results can also correlate very well with these calculated results since the greater the available surface area the more rapid is the commencement and completion of the SMPT.

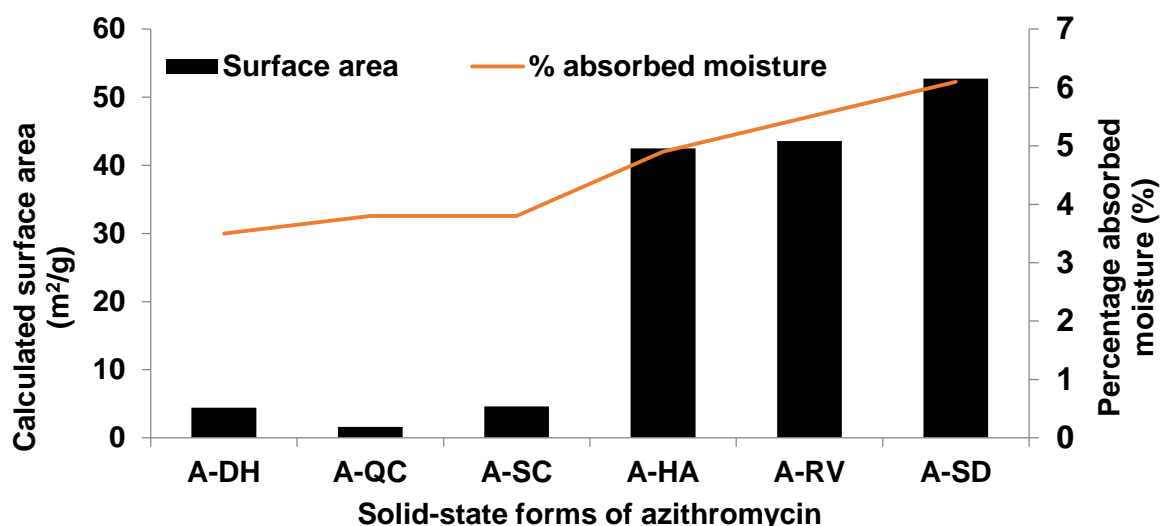


Figure 4.22: The calculated surface area and maximum percentage absorbed moisture for all azithromycin amorphous solid-state forms investigated for SMPT.

The higher calculated surface area of A-HA in comparison with A-QC and A-SC resulted in a more rapid onset of SMPT (after approximately 20 minutes) with completeness of the process after approximately 70 minutes. The significantly higher surface areas of A-RV and A-SD resulted in SMPT so rapid that it was not detectable *via* dissolution testing. The higher surface areas also correlate well with the amount of moisture absorbed during vapour sorption testing (Figure 4.22).

4.4 Conclusion and Summary

The possibility of preparing more than one amorphous form of any given API has not been explored in full and sometimes only one amorphous form has been exploited. Preparing different amorphous forms through different preparative techniques can possibly lead to an API with different physical, chemical and thermodynamic properties. This scope however, is of great importance as this could create the ideal amorphous form with satisfied characteristics such as greater solubility and stability. The principal goal was to investigate the impact and to illustrate the effect of the different preparation methods on the physico-chemical characteristics of each amorphous solid-state form. Preparation methods such as quench cooling of the melt (A-QC), slow cooling of the melt (A-SC), hot-air melting (A-HA), ambient solvent evaporation (A-TB), rapid solvent evaporation (A-RV) and spray-drying (A-SD), all with different intermediary states (melt or solution) were identified and selected.

In the quest to create different amorphous forms of azithromycin, the XRPD results generated in this study positively characterised the amorphous habit of the different preparation techniques. The diffractograms of A-QC, A-SC, A-HA, A-RV and A-SD all showed a halo-like pattern with no indication of sharp or characteristic peaks, illustrating that an amorphous form of azithromycin can indeed be created. A-QC, A-SC, A-HA, A-RV and A-SD can be considered amorphous. The absorbance peaks through FTIR (complimentary to the XRPD results) also confirmed the amorphous forms and A-QC, A-SC, A-HA, A-RV and A-SD all showed broad peaks at 3500 cm^{-1} that indicates a hydroxyl group stretch. There were also no peak at 3251 cm^{-1} that indicates the absence of hydrogen bonded hydroxyl and intramolecular hydrogen bonded hydroxyl functional groups.

The thermodynamic properties were also revealed through DSC analyses. The thermograms revealed that A-QC, A-SC, A-HA, A-RV and A-SD showed no thermally induced recrystallization during the heating process. A great advantage like this, means that all the amorphous forms prepared could stay stable during further processing techniques that require the addition of heat. A-QC, A-SC, A-HA, A-RV and A-SD reflected a fragility index lower than 75 and a high strength parameter which characterised these amorphous forms as non-fragile systems. This usually means that a lower energy is present that will lead to a higher physical stability. All five amorphous forms recorded close to 1.0 reduced glass transition ratio, revealing good glass forming abilities for each preparation technique. A-QC, A-SC and A-HA, with melt intermediary states, were fairly similar in regards with the low fragility index, strength parameter and temperature of zero mobility. On the other hand both A-RV and A-SD with solution intermediary states, showed significantly higher temperatures of zero mobility compared to A-QC, A-SC and A-HA giving these two amorphous forms better physical stability during ambient storage. A-SD had the lowest activation energy for structural relaxation, making this the amorphous form with the least physical stability.

When looking at the sorption isotherms and SEM micrographs, there were also differences between the different amorphous forms. A-QC, A-SC and A-HA showed typical Type III sorption isotherms with SEM micrographs showing smooth 'glassy'-like particle surface. Both A-RV and A-SD showed a typical Type II sorption isotherm with A-RV, a more flake-like and A-SD, a more raisin-like morphology visible on the SEM micrographs. The almost poreless surface from the three melt intermediary states (A-QC, A-SC and A-HA) had the lowest absorbed maximum moisture percentage (A-QC & A-SC both 3.8%; A-HA 4.9%) when compared to the solution intermediary states A-RV and A-SD. The latter had higher absorbed maximum moisture percentage of 5.5% and 6.1% respectively.

From the results generated above, it is evident that it is possible to prepare successfully, more than one amorphous form of azithromycin. However, there are some differences between the different amorphous forms, giving the advantage on selecting the amorphous form with the most desired physical, chemical and thermodynamic properties.

The results and data generated in this chapter will be submitted to *AAPS PharmSciTech*.

4.5 Reference list

Angell, C.A. 1997. Why $C_1 = 16-17$ in the WLF equation is physical-and the fragility of polymers. *Polymer*. 38:6261-6266.

Aucamp, M.E., Liebenberg, W., Strydom, S.J., Van Tonder, E.C. & De Villiers, M.M. 2012. Physicochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. *Journal of the American Association of Pharmaceutical Scientists, PharmSciTech.*, 13:467-476.

Aucamp, M., Odendaal, R., Liebenberg, W. & Hamman, J. 2015. Amorphous azithromycin form with improved aqueous solubility and intestinal membrane permeability. *Drug development and industrial pharmacy*, 41:1100-1108.

Blanco, M., Valdés, D., Bayod, M.S., Fernández-Marí, F. & Llorente, I. 2004. Characterization and analysis of polymorphs by near-infrared spectrometry. *Analytica Chimica Acta*, 502:221–227.

British Pharmacopoeia. 2015. Appendix IF.

Polymorphism, <https://www.pharmacopoeia.com/bp-2016/appendices/appendix-01/appendix-01-f/appendix-i-f--polymorphism.html?published-date=2015-08-03#p2p06455>

Date of access: 07 Nov. 2015.

Burnett, D.J., Thielmann, F., Booth, J. 2004. Determining the critical relative humidity for moisture-induced phase transitions. *International journal of pharmaceuticals*, 287:123-133.

Gilmore, C.J. 2011. X-ray diffraction. (*In Storey, R.A. & Ymén, I., ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 35-70).*

Graeser, K.A., Strachan, C.J., Patterson, J.E., Gordon, K.C. & Rades, T. 2008. Physicochemical properties and stability of two differently prepared amorphous forms of simvastatin. *Crystal growth and design*, 8:128–135.

Jasanada, B., Santos, M., Isidro, L.G. & Felix, F.M. 2001. Preparation of non-crystalline and crystalline dihydrate forms of azithromycin. (Patent: EPO 1 103558 A2).

Li, Z.J. & Trask, A.V. 2005. Crystal forms of azithromycin. (Patent: US 6977243B2).

Lu, Q. & Zografi, G. 1997. Properties of citric acid at the glass transition. *Journal of pharmaceutical sciences*, 86:1374-1378.

Moynihan, C.T. 1993. Correlation between the width of the glass transition region and the temperature dependence of the viscosity of high-T_g glasses. *Journal of the American ceramic society*, 76:1081-1087. Odendaal, R.W., Liebenberg, W. & Aucamp, M. 2013.

Composition comprising an amorphous non-crystalline glass form of azithromycin. (Patent: US 2013/0059805 A1).

Patterson, J.E., James, M.B., Forster, A.H., Lancaster, R.W., Butler, J.M. & Rades, T. 2005. The influence of thermal and mechanical preparative techniques on the amorphous state of four poorly soluble compounds. *Journal of pharmaceutical sciences*, 94:1998-2012.

Ramos, J.J.M, Taveira-Marques, R. & Diogo, H.P. 2003. Estimation of the fragility index of indomethacin by DSC using the heating and cooling rate dependency of the glass transition. *Journal of pharmaceutical sciences*, 93:1503-1507.

Rouquerol, F., Rouquerol, J. & Sing, K.S.W., eds. 1999. Adsorption by powders and porous solids, first ed. London: Academic Press.

Savolainen, M., Kogermann, K., Heinz, A., Aaltonen, J., Peltonen, L., Strachan, C. & Yliruusi, J. 2009. Better understanding of dissolution behaviour of amorphous drugs by *in situ* solid-state analysis using Raman spectroscopy. *European journal of pharmaceutics and biopharmaceutics*, 71:71–79.

Ymén, I. 2011. Introduction to the solid state-physical properties and processes. (In Storey, R.A. & Ymén, I., ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 1-34).

Yu, L. 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Advanced drug delivery reviews*, 48:27-42.

Chapter 5

Instructions for Authors for Drug Development and Industrial Pharmacy

This chapter was submitted to the journal *Drug Development and Industrial Pharmacy*.

Current impact factor: 2.101.

5.1 About the journal

5.1.1 Aims and scope

The aim of *Drug Development and Industrial Pharmacy* is to publish original, peer-reviewed research manuscripts on a variety of relevant topics and research methods related to all facets of pharmaceutical research and development, and industrial pharmacy. This also includes timely critical review papers on related subjects. The research papers must be hypothesis driven and emphasize innovative breakthrough topics in pharmaceuticals and drug delivery.

The topics primarily considered for the journal include:

- Emerging technologies in pharmaceutical development and industrial pharmacy
- *In vitro*–*In vivo* correlations
- Oral controlled release systems
- Drug delivery (oral, inhalation, injectable, buccal, etc.)
- Preclinical drug development, pharmacokinetics and pharmacodynamics
- Drug pharmacokinetics and pharmacodynamics
- Biopharmaceutics and oral absorption
- Aerosols
- Transdermals
- Preformulation and physical pharmacy
- Methodologies, including statistical design/optimisation, if there is clear clinical relevance

5.1.2 Editor-in-chief

Robert O. (Bill) Williams III, PhD

Johnson & Johnson Centennial Professor

Division Head, Pharmaceuticals

University of Texas

Austin, TX

USA

5.2 Manuscript submission

All submissions should be made online at *Drug Development and Industrial Pharmacy's* ScholarOne Manuscripts site. New users should first create an account. Once a user is logged onto the site, submissions should be made via the Author Center. If you experience any problems with your submission or with the site, please contact ScholarOne support through the "get help now" link.

All submissions to the journal must include full disclosure of all relationships that could be viewed as presenting a potential conflict of interest. If there are no conflicts of interest, authors should state that there are none. This must be stated at the point of submission (within the manuscript, after the main text under a subheading "Declaration of interest", and, where available within the appropriate field on the journal's ScholarOne Manuscripts site).

5.3 Rapid publication

Publication in *Drug Development and Industrial Pharmacy* is driven entirely by editorial considerations and independent authoritative peer review. As part of the journal's responsive approach to the publication of timely research results, *Drug Development and Industrial Pharmacy* offers an optional prioritised mode of publication, a fast-track service.

Articles are published online within 5-7 weeks of submission (subject to 1–2 week author revision following initial peer-review, prompt turnaround of proofs and, of course, acceptance of the article).

5.4 Manuscript preparation

5.4.1 File preparation and types

Manuscripts are preferred in Microsoft Word format (.doc files). Documents must be double-spaced, with margins of one inch on all sides. Tables and figures should not appear in the main text, but should be submitted as separate digital files and designated with the appropriate file type on ScholarOne Manuscripts. References should be given in Harvard style (see References section for example).

Manuscripts should be compiled in the following order: title page; abstract; main text; acknowledgments; Declaration of Interest statement; appendices (as appropriate); references; tables with captions (on separate pages); figures; figure captions (as a list).

Drug Development and Industrial Pharmacy publishes the following manuscript types:

- Original papers
- Reviews
- Book reviews

5.4.2 Title page

A title page should be provided comprising the manuscript title plus the full names and affiliations of all authors involved in the preparation of the manuscript. One author should be clearly designated as the corresponding author and full contact information, including phone number and email address, provided for this person. Five to ten key terms that are not in the title should also be included on the title page. The keywords will assist indexers in cross indexing your article.

5.4.3 Abstract

All original articles and reviews should start with an abstract of 250 or fewer words, summarising the central core of knowledge that is the focus of the paper. The recommended format is as a structured abstract, with the following headings for an original article: context, objective, materials and methods, results, discussion and conclusion. For a review article, it should be structured as follows: context, objective, methods (including data sources, study selection and data extraction), results and conclusion. It should be written in an informative style permitting its use, without revision, by abstracting services, give essential details of research findings without further reference to the text, and avoid

generalisations and nonessential information.

5.4.4 Main text

5.4.4.1 Original articles

The body of the article should include the following sections: introduction; methods; results; discussion; conclusions.

Introduction: This section should state the relevance and background to the study, and its rationale and purpose.

Methods: This section should include only information that was available at the time the plan or protocol for the study was being written. You should describe your selection of the observational or experimental participants, identify the methods, apparatus and procedures in sufficient detail to allow others to reproduce the results, and describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. ***Drug Development and Industrial Pharmacy*** requires that studies involving humans, both volunteers and patients, or animals approved by an institutional review board, in accordance with approved published guidelines, prior to actually performing the research and publishing the data. Details including clinical trial registration number must be provided in the methods section if research includes studies conducted on human volunteers.

Results: Present your results in logical sequence in the text, tables, and illustrations.

Discussion: This should include implications of the findings and their limitations, with reference to all other relevant studies and the possibilities these suggest for future research.

Conclusions: This must summarize the main paper. Ensure that extrapolations are reasonable and that conclusions are justified by the data presented, and indicate if the study design can be generalized to a broader study population.

5.4.4.2 Reviews

The body of a review article should be a comprehensive, scholarly evidence-based review of the literature, accompanied by critical analysis and leading to reasonable conclusions. Wherever appropriate details of the literature search methodology should be provided, i.e. the databases searched (normally Medline and at least one or two other databases), the search terms and inclusive dates, and any selectivity criteria imposed.

Wherever possible, use primary resources, avoiding “Data on File”, “Poster” or other unpublished references.

5.4.5 Acknowledgments and declaration of interest sections

Acknowledgments and declaration of interest sections are different, and each has a specific purpose. The Acknowledgments section details special thanks, personal assistance, and dedications. Contributions from individuals who do not qualify for authorship should also be acknowledged here.

Declarations of interest, however, refer to statements of financial support and/or statements of potential conflict of interest. Within this section also belongs disclosure of scientific writing assistance (use of an agency or agency/ freelance writer), grant support and numbers, and statements of employment, if applicable. For a more detailed list of points to include, please see “Declaration of Interest section” below.

5.4.5.1 Acknowledgments section

Any acknowledgments authors wish to make should be included in a separate headed section at the end of the manuscript preceding any appendices, and before the references section. Please do not incorporate acknowledgments into notes or biographical notes.

5.4.5.2 Declaration of interest section

All declarations of interest must be outlined under the subheading “Declaration of interest”. If authors have no declarations of interest to report, this must be explicitly stated. The suggested, but not mandatory, wording in such an instance is: *The authors report no declarations of interest.* When submitting a paper via ScholarOne Manuscripts, the “Declaration of interest” field is compulsory (authors must either state the disclosures or report that there are none). If this section is left empty authors will not be able to progress with the submission.

Please note: for NIH/Wellcome-funded papers, the grant number(s) must be included in the Declaration of Interest statement. For more information regarding DOI and NIH policy, please refer to our Author Resources page here.

5.4.6 References

References should be given in the Vancouver style. Citation in the text is by superscript Arabic numbers outside of the punctuation. References should be arranged in a numbered list at the end of the manuscript following the style below:

- *Journal*: Mei-Tal V, Meyerowitz S, Engel G. The role of psychological process in a somatic disorder: multiple sclerosis. *Psychosom Med* 1970;32:67-89.
- *Book*: Kao F. *An Introduction to Respiratory Physiology*. New York: Elsevier North Holland, 1973:50-61.
- *Contribution to a book*: Tung PS, Fritz IB. Isolation and culture of testicular cells: a morphological characterization. In: Hafez ES, ed. *Techniques of Human Andrology*. Amsterdam: Elsevier North Holland, 1977:125-43.
- *Electronic Resources*: Lin A-S, Shibano M, Nakagawa-Goto K, Tokuda H, Itokawa H, Morris-Natschke, SL, Lee K-H. Cancer Preventive Agents. 7. Antitumor- Promoting Effects of Seven Active Flavonolignans from Milk Thistle (*Silybum marianum*) on Epstein-Barr Virus Activation. *Pharm Biol* [Online] 2007;45:735-738. Available at: <http://www.informapharmascience.com/doi/abs/10.1080/13880200701585592>. Accessed on 12 April 2009.

Periodical abbreviations should follow the style given by Index Medicus.

5.4.7 Tables

Tables should be used only when they can present information more efficiently than running text. Care should be taken to avoid any arrangement that unduly increases the depth of a table, and the column heads should be made as brief as possible, using abbreviations liberally. Lines of data should not be numbered nor run numbers given unless those numbers are needed for reference in the text. Columns should not contain only one or two entries, nor should the same entry be repeated numerous times consecutively. Tables should be grouped at the end of the manuscript on separate pages.

5.4.8 Illustrations

Illustrations (line drawings, halftones, photos, photomicrographs, etc.) should be submitted as digital files for highest quality reproduction and should follow these guidelines:

- 300 dpi or higher

- Sized to fit on journal page
- EPS, JPG, TIFF, or PSD format only
- Submitted as separate files, not embedded in the text
- Legends or captions for figures should be listed on a separate page, double spaced

For information on submitting animations, movie files and sound files or any additional information including indexes and calendars please click [here](#).

Colour figure charges are: US\$1000 per article, a 15% service fee will be applied by Rightslink.

5.4.9 Notes on style

5.4.9.1 General style

Authors are asked to take into account the diverse audience of the journal. Please avoid the use of terms that might be meaningful only to a local or national audience, or provide a clear explanation where this is unavoidable. However, papers that reflect the particularities of a social and cultural system are acceptable. Some specific points on style follow:

1. Authors should write in clear, concise US English. Language and grammar should be consistent with Fowler's English Usage; spelling and meaning of words should conform to Webster's Dictionary. If English is not your native language please ensure the manuscript has been reviewed by a native speaker. Please note: extensive rewriting of the text will not be undertaken by the editorial staff.
2. Latin terminology, including microbiological and species nomenclature, should be italicized.
3. Use standard convention for human and animal genes and proteins: italics for genes and regular font for proteins, and upper case for human products and lower case for animal products.
4. "US" is preferred to "American", "USA" to "United States", and "UK" to "United Kingdom".
5. Double quotation marks rather than single are used unless the "quotation is "within" another".
6. Punctuation of common abbreviations should adhere to the following conventions: "e.g."; "i.e."; "cf.". Note that such abbreviations should not generally be followed by a comma or a (double) point/period.

7. Upper case characters in headings and references should be used sparingly, e.g. only the first word of paper titles, subheadings and any proper nouns begin upper case; similarly for the titles of papers from journals in the references and elsewhere.
8. Apostrophes should be used sparingly. Thus, decades should be referred to as follows: "The 1980s [not the 1980"s] saw ...". Possessives associated with acronyms (e.g. APU), should be written as follows: "The APU"s findings that ..." but note that the plural is "APUs".
9. All acronyms for national agencies, examinations, etc., should be spelled out the first time they are introduced in text or references. Thereafter the acronym can be used if appropriate, e.g. "The work of the Assessment of Performance Unit (APU) in the early 1980s ..." and subsequently, "The APU studies of achievement ...", in a reference "(Department of Education and Science [DES] 1989a)".
10. Brief biographical details of significant national figures should be outlined in the text unless it is quite clear that the person concerned would be known internationally. Some suggested editorial comments in a typical text are indicated in the following with square brackets: "From the time of H. E. Armstrong [in the 19th century] to the curriculum development work associated with the Nuffield Foundation [in the 1960s], there has been a shift from constructivism to heurism in the design of [British] science courses".
11. The preferred local (national) usage for ethnic and other minorities should be used in all papers. For the USA, "African-American", "Hispanic" and "Native American" are used, e.g. "The African-American presidential candidate, Jesse Jackson ..."; for the UK, "Afro-Caribbean" (not "West Indian"), etc.
12. Material to be emphasised by italicisation in the printed version should be italicized in the typescript rather than underlined. Please use such emphasis sparingly.
13. Numbers in text should take the following forms: 300, 3000, 30 000 (not 30,000). Spell out numbers under 10 unless used with a unit of measure, e.g. nine pupils but 9 mm (do not use full stops (periods) within units). For decimals, use the form 0.05 (not .05, x 05 or 0x 05). "%" (not "per cent") should be used in typescripts.
14. Appendices should appear before the references section and after any acknowledgments section. The style of the title is shown by the following example: "Appendix C: The random network generator".

Figures and tables within appendices should continue the sequence of numbering from the main body of the text. Sections within appendices should be numbered, for

example, C.1, C.2. Equations in appendices should be numbered, for example, (C 1), (C 2). If there is only one appendix, it is referred to as “the appendix” and not called “Appendix A”.

5.4.9.2 Abbreviations and nomenclature

For abbreviations and nomenclature, authors should consult the latest edition of the CSE Style Manual available from the Council of Science Editors, 60 Revue Drive, Suite 500 Northbrook, IL, 60062, USA.

5.4.9.3 Footnotes

Footnotes are not to be used except for designation of the corresponding author of the paper or current address information for an author (if different from that shown in the affiliation). Information concerning grant support of research should appear in a separate Declaration of interest section at the end of the paper. Acknowledgements of the assistance of colleagues or similar notes of appreciation belong in a separate Acknowledgements section.

Footnotes to tables should be typed directly below the table and are indicated by the following symbols: * (asterisk or star), † (dagger), ‡ (double dagger), ¶ (paragraph mark), § (section mark), || (parallels), # (number sign). Reinitialize symbol sequence within tables.

5.4.10 Editorial policies

5.4.10.1 Authorship

According to the International Committee on Medical Journal Ethics (ICMJE), an author is defined as one who has made substantial contributions to the conception and development of a manuscript. Informa Pharmaceutical Science adheres to the ICMJE guidelines (<http://www.icmje.org/#author>), which state that “authorship credit should be based on all of the following: 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or advising it critically for important intellectual content; and 3) final approval of the version to be published”¹. All other contributors should be listed as acknowledgements.

All submissions are expected to comply with the above definition. Changes to the authorship list after submission will result in a query from the publisher requesting written explanation.

5.4.10.2 Redundant publication and plagiarism

Drug Development and Industrial Pharmacy considers all manuscripts on the strict condition that they have not been published already, nor are they under consideration for publication or in press elsewhere.

Drug Development and Industrial Pharmacy will not consider, in general, redundant publications, i.e. “publication of a paper that overlaps substantially with one already published in print or electronic media” (International Committee of Publication Ethics: http://www.icmje.org/publishing_4overlap.html). This rule applies whether the overlapping papers are from the same authors or not.

In addition, Informa has a strict policy against plagiarism. We define plagiarism as the use of extracts from another person's work that are not placed in quotation marks, without the permission of that person, and without acknowledgement to that person (using the appropriate reference style), with the result that your article presents these extracts as original to you. By submitting your work to an Informa Healthcare journal, you warrant that it is your original work, and that you have secured the necessary written permission from the appropriate copyright owner or authority for the reproduction of any illustration or other material.

Paraphrasing, defined as “taking portions of text from one or more sources, crediting the author/s, but only changing one or two words or simply rearranging the order, voice (i.e., active vs. passive) and/or tense of the sentences” by the US Office on Research Integrity (<http://ori.dhhs.gov/education/products/plagiarism/7.shtml>), will not be accepted if found to be used extensively and to make up large sections of the manuscript. Extensive paraphrasing will not be deemed original work and will be viewed as redundant publication and/or plagiarism.

5.4.10.3 Peer review

All manuscripts will be subjected to confidential peer review by experts in the field and, on the basis of reviewers’ feedback, papers will be accepted unconditionally, accepted subject to revision or rejected. Authors are required to recommend two peer reviewers during the submission process.

¹ Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication. Available at: <http://www.icmje.org/>

5.4.10.4 Ethics and consent

- Do not use patients' names, initials, or hospital numbers, especially in illustrative material. Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published.
- Papers including animal experiments or clinical trials must be conducted with approval by the local animal care or human subject committees, respectively (see below).
- To comply with FDAAA legislation, Informa Pharmaceutical Science requires trial registration as a condition of publication for all studies involving clinical trials. Trial registration numbers should be included in the abstract, with full details provided in the methods section.
- All manuscripts, except reviews, must include a statement in the Introduction or Methods section that the study was approved by an Investigational Review Board (Human Studies Committee or Ethics Committee or Animal Care and Use Committee), if applicable. Authors who do not have formal ethics review committees should include a statement that their study followed principles in the Declaration of Helsinki (<http://www.wma.net/e/policy/b3.htm>).
- When a product has not yet been approved by an appropriate regulatory body for the use described in the manuscript, the author must specify that the product is not approved for the use under discussion or that the product is still under investigation.

Further information on Ethics and Consent can be found by clicking [here](#)

5.4.10.5 Copyright and permissions

It is a condition of publication that authors assign copyright or license the publication rights in their articles, including abstracts, to Informa UK Ltd. This enables us to ensure full copyright protection and to disseminate the article, and the Journal, to the widest possible readership in print and electronic formats as appropriate. Authors may, of course, use the article elsewhere after publication without prior permission from Informa UK Ltd., provided that acknowledgement is given to the Journal as the original source of publication, and that Informa Pharmaceutical Science is notified so that our records show that its use is properly authorised. Authors retain a number of other rights under the Informa UK Ltd. rights policies documents.

Authors are required to sign an agreement for the transfer of copyright to the publisher. All accepted manuscripts, artwork, and photographs become the property of the publisher. A copyright agreement form can be downloaded by corresponding authors of accepted manuscripts with proofs. This should be signed and returned to Informa Pharmaceutical Science.

Authors are themselves responsible for obtaining permission to reproduce copyright material from other sources.

5.4.10.6 Declaration of interest

It is the policy of all Informa Pharmaceutical Science, to adhere in principle to the Conflict of Interest policy recommended by the ICMJE. All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. It is the sole responsibility of authors to disclose any affiliation with any organization with a financial interest, direct or indirect, in the subject matter or materials discussed in the manuscript (such as consultancies, employment, paid expert testimony, honoraria, speakers bureaus, retainers, stock options or ownership, patents or patent applications or travel grants) that may affect the conduct or reporting of the work submitted. All sources of funding for research are to be explicitly stated. If uncertain as to what might be considered a potential conflict of interest, authors should err on the side of full disclosure.

If there are no declarations, authors should explicitly state that there are none. This must be stated at the point of submission (within the manuscript, after the main text, under a subheading "Declaration of interest", and within the appropriate field on the journal's ScholarOne Manuscripts site). Manuscript submission cannot be completed unless a declaration of interest statement (either stating the disclosures or reporting that there are none) is included.

This will be made available to reviewers and will appear in the published article. If any potential conflicts of interest are found to have been withheld following publication, the journal will proceed according to COPE guidance.

The intent of this policy is not to prevent authors with any particular relationship or interest from publishing their work, but rather to adopt transparency such that reviewers, editors, the publisher, and most importantly, readers can make objective judgements concerning the work product.



Private Bag X6001, Potchefstroom
South Africa 2520
Tel: 018 299-1111/2222
Web: <http://www.nwu.ac.za>

**CENTRE OF EXCELLENCE FOR
PHARMACEUTICAL SCIENCES**
Tel: 018 299-4472
Fax: 087 231-5432
Email: Marique.Aucamp@nwu.ac.za

4 April 2016

Editor-in-Chief,
R.O. Williams III
Division Head
Pharmaceutics
University of Texas
Austin
TX
USA

Hereby I would like to submit the manuscript entitled: ***The stability of amorphous azithromycin: a thermodynamic and kinetic perspective*** for publication in *Drug Development and Industrial Pharmacy*. 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 the physical and chemical stability of amorphous azithromycin. Through this study the recrystallization kinetics of the amorphous to the most stable crystalline dihydrate form of azithromycin was investigated. Typically, solution-mediated phase transformation is a difficult process to quantify. This study showed that through combining dissolution studies with heat of solution determinations it is possible to quantify the process of phase transformation. This study is a first of its kind in applying dissolution, heat of solution data and the temperature dependence of both parameters in the calculation of the recrystallization kinetics of amorphous azithromycin.

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

Kind regards,

A handwritten signature in black ink, appearing to be "M.E. Aucamp".

PROF. M.E AUCAMP

The stability of amorphous azithromycin: a thermodynamic and kinetic perspective

André Joubert^a, Wilna Liebenberg^b, Nicole Stieger^b, Marique Aucamp^{b*}

^aResearch Institute for Industrial Pharmacy (RIIP)[®] incorporating CENQAM[®], North-West University, Potchefstroom Campus, Potchefstroom, South Africa, 2520

^bCentre 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

Keywords: Azithromycin, amorphous, solution-mediated phase transformation, recrystallization kinetics, solubility

Abstract

The use of amorphous solid-state forms of drugs is becoming a more prevalent topic within the pharmaceutical sector. Mostly, the pharmaceutical industry avoids the incorporation of amorphous forms into solid dosage forms due to the inherent instability associated with these higher energy state forms. However, comprehensive stability studies will allow the identification of possible pitfalls beforehand, thereby providing sufficient information which would allow the successful use of amorphous forms in dosage form development. This study focusses on the investigation of the thermodynamic properties of amorphous azithromycin prepared through quench cooling of the melt. Furthermore, to determine the physico-chemical conditions, that could trigger the recrystallization thereof. The thermodynamic properties of amorphous azithromycin were determined from thermal analyses. Possible solvent-mediated phase transformation was investigated through vapor sorption studies, while solution-mediated phase transformation (SMPT) and associated kinetics were evaluated through dissolution studies at varying temperatures and heat of solution determinations. The kinetics and activation energy involved in the SMPT of amorphous to crystalline azithromycin dihydrate was determined through the application of the Arrhenius equation. The least-squares fit showed that the process of SMPT follows first-order reaction rate kinetics. Through applying the Nogami method, the true solubility advantage of amorphous azithromycin was determined to be an 8-fold improvement in distilled water at 25°C. These results showed amorphous azithromycin to be a relative stable solid-state form, with strong Arrhenius-like behavior in terms of physical stability. It was determined that recrystallization of the amorphous form only occur *via* SMPT.

INTRODUCTION

It is a well-known fact that most drugs possess the ability to exist in different solid-state forms and that these different forms can significantly influence the physico-chemical properties of the given drug ^[1]. During the last two decades amorphous solid-state forms of drugs received more and more attention within the pharmaceutical research and development environment. The reason for this being the higher apparent solubility and faster dissolution rates which are attributes of thermodynamically metastable solid-state forms of drugs ^[2]. The general viewpoint exist that the incorporation of a metastable form of any given drug is unacceptable due to possible crystallization and transformation to a more stable solid-state form that might occur during product processing, storage and dissolution. A major drawback with regards to amorphous solid-state forms is the influence that recrystallization can have on the dissolution and resulting bioavailability of the drug ^[3]. Considering this, it is evident that the investigation of all possible triggers which could lead to the recrystallization of an amorphous drug should be investigated prior to any other pre-formulation studies. Temperature and moisture are two factors that can significantly reduce the stability of an amorphous drug. Moisture-induced recrystallization can either occur through solvent- or solution-mediated phase transformations ^[4,5]. The fundamental understanding and characterization of the kinetics involved during the recrystallization of an amorphous solid-state form will allow the possibility to approach the stability issue in a mathematical manner ^[3].

Azithromycin is a 15-membered macrolide antibiotic which has been characterized by previous studies as a drug which exhibits poor aqueous solubility. These studies also proved that azithromycin can be prepared as an amorphous solid-state form ^[6,7]. However, currently in literature no reports exist on the stability of amorphous azithromycin prepared through the quench cooling of the melt method. This study focusses on the investigation of all possible recrystallization mechanisms that might play a part in the stability of amorphous azithromycin. The recrystallization of amorphous azithromycin through the process of solution-mediated phase transformation was modeled in terms of fraction transformed as a function of time. Through applying the data presented in this paper effective crystallization control of amorphous azithromycin can be achieved during further processing and manufacturing steps.

MATERIALS AND METHODS

Materials

Crystalline azithromycin dihydrate was purchased from DB Fine Chemicals (Pty) Ltd (Johannesburg, South Africa). The purity of the raw material was 96.5% (on dried basis). Potassium dihydrogen orthophosphate was obtained from Saarchem (Pty) Ltd (Krugersdorp, South Africa) and all other reagents used were of chromatography grade.

Methods

Preparation of amorphous azithromycin

The preparation of amorphous azithromycin was done by melting azithromycin raw material in a Petri dish by heating the sample to approximately $130 \pm 5^\circ\text{C}$ using an oven (Binder GmbH, Germany). The obtained melt was subsequently quenched cooled on a cold surface. The amorphous azithromycin was removed from the Petri dish and powdered for further use. In order to determine the purity of the prepared amorphous form, an HPLC assay was done and the amorphous habit of the solid-state form was confirmed by X-ray powder diffraction (XRPD).

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 aluminium crimp cells with pierced lids. The heating rate was set to $10^\circ\text{C}/\text{min}$ and the samples were heated from $25 - 250^\circ\text{C}$ with a nitrogen gas purge of $35 \text{ mL}/\text{min}$. The onset temperatures of the thermal events are reported.

X-ray powder diffraction (XRPD)

X-ray powder diffraction measurements were performed to confirm the crystalline or amorphous nature of the azithromycin solid-state forms. 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, 45 kV; current, 40 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; scanning speed, $2^\circ/\text{min}$ (step size, 0.02° ; step time, 1.0 s).

Vapor sorption analysis

The moisture sorption analyses were performed utilizing a VTI-SA vapor sorption analyzer (TA Instruments, 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 a 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 - 95% RH, followed by a decrease in % RH from 95 - 5%. The last absorption phase was set to also ramp from 5 - 95% RH. A drying phase of 40°C with a weight loss criterion of not more than 0.01% weight loss in 2 minutes was set to run prior the % RH ramp program. Care was taken that the temperature of the drying phase was not set to be close to the glass transition (T_g) temperature of amorphous azithromycin. 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.

Determination of degree of crystallinity

Approximately 100 mg of both the azithromycin dihydrate raw material and amorphous azithromycin were weighed into test tubes ($n = 3$). 10 mL distilled water (25, 30 and 35°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 (25, 30 and 35°C). The axis was set to rotate at 54 rpm for a period of 360 minutes. Subsequently, the test tubes were detached from the rotating axis at predetermined time intervals. The powders that did not dissolve were removed from the test tubes for analysis. The heat of solution data were collected for these samples.

The degree of crystallinity of the solid samples, remaining after the solubility or dissolution experiments, was determined by means of heat of solution. For this a Thermal Activity Monitor (TAM III) (TA Instruments, USA) was used. The heat of solution data was collected at 25°C. Approximately 10 mg of the undissolved powder, removed from the test tubes and blotted dry on filter paper, were weighed into glass crushing ampoules. The crushing ampoules were sealed with wax plugs. 20 mL of absolute ethanol was pipetted into the reaction vessel of the solution calorimeter. The crushing ampoule containing the sample was affixed to the gold stirrer of the calorimeter, followed by attaching the reaction vessel containing the solvent. The stirring speed was set to 300 rpm. The complete reaction unit was lowered into the calorimeter. The experiment was started by two electrical calibration steps followed by crushing of the ampoule. The degree of crystallinity was determined by assuming 100% crystallinity for azithromycin dihydrate and 0% crystallinity for amorphous azithromycin prepared *via* quench cooling of the melt.

Powder dissolution testing

A VanKel700 dissolution bath was used for dissolution testing. USP apparatus 2 (paddle) was set up at either 25, 30 or 35°C with a rotational speed of 100 rpm, 900 mL distilled water was added to each dissolution vessel. Approximately 600 mg of powder of either azithromycin dihydrate or the amorphous form were weighed into 10 mL test tubes, to which 300 mg glass beads, $\leq 106 \mu\text{m}$ (Sigma-Aldrich, South Africa) was added. 5 mL of dissolution medium (distilled water maintained at 25, 30 or 35°C) was added to each test tube. The mixtures were agitated for a period of 120 seconds, 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. After withdrawal, the samples were filtered through a 0.45 μm PVDF filter into an HPLC vial. The filtered solutions were analyzed by HPLC.

High-performance liquid chromatography (HPLC)

The samples obtained from the powder dissolution testing or equilibrium solubility studies, were assayed by means of HPLC. A Shimadzu (Kyoto, Japan) UFLC (LC-20AD) chromatographic system was used. 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 0.06 M potassium orthophosphate buffer, pH adjusted to 6.0 with 1.0 M sodium hydroxide solution. The buffer was mixed with acetonitrile in the ratio 700:300. A Luna C₁₈ 150 × 4.6 mm column was used and a flow rate of 1.0 mL/min was used. A wavelength of 205 nm was used for detection^[8]. Both the column and sample tray temperature were set to control the temperature at 25, 30 or 35°C. This was done in order to prevent any recrystallization from solution due to a possible decrease in sample solution temperature.

RESULTS

Characterization and thermodynamic properties of amorphous azithromycin

The habit of amorphous azithromycin obtained through the quench cooling of the melt method was compared to crystalline azithromycin dihydrate through XRPD analysis (Figure 1). Immediately after preparation of amorphous azithromycin a purity analysis by means of HPLC was performed to confirm that the preparation method did not result in the

degradation of the macrolide antibiotic. The purity of amorphous azithromycin and crystalline azithromycin dihydrate was determined to be $96.0 \pm 0.5\%$ and $96.5 \pm 0.7\%$, respectively, thereby indicating that no degradation occurred.

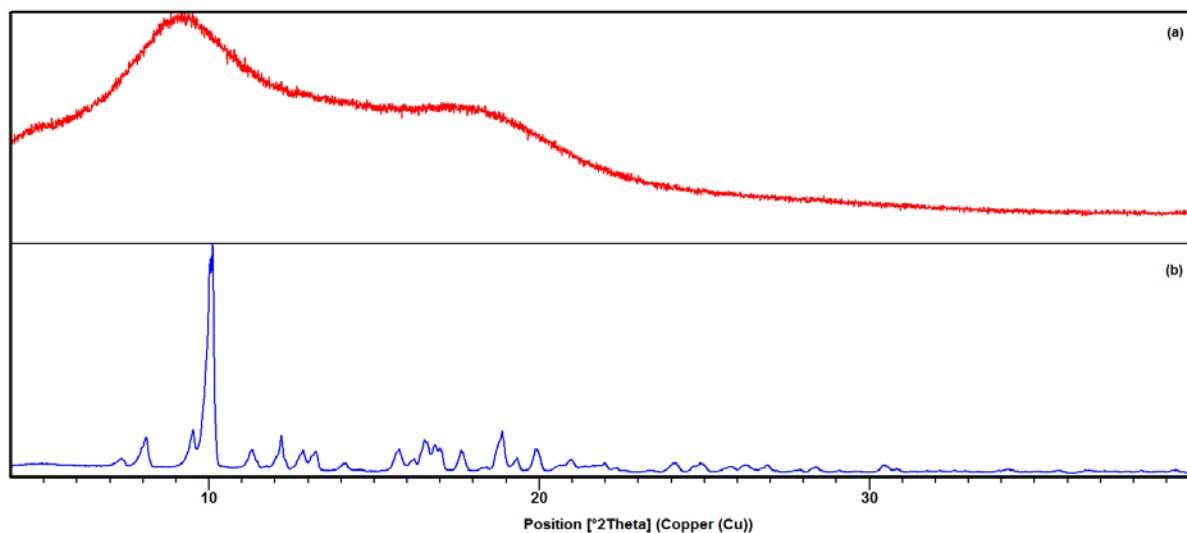


Figure 1: Overlay of XRPD diffractograms obtained for (a) amorphous azithromycin and (b) crystalline azithromycin dihydrate used to prepare the amorphous solid-state form.

Although other studies reported on amorphous azithromycin and that it is possible to prepare a stable amorphous form of this drug ^[6,7], limited information is available on the thermal behavior and subsequent thermodynamics of amorphous azithromycin prepared through quenching of the molten drug. It is however imperative to investigate the thermodynamic properties of an amorphous drug. This will be an enabling tool in knowing and understanding the stability of the amorphous form under investigation ^[9]. Thermal analysis of crystalline azithromycin dihydrate showed a broad melting endotherm in the region 110.6 – 131.0°C (Figure 2) and correlates well with a previous literature report ^[7]. The enthalpy (ΔH_m) and entropy of melting (ΔS_m) were obtained from the integration of the endothermic melting event (Table 1). These parameters are essential in calculating the configurational enthalpy, entropy as well as the available free energy of an amorphous solid-state form. These parameters are therefore useful in characterizing whether an amorphous form of a given drug will exhibit significant physical stability. Physical stability of an amorphous drug is essential in further pharmaceutical processing steps and therefore it is important to have sufficient characterization data which would allow pharmaceutical and formulation scientists to pin-point any possible pitfalls.

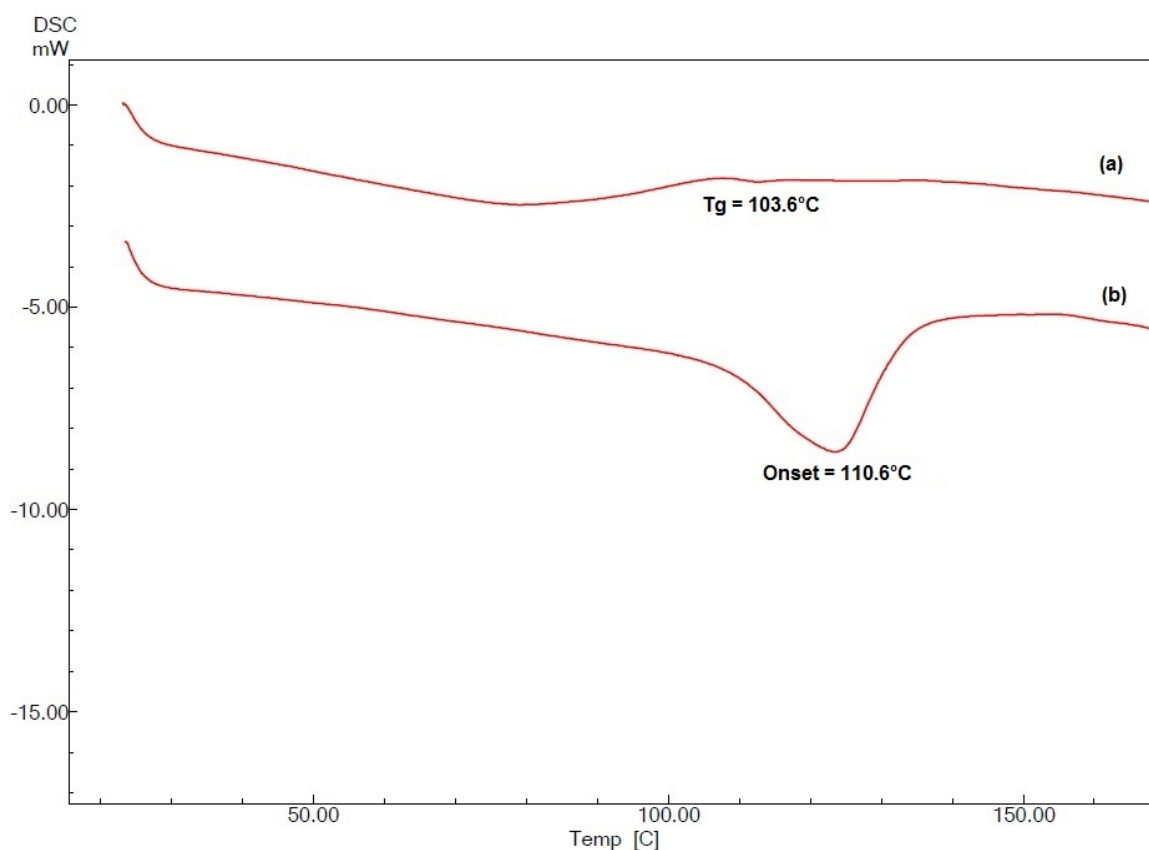


Figure 2: Overlay of DSC thermograms obtained for (a) amorphous azithromycin and (b) crystalline azithromycin dihydrate used to prepare the amorphous solid-state form.

Table 1: Thermodynamic properties calculated for crystalline azithromycin dihydrate and amorphous azithromycin prepared through quench cooling of the melt

	T_m (°C)	ΔH_m (kJ/mol)	ΔS_m (J/mol/K)	T_g (°C)	T_g / T_m
Crystalline azithromycin	110.6	54.1	141.0	-	-
Amorphous azithromycin	-	-	-	103.6	0.9

For amorphous azithromycin a glass transition temperature (T_g) of 103.6°C (Figure 2) was recorded and no thermally induced crystallization of the amorphous form was evident. It is well-known that the T_g of an amorphous solid-state form is greatly dependent on the heating or cooling rate (q) applied during thermal investigations^[10,11,12]. Considering this, it is

therefore possible to determine the activation energy (ΔH^\ddagger) for structural relaxation of any amorphous form from different heating or cooling rates (q) and subsequently using the resulting Arrhenius plot. Figure 3 depicts the plot of $\ln(q)$ versus $1000/T_g$. From this plot, activation energy (E_a) of $380.38 \pm 23.49 \text{ kJ.mol}^{-1}$ was calculated. For this, equation (1) will apply:

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

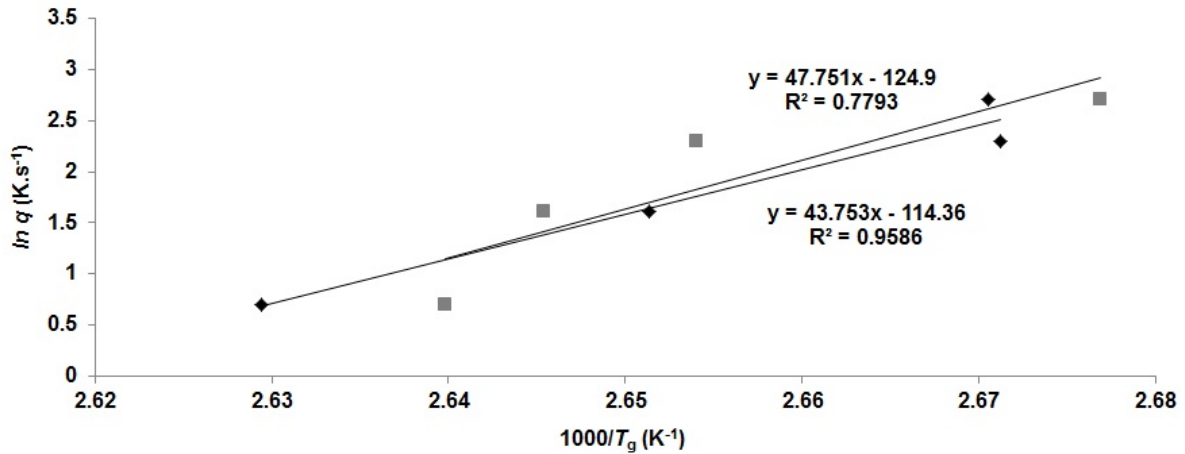


Figure 3: An Arrhenius plot for the glass transition temperature (T_g) affected by varying heating rates (q).

In order to further understand the physical stability of an amorphous solid-state form it is imperative to investigate and quantify the fragility index (m) and the strength parameter (D). The fragility index is defined as a temperature dependent property which allows a quantitative understanding of the chemical, physical or mechanical stability of a specific amorphous form of a drug [13,14,15]. The fragility index is also a dimensionless parameter that 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 [12,16,17]. From this equation 2, defines the fragility index then as follow:

$$m = \left[\frac{d \log_{10} \tau(T)}{d \left(\frac{T_g}{T} \right)} \right] \quad (2)$$

Since the fragility index is a dimensionless attribute which describes the molecular kinetics of an amorphous solid-state form, equation 2 can be expressed in terms of temperature dependence and then equation 3 will apply:

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

By applying equation 2 the fragility index can subsequently be related to the strength parameter (D) (equation 4):

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

assuming that the viscosity of amorphous azithromycin 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 equation 5 [18] :

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

Literature clearly describes that a strong amorphous solid-state form will exhibit a value of $m < 40$ while a fragile system will show a value of $m > 75$. 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 fragile systems [12]. For amorphous azithromycin obtained through quench cooling of the melt, a fragility index of 15.5 and a strength parameter of 447.1 was calculated. Thereby, proving this particular amorphous form of azithromycin possesses strong Arrhenius behavior.

Recrystallization behavior of amorphous azithromycin

Investigation of possible solvent-mediated recrystallization

The thermodynamic properties of an amorphous solid-state form of a drug are mere indications of the mobility of the molecules in the solid-state. If an amorphous solid-state form is characterized to possess strong Arrhenius behavior it does not necessarily imply that the amorphous form of the drug will remain stable throughout all relevant pharmaceutical processing steps. It was therefore important to investigate the stability of amorphous azithromycin when exposed to higher relative humidity. As mentioned, heating of amorphous azithromycin up to a temperature of 250°C proved that no recrystallization of the amorphous solid-state form occurred, thereby indicating it to be stable when exposed to temperatures above the T_g .

Figures 4 and 5 depict vapor sorption isotherms obtained for amorphous azithromycin when exposed to a constant temperature of 25 or 40°C and a relative humidity variation from 0 – 95%, 95 – 5% and 5 – 95%. The resulting isotherms indicated that irrespective of the temperature, the amount of sorbed moisture by amorphous azithromycin remains approximately 3.5%. This correlates well with what literature dictates that amorphous forms

of a drug possess the ability to absorb significantly more water than the crystalline counterpart of the same drug [18,19]. A previous study indicated that crystalline azithromycin dihydrate absorbs an approximate maximum percentage moisture of 1.3% during exposure thereof to an RH ramp of 0 – 95% and a de-ramping of 95 – 0% RH [7].

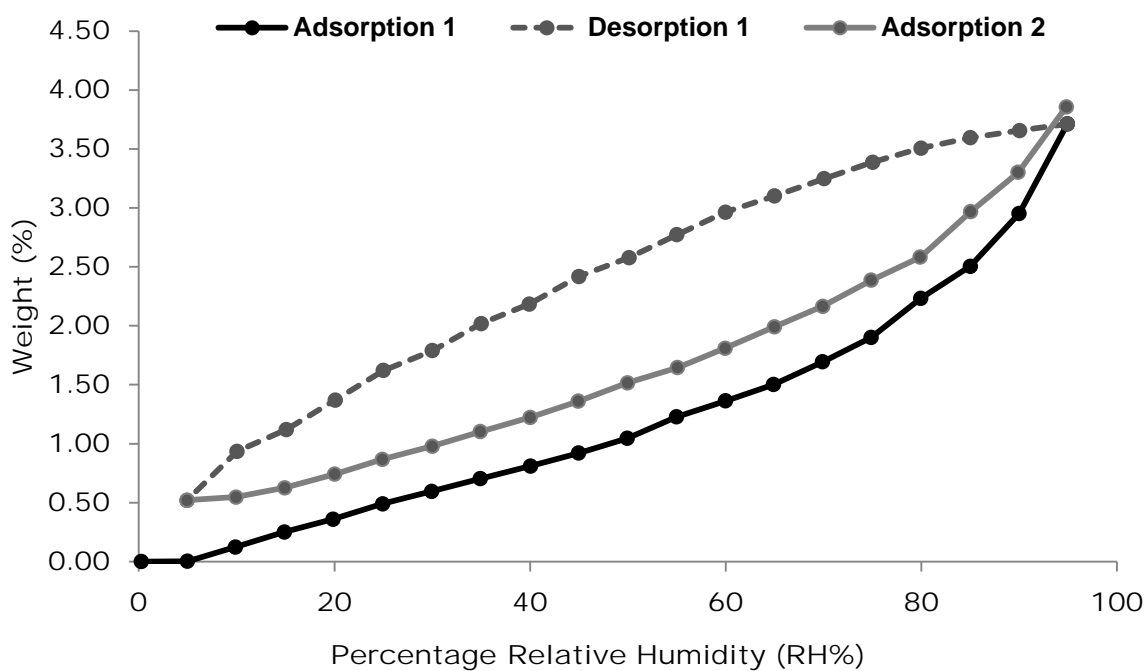


Figure 4: Vapor sorption isotherm obtained for amorphous azithromycin. The isotherms were obtained at 25°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.

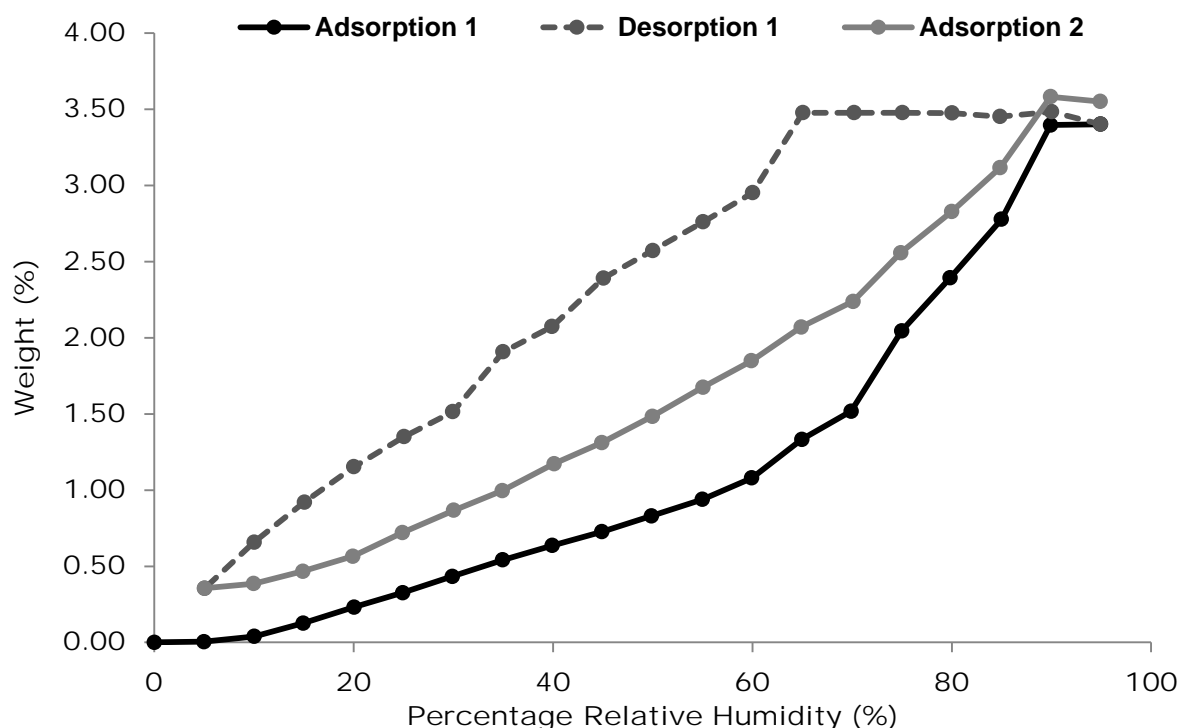


Figure 5: Vapor sorption isotherm obtained for amorphous azithromycin. The isotherms were obtained at 40°C with humidity variation of 0 – 95%, 95 – 5% and 5 – 95% RH.

The moisture isotherms in Figures 4 and 5 indicate some degree of hysteresis, however there is no indication of recrystallization of the amorphous form due to the exposure to a high water vapor environment. The residual sample of each vapor sorption experiment was tested in terms of recrystallization through XRPD analyses. In both cases (i.e. 25 and 40°C), the XRPD analyses confirmed the amorphous habit of the drug. From the vapor sorption studies it is evident that amorphous azithromycin prepared through the quench cooling of the melt does not result in the recrystallization of azithromycin to the most stable dihydrate solid-state form. Thus, no solvent-mediated phase transformation occurs. This is considered to be very positive in terms of pharmaceutical processing, dosage form formulation as well as storage of not only the bulk drug (in amorphous form) but also storage of solid dosage forms containing amorphous azithromycin.

Solution-mediated recrystallization of amorphous azithromycin

The reason why amorphous solid-state forms are so interesting to pharmaceutical researchers lies within the advantages exhibited by these solid-state forms. Currently, a great number of literature reports are available which discuss the pros and cons associated with amorphous solid-state forms. The greatest advantage is probably that of improved aqueous solubility of a drug which can exist in the amorphous form; however this advantage

is mostly counteracted by the disadvantage of the recrystallization of the amorphous form to the most stable crystalline form upon exposure to water or dissolution media.

During this study the recrystallization of amorphous azithromycin was tested during dissolution testing at different dissolution media temperatures. The rationale was to determine if solution-mediated phase transformation will occur during exposure of amorphous azithromycin to a sufficient amount of water that would allow an azithromycin solution. Furthermore, to determine the kinetics involved during a solution-mediated transformation process of amorphous azithromycin.

Figure 6 depicts the dissolution profiles obtained for crystalline azithromycin dihydrate and amorphous azithromycin in distilled water as dissolution medium at 25, 30 and 35°C. From the dissolution profiles it is evident that amorphous azithromycin exhibit typical dissolution behavior of a metastable solid-state form ^[20,21,22,23,24]. Solution-mediated phase transformation occurred at all three temperatures and the rate of the transformation process increased with an increase in temperature.

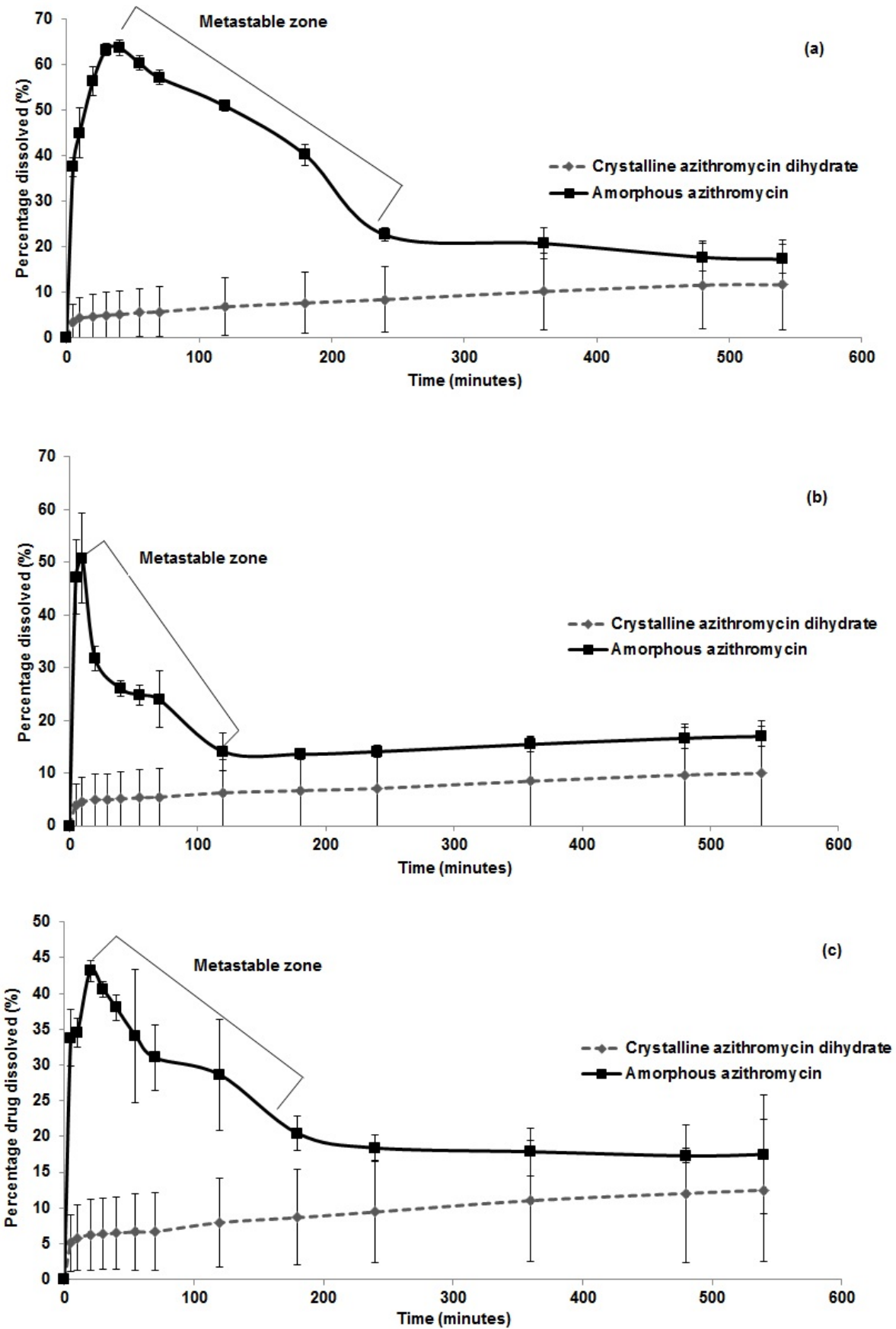


Figure 6: Dissolution profiles of crystalline azithromycin dihydrate and amorphous azithromycin at dissolution medium temperatures of (a) 25, (b) 30 and (c) 35°C.

The dissolution profiles show the typical “spring and parachute” approach as first described by Guszmán *et al.*,^[23]. This approach is being applied to instances where the bioavailability of a drug is limited by the dissolution rate thereof. Azithromycin is classified as a Biopharmaceutics Classification System (BCS) Class II drug, with low solubility and high permeability^[25]. Guszmán *et al.*,^[23] described this approach as a method to promote and maintain supersaturation of drugs in solution. A higher energy state of the drug, in this case the amorphous solid-state form provides the “spring”, which is signified by a significant increase in the dissolution and drug absorption rate. However, crystallization of the amorphous form to the thermodynamically most stable solid-state form of the drug will limit this “spring” effect, which is typically signified by a sharp decrease (within minutes) of the dissolved concentration of the drug. However, literature reports that combination of drugs with suitable polymers, or crystalline drugs either in salt forms or cocrystals will counteract this rapid decrease in drug concentration due to inhibition of nucleation and crystal growth of the most stable crystalline form of the drug^[20,23]. This leads to the “parachute” phase, where precipitation or sudden “crashing out” of the drug is prevented by stabilization of the supersaturated drug solution.

Interestingly, amorphous azithromycin showed a relative long period constituting the “parachute” or metastable zone, without the addition of any stabilizing polymers or additive excipients. From Figure 6 it is evident that the metastable zone ends at approximately 240 minutes for dissolution conditions at ambient temperature and 120 minutes for dissolution temperatures of 30 and 35°C. Therefore, it can be deduced that the peak solubility concentration for amorphous azithromycin remains for a relative long period of time, depending on the temperature at which the supersaturated solution is maintained. This would ultimately give rise to higher dose solubility and possible improved bioavailability due to the fact that the phase transformation from amorphous to the dihydrate form of azithromycin is a slow process within an aqueous environment.

Figure 7 depicts an overlay of the dissolution profiles obtained for amorphous azithromycin as well as the degree of crystallinity determined from heat of solution data at 120 minutes. These results showed that 99.5% of recrystallization of amorphous to the dihydrate form of azithromycin occurred in approximately 120 minutes at a temperature of 30°C, while only 63.7% and 76.9% of recrystallization occurred, within the same time period, at 25 and 35°C, respectively. The heat of solution data obtained during the study was used to construct a plot of degree of conversion (α) versus time (Figure 8). From the plotted graph one can clearly observe the difference in conversion rate as a function of temperature, with a complete conversion of amorphous azithromycin to the stable azithromycin dihydrate in a period of 120 minutes at 35°C. The conversion took substantially longer at 25 and 30°C,

with complete transformation of the amorphous form to the dihydrate at approximately 480 minutes. This conversion is 4 times slower than that obtained at 35°C.

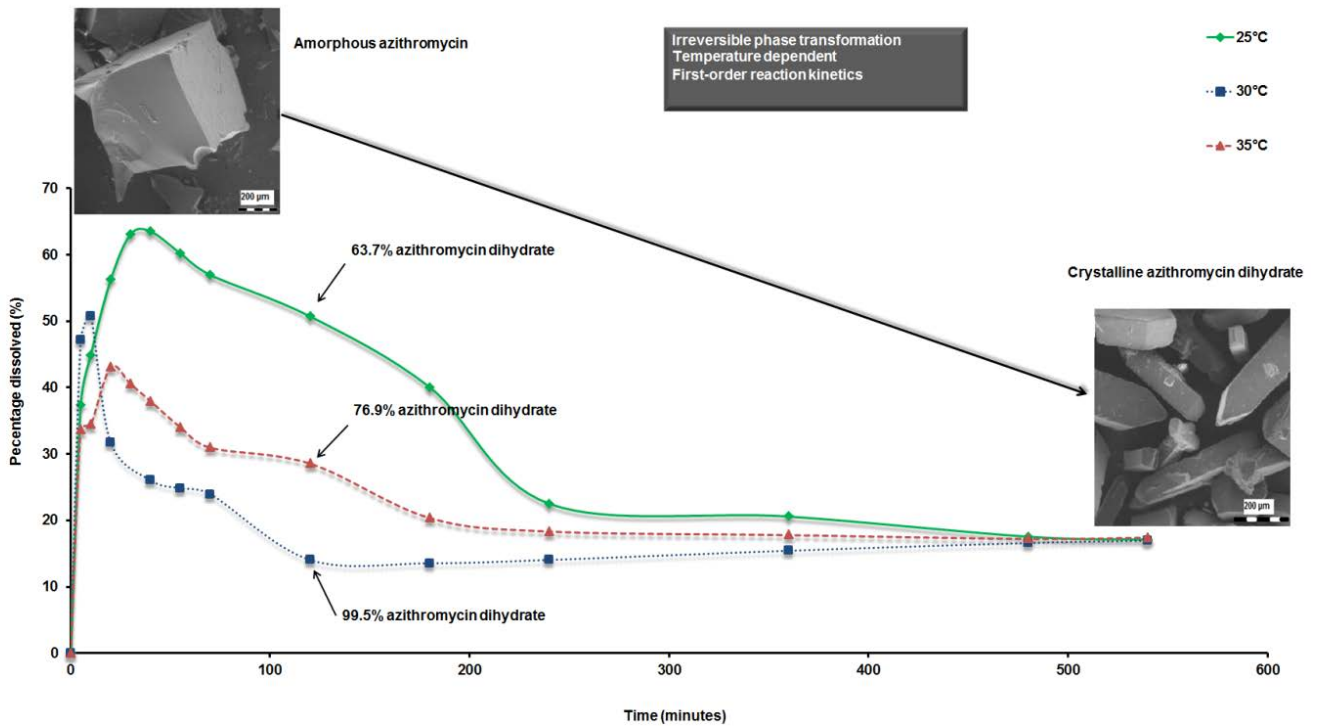


Figure 7: Dissolution profiles of amorphous azithromycin in water obtained at dissolution medium temperatures of 25, 30 and 35°C and (a), (b), (c) denoting the percentage of crystalline azithromycin present as undissolved drug within the dissolution vessels at 120 minutes.

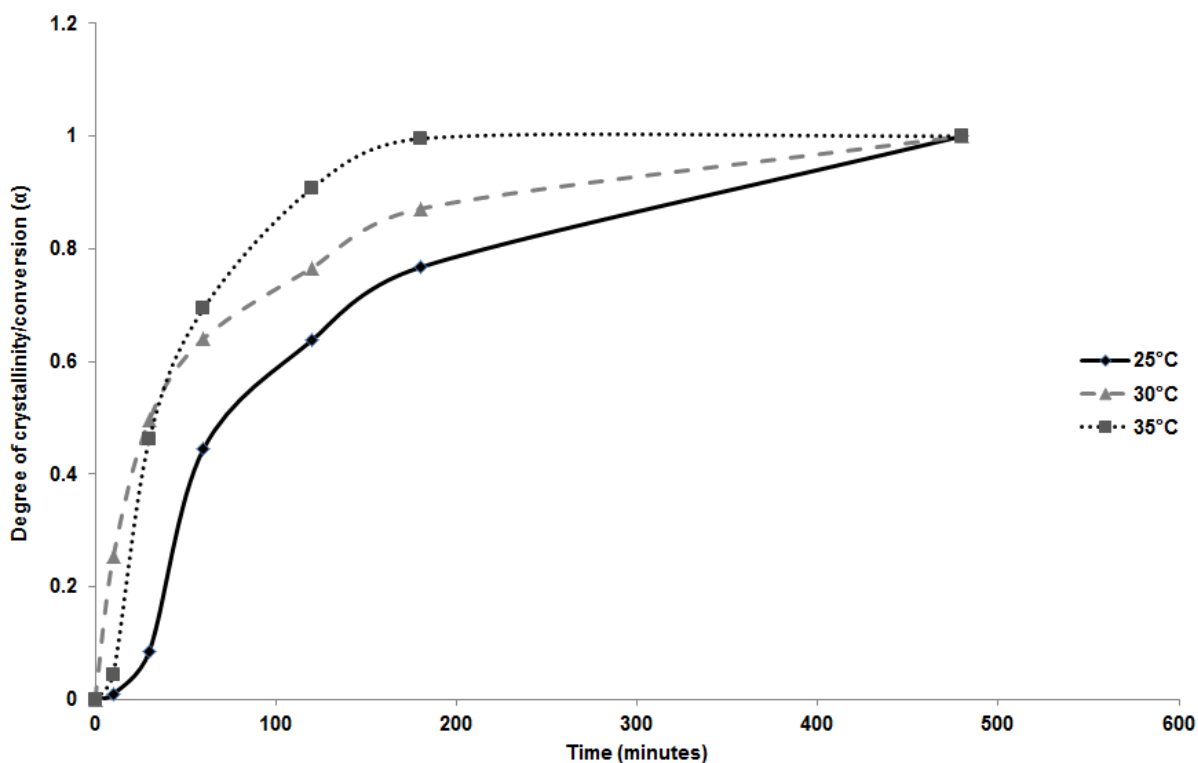


Figure 8: Graph depicting the degree of conversion of amorphous azithromycin to azithromycin dihydrate over a time range of 0 to 480 minutes, in distilled water at temperatures of 25, 30 and 35°C.

Recrystallization kinetics of the solution-mediated transformation of amorphous azithromycin

From the preceding data it is clear that temperature is a key determinant in terms of phase conversion of solid-state forms of drugs. Since it was possible to calculate the degree of crystallinity from heat of solution data, it was also possible to calculate the percentage of the amorphous form retained during the dissolution experiments at different temperatures. Using this data and applying the Arrhenius equation (eq. 6) it was possible to calculate the activation energy (E_a) associated with the transformation process of amorphous to the most stable solid-state form of azithromycin.

$$\ln(k) = [\ln A] - \frac{E_a}{RT} \quad (6)$$

Figure 9 depicts the plots obtained from conversion data as a function of time. A plot of the natural logarithm of the percentage amorphous form retained during solution-mediated phase transformation showed the best linear fit. The slope of each linear plot was used to obtain the rate constants at each individual temperature.

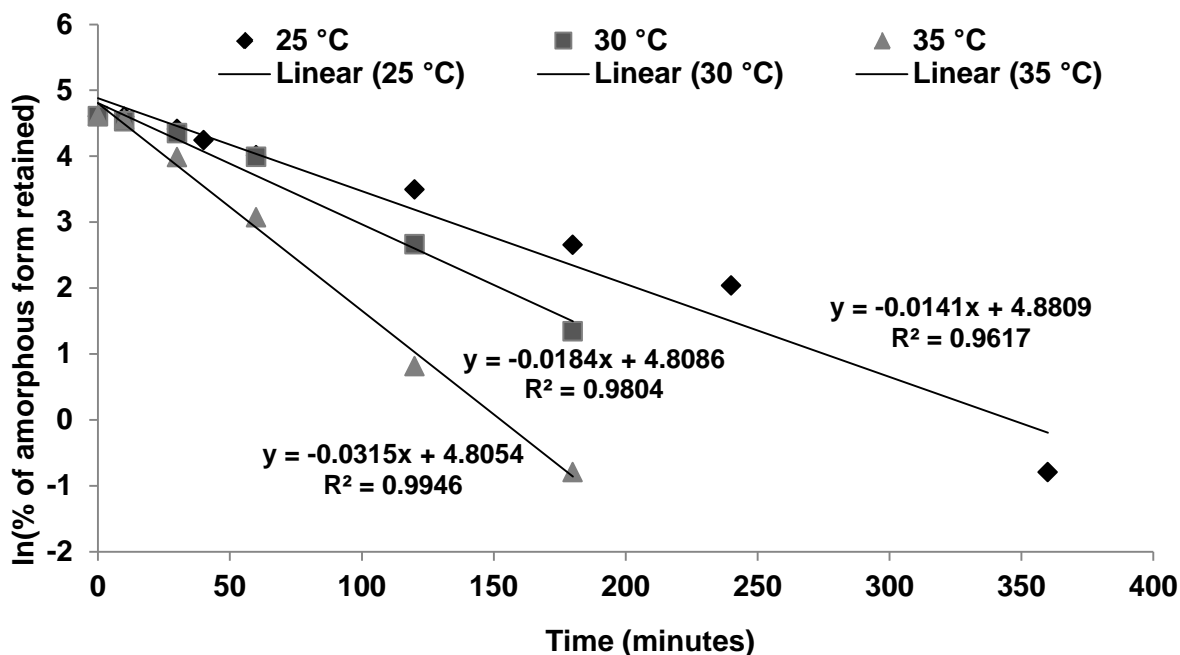


Figure 9: Graph constructed from the calculated percentage of amorphous solid-state form retained as a function of time during solution-mediated studies conducted at 25, 30 and 35 °C.

If the temperatures used during the experiments are converted to inverse absolute temperatures and the logarithms of the rate constants are used, a plot of $\ln[k]$ versus $1000/T$ (Figure 10) can be obtained. By applying the Arrhenius plot, activation energy (E_a) of 61.21 $\text{kJ}\cdot\text{mol}^{-1}$, was calculated for the solution-mediated transformation of amorphous azithromycin to stable azithromycin dihydrate. First-order rate kinetics was found to model the solution-mediated transformation the best.

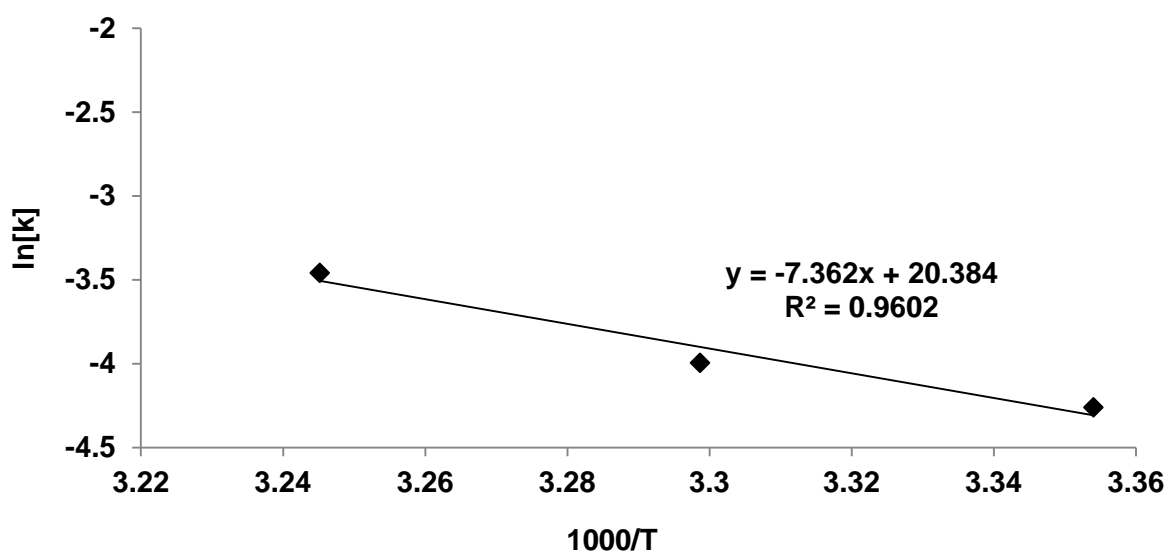


Figure 10: An Arrhenius plot obtained from degree of conversion data as a function of temperature.

The true solubility advantage of amorphous azithromycin

In the case of amorphous solid-state forms of drugs the traditional method of solubility determination, where solvent is added to an excess amount of drug, followed by the agitation of the sample for a sufficiently long enough period of time to obtain equilibrium, is not a suitable method. This is due to the fact that amorphous forms of drugs convert to the thermodynamically stable solid-state form during exposure to sufficient quantities of moisture. Thereby, the true solubility or apparent solubility of an amorphous solid-state form cannot be determined through applying the general equilibrium solubility method. When the use and study of amorphous solid-state forms became more prevalent within the pharmaceutical research field, several approaches have been applied to accurately quantify the solubility advantage of these solid-state forms. A popular approach is that of Hancock and Parks^[24,26] in which measured thermal properties of an amorphous and crystalline form of any given drug is used to determine a solubility ratio. This ratio is then used as an indication of possible improved aqueous solubility.

Another approach is the application of the Nogami method^[21,22,27]. With this method the solubility of a metastable compound is determined from dissolution determinations of samples taken at equal time intervals (δ). With this method the following relationship applies:

$$C(t + \delta) = S[1 - \exp(-k\delta)] + \exp(-k\delta) Ct \quad (7)$$

$$C(t + \delta) = Ct \quad (8)$$

If the concentration dissolved at $t + \delta$ is plotted *versus* the amount at time t , a linear plot will result. From the slope of this plot k can be obtained and if this is inserted in the intercept expression of equation 7, the solubility (S) can be calculated. Using the Nogami method, the solubility (S) for amorphous azithromycin was determined to be 580 $\mu\text{g/ml}$, this is an 8-fold improvement in comparison with the equilibrium solubility of $69.7 \pm 4.9 \mu\text{g/ml}$ determined for azithromycin dihydrate.

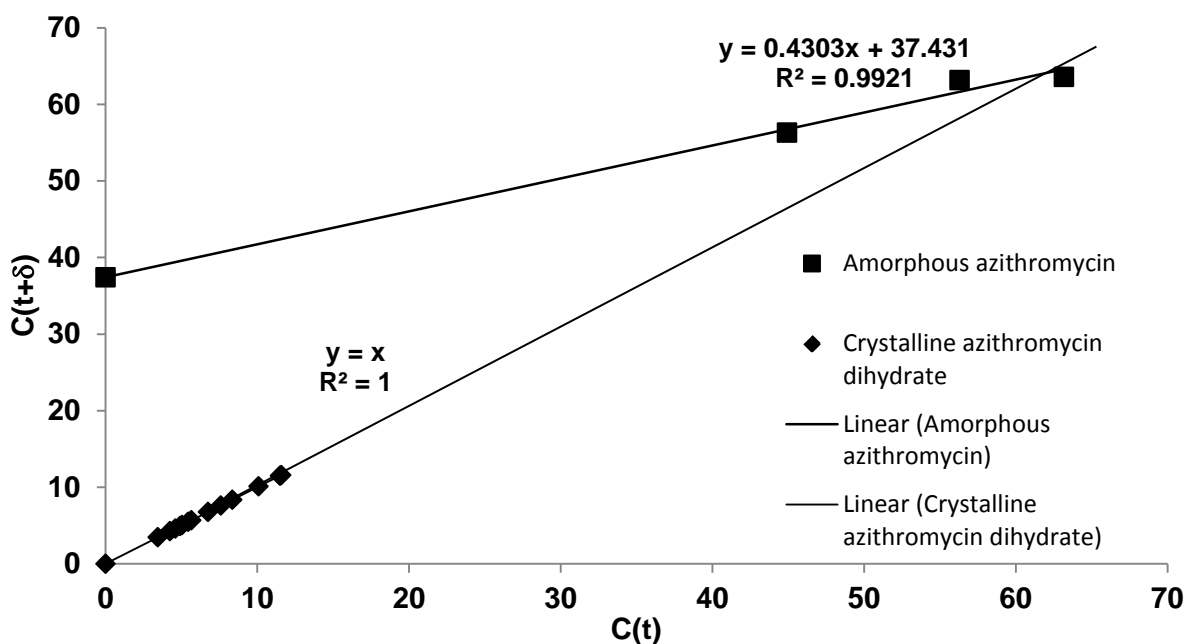


Figure 11: A Nogami plot of the dissolution data obtained for amorphous and crystalline azithromycin dihydrate in water at 25°C.

CONCLUSION

During this study the stability of amorphous azithromycin, prepared through the quench cooling of the melt method was investigated. Both the fragility index and strength parameter proved that amorphous azithromycin exhibit strong glassy behavior. This is considered an advantage especially in terms of pharmaceutical processing steps, i.e. milling, grinding and mixing. However, the fact that a drug shows excellent physical stability does not imply that the amorphous solid-state form will, by default, remain stable throughout an array of processing and storage conditions. With this in mind, it was considered important to investigate the possibility of recrystallization of amorphous azithromycin when exposed to either vapor or solution. Interestingly, a sufficiently high degree of relative humidity (25°C / 95%RH and 40°C / 95%RH) did not induce solvent-mediated recrystallisation of the amorphous drug. This proved that amorphous azithromycin will remain stable even upon exposure to high humidity environments. On the other hand, recrystallization of the amorphous form occurred when exposed to dissolution medium. The recrystallization kinetics involved during the solution-mediated phase transformation of amorphous azithromycin was investigated and it was proved that this phase transformation followed first-order reaction kinetic behavior. Interestingly, the results also showed that although solution-mediated phase transformation occurs, it is not a rapid transformational process. This is also considered to be an advantageous characteristic of amorphous azithromycin, due to the fact

that a sufficient amount of time is still available for dissolution and solubility of the drug to occur before the transformation process to the less soluble solid-state form will commence. Thereby, indicating that the amorphous form of azithromycin will provide a higher dissolved concentration of the drug when administered orally and that this higher dissolved concentration will remain for a sufficiently long enough period of time, which in turn could ensure improved bioavailability. The true solubility advantage of amorphous azithromycin was determined to be an 8-fold improvement. In terms of solubility, dissolution and bioavailability, this is considered an extremely significant solubility improvement. Overall, amorphous azithromycin exhibit exceptional stability properties, which in some cases is somewhat unexpected and the opposite of what is observed with amorphous states of other macrolide antibiotics and other drug classes.

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 (Pharmacén) at the North-West University, Potchefstroom, South Africa. Without their contribution, this study would not have been possible.

DECLARATION OF INTEREST

The authors declare no conflict of interest.

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.

REFERENCES

- [1] Blagden N, de Matas M, Gavan PT, York P. Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates. *Adv. Drug Deliv. Rev.* 2007; 59: 617 – 630.
- [2] Savolainen M, Kogermann K, Heinz A, Aaltonen J, Peltonen L, Strachan C, Yliruusi J. Better understanding of dissolution behaviour of amorphous drugs by in situ solid-state analysis using Raman spectroscopy. *Eur. J. Pharm. Sci.* 2007; 71: 71 – 79.
- [3] Sinclair W, Leane M, Clarke G, Dennis G, Dennis A, Tobyn M, Timmins P. Physical stability and recrystallization kinetics of amorphous ibipinabant drug product by fourier transform raman spectroscopy. *J. Pharm. Sci.* 2011; 100(11):4867 – 4699.
- [4] Cardew PT, Davey RJ. The kinetics of solvent-mediated phase transformations. *Proc. R. Soc. London*, 1985; A398, 415 – 428.
- [5] Greco K, Bogner R. Solution-mediated phase transformation: significance during dissolution and implications for bioavailability. *J. Pharm. Sci.* 2012; 101:2996 – 3018.
- [6] Adrjanowicz K, Zakowiecki D, Kaminski K, Hawelek L, Grzybowska K, Tarnacka M, Paluch M, Cal K. Molecular dynamics in supercooled liquid and glassy states of antibiotics: azithromycin, clarithromycin and roxithromycin studied by dielectric spectroscopy. Advantages given by the amorphous state. *Mol. Pharm.* 2012; 9:1748 – 1763.
- [7] Aucamp M, Odendaal R, Liebenberg W, Hamman J. Amorphous azithromycin with improved aqueous solubility and intestinal membrane permeability. *Drug Dev Ind Pharm.* 2015; 41(7):1100 – 1108.
- [8] Odendaal RW, Liebenberg W, Aucamp ME. A novel reversed-phase LC method for quantitative detection of azithromycin in bulk drug and tablet formulations in various aqueous media. *Pharmazie*, 2012; 67:984-986.
- [9] Kaushal AM, Bansal KA. Thermodynamic behavior of glassy state of structurally related compounds. *Eur. J. Pharm. Biopharm.* 2008; 69:1067–1076.
- [10] Moynihan CT, Eastal AJ, Wilder J, Tucker J. Dependence of the glass transition temperature on heating and cooling rate. *J. Phys. Chem.* 1974; 78(26):2673 - 2677.
- [11] Ramos JJM, Taveira-marques R, Diogo HP. Estimation of the fragility index of indomethacin by DSC using the heating and cooling rate dependency of the glass transition. *J. Pharm. Sci.* 2003; 93(6):1503 - 1507

- [12] Yu L, Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Adv. Deliv. Rev.*, 2001; (48) :27-42.
- [13] Shalaev EY, Zografi G. How does residual water effect the solid-state degradation of drugs in the amorphous state? *J. Pharm. Sci.* 1996; 85, 1137-1141.
- [14] Hancock BC, Zografi G. Characteristics and significance of the amorphous state in pharmaceutical systems. *J. Pharm. Sci.* 1997; 1(86): 1-12.
- [15] Hancock BC, Dalton CR, Pikal MJ, Shamblin, SL. A pragmatic test of a simple calorimetric method for determining the fragility of some amorphous pharmaceutical materials. *Pharmaceut. Res.* 1998; (15): 762-767.
- [16] Angell CA. Why $C_1 = 16 - 17$ in the WLF equation is physical-and the fragility of polymers. *Polymer.* 1997; 38(26): 6261-6266.
- [17] Lu Q, Zografi G. Properties of citric acid at the glass transition. *J. Pharm. Sci.* 1997; 86(12):1374-1378.
- [18] Burnett DJ, Thielmann F, Booth J. Determining the critical relative humidity for moisture-induced phase transitions. *Int. J. Pharm.* 2004; 287:123 – 133.
- [19] Hancock BC, Zografi G. The use of solution theories for predicting water vapor absorption by amorphous pharmaceutical solids: A test of the Flory-Huggins and Vrentas models. *Pharmaceut. Res.* 1993; 10(9): 1262 – 1267.
- [20] Babu NJ, Nangia A. Solubility Advantage of Amorphous Drugs and Pharmaceutical Cocrystals. *Cryst. Growth Des.* 2011; 11:2662 – 2679.
- [21] Carstensen JT. Preformulation. In: Swarbrick J. ed. *Drug stability: principles and practices.* New York: Marcel Dekker, 1990:263 – 269.
- [22] Carstensen JT. Preformulation. In: Carstensen JT, Rhodes CT. eds. *Drug stability: principles and practices.* New York: Informa Healthcare, 2007:237 – 249.
- [23] Guszmán HR, Tawa M, Zhang Z, Ratananbanangkoon P, Shaw P, Gardner CL, Chen H, Moreau JP, Almarsson Ö, Remenar JF. Combined use of crystalline salt forms and precipitation inhibitors to improve oral absorption of celecoxib from solid oral formulations. *J. Pharm. Sci.* 2007; 96, 2686-2702.
- [24] Hancock BC, Parks M. What is the true solubility advantage for amorphous pharmaceuticals?. *Pharmaceut. Res.* 1999; 17(4): 397-404.
- [25] Adebayo AS, McFarlane N. Model-based bioequivalence assessment of a commercial Azithromycin capsule against Pfizer Zithromax® Tablet marketed in Jamaica. *JAPS.* 2014; 4(10):062 – 068.

- [26] Murdande SB, Pikal MJ, Shanker RM, Bogner RH. Solubility advantage of amorphous pharmaceuticals: I. A thermodynamic analysis. *J. Pharm. Sci.* 2010; 99(3):1254 – 1264.
- [27] Nogami H, Nagai T, Suzuki A. Studies on powdered preparations. XVII. Dissolution rate of sulfonamides by rotating disk method. *Chem. Pharm. Bull.* 1966; 14(4):329 – 338.

Chapter 6

Concluding remarks

Azithromycin is a 15-membered derivative (azolide) macrolide that is chemically modified from the 14-membered erythromycin macrolide. This chemical modification increased the efficacy and by that, giving it a greater advantage above other macrolide antibiotics, making azithromycin the most prescribed and used macrolide antibiotic worldwide. With every advantage in life there is also a disadvantage present. The biggest disadvantage of azithromycin is surely its poor water solubility, giving an oral bioavailability of about 37% after oral administration. Poor water solubility of an active pharmaceutical ingredient (API) is seen as a critical factor, which can have a detrimental effect on not only the bioavailability of the API, but also the effective treatment of patients.

An API may exist in different solid-state forms such as crystalline polymorphs, solvates, hydrates, co-crystals, salts and even non-crystalline amorphous forms. These different forms have the same chemical structure and composition but can display different physico-chemical and thermodynamic properties. These differences can further influence properties such as API stability, solubility, dissolution rate, bioavailability, particle morphology, powder flow, powder colour and tableting behaviour (Holzgrabe *et al.*, 1999). It is therefore of great importance during the drug development and pre-formulation processes to do a complete physico-chemical characterisation of an API. Not only does this step assist in identifying the better water soluble solid-state form of a given API, it might also help to choose the solid-state form with the best stability characteristics. Usually, the most thermodynamic stable form is picked for the development of final pharmaceutical products (Craig *et al.*, 1999; O'Neil & Edwards, 2011).

The physiological absorption of a solid dosage form involves the dissolution of the API in the gastrointestinal tract (GIT) and the rate and extent of that dissolution can greatly influence the successful treatment of a patient (Bernstein, 2002). The better the dissolution rate of an API, the better is the absorption from the GIT, leading to improved bioavailability of the API. The most important reason for the research and investigation of the preparation, characteristics and stability of amorphous solid-state forms is that of possible improved bioavailability of a poorly water soluble API. One very easy and effective method to improve the aqueous solubility of any given API is to prepare an amorphous solid-state form of that API. The higher free energy and higher degree of molecular mobility of amorphous solid-state forms lead to an increased dissolution rate (Craig *et al.*, 1999). However, the advantage of improved solubility and dissolution rate due to the amorphous state of an API

is usually countered by the inherent instability of these solid-state forms. Although the higher molecular free energy is a property that is sought after, it is also that aspect which makes amorphous solid-state forms unstable, especially when exposed to solutions.

Preparing different amorphous solid-state forms of any given API is still a much debated topic within the field of solid-state properties of API's. The scope of this study was therefore of great importance as different amorphous forms of azithromycin could create the opportunity to choose the ideal amorphous form of azithromycin. The principal goal was to investigate the impact and to illustrate the effect of the different preparation methods on the physico-chemical characteristics of each obtained amorphous solid-state form of azithromycin. Preparation methods such as quench cooling of the melt, slow cooling of the melt, hot-air melting, ambient solvent evaporation, rapid solvent evaporation and spray-drying, all with different intermediary states (melt or solution), were identified and selected. The possibility of solution-mediated, solvent-mediated and solid-solid phase transformations of amorphous azithromycin was also investigated.

The amorphous habit of the different preparation techniques in this study were positively characterised by means of XRPD and FTIR and clearly showed that each mentioned preparation method can indeed create amorphous forms of azithromycin. The thermodynamic properties of all amorphous forms were also explored and the results purvey that none of these forms showed any thermally induced recrystallization during the heating process and they all reflected a fragility index (m) lower than 75 and a high strength parameter (D), meaning that a lower energy is present that will lead to a higher physical stability. As mentioned, the different preparation methods had two different intermediary states, namely melt and solution. The amorphous solid-state forms that were prepared from a melt intermediary state all showed similar fragility indices, strength parameters and temperature of zero mobility. Those two amorphous forms prepared from a solution intermediary state showed significantly higher temperatures of zero mobility making them more physically stable during ambient storage conditions. On the other hand, the amorphous form prepared from a spray-drying technique also showed the lowest activation energy for structural relaxation, thereby contradicting the temperature of zero mobility finding of this amorphous form.

The stability amorphous forms can also be influenced by means of solid-solid phase transformation, solvent-mediated phase transformation and solution-mediated phase transformation (SMPT). These methods can trigger the crystallisation of an amorphous form to the most thermodynamically stable crystalline form. None of the amorphous forms investigated in this study showed the occurrence of solid-solid phase transformations. Solvent-mediated phase transformation was also investigated through vapour sorption

experiments and it was proven that the different amorphous forms are not influenced by solvent-mediated phase transformations. An easy and effective method, namely dissolution was used to investigate the possibility of SMPT of the amorphous forms. Nucleation and crystal growth of a more stable solid-state form of azithromycin (A-DH) was identified during dissolution testing. It was concluded that SMPT occurred with each amorphous solid-state form of azithromycin. From the dissolution profiles it became also evident that there exist significant differences between the different amorphous forms in terms of the SMPT process and rates as well as with regards to the dissolution rate of the amorphous forms. The rates of SMPT differed and in some instances the transformation was not visible. This study showed how the dissolution of amorphous azithromycin is detrimentally affected due to the metastability of the prepared amorphous forms. The dissolution behaviour of each amorphous form was brought into context with surface area and particle morphology.

Another part of the study was the investigation of the recrystallization kinetics of amorphous azithromycin prepared by the quench cooling of the melt. Through investigation of the recrystallization behaviour of amorphous quench cooled azithromycin, it became evident that the recrystallization process followed a first-order reaction rate. An 8-fold solubility enhancement in comparison with the solubility of crystalline azithromycin was determined by applying a Nogami plot.

From the results generated, it is evident that more than one amorphous form of azithromycin can be successfully prepared. However, there are some differences between the different amorphous forms, giving the advantage on selecting the amorphous form with the most desired physical, chemical and thermodynamic properties. However, the fact that a drug shows excellent physical stability does not imply that the amorphous solid-state form will, by default, remain stable throughout an array of processing and storage conditions. Overall, amorphous azithromycin exhibit exceptional stability properties, which in some cases is somewhat unexpected and the opposite of what is observed with amorphous states of other macrolide antibiotics and other drug classes.

References list

Bernstein, J. 2002. Polymorphism in molecular crystals. Clarendon, Oxford: Oxford University press.

Craig, D.Q.M., Royall, P.G., Kett, V.L. & Hopton, M.L. 1999. The relevance of the amorphous state to pharmaceutical dosage forms: glassy drugs and freeze dried systems. *International journal of pharmaceutics*, 179:179-207.

Holzgrabe, U., Wawer, I. & Diehl, B. eds. 1999. NMR spectroscopy in drug development and analysis. Weinheim:Wiley.

O'Neil, A. & Edwards, H. 2011. Spectroscopic characterization. (*In* Storey, R. A. & Ymén, I., ed. Solid state characterization of pharmaceuticals. West Sussex, United Kingdom: Wiley. p. 71-134).