

Investigation of amorphous solid-state forms of spiramycin and clarithromycin

RM Katsidzira
21252998

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

Promoter: Prof M Aucamp
Co-promoter: Prof W Liebenberg

May 2017

TABLE OF CONTENTS

TABLE OF CONTENTS.....	I
ACKNOWLEDGEMENTS	IV
ABBREVIATIONS	V
ABSTRACT	VII
CHAPTER 1	1
PRINCIPLES OF THE SOLID-STATE PROPERTIES OF PHARMACEUTICAL COMPOUNDS	1
1.1 INTRODUCTION	1
1.2 PHARMACEUTICAL SOLID-STATE FORM CLASSIFICATION	1
1.2.1 <i>The principles of the crystalline solid-state form</i>	3
1.2.1.1 Polymorphs.....	4
1.2.1.2 Solvates	7
1.2.1.3 Hydrates	8
1.2.1.4 Co-crystals	9
1.2.1.5 Salts	10
1.2.2 <i>The principles of the amorphous solid-state form</i>	11
1.2.2.1 Classification of solid dispersions	14
1.2.2.2 Amorphous solid dispersions (ASDs)	15
1.3 CONCLUSION	15
REFERENCES.....	17
CHAPTER 2	27
MACROLIDES ANTIBIOTICS: CLARITHROMYCIN AND SPIRAMYCIN	27
2.1 INTRODUCTION	27
2.2 MACROLIDES ANTIBIOTICS	27
2.3 PHYSICO-CHEMICAL PROPERTIES OF CLARITHROMYCIN	30
2.4 PHYSICO-CHEMICAL PROPERTIES OF SPIRAMYCIN.....	32
2.5 CONCLUSION	35
REFERENCES.....	37
CHAPTER 3	47
RESEARCH METHODOLOGY	47
3.1 INTRODUCTION	47
3.2 MATERIALS AND METHODS.....	47
3.2.1 <i>Materials</i>	47

3.2.2 Preparation of neat amorphous solid-state form of clarithromycin	47
3.2.3 Preparation of ASDs of clarithromycin and spiramycin.....	48
3.2.3.1 Miscibility determination	48
3.2.3.2 Preparation of ASDs.....	48
3.2.4 Characterisation methods.....	49
3.2.4.1 Differential scanning calorimetry (DSC).....	49
3.2.4.2 Hot-stage microscopy (HSM)	50
3.2.4.3 Fourier-Transform Infrared spectroscopy (FTIR)	51
3.2.4.4 X-Ray Powder Diffraction (XRPD).....	52
3.2.4.5 Scanning electron microscopy (SEM)	52
3.2.4.6 High-performance liquid chromatography (HPLC)	53
3.2.4.7 Solubility studies.....	54
3.2.4.8 Dissolution studies.....	55
3.2.4.9 Vapour sorption analysis	55
3.3 CONCLUSION	56
REFERENCES.....	57
CHAPTER 4	62
THE PHYSICO-CHEMICAL AND THERMOKINETIC PROPERTIES OF CRYSTALLINE AND AMORPHOUS CLARITHROMYCIN	62
4.1 INTRODUCTION	62
4.2 PREPARATION OF CLARITHROMYCIN NEAT AMORPHOUS FORM	62
4.3 PHYSICO-CHEMICAL CHARACTERISATION OF CRYSTALLINE CLARITHROMYCIN AND THE NEAT AMORPHOUS FORM	63
4.3.1 Differential scanning calorimetry.....	63
4.3.2 X-ray power diffraction	64
4.3.3 Fourier-transform infrared spectroscopy (FT-IR).....	67
4.3.4 Scanning electron microscopy.....	69
4.3.5 Hot-stage microscopy (HSM)	70
4.3.6 Non-isothermal crystallisation kinetics of amorphous clarithromycin.....	73
4.3.7 Vapour sorption analyses.....	76
4.3.8 Solubility studies	77
4.3.9 Dissolution studies	78
4.3.10 Discussion.....	79
4.4 PREPARATION OF CLARITHROMYCIN PHYSICAL MIXTURES	80
4.5 MISCIBILITY STUDIES OF CLARITHROMYCIN-POLYMER PHYSICAL MIXTURES.....	80
4.5.1 Differential scanning calorimetry.....	81
4.5.2 Hot-stage microscopy (HSM)	85
4.5.3 Discussion.....	89
4.6 PHYSICO-CHEMICAL CHARACTERIZATION OF SOLID DISPERSIONS OF CLARITHROMYCIN	90

4.6.1 Differential scanning calorimetry.....	90
4.6.2 X-ray powder diffraction	92
4.6.3 Infrared spectroscopy.....	94
4.6.4 Scanning electron microscopy.....	95
4.6.5 Dissolution studies	96
4.7 CONCLUSION	98
REFERENCES.....	100
CHAPTER 5	106
AMORPHOUS SOLID DISPERSIONS OF SPIRAMYCIN	106
5.1 INTRODUCTION	106
5.2 PHYSICO-CHEMICAL CHARACTERISATION OF SPIRAMYCIN RAW MATERIAL	106
5.2.1 Discussion.....	114
5.3 SPIRAMYCIN / POLYMER MISCIBILITY STUDIES	114
5.3.1 Differential scanning calorimetry.....	114
5.3.2 Hot-stage microscopy	117
5.3.3 Discussion.....	121
5.4 PHYSICO-CHEMICAL CHARACTERISATION OF AMORPHOUS SOLID DISPERSIONS OF SPIRAMYCIN.....	122
5.4.1 Differential scanning calorimetry.....	122
5.4.2 X-ray powder diffraction	124
5.4.3 Infrared spectroscopy.....	126
5.4.4 Scanning electron microscopy.....	129
5.4.5 Dissolution studies	130
5.4.6 Discussion.....	132
5.5 CONCLUSION	132
REFERENCES.....	134
CONCLUSION AND RECOMMENDATIONS	137
6.1 CONCLUSION	137
6.2 RECOMMENDATIONS.....	140
REFERENCES.....	141
ANNEXURE A	142

ACKNOWLEDGEMENTS

- I thank God Almighty for making this research project a success. Glory be to God for being my source of strength, hope, courage and perseverance.
- My sincere appreciation goes to Prof Marique Aucamp, my enthusiastic research supervisor, for her priceless mentorship, immense knowledge, support, precious time, patience, assistance in planning and development of this research work.
- My deep gratitude goes to Prof Wilna Liebenberg my research co-supervisor for her useful critics, advice, support and assistance during this research work.
- I am profoundly grateful to the faculty members of the Pharmaceutics North-West University especially to Prof Jeanetta DuPlessis, Prof Nicole Stieger and Neil Barnard for their valuable support.
- Many thanks to Mrs Elzabe Bekker for her encouragement and support.
- Financial assistance provided by General Board of Global Ministries (GBGM) and the National Research Foundation (NRF) is greatly appreciated.
- Most importantly, I would have not made it without constant prayers, love and support of my dear and lovely family: parents, brothers, sisters, brothers/sisters in law and friends. Special thanks to Phillip for the unwavering support, words can not express how grateful I am.

ABBREVIATIONS

API	active pharmaceutical ingredient
ARVs	antiretroviral drugs
ASDs	amorphous solid dispersions
BCS	biopharmaceutical classification system
CLAM	clarithromycin neat amorphous form
<i>D</i>	strength parameter
DSC	differential scanning calorimetry
DVS	dynamic vapour sorption
FDA	The U.S Food and Drugs Administration
FTIR	Fourier-transform infrared spectroscopy
HAART	Highly Active Antiretroviral therapy
HIV	human immunodeficiency virus
HPLC	High-performance liquid chromatography
HSM	hot-stage microscopy
Kollidon® VA-64	Vinylpyrrolidone-vinyl acetate copolymer
<i>m</i>	fragility index
MAC	<i>Mycobacterium avium complex</i>
mRNA	messenger RNA
OIs	opportunistic infections
PEG 8000	Polyethylene glycol 8000
PF-127	Pluronic® F-127
PLHIV	People Living with HIV
PM	Physical mixture
PVP K25	Polyvinylpyrrolidone K25
PVP K30	Polyvinylpyrrolidone K30
RH	relative humidity

rRNA	ribosomal ribonucleic acid
SEM	scanning electron microscopy
TGA	thermogravimetric analysis
T_g	glass transition temperature
T_K	Kauzmann temperature
T_m	melting temperature
tRNA	transfer RNA
T_0	zero mobility temperature
XRPD	X-ray powder diffraction
τ	relaxation time

ABSTRACT

Recent studies have shown OIs remain a significant cause of approximately 90% of the morbidity and mortality in PLHIV even in the era of HAART. MAC and cryptosporidiosis are amongst the most prevalent and life threatening OIs among PLHIV. Treatment of these OIs has shown poor outcomes across the globe together with recurring infections. There is dire need to reduce the burden of OIs by optimising treatments. In order to accomplish this, solid-state properties of clarithromycin and spiramycin were investigated. The drugs are indispensable in treatment of MAC and cryptosporidiosis especially in PLHIV. However, they have shown to be poorly water soluble and the culprit could be their poor physico-chemical properties, making investigation of these properties paramount. No other solid state forms of both drugs that might improve the poor aqueous solubility have been reported. Thorough investigation and alteration of physico-chemical properties of clarithromycin and spiramycin were therefore considered a solution for improving aqueous solubility and subsequently bioavailability to achieve optimum treatment outcomes. Of late the use of ASDs has been employed to improve aqueous solubility and stability of poorly soluble drugs.

The aim of the study was to prepare neat amorphous forms and ASDs of spiramycin and clarithromycin. HPLC method was developed and validated for identification and quantification of these drugs. Miscibility of clarithromycin and spiramycin in all available polymers was investigated for preparation of optimised ASDs. The quench cooling of the melt method was employed for preparation of CLAM and ASDs. Physical and chemical properties of spiramycin and clarithromycin raw materials, the prepared amorphous form of clarithromycin (CLAM), physical mixtures of the API with polymers (PMs) and prepared amorphous solid dispersions (ASDs) were investigated and reported on. The following characterisation techniques were used: DSC, FTIR, XRPD, SEM, HSM, vapour sorption analysis, equilibrium solubility and dissolution profiles of the macrolide antibiotics.

CLAM was physically stable at room temperature and high moisture content. Overall, the dissolution rate of clarithromycin was improved by approximately 6.5 times by ASDs. This will have a positive impact on its aqueous solubility. It was concluded that ASDs successfully enabled better control over the solid-state chemistry of clarithromycin by maintaining the API in a stable amorphous state and enhancing dissolution / solubility which will ultimately lead to improved treatment outcomes.

Results proved that spiramycin has a high dissolution rate (90%) due to its amorphous nature. ASDs improved dissolution rate of spiramycin to 100%. Although the 10% increase in dissolution might imply ASDs enhanced aqueous solubility of spiramycin to some extent, it was concluded that the poor treatment outcomes of spiramycin cannot be attributed to a slow

dissolution rate. Future studies on spiramycin will be necessary to clarify the discrepancies between current literature sources and data on the successful treatment of cryptosporidiosis.

Keywords: clarithromycin, spiramycin, macrolide antibiotics, solid-state forms, amorphous solid-state forms, amorphous solid dispersions, polymers, polymer mixture

CHAPTER 1

PRINCIPLES OF THE SOLID-STATE PROPERTIES OF PHARMACEUTICAL COMPOUNDS

1.1 Introduction

Pharmaceutical dosage forms can exist in different forms such as, liquids, solids and semi-solids. Even when formulated as liquids, an API (active pharmaceutical ingredient) is preferably manufactured as a solid form due to reasons such as ease of handling, better chemical stability and ease of purification by crystallisation (Vippagunta *et al.*, 2001). Whether as pure drug substances or in formulated pharmaceutical products, APIs can exist in various solid-state forms. These solid-state forms can be categorised as follows: crystalline (polymorphs, hydrates, solvates, salts and co-crystals) and non-crystalline (amorphous) (Baghel *et al.*, 2016). These different forms of the same API may present different physico-chemical properties (Karpinski, 2006), which in turn may have a direct impact on pharmacokinetic and pharmacodynamic properties of the API (Kesisoglou & Wu, 2008). APIs should have suitable pKa, aqueous solubility, permeability, stability and lipophilicity, to obtain optimum pharmacodynamic properties, such as bioavailability (Jampílek & Dohnal, 2012). Pharmaceutical excipients and processes applied during the drug development process can further influence physico-chemical properties of APIs (Dengale *et al.*, 2014; Feng *et al.*, 2015). It is a well-known fact that physical and chemical interactions between APIs and excipients can affect the chemical nature, stability, bioavailability and ultimately, the therapeutic efficacy of APIs (Bharate *et al.*, 2010). It is therefore paramount to be knowledgeable about the solid-state properties of an API in the initial stage of drug development to avoid problems during and after the manufacturing of a pharmaceutical product. Solubility and stability remain the most important pharmacokinetic properties influenced by the solid-state of APIs. This chapter will provide background and an in-depth discussion on the solid-state properties of APIs and how that is being influenced by different solid-state forms of an API. Furthermore, this chapter will also discuss all the strategies and methods currently used within the pharmaceutical industry in an endeavour to improve physico-chemical properties of an API.

1.2 Pharmaceutical solid-state form classification

Pharmaceutical solids consist of an external and internal structure. The external structure is characterised by the morphology, shape and habit of the particles and does not determine the solid-state form (Moynihan & Crean, 2009; O'Keeffe, 2012). On the other hand, the internal structure determines the solid-state form and is characterised by the order and degree of

molecular packing which can further be classified into two major categories i.e. crystalline and non-crystalline (amorphous) (Qiu *et al.*, 2009). Crystalline molecules are tightly bound to each other in an organised / orderly geometric lattice. This results into well-defined, long range order of repeating unit cells. The repetition of the unit cells forms the basis of each and every crystal of an API. In contrast, the amorphous solid-state form is defined by lack of such long range order, with the molecules arranged in a disorderly manner (Chieng *et al.*, 2011; Jójárt-Laczkovich & Szabó-Révész, 2011; Vippagunta *et al.*, 2001).

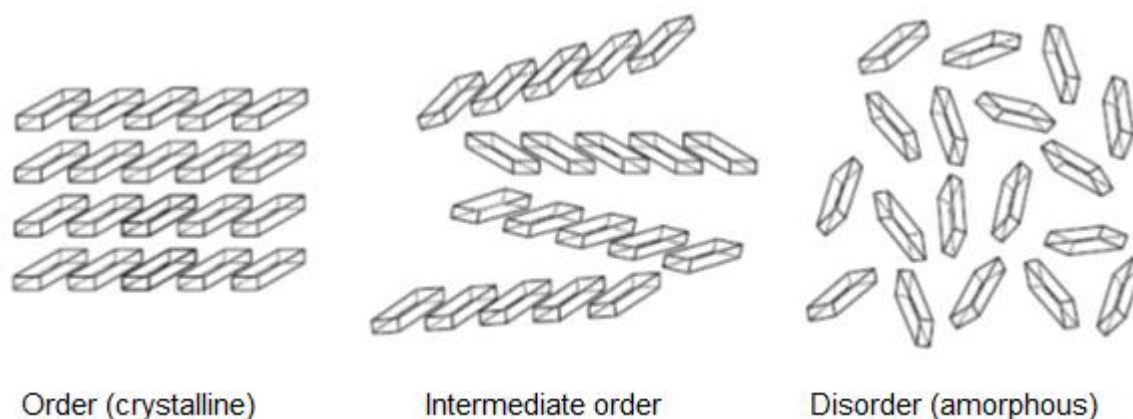


Figure 1.1: Molecular order in the solid-state (Newman & Byrn, 2003).

When the amorphous state exhibit traces of crystallinity or regions of heterogeneity, it is then comprised of a mixture of two discrete phases i.e. crystalline and amorphous (Iuraş *et al.*, 2016). Partially ordered systems can also be an indication of a mesophase, a phase consisting of some degree of orientational and sometimes positional order (Mugheirbi *et al.*, 2016; Pérez *et al.*, 2016). Both crystalline and amorphous solids, might exhibit mesophase behaviour under conditions of temperature / solvent change and dissolution (Chakravarty *et al.*, 2013). It is also quite possible that the three solid-state forms can co-exist independently of each other (Elder *et al.*, 2015). Thereby, in the broadest sense, APIs can be characterised into either crystalline or amorphous forms and the next few paragraphs will discuss the different forms in much greater detail.

1.2.1 The principles of the crystalline solid-state form

An ideal crystal is characterised by a three dimensional structure built up of identical and repeating unit cells (Brittain *et al.*, 2009; Storey & Ymen, 2011). Each unit cell is defined by the lengths of the crystal axis a , b and c and by the respective angles between these axes: α (between sides b and c), β (between sides a and c) and γ (between sides a and b) (Brittain *et al.*, 2009; Vippagunta *et al.*, 2001). Combination of these axis and angles gives rise to seven basic crystal unit cells i.e. cubic, trigonal, orthorhombic, triclinic, hexagonal, tetragonal and monoclinic, which are shown in Figure 1.2.

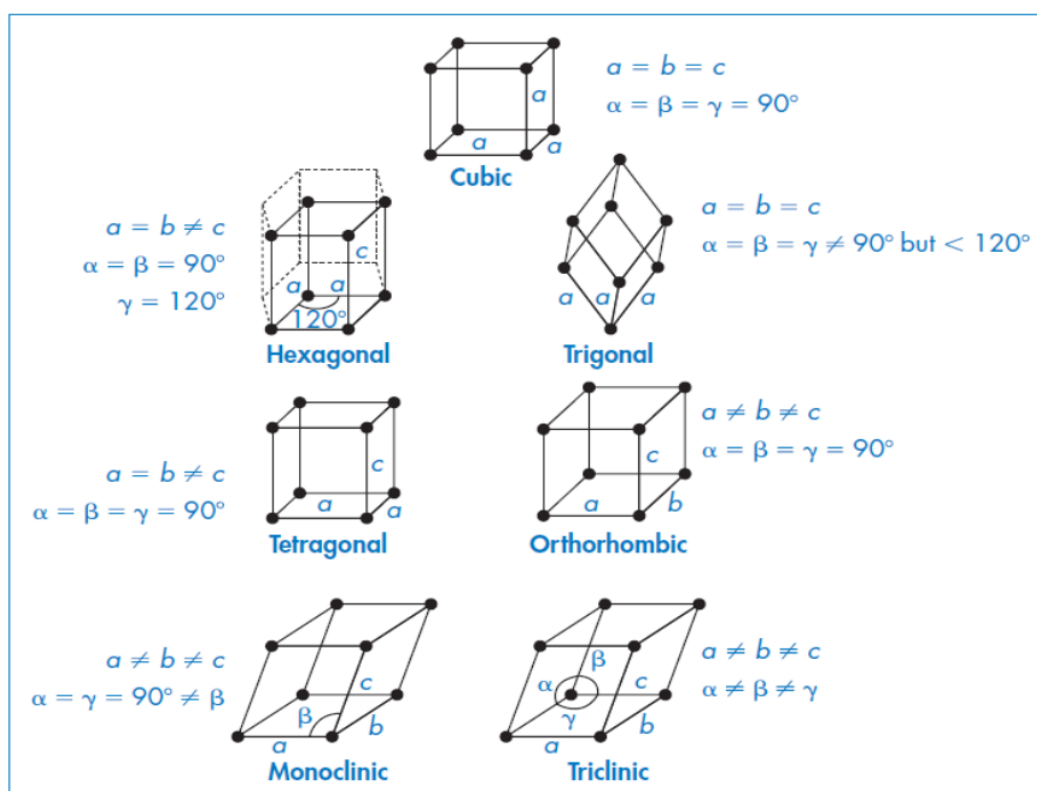


Figure 1.2: The seven possible primitive unit cells (Florence & Attwood, 2015).

Symmetry of the unit cell contents give rise to a total of 14 possible lattices termed the Bravais lattices which occur as a result of variation in cubic, orthorhombic, tetragonal and monoclinic unit cells (Brittain, 2009) as follow: i) monoclinic and orthorhombic unit cells may be end-centred whilst, ii) cubic and orthorhombic unit cells may be face centred and iii) cubic, tetragonal and orthorhombic unit cells may be body centred (Florence & Attwood, 2015). Drug molecules usually form only the triclinic, monoclinic or orthorhombic unit cells (Florence & Attwood, 2015). It is therefore the structural differences and or inclusions in these API crystal lattices that can further define, characterise and classify different solid forms of crystalline APIs as shown in Figure 1.3.

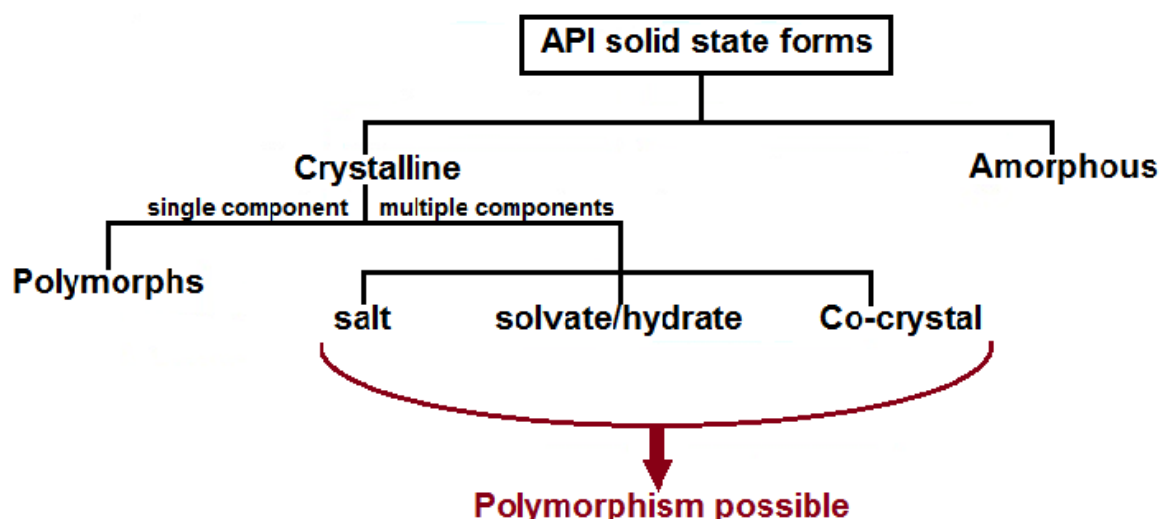


Figure 1.3: Classification of API solid forms as adapted from Steed, 2013.

It has been hitherto, unclear among researchers on the actual number of classes of pharmaceutical solids due to the variation and ambiguity of API solid forms (Aitipamula *et al.*, 2012; Grothe *et al.*, 2016). In this study, crystalline APIs have been sub-classified into (i) single-component APIs i.e. polymorphs, and (ii) multicomponent APIs i.e. salts, solvates or hydrates and co-crystals as discussed below.

1.2.1.1 Polymorphs

Up to date, literature provides numerous definitions of polymorphism. The U.S Food and Drugs Administration (FDA) as well as other authors use the term more broadly including solvates, hydrates and amorphous forms (Aitipamula *et al.*, 2012; Brog *et al.*, 2013). However, the definition that has survived the test of time defines polymorphism as “*the ability of a compound with an identical chemical composition to exist as two or more crystalline phases that have different arrangements and / or conformations of molecules within the crystal lattice*” (Brog *et al.*, 2013; Nagai *et al.*, 2014). Crystal polymorphs are single component solvent-free molecules characterised by structural differences in their crystal lattice (Braga *et al.*, 2009). They are formed when unit cells consisting of one chemical specie of molecules, crystallise in different crystal lattices, giving rise to different molecular packing or conformations as shown in Figure 1.4 (Brog *et al.*, 2013; Jampílek & Dohnal, 2012; Vippagunta *et al.*, 2001). Package polymorphs consist of rigid molecules with same / specific conformations that possess different intermolecular interactions such that they pack into different three-dimensional structures (Brog *et al.*, 2013; Jampílek & Dohnal, 2012). In contrast, conformation polymorphs consist of flexible molecules that crystallise differently due to possible rotation about single bonds in a molecule leading to different conformations / shapes (Brog *et al.*, 2013; Jampílek & Dohnal, 2012).

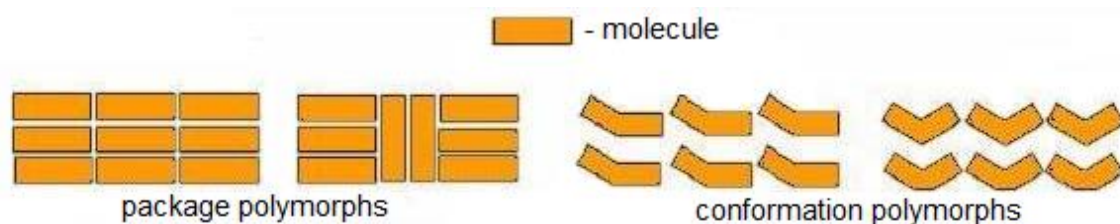


Figure 1.4: Illustration of packaging and conformational polymorphism (Jampílek & Dohnal, 2012).

Based on differences in the thermodynamic properties, polymorphs can be divided into two other categories i.e. enantiotropic and monotropic polymorphism (Vippagunta *et al.*, 2001) depending on their stability (Brittain, 2009). Enantiotropic polymorphs are formed when a reversible polymorphic transition from a metastable to a stable form occurs at a definite transition temperature below melting point (Brittain, 2009; Vippagunta *et al.*, 2001). In contrast, a pair is termed monotropic when it does not exhibit such a reversible polymorphic transition below its melting point. In other words, one polymorph remains stable whilst the other is unstable below melting point (Patel *et al.*, 2015). Even when the difference in free energy of polymorphs may be as little as 0.5 kcal/mol up to a maximum of about 8 kcal/mol (Purohit & Venugopalan, 2009), one form can be slightly unstable compared to other (Upadhyay *et al.*, 2012). As a result, any difference in crystal packing, molecular conformation, lattice energy and entropy (Lohani & Grant, 2006) may have significant impact on physical and chemical properties of an API (solubility and dissolution included) (Mazurek *et al.*, 2016).

Ideally, the more stable polymorph should be used in the final drug product (Censi & Di Martino, 2015), but metastable forms are more soluble than their corresponding stable polymorphic forms (Aulton & Taylor, 2013). However, differences between the solubility of two polymorphs may be insignificant thereby having no advantage in choosing the more soluble polymorph over the original compound (Censi & Di Martino, 2015). Controlling or avoiding polymorphism therefore remains a crucial but a challenging dilemma in pharmaceutical drug development. It is very critical to thoroughly understand the origins of polymorphism, its prediction as well as characterisation (Nagai *et al.*, 2014; Vippagunta *et al.*, 2001). Possible polymorphic forms can be determined by the aid of necessary identification steps for polymorphism in crystalline APIs as shown in Figure 1.5 (Brog *et al.*, 2013; Lu & Rohani, 2009). These polymorphic forms may have different lattice energies and may display different physical and chemical properties which may result in significantly different stability, solubility and bioavailability (Lu & Rohani, 2009). Thus, polymorph screening assist and enable selection and manufacture of the most desired polymorph that is thermodynamically stable, with good solubility and dissolution rate, non-hygroscopic, high melting point, compact morphology and unproblematic manufacturing process (Karpinski *et al.*, 2006).

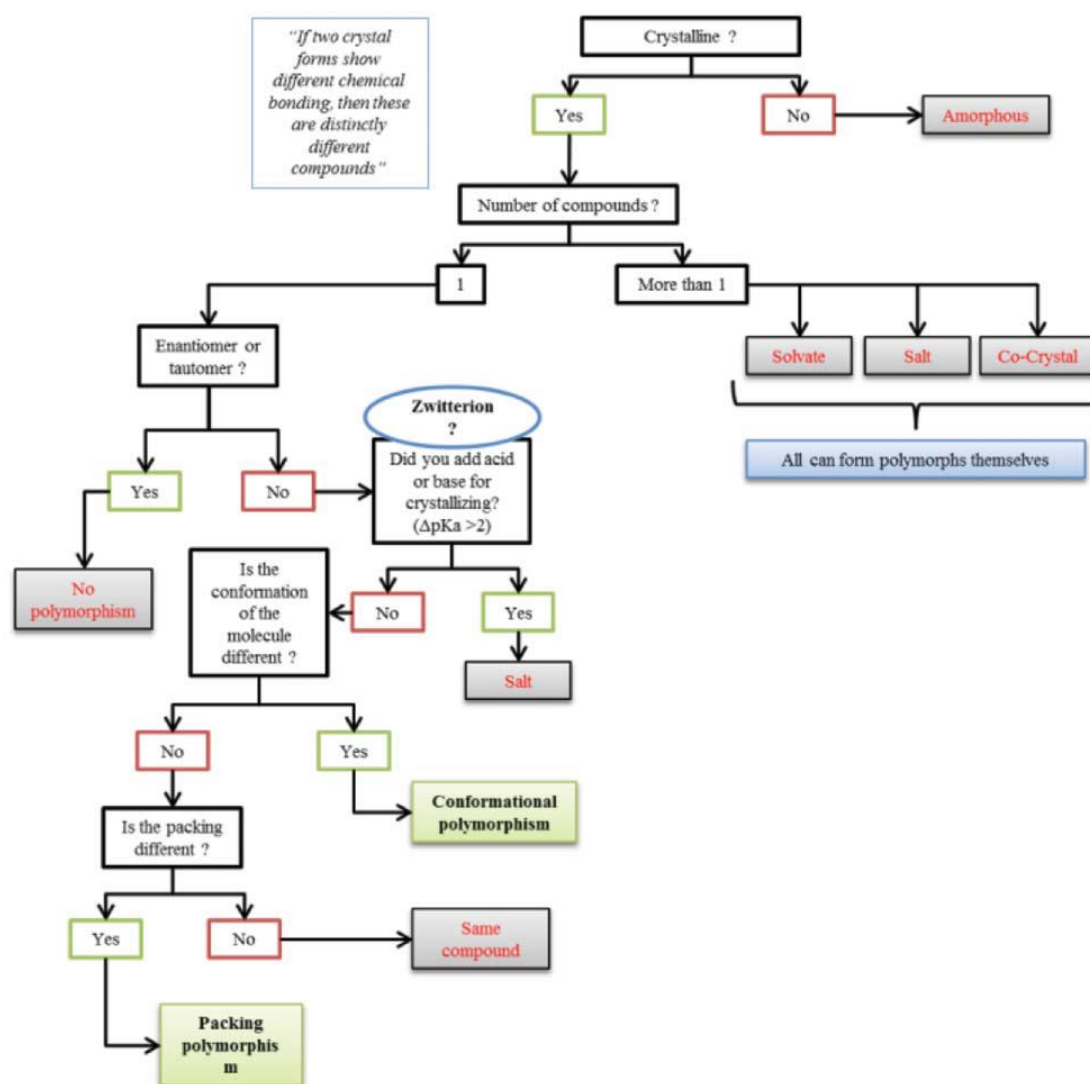


Figure 1.5: Pathway of polymorphism determination in organic compounds (Brog et al., 2013).

Energetics of pharmaceutical processing may further affect the selected polymorph whereby an unintentional conversion of one polymorph to the other may occur due to differences in the heats of fusion (Brittain, 2009). This may influence formulation development and manufacturability of a more soluble polymorph of a given API (Brittain, 2009; Nagai *et al.*, 2014). A thorough research and characterisation of polymorphs is therefore essential so as to prevent any possible polymorphic transformation during processing or storage and also to consistently manufacture the most desired polymorphic form (Newman & Byrn, 2003). This however can be a challenging task during improvement of aqueous solubility of a given API since the more soluble polymorph is always the metastable one. To counteract such a challenging aspect, the desired polymorph can be altered by the inclusion of other molecules in its crystal lattice (Hilfiker, 2006; Sekhon, 2009). These can be either an organic solvent (to

form a solvate) or water (to form a hydrate), or another crystalline solid (to form co-crystals and salts) (Yadav *et al.*, 2009).

1.2.1.2 Solvates

Drugs are often exposed to a solvent during both production and processing, resulting in the entrapment of the particular solvent in the crystal structure upon crystallisation (Cains, 2011). This results in formation of two or more crystalline phases of the same API but with different elemental composition due to the inclusion of one or more solvent molecules in the crystal lattice (Brittain, 2010). The included solvent can be either in a stoichiometric or non-stoichiometric manner. In stoichiometric solvates, as depicted in Figure 1.6(a), the solvent participates in the hydrogen-bonding network within the crystal structure, forming an integral part of the crystal unit cell (Brittain, 2010; Cains, 2011). Differences in solvation states can yield distinctly different crystal structures thereof (Lee *et al.*, 2013). These solvates are physically stable above the critical partial pressure of the solvent but below that pressure, desolvation occurs (Byard *et al.*, 2012). As the solvate is heated above the boiling point of the included solvent or above and beyond the melting temperature of the solvate, desolvation can occur with structural disruption resulting in formation of an amorphous form and / or re-crystallisation to a new unsolvated (polymorphic) form (Cains, 2011; Florence & Attwood, 2011).

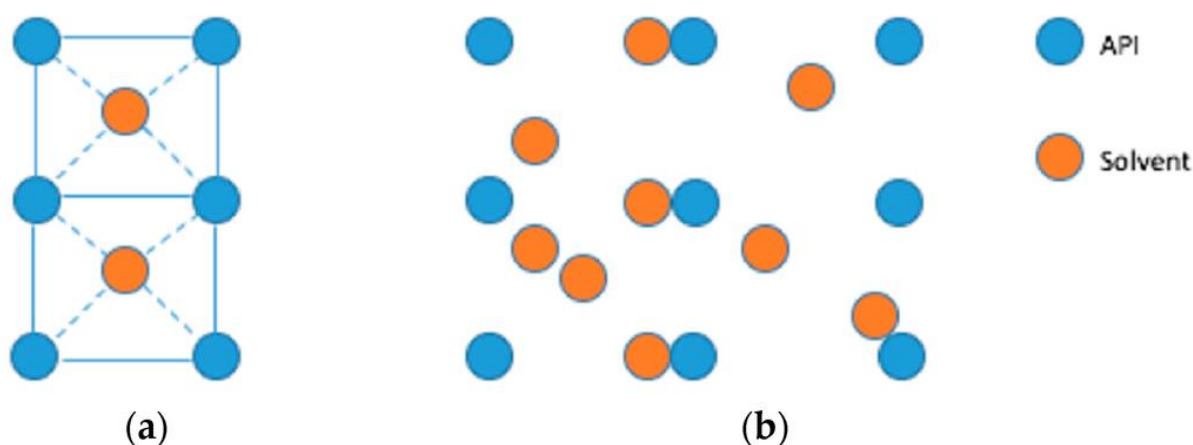


Figure 1.6: A simplified visualisation of (a) a “true” solvate vs. (b) a non-stoichiometric solvate, where the solvent can be one or more types of solvent (Skieneh *et al.*, 2016).

In non-stoichiometric solvates, the solvent content varies gradually and is accompanied by subtle, anisotropic, changes in the crystal structure (Brittain, 2010). The solvent does not form part of the crystal lattice but remains loosely bound on the surface or trapped in void spaces / channels within the crystal lattice, as depicted in Figure 1.6(b) (Brittain, 2010). By doing so, the packing motif of the host molecule remains unchanged (Lee *et al.*, 2013) and desolvation occurs more readily without destruction of the crystal lattice (Florence & Attwood 2015). As a

result, a low-density structure (isomorphous desolvate) and / or different polymorphic forms of the desolvate can be formed (Byard *et al.*, 2012). Such desolvated crystals retain the initial solvate crystal symmetry and are characterised by empty void spaces or channels that were once occupied by the solvent molecules (Braun *et al.*, 2016). There can also be changes in the stoichiometry of the solvent i.e. different solvent or a mixture of solvents without necessarily changing the crystal structure of the desolvated form. Several different solvates of the same compound have been identified and characterised (Byard *et al.*, 2012). Even so, oxygen molecules can diffuse into the lattice through the vacated tunnels and react with the host molecules. Crystal structures of these systems are therefore considered to be isostructural (Nath & Nangia, 2012). However, in some cases structural disruption may occur depending on the thermal stability and the extent of mechanical effects of removing solvent molecules from the depth of the crystal lattice yielding amorphous forms (Cains, 2011; Florence & Attwood, 2011).

Isomorphic desolvates may exhibit reduced chemical stability than the original solvate (de Villiers *et al.*, 2004). On the other hand, the greater the solvation of the crystals, the lower their solubility and dissolution rate in the same solvent (Aulton & Taylor, 2013). For different solvates, properties such as melting point, solubility, dissolution rate and bioavailability can differ significantly (Censi & Di Martino, 2015; Chadha *et al.*, 2012). However, it is unfavourable to use solvates for pharmaceutical APIs as they may be toxic for human use and only a few are harmless (Censi & Di Martino, 2015). Instead, solvates are used as an intermediary step for reasons that include i) production of new unsolvated / desolvated polymorphic forms of the API, ii) purification and, iii) particle size control (Minkov *et al.*, 2014). To a large extent, the behaviour of solvates may also apply to hydrates.

1.2.1.3 Hydrates

Hydrates are the most commonly identified subclass of crystalline solid-state forms, where the solvent included into the molecular structure is nothing other than water (Elder *et al.*, 2015). The hydrogen bonds formed ranges from extremely weak to extremely strong interaction energies (Paisana *et al.*, 2016). Hydrates can be categorised into three subclasses: isolated site hydrates, channel hydrates (planar or expanded) and metal ion associated hydrates (Vippagunta *et al.*, 2001). In the isolated site hydrates, water molecules are isolated from direct contact with each other such that they do not form a hydrogen bond or Van der Waals interactions with other water molecules but with the API alone (Cains, 2011; Vippagunta *et al.*, 2001). In channel hydrates, water molecules form intermolecular interactions with each other and not the API (Cains, 2011; Vippagunta *et al.*, 2001), thus maintaining the crystal structure but forming channels throughout the crystal (Stokes *et al.*, 2014). This class can further be

sub-classified into i) planar hydrates, whereby water is localised in a two-dimensional order or plane in the channel and ii) the expanded channel i.e. non-stoichiometric hydrates (Cains, 2011; Kratochvíl, 2011; Vipagunta *et al.*, 2001). Non-stoichiometric hydrates are formed when exposed to high humidity, with additional moisture effecting changes on the dimensions of the unit cell (Brittain *et al.*, 2009). The amount of water in the crystal lattice may depend on partial pressure of the atmospheric water (Byard *et al.*, 2012). Storing a non-stoichiometric solvate in the presence of water vapour can also result in the formation of a non-stoichiometric hydrate (Pikal *et al.*, 1983). For ion-associated hydrates, the water molecules are bound directly to a metal ion either as part of a coordination complex or through strong ionic bonds (Cains, 2011; Vipagunta *et al.*, 2001). Due to the high bond strength, desolvation of these ion-associated hydrates requires high dehydration energies.

Differences between the physico-chemical properties in particular solubility and dissolution rate of the dehydrated form and that of the hydrate can be observed (Cains, 2011). More frequently, hydrates show a slower dissolution rate than the anhydrous form (Censi & Di Martino, 2015). Transitions from the anhydrous to the hydrated form can easily occur during dissolution at the drug / medium interface hence affecting the rate thereof. The need of evaluating the physico-chemical properties and the hydration or dehydration mechanisms of hydrates remain of importance during the pharmaceutical drug design and development process (Cains, 2011; Florence & Attwood, 2011; Kratochvíl, 2011). The inclusion of a crystalline solid in the crystal lattice to form co-crystals and salts may result in even a better stability and solubility than solvates and hydrates (Sarmah *et al.*, 2015; Shan & Zaworokto, 2008; Yadav *et al.*, 2009).

1.2.1.4 Co-crystals

There appears to be no universally agreed definition of co-crystals as a consequence of ambiguity in their classification (Grothe *et al.*, 2016; Tilborg *et al.*, 2014). The FDA's draft guidance proposed a definition of co-crystals as, "*Solids that are crystalline materials composed of two or more molecules in the same crystal lattice*" and classified them as dissociable "*API-excipient*" molecular complexes (FDA, 2013). Aitipamula *et al.* (2012) points out that this definition limits co-crystals to molecular components and suggested that they should be grouped with salts. Nonetheless, the widely accepted and most useful definition by authors defines a co-crystal as a homogeneous crystalline solid that contains stoichiometric amounts of discrete neutral molecular species that are solids under ambient conditions (Brittain, 2012; Jampílek & Dohnal, 2012). As the debate continues, it was pointed out that there are cases where pharmaceutical drugs were approved and marketed as salts yet are in fact co-crystals (Elder *et al.*, 2015). To avoid overlapping with salts as well as other well-

known solid forms, it is further suggested that co-crystals should be subclassified as (i) "simple" co-crystals; (ii) solvated (hydrated) co-crystals; (iii) salt co-crystals; (iv) solvated salt co-crystals; and (v) polymorphs of all previous types of co-crystals (Grothe *et al.*, 2016; Jampílek & Dohnal, 2012) as shown in Figure 1.7.

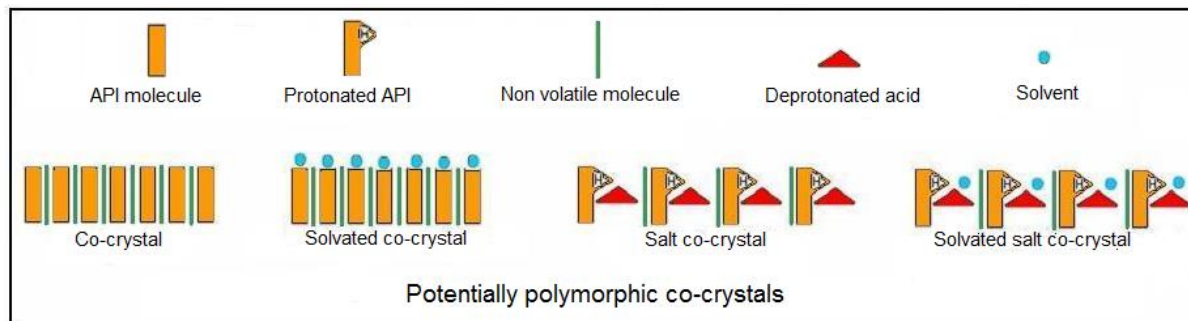


Figure 1.7: Potentially polymorphic multicomponent co-crystals (Jampílek & Dohnal, 2012).

Co-crystals have shown to be more stable to heat than solvates and hydrates (Shan & Zaworokto, 2008). They offer enhancement of physical and technical properties of drugs such as solubility (4 to 20-fold), dissolution rate, stability, hygroscopicity, compressibility and bioavailability without changing the chemical composition and / or pharmacological behaviour of the API (Aitipamula *et al.*, 2014; Brittain, 2012; Duggirala *et al.*, 2016; Sekhon, 2009). Proper understanding of co-crystals thus remains important in the selection of the most appropriate form with best physico-chemical properties e.g. best solubility.

1.2.1.5 Salts

Whilst co-crystals are neutral and interact *via* non-ionic interactions, salts require an ionisable API that forms strong ionic interactions with an oppositely charged counter ion by an acid / base reaction as depicted in Figure 1.8 (Savjani, 2015).



Figure 1.8: Difference between salts and co-crystals (Elder *et al.*, 2013).

Among several other methods used to improve solubility of poorly aqueous soluble APIs, salt formation is the most commonly used technique due to high solubility (500 – 1000-fold) relative to the pure API, dissolution rate and purity (Sarmah *et al.*, 2015). Over half of all medicines on the market are administered as salts (Surov *et al.*, 2016; Makary, 2014). Salt formation

can result in a significant improvement of permeability, efficacy, chemical and physical stability as well as organoleptic properties of the API (Elder *et al.*, 2013). However, only 20 – 30% of new molecules form salts easily (Brough & Williams, 2013; Serajuddin & Pudipeddi, 2008). More so, the best salt is not necessarily the most soluble form (Elder *et al.*, 2013). There can be significant polymorphic / solvation / hydration tendencies in the salt of optimum solubility. This makes it even more challenging to predict which salt have the greatest tendency to exist in other forms so as to provide the best desired physico-chemical properties (Makary, 2014; Surov *et al.*, 2016).

Salts and all the above mentioned and discussed crystalline forms have the potential to improve the physico-chemical properties of APIs. The possibility of each of these crystalline forms to display polymorphism may further lead to a significant improvement in solubility and bioavailability of poorly soluble drugs (Brog *et al.*, 2013; Newman & Wenslow, 2016). However, non-crystalline (amorphous) forms have shown even a greater solubility advantage, but lack the ideal thermodynamic stability that is essential in the final drug product and during drug formulation. The amorphous state will be discussed in more detail in the paragraphs below.

1.2.2 The principles of the amorphous solid-state form

The amorphous state is characterised by a second order glass transition temperature (T_g) which is dependent on molecular mobility (Ghosh, 2006). T_g is defined as a continuous transition from the equilibrium supercooled liquid state to the non-equilibrium glass state (Keys *et al.*, 2013). Thermal transition from the crystalline to amorphous form by quench cooling is shown in Figure 1.9. It is well known that the first order phase transition of the crystalline form to liquid state occurs at melting temperature (T_m) (Einfalt *et al.*, 2013). When this melt is cooled slowly, the molecules nucleate slowly and regenerate back into a crystalline structure (Ghosh, 2006). On the contrary, sudden cooling of this melt prevents crystallisation and yields a supercooled liquid state below T_m , whereby enthalpy and volume will be in equilibrium with the molten drug (Kolodziejczyk *et al.*, 2013). On further cooling, equilibrium state continues until T_g is reached at which the system solidifies and falls out of equilibrium (Baghel *et al.*, 2016). However, in the case that T_g does not occur, equilibrium continues and the entropy volume of the amorphous state equals that of the crystalline state at the Kauzmann temperature (T_K) (Einfalt *et al.*, 2013; Qiu *et al.*, 2009). Below T_K , the amorphous state violates the third law of thermodynamics which states that the entropy of a perfect crystal at absolute zero is exactly equal to zero. On a similar note, below T_g , equilibrium thermodynamics cannot be applied. Thus, T_g marks as the characterisation parameter of the amorphous state at which molecular mobility slows down (Graeser *et al.*, 2010).

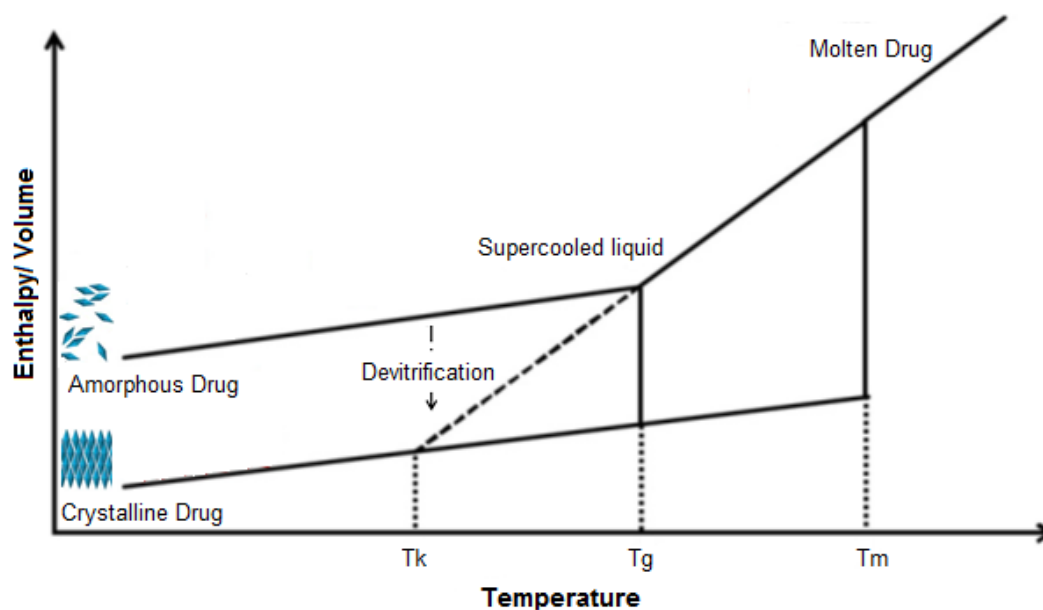


Figure 1.9: Thermodynamic phase transition of crystalline to amorphous form by quench cooling as adopted from Hancock & Zografi, 1997.

Several studies have reported different physico-chemical properties above and below the T_g . Below T_g , the amorphous materials are brittle but above it, they are liquid or rubbery (Donth, 2013). Over a period of time, mobility can still occur below T_g whereby the amorphous state relaxes and its enthalpy and entropy decrease towards the equilibrium glassy state (devitrification) (Graeser *et al.*, 2010). On that note, amorphous forms can exist in different kinetic states and may further possess different physico-chemical properties influenced by the storage period and thermal history (Skotnicki *et al.*, 2015). This amorphous system can easily sorb large amounts of solvent and / or water which lowers its T_g and in turn facilitate crystallisation hence further decreasing its physical stability (Figure 1.10) (Mehta *et al.*, 2016; Szakonyi & Zelkó, 2012). Although the sorbed water or solvent may be of variable quantities, amorphous forms do not form stoichiometric hydrates or solvates (Elder *et al.*, 2015). However, they can form salts which possess higher T_g values leading to improved physical and chemical stability (Tong *et al.*, 2002; Lee *et al.*, 2015).

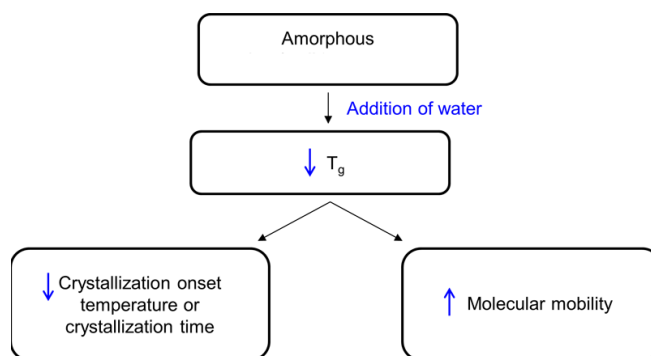


Figure 1.10: Effect of water on molecular mobility and physical stability of the amorphous state (Mehta *et al.*, 2016).

The amorphous state may exist in one or more amorphous phases separated by a clear phase transition (Hancock *et al.*, 2002). Recently, two different amorphous precursor states have been reported for paracetamol (Thi *et al.*, 2015). The use of the term polyamorphism in such cases and on the occurrence of amorphous – amorphous phase transitions or existence of different amorphous forms of the same API remains controversial (Guinet *et al.*, 2016). Different methods of preparation may also result in different amorphous solids of the same API (Kratochvíl, 2011), which may further exhibit significantly different physico-chemical properties (An & Sohn, 2009). Significant differences in dissolution between the two amorphous forms of valsartan have been noted (Skotnicki *et al.*, 2015). In another study by Milne *et al.* (2016), it was reported that different amorphous forms of a macrolide antibiotic roxithromycin were obtained as a result of different preparation methods. They concluded that such amorphous forms do not convert from one amorphous state to another *via* a first order phase transition hence the term polyamorphism is not an appropriate term to use in such circumstances. Ultimately, it is the amorphous phase of best physico-chemical properties that should be selected.

In summary, the higher enthalpy, entropy, free energy and volume of the amorphous state (Figure 1.9) are responsible for its higher solubility and reactivity in comparison to its crystalline counterpart (Bhugra & Pikal, 2008). On the other hand, the very same thermodynamic properties are the culprits for the physical instability of the amorphous state which has a tendency to convert back to a more stable crystalline state thus, in turn, influencing the solubility advantage detrimentally (Gupta & Bansal, 2005). Amongst other, several approaches used to preserve the solubility advantage of this metastable solid-state, stabilisation by the use of solid dispersions of the API in a pharmaceutically acceptable polymer remains one of most promising strategies (Kavanagh *et al.*, 2012; Yu, 2001).

1.2.2.1 Classification of solid dispersions

By definition, a solid dispersion is the dispersion of an API or APIs in an inert carrier or matrix at solid-state (Ha *et al.*, 2014). Basing on the carrier system composition, solid dispersions can be classified into different generations as shown in Figure 1.11 (Vasconcelos *et al.*, 2007; Vo *et al.*, 2013). The first generation comprises of API and highly water soluble crystalline carriers (Kumari *et al.*, 2013). Although thermodynamically more stable, drug release of this generation is slower than the amorphous ones of the second generation (Kapoor *et al.*, 2012). The use of polymeric carriers in the second generation offer even better dispersibility and wettability as a result of supersaturation of the API (Zecevic *et al.*, 2014). However, drug precipitation and recrystallisation may still occur in amorphous solid dispersions (Vo *et al.*, 2013). Inclusion of surface active or self-emulsifying agents provides improved dissolution and stability in the third generation (Yu *et al.*, 2011). This generation is composed of a surfactant or a mixture of amorphous polymers and surfactants (Kapoor *et al.*, 2012; Vasconcelos *et al.*, 2007) In the fourth generation, water insoluble or swellable polymers are used to sustain drug release for a prolonged therapeutic effect (Vo *et al.*, 2013). Amongst these, amorphous solid dispersions (ASDs) have been considered as the major advancement for poorly soluble drugs and have shown greater physical stability than the amorphous drug alone (Brittain, 2014; Zecevic *et al.*, 2014).

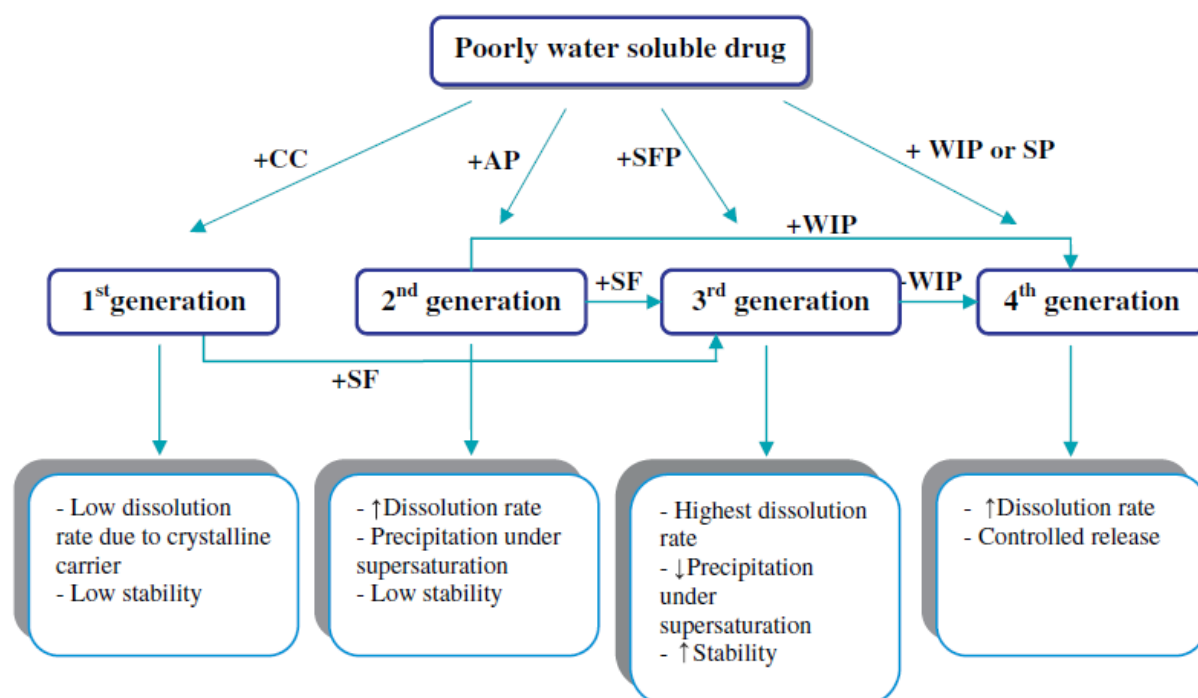


Figure 1.11: Solid dispersions composition and classification. CC: crystalline carrier, AP: amorphous polymer, SFP: surfactant polymer, WIP: water insoluble polymer, SP: swellable polymer, SF: surfactant, (↑): increase, (↓): decrease (Vo *et al.*, 2013).

1.2.2.2 Amorphous solid dispersions (ASDs)

ASDs are defined as molecular mixtures of poorly water soluble drugs in hydrophilic carriers, which present a drug release profile that is driven by polymer properties (Vasconcelos *et al.*, 2007). They have shown to improve drugs' poor water solubility by devitrification of undissolved amorphous drug (Dani *et al.*, 2014) and may also display supersaturation effects which are beneficial in overcoming solubility-limited absorption (Dani *et al.*, 2014; Zecevic *et al.*, 2014). ASDs therefore represent a promising formulation approach to alter solid-state properties of poorly soluble APIs. However, several factors such as moisture, miscibility, and nature of polymer can still influence the physico-chemical properties of ASDs. To obtain maximum solubility and stability, API–polymer miscibility and molecular interactions remain of utmost importance in the design of ASDs (Huang & Dai, 2014; Meng *et al.*, 2015). The API and the carrier of the solid dispersion should form a chemically homogeneous phase at molecular level for maximum stability (Baird & Taylor, 2012; Ivanisevic, 2010; Marsac *et al.*, 2009). Properties of the polymer (hydrophilicity, hygroscopicity and molecular weight) together with API–polymer interactions (plasticizing effect or hydrogen bonds formation) can greatly influence dissolution and physical / chemical stability of the ASD (Kapoor *et al.*, 2012; Vo *et al.*, 2013; Zhaojie *et al.*, 2014). Ultimately, optimised ASDs can improve bioavailability, effectiveness of treatment through reduced doses as well as patient compliance (Newman *et al.*, 2012; Rumondor *et al.*, 2016; Tiwari *et al.*, 2009).

1.3 Conclusion

APIs can be characterised by the order and degree of molecular packing i.e. crystalline (order), amorphous forms (disorder) or mesophase (partially ordered) systems. The mesophase resembles amorphous state in relation to molecular mobility and T_g (Shalaev *et al.*, 2016). The three solid-state forms can co-exist independently of each other but the more energetic, disordered states will eventually change to the lower energy ordered states over time (Elder *et al.*, 2015). Ideally, the lower energy state, i.e. the more stable form is preferred in the final drug product but the more energetic i.e. the metastable forms are always more soluble due to increased mobility (Aulton & Taylor, 2013; Censi & Di Martino, 2015). Several studies have proved amorphous forms have the potential to improve solubility and dissolution rates significantly higher than their crystalline counterparts (> 10 times higher) (Kavanagh *et al.*, 2012; Nagapudi & Jona 2008; Vo *et al.*, 2013). Thus, optimised amorphous forms may have the ability to overcome the growing challenges in oral bioavailability (Paudel *et al.*, 2014). However, the existence of a pure amorphous drug alone is highly unlikely due to its high free energy. The inclusion of excipients e.g. i) polymeric and ii) non-polymeric (mesoporous silica based and co-amorphous formulations) can be employed to stabilise these metastable forms

(Kasten *et al.*, 2016). By stabilising the metastable state in an ASD, improved dissolution rate, solubility and a longer shelf-life can therefore be achieved (Kavanagh *et al.*, 2012; Yu, 2001; Zecevic *et al.*, 2014). Thus for the correct design and development of a pharmaceutical drug, the ultimate focus should be controlling the solid-state structure of the API to guarantee its physico-chemical properties (Brog *et al.*, 2013). For the purpose of this study, the investigation focuses on the solid-state properties of two macrolide antibiotics (spiramycin and clarithromycin) typically used in the treatment of opportunistic co-infections in patients suffering from HIV/AIDS.

References

- Aitipamula, S., Banerjee, R., Bansal, A.K., Biradha, K., Cheney, M.L., Choudhury, A.R., Desiraju, G.R., Dikundwar, A.G., Dubey, R., Duggirala, N. & Ghogale, P.P.** 2012. Polymorphs, salts, and co-crystals: what's in a name?. *Crystal growth & design*, 12:2147-2152.
- An, S.G. & Sohn, Y.T.** 2009. Crystal forms of atorvastatin. *Archives of pharmacal research*, 32:933-936.
- Aulton, M.E. & Taylor, K.M.** 2013. Aulton's pharmaceuticals: the design and manufacture of medicines. 4th ed. New York: Churchill Livingstone.
- Baghel, S., Cathcart, H. & O'Reilly, N.J.** 2016. Polymeric amorphous solid dispersions: a review of amorphization, crystallization, stabilization, solid-state characterization, and aqueous solubilization of biopharmaceutical classification system class II drugs. *Journal of pharmaceutical sciences*, 105: 2527-2544.
- Baird, J.A. & Taylor, L.S.** 2012. Evaluation of amorphous solid dispersion properties using thermal analysis techniques. *Advanced drug delivery reviews*, 64:396-421.
- Bharate, S.S., Bharate, S.B. & Bajaj, A.N.** 2010. Incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a comprehensive review. *Journal of excipients and food chemicals*, 1: 3-26.
- Bhugra, C. & Pikal, M.J.** 2008. Role of thermodynamic, molecular, and kinetic factors in crystallization from the amorphous state. *Journal of pharmaceutical sciences*, 97:1329-1349.
- Braga, D., Grepioni, F., Maini, L. & Polito, M.** 2009. Crystal polymorphism and multiple crystal forms. (In Hossein, M.W., ed. *Molecular Networks*. Berlin Heidelberg: Springer. p. 87-95).
- Braun, D.E., Gelbrich, T., Wurst, K. & Griesser, U.J.** 2016. Computational and experimental characterization of five crystal forms of thymine: packing polymorphism, polytypism/disorder and stoichiometric 0.8-hydrate. *Crystal growth & design*, 16:3480-3496.
- Brittain, H.G.** 2009. Polymorphism in pharmaceutical solids. 2nd ed. New York: Informa healthcare.
- Brittain, H.G., Morris, K.R. & Boerrigter, S.X.** 2009. Structural aspects of solvatomorphic systems. (In Brittain, H.G., ed. *Polymorphism in pharmaceutical solids*. New York: Informa healthcare. p. 233-281).
- Brittain, H.G.** 2010. Polymorphism and solvatomorphism. *Journal of pharmaceutical sciences*, 99:3648-3664.

- Brittain, H.G.** 2012. Polymorphism and solvatomorphism. *Journal of pharmaceutical sciences*, 101:464-484.
- Brittain, H.G.** 2014. Spectroscopic studies of solid-state forms of donepezil free base and salt forms with various salicylic acids. *Journal of molecular structure*, 1078:207-212.
- Brog, J.P. Chanez, C.I Crochet, A. & Fromm, K.M.** 2013. Polymorphism, what it is and how to identify it: a systematic review. *Royal Society of Chemistry Advances*, 3:16905-16931.
- Brough, C. & Williams, R.O.** 2013. Amorphous solid dispersions and nano-crystal technologies for poorly water-soluble drug delivery. *International journal of pharmaceutics*, 453:157-166.
- Byard, S., Abraham, A., Boulton, T., Paul, J., Harris, R.K. & Hodgkinson, P.** 2012. A multi-technique approach to the study of structural stability and desolvation of two unusual channel hydrate solvates of finasteride. *Journal of pharmaceutical sciences*, 101:176-186.
- Cains, P.W.** 2011. Classical methods of preparation of polymorphs and alternative solid forms. *ChemInform*, 42:1.
- Censi, R. & Di Martino, P.** 2015. Polymorph impact on the bioavailability and stability of poorly soluble drugs. *Molecules*, 20:18759-18776.
- Chadha, R., Kuhad, A., Arora, P. & Kishor, S.** 2012. Characterisation and evaluation of pharmaceutical solvates of atorvastatin calcium by thermoanalytical and spectroscopic studies. *Chemistry central journal*, 6:114.
- Chakravarty, P., Bates, S. & Thomas, L.** 2013. Identification of a potential conformationally disordered mesophase in a small molecule: experimental and computational approaches. *Molecular pharmaceutics*, 10:2809-2822.
- Chieng, N., Rades, T. & Aaltonen, J.** 2011. An overview of recent studies on the analysis of pharmaceutical polymorphs. *Journal of pharmaceutical and biomedical analysis*, 55:618-644.
- Dani, P., Puri, V. & Bansal, A.K.** 2014. Solubility advantage from amorphous etoricoxib solid dispersions. *Drug development and industrial pharmacy*, 40:92-101.
- de Villiers, M.M., Mahlatji, M.D., van Tonder, E.C., Malan, S.F., Lötter, A.P. & Liebenberg, W.** 2004. Comparison of the physical and chemical stability of niclosamide crystal forms in aqueous versus nonaqueous suspensions. *Drug development and industrial pharmacy*, 30: 581-592.
- Dengale, S.J., Ranjan, O.P., Hussen, S.S., Krishna, B.S.M., Musmade, P.B., Shenoy, G.G. & Bhat, K.** 2014. Preparation and characterization of co-amorphous ritonavir–indomethacin

systems by solvent evaporation technique: Improved dissolution behavior and physical stability without evidence of intermolecular interactions. *European journal of pharmaceutical sciences*, 62:57-64.

Donth, E.J. 2013. The glass transition: relaxation dynamics in liquids and disordered materials. Berlin Heidelberg: Springer.

Duggirala, N.K., Perry, M.L., Almarsson, Ö. & Zaworotko, M.J. 2016. Pharmaceutical co-crystals: along the path to improved medicines. *Chemical communications*, 52:640-655.

Einfalt, T., Planinšek, O. & Hrovat, K. 2013. Methods of amorphization and investigation of the amorphous state. *Acta pharmaceutica*, 63:305-334.

Elder, D.P., Holm, R. & de Diego, H.L. 2013. Use of pharmaceutical salts and cocrystals to address the issue of poor solubility. *International journal of pharmaceuticals*, 453:88-100.

Elder, D.P., Patterson, J.E. & Holm, R. 2015. The solid-state continuum: a perspective on the interrelationships between different solid-state forms in drug substance and drug product. *Journal of pharmacy and pharmacology*, 67:757-772.

Feng, X., Vo, A., Patil, H., Tiwari, R.V., Alshetali, A.S., Pimparade, M.B. & Repka, M.A. 2015. The effects of polymer carrier, hot melt extrusion process and downstream processing parameters on the moisture sorption properties of amorphous solid dispersions. *Journal of pharmacy and pharmacology*, 68:692-704.

Florence, A.T. & Attwood, D. 2011. Physicochemical principles of pharmacy. 5th ed. London: Pharmaceutical Press.

Florence, A.T. & Attwood, D. 2015. Physicochemical principles of pharmacy in manufacture, formulation and clinical use. 6th ed. London: Pharmaceutical Press.

Food and Drug Administration. See FDA.

FDA. 2007. Guidance for industry. ANDAs: pharmaceutical. solid polymorphism. chemistry, manufacturing and controls information. Center for drug evaluation and research. US: Silver Spring.

FDA. 2013. Guidance for Industry: Regulatory classification of pharmaceutical co-crystals. Center for drug evaluation and research. US: Silver Spring.

Ghosh, P. 2006. Fundamentals of polymer science thermal: transitions in polymers <http://nsdl.niscair.res.in/jspui/bitstream/123456789/407/2/Thermal%20transition.pdf>. Date of access: 20 May. 2016.

- Graeser, K.A., Patterson, J.E., Zeitler, J.A. & Rades, T.** 2010. The role of configurational entropy in amorphous systems. *Pharmaceutics*, 2:224-244.
- Grothe, E., Meekes, H., Vlieg, E., ter Horst, J H. & de Gelder, R.** 2016. Solvates, salts and cocrystals: a proposal for a feasible classification system. *Crystal growth & design*, 16:3237-3243.
- Guinet, Y., Paccou, L., Danède, F., Willart, J. F., Derollez, P. & Hédoux, A.** 2016. Comparison of amorphous states prepared by melt-quenching and cryomilling polymorphs of carbamazepine. *International journal of pharmaceutics*, 509:305-313.
- Gupta, P. & Bansal, A.K.** 2005. Devitrification of amorphous celecoxib. *AAPS PharmSciTech*, 6:223-230.
- Ha, E.S., Baek, I.H., Cho, W., Hwang, S.J. & Kim, M.S.** 2014. Preparation and evaluation of solid dispersion of atorvastatin calcium with Soluplus® by spray drying technique. *Chemical and pharmaceutical bulletin*, 62:545-551.
- Hancock, B.C. & Zografi, G.** 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. *Journal of pharmaceutical sciences*, 86:1-12.
- Hancock, B.C. Shalaev E.Y & Shamblin, S.L.** 2002. Polyamorphism: a pharmaceutical science perspective. *Journal of pharmacy and pharmacology*, 54:1151-1152.
- Hilfiker, R.** 2006. Polymorphism: in the pharmaceutical industry. Germany: John Wiley & Sons.
- Huang, Y. & Dai, W.G.** 2014. Fundamental aspects of solid dispersion technology for poorly soluble drugs. *Acta pharmaceutica sinica b*, 4:18-25.
- Ivanisevic, I.** 2010. Physical stability studies of miscible amorphous solid dispersions. *Journal of pharmaceutical sciences*, 99:4005-4012.
- Jampílek, J. & Dohnal, J.** 2012. Investigation of carbohydrates and their derivatives as crystallization Modifiers. *INTECH open access publisher*.
- Jórárt-Laczkovich, O. & Szabó-Révész, P.** 2011. Formulation of tablets containing an 'in-process' amorphized active pharmaceutical ingredient. *Drug development and industrial pharmacy*, 37:1272-1281.
- Kapoor, B., Kaur, R., Kour, S., Behl, H. & Kour, S.** 2012. Solid dispersion: an evolutionary approach for solubility enhancement of poorly water soluble drugs. *International journal of recent advances in pharmaceutical research*, 2:1-16.

- Karpinski, P.H.** 2006. Polymorphism of active pharmaceutical ingredients. *Chemical engineering & technology*, 29:233-237.
- Kasten, G., Grohgan, H., Rades, T. & Löbmann, K.** 2016. Development of a screening method for co-amorphous formulations of drugs and amino acids. *European journal of pharmaceutical sciences* (In press).
- Kavanagh, A., McConvey, I., McCabe, J., Blade, H.S. & Cosgrove, S.** 2012. Amorphous pharmaceuticals-developing amorphous pharmaceuticals: opportunity and necessity. *American pharmaceutical review*, 15:10.
- Kesisoglou, F. & Wu, Y.** 2008. Understanding the effect of API properties on bioavailability through absorption modeling. *The American Association of Pharmaceutical Scientists Journal*, 10:516-525.
- Keys, A.S., Garrahan, J.P. & Chandler, D.** 2013. Calorimetric glass transition explained by hierarchical dynamic facilitation. *Proceedings of the national academy of sciences*, 110: 4482-4487.
- Kolodziejczyk, K., Paluch, M., Grzybowska, K., Grzybowski, A., Wojnarowska, Z., Hawelek, L. & Ziolo, J.D.** 2013. Relaxation dynamics and crystallization study of sildenafil in the liquid and glassy states. *Molecular pharmaceuticals*, 10:2270-2282.
- Kratochvíl, B.** 2011. Solid forms of pharmaceutical molecules. (In Šesták, J., Mareš, J.J. & Hubík, P., eds. *Glassy, amorphous and nano-crystalline materials: Thermal physics, analysis, structure and properties*. Netherlands: Springer. p. 129-140.)
- Kumari, R., Chandel, P. & Kapoor, A.** 2013. Paramount role of solid dispersion in enhancement of solubility. *Indo global journal of pharmaceutical sciences*, 3:78-89.
- Lee, J., Boerrigter, S.X., Jung, Y.W., Byun, Y., Yuk, S.H., Byrn, S.R. & Lee, E.H.** 2013. Organic vapor sorption method of isostructural solvates and polymorph of tenofovir disoproxil fumarate. *European journal of pharmaceutical sciences*, 50:253-262.
- Lee, S.H., Bae, J.H., Park, Y., Adhikari, B.R., Mao, C., Kim, D., Kim, K.I., Kang, S.K. & Lee, E.H.** 2015. Sulfonic acid salts of donepezil and stabilization of amorphous donepezil via formation of amorphous salts. *Crystal growth & design*, 15:3123-3130.
- Lohani, S. & Grant, D.J.** 2006. Thermodynamics of polymorphs. (In Hilfiker, R., ed. *Polymorphism: in the pharmaceutical industry*. Germany: John Wiley & Sons. p. 21-42).
- Lu, J. & Rohani, S.** 2009. Polymorphism and crystallization of active pharmaceutical ingredients (APIs). *Current medicinal chemistry*, 16:884-905.

- Iuraş, A., Scurr, D.J., Boissier, C., Nicholas, M.L., Roberts, C.J. & Alexander, M.R.** 2016. Imaging of crystalline and amorphous surface regions using time-of-flight secondary-ion mass spectrometry (ToF-SIMS): application to pharmaceutical materials. *Analytical chemistry*, 88:3481-3487.
- Makary, P.** Principles of salt formation. 2014. *UK journal of pharmaceutical and biosciences*, 2:01-04.
- Marsac, P.J., Li, T. & Taylor, L.S.** 2009. Estimation of drug–polymer miscibility and solubility in amorphous solid dispersions using experimentally determined interaction parameters. *Pharmaceutical research*, 26:139-151.
- Mazurek, J., Hoffmann, M., Fernandez Casares, A., Cox, D.P., Minardi, M.D. & Sasine, J.** 2016. Two orthorhombic polymorphs of hydromorphone. *Acta crystallographica section e: crystallographic communications*, 72:730-733.
- Mehta, M., Kothari, K., Ragoonanan, V. & Suryanarayanan, R.** 2016. Effect of water on molecular mobility and physical stability of amorphous pharmaceuticals. *Molecular pharmaceutics*, 13:1339-1346.
- Meng, F., Trivino, A., Prasad, D. & Chauhan, H.** 2015. Investigation and correlation of drug polymer miscibility and molecular interactions by various approaches for the preparation of amorphous solid dispersions. *European journal of pharmaceutical sciences*, 71:12-24.
- 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.
- Minkov, V.S., Beloborodova, A.A., Drebuschak, V.A. & Boldyreva, E.V.** 2014. Furosemide solvates: can they serve as precursors to different polymorphs of furosemide?. *Crystal growth & design*, 14:513-522.
- Moynihan, H. & Crean, A.** 2009. Physicochemical basis of pharmaceuticals. New York: Oxford University Press.
- Mugheirbi, N. A., Fleischer, K. & Tajber, L.** 2016. A rare case of mesomorphic behavior–molecular reorientation of Itraconazole liquid crystal induced by a hygrothermal treatment. *Crystal growth & design*, 16:1329-1336.
- Nagai, K., Ushio, T., Miura, H., Nakamura, T., Moribe, K. & Yamamoto, K.** 2014. Four new polymorphic forms of suplatast tosilate. *International journal of pharmaceutics*, 460:83-91.

- Nagapudi, K. & Jona, J.** 2008. Amorphous active pharmaceutical ingredients in preclinical studies: preparation, characterization, and formulation. *Current bioactive compounds*, 4: 213-224.
- Nath, N.K. & Nangia, A.** 2012. Isomorphous crystals by chloro–methyl exchange in polymorphic fuchsones. *Crystal growth & design*, 12:5411-5425.
- Newman, A. W. & Byrn, S.R.** 2003. Solid-state analysis of the active pharmaceutical ingredient in drug products. *Drug discovery today*, 8:898-905.
- Newman, A., Knipp, G. & Zografi, G.** 2012. Assessing the performance of amorphous solid dispersions. *Journal of pharmaceutical sciences*, 101:1355-1377.
- Newman, A. & Wenslow, R.** 2016. Solid form changes during drug development: good, bad, and ugly case studies. *American Association of Pharmaceutical Scientists Open*, 2:1.
- O'Keeffe, M.** 2012. Structure and bonding in crystals. New York: Academic press.
- Paisana, M.C., Wahl, M.A. & Pinto, J.F.** 2016. Role of moisture on the physical stability of polymorphic olanzapine. *International journal of pharmaceutics*, 509:135-148.
- Patel, J., Jagia, M., Bansal, A.K. & Patel, S.** 2015. Characterization and thermodynamic relationship of three polymorphs of a xanthine oxidase inhibitor, febuxostat. *Journal of pharmaceutical sciences*, 104:3722-3730.
- Paudel, A., Geppi, M. & Den Mooter, G.V.** 2014. Structural and dynamic properties of amorphous solid dispersions: the role of solid-state nuclear magnetic resonance spectroscopy and relaxometry. *Journal of pharmaceutical sciences*, 103:2635-62.
- Pérez, E., Cerrada, M.L., García-Peñas, A., Gómez-Elvira, J.M., Fernández-Blázquez, J.P., Martínez-Gómez, A., López-Velázquez, D. & Martínez, J.C.** 2016. The role of mesophases in the ordering of polymers. *European polymer journal*, 81:661-673.
- Pikal, M.J., Lang, J.E. & Shah, S.** 1983. Desolvation kinetics of cefamandole sodium methanolate: the effect of water vapor. *International journal of pharmaceutics*, 17:237-262.
- Purohit, R. & Venugopalan, P.** 2009. Polymorphism: an overview. *Resonance*, 14:882-893.
- Qiu, Y., Chen, Y., Zhang, G. G., Liu, L. & Porter, W., eds.** 2009. Developing solid oral dosage forms: pharmaceutical theory & practice. New York: Academic press.
- Rumondor, A.C., Dhareshwar, S.S. & Kesisoglou, F.** 2016. Amorphous solid dispersions or prodrugs: complementary strategies to increase drug absorption. *Journal of pharmaceutical sciences*, 105:2498-2508.

- Sarmah, K.K., Sarma, A., Roy, K., Rao, D.R. & Thakuria, R.** 2015. Olanzapine salts and diversity in molecular packing. *Crystal growth & design*, 16:1047-1055.
- Savjani, J.** 2015. Co-crystallization: an approach to improve the performance characteristics of active pharmaceutical ingredients. *Asian journal of pharmaceutics*, 1:147-151.
- Sekhon, B.S.** 2009. Pharmaceutical co-crystals-a review. *ARS phamaceutica*, 50:99-117.
- Serajuddin, A.T. & Pudipeddi, M.** 2008. Salt-selection strategies. (In Stahl P.H., Wermuth, G., eds. *Handbook of Pharmaceutical salts properties, selection and use*. Weinheim: Wiley-VCH. p. 135-160).
- Shalaev, E., Wu, K., Shamblin, S., Krzyzaniak, J. F. & Descamps, M.** 2016. Crystalline mesophases: structure, mobility, and pharmaceutical properties. *Advanced drug delivery reviews*, 100:194-211.
- Shan, N. & Zaworotko, M.J.** 2008. The role of cocrystals in pharmaceutical science. *Drug discovery today*, 13:440-446.
- Skieneh, J., Khalili Najafabadi, B., Horne, S. & Rohani, S.** 2016. Crystallization of esomeprazole magnesium water/butanol solvate. *Molecules*, 21: 544.
- Skotnicki, M., Apperley, D.C., Aguilar, J.A., Milanowski, B., Pyda, M. & Hodgkinson, P.** 2015. Characterization of two distinct amorphous forms of valsartan by solid-state NMR. *Molecular pharmaceutics*, 13:211-222.
- Steed, J.W.** 2013. The role of co-crystals in pharmaceutical design. *Trends in pharmacological sciences*, 34:185-193.
- Stokes, S.P., Seaton, C.C., Eccles, K.S., Maguire, A.R. & Lawrence, S.E.** 2014. Insight into the mechanism of formation of channel hydrates via templating. *Crystal growth & design*, 14:1158-1166.
- Storey, R.A. & Ymen, I., eds.** 2011. *Solid state characterization of pharmaceuticals*. Chichester: Wiley & sons.
- Surov, A.O., Voronin, A.P., Simagina, A.A., Churakov, A.V. & Perlovich, G.L.** 2016. Pharmaceutical salts of biologically active hydrazone compound salinazid: crystallographic, solubility and thermodynamic aspects. *Crystal growth & design*, 16:2605-17.
- Szakonyi, G. & Zelkó, R.** 2012. The effect of water on the solid state characteristics of pharmaceutical excipients: molecular mechanisms, measurement techniques, and quality aspects of final dosage form. *International journal of pharmaceutical investigation*, 2:18-25.

- Thi, Y.N., Rademann, K. & Emmerling, F.** 2015. Direct evidence of polyamorphism in paracetamol. *Crystal engineering communications*, 17:9029-9036.
- Tilborg, A., Norberg, B. & Wouters, J.** 2014. Pharmaceutical salts and cocrystals involving amino acids: A brief structural overview of the state-of-art. *European journal of medicinal chemistry*, 74:411-426.
- Tiwari, R., Tiwari, G., Srivastava, B. & Rai, A.K.** 2009. Solid dispersions: an overview to modify bioavailability of poorly water soluble drugs. *International journal of pharmtech research*, 1:1338-1349.
- Tong, P., Taylor, L.S. & Zografi, G.** 2002. Influence of alkali metal counterions on the glass transition temperature of amorphous indomethacin salts. *Pharmaceutical research*, 19:649-654.
- Upadhyay, P., Dantuluri, A.K., Kumar, L. & Bansal, A.K.** 2012. Estimating relative stability of polymorphs by generation of configurational free energy phase diagram. *Journal of pharmaceutical sciences*, 101:1843-1851.
- Vasconcelos, T., Sarmiento, B. & Costa, P.** 2007. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. *Drug discovery today*, 12:1068-1075.
- Vippagunta, S.R., Brittain, H.G. & Grant, D.J.** 2001. Crystalline solids. *Advanced drug delivery reviews*, 48:3-26.
- Vo, C. L.N., Park, C. & Lee, B.J.** 2013. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. *European journal of pharmaceuticals and biopharmaceutics*, 85:799-813.
- Yadav, A.V., Shete, A.S., Dabke, A.P., Kulkarni, P.V. & Sakhare, S.S.** 2009. Co-crystals: a novel approach to modify physicochemical properties of active pharmaceutical ingredients. *Indian journal of pharmaceutical sciences*, 71:359.
- Yu, L.** 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Advanced drug delivery reviews*, 48:27-42.
- Yu, D.G., Zhu, L.M., Branford-White, C.J., Yang, J.H., Wang, X., Li, Y. & Qian, W.** 2011. Solid dispersions in the form of electrospun core-sheath nanofibers. *International journal of nanomedicine*, 6:3271-3280.
- Zecevic, D.E., Meier, R., Daniels, R. & Wagner, K.** 2014. Site specific solubility improvement using solid dispersions of HPMC-AS/HPC SSL-Mixtures. *European journal of pharmaceuticals and biopharmaceutics*, 87:264-270.

Zhaojie, M., Ming, Z., Shengnan, W., Xiaojia, B., Hatch, G.M., Jingkai, G. & Li, C. 2014. Amorphous solid dispersion of berberine with absorption enhancer demonstrates a remarkable hypoglycemic effect via improving its bioavailability. *International journal of pharmaceutics*, 467:50-59.

CHAPTER 2

MACROLIDES ANTIBIOTICS: CLARITHROMYCIN AND SPIRAMYCIN

2.1 Introduction

Recent studies have shown that opportunistic infections (OIs) remain a significant cause of approximately 90% of the morbidity and mortality in People Living with HIV (PLHIV), even in the era of HAART (Highly Active Antiretroviral therapy) (Iroezindu, 2016; Rubaihayo *et al.*, 2015). Although OIs are mainly a consequence of HIV infection, they can still occur in other immuno-compromised individuals like cancer and organ transplant patients (Jordan *et al.*, 2015; Rali *et al.*, 2016). Other conditions like asthma, inflammatory bowel disease, and rheumatoid arthritis being treated with cytotoxic or with anti-inflammatory monoclonal agents may further put individuals at risk of developing OIs (Berg *et al.*, 2014; Sepkowitz, 2002; Yomota *et al.*, 2012). On this note, *Mycobacterium tuberculosis* (TB), *Pneumocystis jirovecii* pneumonia (PCP), *Mycobacterium avium* complex (MAC) and Cryptosporidiosis are amongst the most prevalent and life threatening OIs among PLHIV in different populations (Iroezindu, 2016; Sepkowitz, 2002). A comprehensive quantitative microbial risk assessment in China showed that cryptosporidiosis alone could result in 70% mortality rate of PLHIV due to *cryptosporidium* in drinking water (Xiao *et al.*, 2012). Another study conducted in South Africa indicated that MAC alone is responsible for 14% deaths of adults using antiretroviral drugs (ARVs) (Karstaedt, 2012). Treatment of these two OIs (MAC and cryptosporidiosis) is currently provided by the macrolide antibiotics (clarithromycin, azithromycin, spiramycin and roxithromycin). This chapter will focus on the two macrolide antibiotics, clarithromycin and spiramycin and their importance in the treatment of life-threatening OIs as well as the current drawbacks in terms of the physico-chemical properties associated with both antibiotics.

2.2 Macrolides antibiotics

In general, macrolides are bacteriostatic, but can also be bactericidal depending on bacterial sensitivity and antibiotic concentration (Abu-Gharbieh *et al.*, 2004). They inhibit protein synthesis of susceptible microorganisms, both *in vivo* and *in vitro* with varying potencies. Macrolides reversibly bind to 23S ribosomal ribonucleic acid (rRNA) in the 50S subunit and inhibit translocation messenger RNA (mRNA) directed protein synthesis (Phan *et al.*, 2004; Retsema & Fu, 2001). They induce dissociation of peptidyl transfer RNA (tRNA) from the ribosome during translocation, suppressing RNA-dependent protein synthesis (Abu-Gharbieh *et al.*, 2004).

Macrolides are less active against gram-negative organisms due to their hydrophobic nature making it difficult to penetrate both the inner and outer membranes of gram-negative bacteria (Brisson-Noël *et al.*, 1988). Their overall antimicrobial spectrum includes i) gram-positive aerobes: *staphylococci*, *streptococci*; ii) gram-negative aerobes: *haemophilus influenzae*, *M. catarrhalis*; iii) anaerobes: *legionella pneumophila*, *chlamydia sp.* and iv) other bacteria: MAC, *treponemapallidum* (Alzolibani & Zedan, 2012; Steel *et al.*, 2012). In addition to antibacterial properties, macrolides possess anti-inflammatory and immuno-modulatory effects utilised in the treatment of rheumatoid arthritis, asthma, lupus erythematosus to mention but a few (Kwiatkowska *et al.*, 2013; Shimane *et al.*, 2001). They possess anticancer, antiviral, antimalarial activity and are also used in organ transplantation and interventional cardiology (Andersen *et al.*, 1998; Čulić *et al.*, 2001; Kwiatkowska *et al.*, 2013; Saviola *et al.*, 2013; Steel *et al.*, 2012; Rodriguez-Cerdeira *et al.*, 2012). Such a broad activity and indication spectrum promote the significance of macrolide antibiotics amongst other therapeutic agents and / or antibiotics (Minas, 2010).

Macrolide antibiotics are more active in slightly alkaline conditions, chemically stable at neutral pH but unstable in acidic conditions (Kosugi *et al.*, 2015; Nie *et al.*, 2013). With a molecular weight of >700 and hydrogen bond acceptors of >10, they do not fit into Lipinski's rule of five and consequently possess poor drug-like properties (Lipinski, 2000; Stepanic *et al.*, 2011). The poor physico-chemical properties remain their largest obstacle from obtaining optimum bioavailability, therapeutic response and effectiveness. The current poor treatment outcomes of MAC and cryptosporidiosis infections, may therefore be directly linked to poor aqueous solubility of clarithromycin and spiramycin.

In addition to the already poor physico-chemical properties and poor treatment outcomes of macrolides, antibiotic resistance continue to be problematic. Bacteria resist macrolide antibiotics in 3 ways: (i) through target-site modification by methylation or mutation; (ii) through efflux of the antibiotic, and (iii) by drug inactivation (Sener *et al.*, 2005). Sadly, resistance cannot be stopped, but can be combated by strategies which includes the design of new and novel antibiotics or the improvement of the physico-chemical properties of the existing ones (Sener *et al.*, 2005; Sharma *et al.*, 2012; Ranghar *et al.*, 2014). Due to difficulties in designing new and novel antibiotics over the decades, modification and development of existing antibiotics remains paramount in combating both antibiotic resistance together with the poor physico-chemical properties thereof (Coates *et al.*, 2011; Singh, 2014; Theuretzbacher *et al.*, 2015).

Macrolide antibiotics belong to natural polyketides first isolated from *streptomyces erythreus* (Avisar & Gozlan, 2013; Ding *et al.*, 2015; Katz & Baltz, 2016). Structural differences of macrolides arise from the amount of carbon atoms in the lactone ring together with the amount

and nature (neutral or basic) of sugar moieties attached (Or *et al.*, 2002; Phan *et al.*, 2004). An important class of all times comprise of 14-, 15- and 16- membered macrocyclic lactone ring with one or more sugar moieties and amino sugars attached (Čulić *et al.*, 2001; Jain & Danziger, 2004; Omura, 2002).

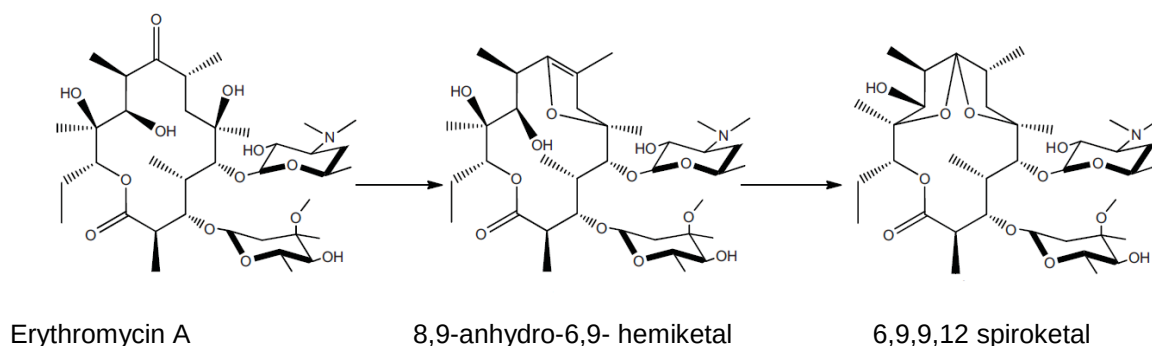
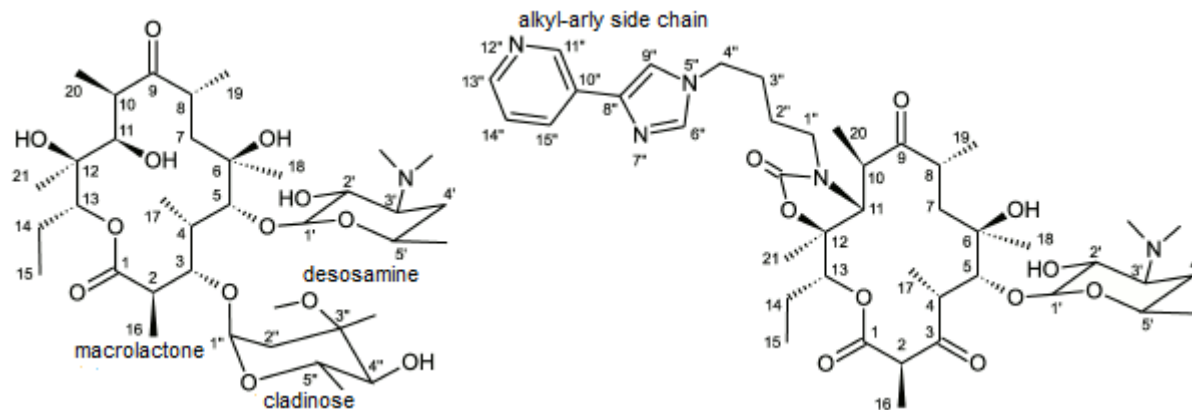


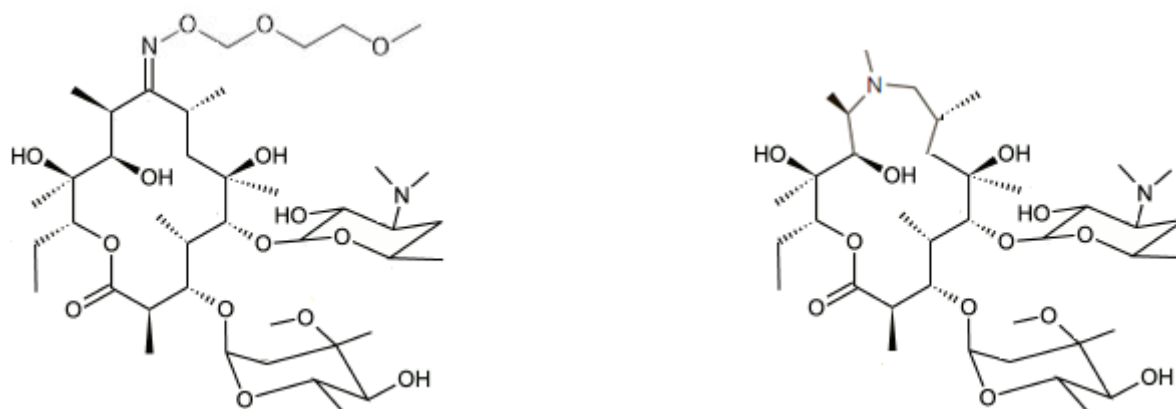
Figure 2.1: Degradation of erythromycin A under acidic conditions.

The prototype erythromycin A (Figure 2.1) is a 14-membered lactone ring with two sugar moieties, desosamine and cladinose (Douthwaite, 2001). However, erythromycin A is associated with gastrointestinal adverse effects as a consequence of the intermediate 8,9-*anhydro-6,9- hemiketal* during stomach acid degradation (Zuckerman, 2004). Such acidic instability together with narrow spectrum and short elimination half-life has led to the development of semisynthetic derivatives of erythromycin with improved antimicrobial spectrum, pharmacokinetic and pharmacodynamics properties (Jain & Danziger, 2004). Depending on chemical modification of erythromycin A core structure, these semisynthetic derivatives fall into three groups: i) compounds obtained by modification of the C-3 α -L-cladinose e.g telithromycin (Figure 2.2); ii) compounds with substituent modifications e.g roxithromycin (Figure 2.2) and iii) compounds obtained from modifications of the glycone A, e.g. azithromycin (Figure 2.2) (Retsema & Fu, 2001).



Erythromycin A

Telithromycin



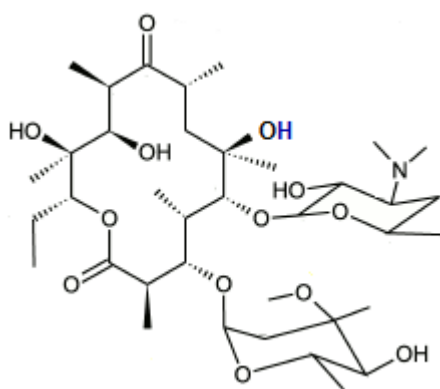
Roxithromycin

Azithromycin

Figure 2.2: Structural differences of erythromycin A and its derivatives.

2.3 Physico-chemical properties of clarithromycin

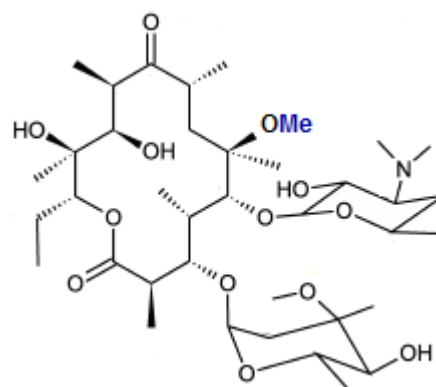
Clarithromycin (6-O-methyl-erythromycin) is effective against gram-positive, some gram-negative, anaerobic, *mycoplasma*, chlamydia and *helicobacter pylori* organisms (Adachi & Morimoto, 2002; Noguchi *et al.*, 2012; Wibawa *et al.*, 2003). Beneficially, clarithromycin works in synergy with its primary metabolite, 14-hydroxy 6-O-methyl- erythromycin which also has antimicrobial activity (Alder *et al.*, 1993; LeBel, 1993). Clarithromycin differs from erythromycin at the 6-position whereby a methoxy group replaces the hydroxyl group (Derakhshandeh *et al.*, 2014). In comparison, it presents improved acid stability, bioavailability and antibiotic activity than the erythromycin A core structure (Liang & Han, 2013; Nakagawa *et al.*, 1992).



Molecular formula: $C_{37}H_{67}NO_{13}$

Molecular weight: 733.93 g/mol

Erythromycin A



Molecular formula: $C_{38}H_{69}NO_{13}$

Molecular weight: 747.95 g/mol

Clarithromycin

Figure 2.3: Chemical structure and molecular formula of erythromycin and clarithromycin.

Literature shows that the macrolide antibiotic clarithromycin remains the cornerstone drug in the treatment of MAC (Andréjak *et al.*, 2015, Kadota *et al.*, 2016; Martins *et al.*, 2005; Nobre *et al.*, 2003) but MAC lung disease is well known for its high treatment failure rates (Aksamit *et al.*, 2014; Griffith & Winthrop, 2012). With propriety names *Biaxin*[®], *Klacid*[®], *Klaricid*[®], clarithromycin is a white to off-white crystalline odourless powder (Van Rooyen *et al.*, 2002). It is a weak base (pK_a 8.76) and soluble in organic solvents: methanol, acetonitrile, acetone and methylene chloride, but possess a low aqueous solubility (Inoue *et al.*, 2007; Lebedeva *et al.*, 2014). Clarithromycin possesses dissolution rate limited absorption and is classified under biopharmaceutical classification system (BCS) class II (low solubility and high permeability) (Jadhav *et al.*, 2012; Morakul *et al.*, 2013). Solubility of clarithromycin in water and in phosphate buffer solutions is temperature and pH-dependent respectively. As the temperature or pH increases, solubility decreases (Nakagawa *et al.*, 1992; Pereira *et al.*, 2013). This can be attributed to the instability of clarithromycin in acidic conditions for example, below pH of 3.0. Hydrolysis can occur, forming a microbiologically inactive hydrolysed clarithromycin form (Nakagawa *et al.*, 1992; Noguchi *et al.*, 2014).

Different solid-state forms has been reported on clarithromycin and therefore is known to exist in different crystalline forms. Up to this point in time, eight crystalline solid-state forms of clarithromycin have been prepared and characterised. “Form 0” is an ethanol solvate. Form I can be obtained from the desolvation of “Form 0” at ambient up to 50°C. Form II is an anhydrate solid-state form of this macrolide. Form III (acetonitrile solvate) and Form IV (hydrate) can easily convert to Form II at desolvation or dehydration temperatures of 100°C and higher (Avrutov *et al.*, 2003, Iwasaki *et al.*, 1993; Liang & Yao, 2008; Lui *et al.*, 2003;

Spanton *et al.*, 1999). Clarithromycin can also exist as a Form V (Gruss, 2008), a hydrochloride salt (Parvez *et al.*, 2000) and lastly a methanol solvate (Iwasaki *et al.*, 1993).

The anhydrate polymorphs, Form I and Form II are considered monotropic (Kuncham *et al.*, 2014) and Form I is metastable comparing to Form II (Sohn *et al.*, 2000; Spanton *et al.*, 1999). It was believed that clarithromycin Form II was more thermodynamically stable and had been included into the drug formulations on the market (Liu & Riley, 1998; Lee *et al.*, 2003). In 2008 Liang & Yao discovered that Form III is more stable than the anhydrate Form II, however up to this point in time clarithromycin containing products are formulated containing Form II (Kuncham *et al.*, 2014). This is probably due to the fact that the pharmaceutical industry steers away from including solvates into dosage forms, due to the inherent toxicity associated with solvents, such as acetonitrile.

Another recent study by Noguchi and co-workers (2014), points out that clarithromycin Form II can undergo polymorphic transformation from Form II to a Form A and Form B under acidic conditions. Furthermore, it is of concern that Form II may undergo crystal transition to its metastable form during the manufacturing process (Nozawa *et al.*, 2015). On another note, Form II can also absorb water without crystal lattice disruption or change, resulting in a new crystalline form (clarithromycin hemihydrate) which is metastable at room temperature (Tian *et al.*, 2009). The above mentioned different crystalline forms of clarithromycin may have different thermal stability, dissolution characteristics and bioavailability which in turn affect treatment outcomes.

A study by Yonemochi *et al.* (1999) proved that preparation methods such as grinding or spray drying could lead to the formation of partially amorphous clarithromycin. Another fairly recent study by Adrjanowicz *et al.* (2012) proved that a glassy state of clarithromycin can be prepared through the process of super-cooling of the molten drug. However, none of these studies reflected on the true aqueous solubility advantage that such a solid-state form of clarithromycin may have.

2.4 Physico-chemical properties of spiramycin

In comparison to the 14-membered erythromycin A derivatives like clarithromycin, the 16-membered macrolides shows better pharmacokinetic properties (LeBel, 1993). Structurally, the 16-membered macrolides comprise of an amino disaccharide-4-O-(L-mycarosyl)-D-mycaminose and/or D-desosamine (Or *et al.*, 2002). Basing on their glycosylation pattern, they can be classified as tylosins and leucomycins / spiramycins (Gebhardt *et al.*, 2005). Both tylosins and leucomycins can be further classified as natural or semi-synthetic derivatives. The tylosins family include tylosin and its semi-synthetic derivatives tilmicosin and tildipirosin (Cundliffe *et al.*, 2001; Marinelli & Genilloud, 2013; Or *et al.*, 2002). Semi-synthetic

leucomycins include rokitamycin and miocamycin whereas the natural molecules include kitasamycins, midecamycins and spiramycins (Przybylski, 2011).

Isolated from *streptomyces ambofaciens*, spiramycin is a 16-membered lactone (platenolide), which consist of two amino sugars (D-mycaminose and D-forosamine) and one neutral sugar (L-mycarose) (Aigle *et al.*, 2014; Zhu *et al.*, 2014). It is a mixture of three major (I, II, III), three minor components (IV, V, VI), two other spiramycins; 18-deoxy-18-dihydrospiramycin (DSPM) and 17-methylene spiramycin (Shi *et al.*, 2004; Chepkwony *et al.*, 2001; Or *et al.*, 2002). Although spiramycin I is the main component, quantity of each component varies according to the manufacturer (Maher *et al.*, 2008; Oka *et al.*, 2000; Wang *et al.*, 2010). Spiramycin I content is usually very high such that it is very difficult to isolate spiramycin II and III from the mixture (Leon & Jean, 1960). Molecular formulas and mass of the three major spiramycins are: spiramycin I ($C_{43}H_{74}N_2O_{14}$) 843.1 g/mol, spiramycin II ($C_{45}H_{76}N_2O_{15}$) 885.1 g/mol and spiramycin III ($C_{46}H_{78}N_2O_{15}$) 899.1g/mol (Ounnar *et al.*, 2016). Their melting points ranges between 133-137°C for spiramycin I, 130-133°C for spiramycin II and 128-131°C for spiramycin III (Omura & Nakagawa, 1975).

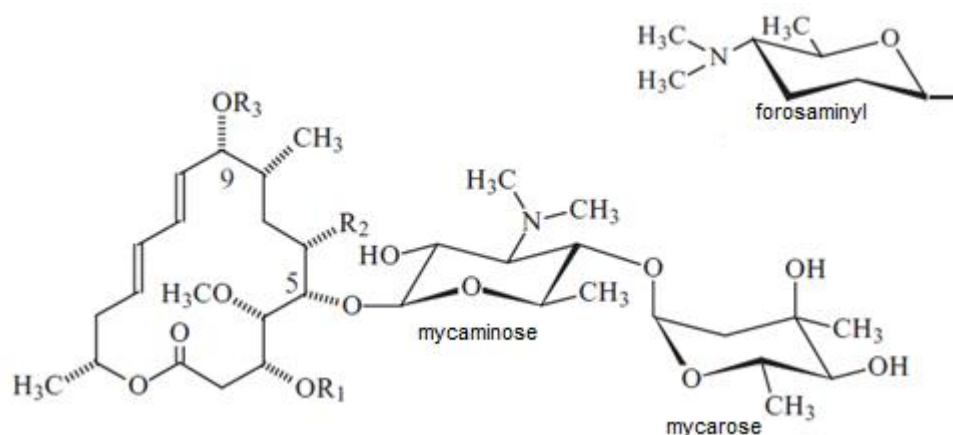


Figure 2.4: Chemical structure and molecular formula of spiramycins adapted from (Pendela *et al.*, 2007).

Table 2.1: Components of spiramycin

Compound	R₁	R₂	R₃	Mycarose
<i>Spiramycin I</i>	H	CH ₂ CHO	forosaminyI	+
<i>Spiramycin II</i>	COCH ₃	CH ₂ CHO	forosaminyI	+
<i>Spiramycin III</i>	COCH ₂ CH ₃	CH ₂ CHO	forosaminyI	+
<i>Spiramycin IV</i>	H	CH ₂ CH ₂ OH	forosaminyI	+
<i>Spiramycin V</i>	H	CH ₂ CHO	mycarosyl	+
<i>Spiramycin VI</i>	H	CH ₂ CH ₂ OH	mycarosyl	+
18-deoxy-18-dihydrospiramycin	H	CH ₂ CH ₃	forosaminyI	+
17-methylenespiramycin	H	C(CH ₂)CHO	forosaminyI	+
Neospiramycin I	H	CH ₂ CHO	forosaminyI	—
Neospiramycin II	COCH ₃	CH ₂ CHO	forosaminyI	—
Neospiramycin III	COCH ₂ CH ₃	CH ₂ CHO	forosaminyI	—

For spiramycin I, II and III, a disaccharide (mycarose and mycaminose) and a monosaccharide (forosamine) are attached at positions 5 and 9, respectively, via β -glycosidic bonds (Pendela, *et al.*, 2007). They possess different substituents at position 3, as follows: spiramycin I (3-OH), spiramycin II (3-O-acetyl) and spiramycin III (3-O-propionyl) (Maher *et al.*, 2008; Nguyen *et al.*, 2010; Or *et al.*, 2002; Przybylski, 2011). Spiramycin IV and spiramycin VI consist of a primary alcohol at position C-6 instead of an aldehyde group (Pendela *et al.*, 2007). The mycarosyl of spiramycin V and VI is replaced by forosaminyI in spiramycin I and IV (Pendela *et al.*, 2007). 18-deoxy-18-dihydrospiramycin (DSPM) and 17-methylenespiramycin consist of different substituents at position C-6. All neospiramycins lack the mycarose sugar (Omura, 2002; Or *et al.*, 2002; Pendela *et al.*, 2007).

Spiramycin is one of the macrolide antibiotics recommended for treating cryptosporidiosis among immuno-suppressed individuals (Huang *et al.*, 2015; Wang & Zhang, 2014). Yet, the drug is not commercially available in South Africa (Davis, 2011), which is one of the countries with a very high HIV burden in the world (Gómez-Olivé *et al.*, 2013). Although spiramycin is

recommended in PLHIV, studies have shown that its poor oral absorption rate and stability cause poor treatment outcomes (Huang *et al.*, 2015). Proprietary names of spiramycin include: *Acetylspiramycin*[®], *Dicorvin*[®], *Osmycin*[®], *Rovadin*[®], *Rovamicina*[®], *Rovamycin*[®], *Rovamycin Forte*[®], *Rovamycine*[®], *Rovamycin*[®] [inj.], *Selectomycin*[®], *Spirabiotic*[®], *Spiradan*[®], *Spiranter*. There is significant variation in the amounts (purity) of these components depending on the manufacturing process. In France, spiramycin I is > 85% whilst spiramycins II and III are < 5 and 10% respectively (Mourier & Brun, 1997). In China, spiramycin III is the main component > 60% whilst spiramycins I and II are < 10 and 30% respectively (Liu *et al.*, 1997). However, the antimicrobial activity of spiramycins I, II and III are more or less the same (Leon & Jean, 1960). Spiramycins II and III possess lower toxicity in comparison to spiramycin I (Leon & Jean, 1960).

Spiramycin is a slightly hygroscopic white or yellowish powder (Omura *et al.*, 1979). It is soluble in acetone, ethanol, methanol and slightly soluble in water. As a weak base (pKa of 8), spiramycin can be protonated and / or deprotonated depending on pH (Ounnar *et al.*, 2016). Under acidic conditions, the mycarose is lost to form the inactive neospiramycins that can further lose forosamine to form forocidin (Ramu *et al.*, 1995; Feng *et al.*, 1997). These acid unstable properties of spiramycin can lead to its demycarsolation into neospiramycin in the stomach (Or *et al.*, 2002). Although the three components of spiramycin are very similar in properties (Leon & Jean, 1960), it is well known that physico-chemical differences are common for each component in the mixture. The ability of spiramycin to exist as a mixture may therefore influence its physico-chemical properties. The acetylated derivative of spiramycin (acetylspiramycin) shows improved oral absorption rate and stability than spiramycin (Hong *et al.*, 2000; Huang *et al.*, 2015). Limited literature reports spiramycin as existing in an amorphous state (Abou-Zeid, *et al.*, 1980; Leon & Jean, 1961). However, during thorough literature searches little information was obtained on the physico-chemical properties of this drug.

2.5 Conclusion

In the era of HAART, OIs remain a major public health problem (Perbost *et al.*, 2005; Coelho, 2014) and measures to prevent and effectively treat them, remains very essential. Literature has proven there is no specific treatment targeting MAC and *cryptosporidium* directly (Gopinath & Singh, 2010; Huston *et al.*, 2015, Vandenberg *et al.*, 2012). However, macrolide antibiotics have shown to be the cornerstone in the treatment of MAC and *Cryptosporidiosis* but several studies have shown poor treatment outcomes of the selected OIs. Spiramycin, which is believed to be amorphous, has shown to be partially effective due to its poor oral absorption rate and stability (Huang *et al.*, 2015; Sinkala *et al.*, 2011). On a similar note,

clarithromycin Form II currently on the market, has been shown to be prone to solid-state transition during typical pharmaceutical processing steps (Nozawa *et al.*, 2015). A transition to a more stable solid-state form might cause a decrease in the solubility of clarithromycin, possibly influencing the bioavailability detrimentally. It is therefore evident that the particular solid-state form included into a solid dosage form exhibits poor physico-chemical properties and must be addressed in the cases of clarithromycin and spiramycin. Therefore, it might be useful to investigate the advantages that these two macrolides may hold when formulated into amorphous solid dispersions. In this regard, different solid-state analytical techniques were employed in the characterisation of both crystalline and amorphous forms of these macrolide antibiotics.

References

- 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.
- Abou-Zeid, A.Z. A., Abd-El-Galil, M.K. & Rabei, M.** 1980. Spiramycin, a macrolide antibiotic. *Zentralblatt für bakteriologie, parasitenkunde, infektionskrankheiten und hygiene. zweite naturwissenschaftliche abteilung: mikrobiologie der landwirtschaft, der technologie und des umweltschutzes*, 135:443-453.
- Adachi, T. & Morimoto, S.** 2002. Clarithromycin and new derivatives of erythromycin. (In Schönfeld, W. & Kirst, H.A., eds. *Macrolide antibiotics*. Switzerland: Birkhäuser Basel. p. 53-72).
- 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.
- Aigle, B., Lautru, S., Spitteller, D., Dickschat, J.S., Challis, G.L., Leblond, P. & Pernodet, J.L.** 2014. Genome mining of streptomyces ambofaciens. *Journal of industrial microbiology & biotechnology*, 41:251-263.
- Aksamit, T.R., Philley, J.V. & Griffith, D.E.** 2014. Nontuberculous mycobacterial (NTM) lung disease: the top ten essentials. *Respiratory medicine*, 108:417-425.
- Alder, J., Jarvis, K., Mitten, M., Shipkowitz, N.L., Gupta, P. & Clement, J.** 1993. Clarithromycin therapy of experimental treponema pallidum infections in hamsters. *Antimicrobial agents and chemotherapy*, 37:864-867.
- Alzolibani, A.A. & Zedan, K.** 2012. Macrolides in chronic inflammatory skin disorders. *Mediators of inflammation*, 2012:159354-159354.
- Andersen, S.L., Oloo, A.J., Gordon, D.M., Ragama, O.B., Aleman, G.M., Berman, J.D., Tang, D.B., Dunne, M.W. & Shanks, G.D.** 1998. Successful double-blinded, randomized, placebo-controlled field trial of azithromycin and doxycycline as prophylaxis for malaria in Western Ken. *Clinical infectious diseases*, 26:146-150.
- Andréjak, C., Almeida, D.V., Tyagi, S., Converse, P.J., Ammerman, N.C. & Grosset, J.H.** 2015. Characterization of mouse models of mycobacterium avium complex infection and evaluation of drug combinations. *Antimicrobial agents and chemotherapy*, 59:2129-2135.

- Avisar, D. & Gozlan, I.** 2013. Identification and detection of antibiotic drugs and their degradation products in aquatic samples. *Advances in chromatography*, 51:193-214.
- Avrutov, I., Lifshitz, I., Borochovit, R., Masarwa, B. & Schwartz, E.** 2003. Processes for preparing clarithromycin polymorphs and novel polymorph IV. (Patent: US. 10/459,124).
- Berg, G., Erlacher, A., Smalla, K. & Krause, R.** 2014. Vegetable microbiomes: is there a connection among opportunistic infections, human health and our 'gut feeling'?. *Microbial biotechnology*, 7:487-495.
- Brisson-Noël, A., Trieu-Cuot, P. & Courvalin, P.** 1988. Mechanism of action of spiramycin and other macrolides. *Journal of antimicrobial chemotherapy*, 22:13-23.
- Chepkwony, H. K., Vermaelen, A., Roets, E. & Hoogmartens, J.** 2001. Development and validation of an reversed-phase liquid chromatographic method for analysis of spiramycin and related substances. *Chromatographia*, 54:51-56.
- Coates, A. R., Halls, G. & Hu, Y.** 2011. Novel classes of antibiotics or more of the same?. *British journal of pharmacology*, 163:184-194.
- Coelho, L., Veloso, V.G., Grinsztejn, B. & Luz, P.M.** 2014. Trends in overall opportunistic illnesses, pneumocystis carinii pneumonia, cerebral toxoplasmosis and mycobacterium avium complex incidence rates over the 30 years of the HIV epidemic: a systematic review. *Brazilian journal of infectious diseases*, 18:196-210.
- Čulić, O., Eraković, V. & Parnham, M. J.** 2001. Anti-inflammatory effects of macrolide antibiotics. *European journal of pharmacology*, 429:209-229.
- Cundliffe, E., Bate, N., Butler, A., Fish, S., Gandeche, A. & Merson-Davies, L.** 2001. The tylosin-biosynthetic genes of streptomyces fradiae. *Antonie van leeuwenhoek*, 79: 229-234.
- Davis, S.** 2011. Macrolides and ketolides: more than just antimicrobials. *SA pharmaceutical journal*, 78:24-27.
- Derakhshandeh, K., Bahrami, G., Mohammadi, B. & Alizadeh, E.** 2014. Oral bioavailability and pharmacokinetic study of clarithromycin in different dosage forms in Iranian healthy volunteers. *Journal of bioequivalence & bioavailability*, 6:206-211.
- Ding, Q.L., Lv, D., Wang, B.J., Zhang, Q.L., Yu, Y.M., Sun, S.F., Chen, Z.B., Ma, H.Y. & Deng, Z.C.** 2015. Macrolide therapy in cryptogenic organizing pneumonia: a case report and literature review. *Experimental and therapeutic medicine*, 9:829-834.
- Douthwaite, S.** 2001. Structure–activity relationships of ketolides vs. macrolides. *Clinical microbiology and infection*, 7:11-17.

Feng, W. Z., Qi, P. Y., Zhou, T., Miao, Y. & Duan, X. B. 1997. Study on the degradation kinetics of spiramycin in acid and alkaline solutions. *Yao xue xue bao= Acta pharmaceutica sinica*, 32:934-937.

Gebhardt, P., Gräfe, U., Möllmann, U. & Hertweck, C. 2005. Semisynthetic preparation of leucomycin derivatives: Introduction of aromatic side chains by reductive amination. *Molecular diversity*, 9:27-32.

Gómez-Olivé, F.X., Angotti, N., Houle, B., Klipstein-Grobusch, K., Kabudula, C., Menken, J., Williams, J., Tollman, S. & Clark, S.J. 2013. Prevalence of HIV among those 15 and older in rural South Africa. *AIDS care*, 25:1122-1128.

Gopinath, K. & Singh, S. 2010. Non-tuberculous mycobacteria in TB-endemic countries: are we neglecting the danger?. *Plos neglected tropical diseases*, 4:615-615.

Griffith, D.E. & Winthrop, K.L. 2012. Mycobacterium avium complex lung disease therapy. *American journal of respiratory and critical care medicine*, 186:477-479.

Gruss, M. 2008. Polymorph of clarithromycin (form V). (Patent: US 12/060,370).

Hong, J., Liangzhen, W. & Xiazhen, L. 2000. The clinical evaluation of curative effect of acetylspiramycin on toxoplasmosis infection during pregnancy. *Chinese journal of practical gynecology and obstetrics*, 2:024.

Huang, M.Z., Li, J., Guan, L., Li, D. Q., Nie, X. M., Gui, R. & Chen, X. 2015. Therapeutic effects of acetylspiramycin and garlicin on cryptosporidiosis among drug users. *International journal for parasitology: drugs and drug resistance*, 5:185-190.

Huston, C.D., Spangenberg, T., Burrows, J., Willis, P., Wells, T.N. & van Voorhis, W. 2015. A proposed target product profile and developmental cascade for new cryptosporidiosis treatments. *Plos neglected tropical diseases*, 9:0003987.

Inoue, Y., Yoshimura, S., Tozuka, Y., Moribe, K., Kumamoto, T., Ishikawa, T. & Yamamoto, K. 2007. Application of ascorbic acid 2-glucoside as a solubilizing agent for clarithromycin: solubilization and nanoparticle formation. *International journal of pharmaceutics*, 331:38-45.

Iroezindu, M.O. 2016. Disparities in the magnitude of human immunodeficiency virus-related opportunistic infections between high and low/middle-income countries: Is highly active antiretroviral therapy changing the trend?. *Annals of medical and health sciences research*, 6:4.

Iwasaki, H., Sugawara, Y., Adachi, T., Morimoto, S. & Watanabe, Y. 1993. Structure of 6-O-methylerythromycin A (clarithromycin). *Acta crystallographica*, 49:1227-1230.

- Jadhav, K.R., Pacharane, S.S., Pednekar, P.P., Koshy, P.V & J Kadam, V.J** 2012. Approaches to stabilize amorphous form-A review. *Current drug therapy*, 7: 255-262.
- Jain, R. & Danziger, L.H.** 2004. The macrolide antibiotics: a pharmacokinetic and pharmacodynamic overview. *Current pharmaceutical design*, 10:3045-3053.
- Jordan, C.L., Taber, D.J., Kyle, M.O., Connelly, J., Pilch, N.W., Fleming, J., Meadows, H.B., Bratton, C.F., Nadig, S.N., McGillicuddy, J.W. & Chavin, K.D.** 2015. Incidence, risk factors, and outcomes of opportunistic infections in pediatric renal transplant recipients. *Pediatric transplantation*, 20:44-8.
- Kadota, T., Matsui, H., Hirose, T., Suzuki, J., Saito, M., Akaba, T., Kobayashi, K., Akashi, S., Kawashima, M., Tamura, A. & Nagai, H.** 2016. Analysis of drug treatment outcome in clarithromycin-resistant *Mycobacterium avium* complex lung disease. *BMC infectious diseases*, 16:1.
- Karstaedt, A.S.** 2012. Profile of cause of death assigned to adults on antiretroviral therapy in Soweto. *South African medical journal*, 102:680-682.
- Katz, L. & Baltz, R.H.** 2016. Natural product discovery: past, present, and future. *Journal of industrial microbiology & biotechnology*, 1-22.
- Kosugi, Y., Yamamoto, S., Sano, N., Furuta, A., Igari, T., Fujioka, Y. & Amano, N.** 2015. Evaluation of acid tolerance of drugs using rats and dogs controlled for gastric acid secretion. *Journal of pharmaceutical sciences*, 104:2887-2893.
- Kuncham, S., Shete, G. & Bansal, A.K.** 2014. Quantification of clarithromycin polymorphs in presence of tablet excipients. *Journal of excipients and food chemicals*, 5:65-78.
- Kwiatkowska, B., Maslinska, M., Przygodzka, M., Dmowska-Chalaba, J., Dabrowska, J. & Sikorska-Siudek, K.** 2013. Immune system as a new therapeutic target for antibiotics. *Advances in bioscience and biotechnology*, 4:91-101.
- Lebedeva, M.V., Prokhorova, A.F., Shapovalova, E.N. & Shpigun, O.A.** 2014. Clarithromycin as a chiral selector for enantioseparation of basic compounds in nonaqueous capillary electrophoresis. *Electrophoresis*, 35:2759-2764.
- LeBel, M.** 1993. Pharmacokinetic properties of clarithromycin: a comparison with erythromycin and azithromycin. *The Canadian journal of infectious diseases*, 4:148.
- Lee, T.S., Lee, J.C., Lee, K.I., Lee, G.S. & Kim, W.J.** 2003. Method of preparing form II crystals of clarithromycin. (Patent: US 6,506,886).
- Leon, N. & Jean, V.** 1960. Preparation of spiramycin iii. (Patent: US. 2,943,024).

- Leon, N. & Jean, P.H.** 1961. Spiramycins and their production. (Patent: US 3,000,78).
- Liang, J. H. & Yao, G. W.** 2008. A new crystal structure of clarithromycin. *Journal of chemical crystallography*, 38:61-64.
- Liang, J.H. & Han, X.** 2013. Structure-activity relationships and mechanism of action of macrolides derived from erythromycin as antibacterial agents. *Current topics in medicinal chemistry*, 13:3131-3164.
- Lipinski, C.A.** 2000. Drug-like properties and the causes of poor solubility and poor permeability. *Journal of pharmacological and toxicological methods*, 44:235-249.
- Liu, J.H. & Riley, D.A.** 1998. Preparation of crystal form II of clarithromycin. (Patent: US 5,844,105).
- Liu, L., Roets, E. & Hoogmartens, J.** 1997. *Journal of chromatography a*, 764:43–53.
- Lui, J.H., Henry, R.F., Spanton, S.G. & Riley, G.D.A.** 2003. 6-O-methylerythromycin A crystal form III. (Patent: US 6,627,743).
- Maher, H.M., Youssef, R.M., Khalil, R.H. & El-Bahr, S.M.** 2008. Simultaneous multiresidue determination of metronidazole and spiramycin in fish muscle using high performance liquid chromatography with UV detection. *Journal of chromatography b*, 876:175-181.
- Marinelli, F. & Genilloud, O., eds.** 2013. Antimicrobials: new and old molecules in the fight against multi-resistant bacteria. New York: Springer.
- Martins, A.B., Matos, E.D. & Lemos, A.C.M.** 2005. Infection with the mycobacterium avium complex in patients without predisposing conditions: a case report and literature review. *Brazilian journal of infectious diseases*, 9:173-179.
- Minas, W.** 2010. Erythromycins. (*In Encyclopedia of industrial biotechnology*).
- Morakul, B., Suksiriworapong, J., Leanpolchareanchai, J. & Junyaprasert, V. B.** 2013. Precipitation-lyophilization-homogenization (PLH) for preparation of clarithromycin nanocrystals: influencing factors on physicochemical properties and stability. *International journal of pharmaceutics*, 457:187-196.
- Mourier, P. & Brun, A.** 1997. Study of the metabolism of spiramycin in pig liver. *Journal of Chromatography b: biomedical sciences and applications*, 704:197–205.
- Nakagawa, Y., Itai, S., Yoshida, T. & Nagai, T.** 1992. Physicochemical properties and stability in the acidic solution of a new macrolide antibiotic, clarithromycin, in comparison with erythromycin. *Chemical and pharmaceutical bulletin*, 40:725-728.

- Nguyen, H.C., Karray, F., Lautru, S., Gagnat, J., Lebrihi, A., Huynh, T.D.H. & Pernodet, J.L.** 2010. Glycosylation steps during spiramycin biosynthesis in streptomyces ambofaciens: involvement of three glycosyltransferases and their interplay with two auxiliary proteins. *Antimicrobial agents and chemotherapy*, 54:2830-2839.
- Nie, Y., Sun, Y. & You, Q.D.** 2013. Synthesis and antibacterial activity of novel 10, 11-epoxy acylide erythromycin derivatives. *Chinese chemical letters*, 24:183-185.
- Nobre, V., Braga, E., Rayes, A., Serufo, J. C., Godoy, P., Nunes, N., Antunes, C.M. & Lambertucci, J. R.** 2003. Opportunistic infections in patients with AIDS admitted to an university hospital of the Southeast of Brazil. *Revista do instituto de medicina tropical de são paulo*, 45:69-74.
- Noguchi, S., Miura, K., Fujiki, S., Iwao, Y. & Itai, S.** 2012. Clarithromycin form I determined by synchrotron X-ray powder diffraction. *Acta crystallographica section C: crystal structure communications*, 68:41-44.
- Noguchi, S., Takiyama, K., Fujiki, S., Iwao, Y., Miura, K. & Itai, S.** 2014. Polymorphic transformation of antibiotic clarithromycin under acidic condition. *Journal of pharmaceutical sciences*, 103:580-586.
- Nozawa, K., Iwao, Y., Noguchi, S. & Itai, S.** 2015. Effect of surfactants or a water soluble polymer on the crystal transition of clarithromycin during a wet granulation process. *International journal of pharmaceutics*, 495:204-217.
- Oka, H., Harada, K. I., Suzuki, M. & Ito, Y.** 2000. Separation of spiramycin components using high-speed counter-current chromatography. *Journal of chromatography a*, 903:93-98.
- Omura, S., Ikeda, H., & Kitao, C.** 1979. Isolation and Properties of spiramycin I 3-Hydroxyl acylase from streptomyces ambofaciens. *Journal of biochemistry*, 86:753-758.
- Omura, S. & Nakagawa, A.** 1975. Chemical and biological studies on 16-membered macrolide antibiotics. *The journal of antibiotics*, 28:401-433.
- Omura, S.** 2002. Macrolide antibiotics: chemistry, biology, and practice. 2nd ed. San Diego: Academic Press.
- Or, Y. S., Binet, S. & Phan, L.T.** 2002. Bicyclic leucomycins. (Patent: US 6,462,026).
- Ounnar, A., Favier, L., Bouzaza, A., Bentahar, F. & Trari, M.** 2016. Kinetic study of spiramycin removal from aqueous solution using heterogeneous photocatalysis. *Kinetics and catalysis*, 57:200-206.
- Parvez, M., Arayne, M. S., Sabri, R. & Sultana, N.** 2000. Clarithromycin hydrochloride 3.5-hydrate. *Acta crystallographica section C: crystal structure communications*, 56:398-399.

- Perbost, I., Malafronte, B., Pradier, C., Santo, L.D.I., Dunais, B., Counillon, E., Vinti, H., Enel, P., Fuzibet, J.G., Cassuto, J.P. & Dellamonica, P.** 2005. In the era of highly active antiretroviral therapy, why are HIV-infected patients still admitted to hospital for an inaugural opportunistic infection?. *HIV medicine*, 6:232-239.
- Pendela, M., Govaerts, C., Diana, J., Hoogmartens, J., Van Schepdael, A. & Adams, E.** 2007. Characterization of impurities in spiramycin by liquid chromatography/ion trap mass spectrometry. *Rapid communications in mass spectrometry*, 21:599-613.
- Pereira, J.M., Mejia-Ariza, R., Ilevbare, G.A., McGettigan, H.E., Sriranganathan, N., Taylor, L.S., Davis, R.M. & Edgar, K.J.** 2013. Interplay of degradation, dissolution and stabilization of clarithromycin and its amorphous solid dispersions. *Molecular pharmaceuticals*, 10:4640-4653.
- Phan, L.T., Jian, T., Chen, Z., Qiu, Y.L., Wang, Z., Beach, T., Polemeropoulos, A. & Or, Y.S.** 2004. Synthesis and antibacterial activity of a novel class of 4'-substituted 16-membered ring macrolides derived from tylosin. *Journal of medicinal chemistry*, 47:2965-2968.
- Przybylski, P.** 2011. Modifications and biological activity of natural and semisynthetic 16-membered macrolide antibiotics. *Current organic chemistry*, 15:328-374.
- Rali, P., Veer, M., Gupta, N., Singh, A.C. & Bhanot, N.** 2016. Opportunistic pulmonary infections in immunocompromised hosts. *Critical care nursing quarterly*, 39:61-175.
- Ramu, K., Shringarpure, S. & Williamson, J.S.** 1995. A solution conformation analysis of forocidins I and isoforocidins I using NMR and molecular modeling. *Pharmaceutical research*, 12:621-629.
- Ranghar, S., Sirohi, P., Verma, P. & Agarwal, V.** 2014. Nanoparticle-based drug delivery systems: promising approaches against infections. *Brazilian archives of biology and technology*, 57:209-222.
- Retsema, J. & Fu, W.** 2001. Macrolides: structures and microbial targets. *International journal of antimicrobial agents*, 18: 3-10.
- Rodriguez-Cerdeira, C., Sanchez-Blanco, E. & Molares-Vila, A.** 2012. Clinical application of development of nonantibiotic macrolides that correct inflammation-driven immune dysfunction in inflammatory skin diseases. *Mediators of inflammation*, 2012.
- Rubaihayo, J., Tumwesigye, N. M. & Konde-Lule, J.** 2015. Trends in prevalence of selected opportunistic infections associated with HIV/AIDS in Uganda. *BMC infectious diseases*, 15:1.

Saviola, G., Abdi-Ali, L., Campostrini, L., Sacco, S., Baiardi, P., Manfredi, M., Benucci, M., Bucci, M. & Cirino, G. 2013. Clarithromycin in rheumatoid arthritis: the addition to methotrexate and low-dose methylprednisolone induces a significant additive value—a 24-month single-blind pilot study. *Rheumatology international*, 33:2833-2838.

Sener, B., Köseoglu, Ö., Gür, D. & Bryskier, A. 2005. Mechanisms of macrolide resistance in clinical pneumococcal isolates in a university hospital, Ankara, Turkey. *Journal of chemotherapy* 17:31-35.

Sepkowitz, K. A. 2002. Opportunistic infections in patients with and patients without acquired immunodeficiency syndrome. *Clinical infectious diseases*, 34:1098-1107.

Sharma, A., Kumar Arya, D., Dua, M., Chhatwal, G.S. & Johri, A.K. 2012. Nano-technology for targeted drug delivery to combat antibiotic resistance. *Expert opinion on drug delivery*, 9:1325-1332.

Shi, X., Zhang, S., Fawcett, J. P. & Zhong, D. 2004. Acid catalysed degradation of some spiramycin derivatives found in the antibiotic bitespiramycin. *Journal of pharmaceutical and biomedical analysis*, 36:593-600.

Shimane, T., Asano, K., Suzuki, M., Hisamitsu, T. & Suzaki, H. 2001. Influence of a macrolide antibiotic, roxithromycin, on mast cell growth and activation in vitro. *Mediators of inflammation*, 10:323-332.

Sinkala, E., Katubulushi, M., Sianongo, S., Obwaller, A. & Kelly, P. 2011. In a trial of the use of miltefosine to treat HIV-related cryptosporidiosis in Zambian adults, extreme metabolic disturbances contribute to high mortality. *Annals of tropical medicine & parasitology*, 105:129-134.

Singh, S.B. 2014. Confronting the challenges of discovery of novel antibacterial agents. *Bioorganic & medicinal chemistry letters*, 24:3683-3689.

Sohn, Y.T., Rhee, J.K. & Im, W.B. 2000. Polymorphism of clarithromycin. *Archives of pharmacal research*, 23:381-384.

Spanton, S.G., Henry, R.F., Riley, D.A. & Liu, J.H. 1999. Crystal form O of clarithromycin. (Patent: US 5,945,405).

Steel, H.C., Theron, A.J., Cockeran, R., Anderson, R. & Feldman, C. 2012. Pathogen-and host-directed anti-inflammatory activities of macrolide antibiotics. *Mediators of inflammation*, 2012.

Stepanic, V., Kostrun, S., Malnar, I., Hlevnjak, M., Butkovic, K., Caleta, I., Dukši, M., Kragol, G., Makaruha-Stegic, O., Mikac, L. & Ralic, J. 2011. Modeling cellular

pharmacokinetics of 14-and 15-membered macrolides with physicochemical properties. *Journal of medicinal chemistry*, 54:719-733.

Theuretzbacher, U., Van Bambeke, F., Cantón, R., Giske, C.G., Mouton, J.W., Nation, R.L., Paul, M., Turnidge, J.D. & Kahlmeter, G. 2015. Reviving old antibiotics. *Journal of antimicrobial chemotherapy*, 70:2177-2181.

Tian, J., Thallapally, P. K., Dalgarno, S. J. & Atwood, J.L. 2009. Free transport of water and CO₂ in nonporous hydrophobic clarithromycin form II crystals. *Journal of the American chemical society*, 131:13216-13217.

Vandenberg, O., Robberecht, F., Dauby, N., Moens, C., Talabani, H., Dupont, E., Menotti, J., Van Gool, T. & Levy, J. 2012. Management of a cryptosporidium hominis outbreak in a day-care center. *The pediatric infectious disease journal*, 31:10-15.

Van Rooyen, G.F., Smit, M.J., De Jager, A.D., Hundt, H.K.L., Swart, K.J. & Hundt, A.F. 2002. Sensitive liquid chromatography–tandem mass spectrometry method for the determination of clarithromycin in human plasma. *Journal of chromatography b*, 768:223-229.

Wang, M.J., Pendela, M., Hu, C.Q., Jin, S.H., Hoogmartens, J., Van Schepdael, A. & Adams, E. 2010. Impurity profiling of acetylspiramycin by liquid chromatography–ion trap mass spectrometry. *Journal of chromatography a*, 1217:6531-6544.

Wang, D. & Zhang, Y.Y. 2014. Intestinal pathological changes of Kunming mice infected by *Cryptosporidium* and the therapeutic efficacy of spiramycin on infected mice. *Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi= Chinese journal of parasitology & parasitic diseases*, 32:225-228.

Wibawa, J.I.D., Shaw, P.N. & Barrett, D.A. 2003. Quantification of clarithromycin, its 14-hydroxy and decladinose metabolites in rat plasma, gastric juice and gastric tissue using high-performance liquid chromatography with electrochemical detection. *Journal of chromatography b*, 783:359-366.

Xiao, S., An, W., Chen, Z., Zhang, D., Yu, J. & Yang, M. 2012. The burden of drinking water-associated cryptosporidiosis in China: the large contribution of the immunodeficient population identified by quantitative microbial risk assessment. *Water research*, 46:4272-4280

Yonemochi, E., Kitahara, S., Maeda, S., Yamamura, S., Oguchi, T. & Yamamoto, K. 1999. Physicochemical properties of amorphous clarithromycin obtained by grinding and spray drying. *European journal of pharmaceutical sciences*, 7:331-338.

Yomota, M., Amano, I., Horita, N., Takezawa, T., Arai, T., To, M. & To, Y. 2012. Serum immunoglobulin G is a marker for the risk of opportunistic infection in steroid-dependent severe asthmatic patients. *Internal medicine*, 51:2715-2719.

Zhu, P., Chen, D., Liu, W., Zhang, J., Shao, L., Li, J. A. & Chu, J. 2014. Hydroxylation and hydrolysis: two main metabolic ways of spiramycin I in anaerobic digestion. *Bioresource technology*, 153, 95-100.

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

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Introduction

Typical solid-state studies of pharmaceutical ingredients involve a combination of several techniques rather than focussing on single techniques or methods. This study used a range of methods in the physico-chemical characterisation of the purchased raw materials of spiramycin and clarithromycin, the neat amorphous solid-state form of clarithromycin as well as amorphous solid dispersions (ASDs) of both macrolides.

3.2 Materials and methods

3.2.1 Materials

Clarithromycin and spiramycin raw materials were purchased from DB Fine Chemicals (Johannesburg, South Africa). Polymers: Polyvinylpyrrolidone K25 (PVP K25), Polyvinylpyrrolidone K30 (PVP K30), Polyethylene glycol 8000 (PEG 8000), Pluronic® F-127 (PF-127) and Vinylpyrrolidone-vinyl acetate copolymer (Kollidon® VA-64) were purchased from DB Fine Chemicals (Johannesburg, South Africa). Other excipients: microcrystalline cellulose, lactose, magnesium stearate and talc were a kind donation from the Department of Pharmaceutics, School of Pharmacy, North-West University, Potchefstroom. Di-potassium hydrogen orthophosphate, potassium chloride, boric acid, potassium bipthalate, monobasic potassium phosphate, acetic acid, sodium acetate, ammonium acetate, hydrochloric acid and sodium hydroxide were purchased from ACE Chemicals (Johannesburg, South Africa) and all were of analytical grade. Acetonitrile and methanol were purchased from ACE Chemicals (Johannesburg, South Africa) and were of chromatography grade. Potassium bromide and glass beads were purchased from Sigma-Aldrich (Johannesburg, South Africa).

3.2.2 Preparation of neat amorphous solid-state form of clarithromycin

Various methods used for the preparation of amorphous forms include: quenching of melts, rapid precipitation by anti-solvent addition, freeze drying, spray drying, compression, rapid solvent evaporation, lyophilisation and many others (Yu, 2001). In this study, the method of quench cooling of the melt of the drug was used as preparation method for the amorphous solid-state form of clarithromycin. In this method, the crystalline raw material was spread out thinly and evenly on the surface of a foil paper. The prepared foil paper was then placed on a hot plate. The temperature at which the hot plate was set for the melting were deduced from differential scanning calorimetry (DSC) results obtained with the crystalline clarithromycin raw

material. Subsequently, the molten product was removed from the hot plate and quenched cooled on a cold surface. The amorphous product was removed from the foil paper and characterised to ensure the amorphous habit thereof.

3.2.3 Preparation of ASDs of clarithromycin and spiramycin

Miscibility between the API and the carrier of the solid dispersion has proven to have a clear impact on physical stability. The two should form a chemically homogeneous phase at molecular level (Baird & Taylor, 2012; Marsac *et al.*, 2009, Ivanisevic, 2010). API / polymer miscibility determination was therefore done so as to prepare ASDs of optimal stability. In this study miscibility of the clarithromycin and spiramycin in all available polymers i.e. PVP K25, PVP K30, PEG 8000, PF-127 and Kollidon® VA-64 was investigated. The melting point depression was determined so as to indicate not only the best API / polymer ratio but also the most suitable polymer grade of polyvinylpyrrolidone used.

3.2.3.1 Miscibility determination

Different weight / weight (w/w) ratios (1:1, 1:2, 1:3, 2:1, 2:3, 3:1 and 3:2) of the API to the polymer were prepared by mixing either clarithromycin or spiramycin and the corresponding polymer using a mortar and pestle. Each mixture was mixed for 3 minutes. The observation of the melting point depression of API / polymer PMs was done using DSC analysis. Miscible mixtures should show various degree of melting point depression whilst immiscible or partially miscible mixtures should show little or no melting point depression (Bikiaris *et al.*, 2005; Marsac *et al.*, 2009). To exclude or confirm any miscibility uncertainties of the melting point depression method, HSM was used as a supplementary method (as discussed in paragraph 3.24 (c)). Certain uncertainties in terms of miscibility were excluded or confirmed *via* more visual HSM analyses.

3.2.3.2 Preparation of ASDs

Techniques commonly used for the preparation of ASDs are: the quenching of melt method, solvent method, supercritical fluid method and hot melt extrusion method. Different methods of preparation may result in different amorphous solids of the same API (Kratochvíl, 2011) which may further exhibit significantly different physico-chemical properties (An & Sohn, 2009). In this study, several methods were employed in an effort to prepare optimised clarithromycin ASDs. These methods include: spray drying and solvent evaporation in several organic solvents such as acetonitrile, chloroform, propan-1-ol, dichloromethane, isopropanol etc. Following suit to the preparation method of neat amorphous forms, the quench cooling of the melt method proved again to be the best. Only the quenching of melt method was therefore used for the preparation of ASDs of both drugs. A physical mixture of either

spiramycin or clarithromycin and the chosen polymer was made. The physical mixture was evenly spread on a foil paper, followed by the heating of the mixture until a molten product was obtained. The melted mixture was then removed from the hot plate and subsequently cooled rapidly on a cold surface. The ASD product was then removed from the foil paper for physico-chemical characterisation and testing.

3.2.4 Characterisation methods

Several methods were employed in order to characterise the neat drugs (crystalline or amorphous solid-state forms) as well as the ASDs. These techniques included: differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), hot-stage microscopy (HSM), Fourier-transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRPD), scanning electron microscopy (SEM), non-isothermal crystallisation kinetics, solubility and dissolution as well as vapour sorption analysis.

3.2.4.1 Differential scanning calorimetry (DSC)

DSC is a thermal analysis technique which is used to measure the temperatures and heat flows associated with transitions in materials as a function of time and temperature (Gill *et al.*, 1993). In this analysis, energy is introduced simultaneously into a sample and a reference pan. Temperatures of both pans are then raised identically over time (Brittain, 2009). The amount of heat radiated (exothermic process) or absorbed (endothermic process) by the sample due to temperature difference between the sample and the reference is then measured (Gill *et al.*, 2010). These energy changes can therefore be used to determine thermal stability, structural-phase transition temperatures, latent heat of melting / fusion, reaction kinetics, crystallisation kinetics, degree of crystallinity, specific heat or heat capacity, purity and excipient compatibility (Brittain, 2009; Chieng *et al.*, 2011; Gill *et al.*, 2010; Höhne *et al.*, 2003; Storey & Ymen, 2011).

For amorphous forms, DSC analyses can indicate the glass transition temperature (T_g) and the subsequent crystallisation temperature of the amorphous form back to the thermodynamically most stable crystalline form. Dehydration or desolvation, recrystallisation and melting temperatures can be determined for crystalline forms (Chieng *et al.*, 2011). DSC analyses can therefore act as an indication whether a drug is crystalline or amorphous. In addition, amorphous solid forms can age or relax over time, thus the DSC for amorphous forms should show an enthalpy relaxation endotherm (Shamblin & Zografi, 1998; Surana *et al.*, 2004). DSC can also be used to assess the molecular mobility of the amorphous solids (Brittain, 2009).

In this study, a Shimadzu (Kyoto, Japan) DSC-60 instrument was used to record the DSC thermograms. Samples (3 – 5 mg) were weighed and sealed in aluminium crimp cells with pierced lids. The samples were heated from 25°C to 250°C with a heating rate of 10°C/min and a nitrogen gas purge of 35 ml/min. The onset temperatures of the thermal events were reported. Depending on the drug used or the type of thermal parameter to be determined, the heating rate as well as the end heating temperature was varied. In this study, the direction of heat flow for an exothermic event was recorded up the y axis whilst that of an endothermic event was recorded down the y axis.

- **Non isothermal crystallisation kinetics**

Recrystallisation of amorphous forms can be influenced by several factors such as T_g , melting and crystallisation enthalpy/entropy, molecular mobility and viscosity (Descamps & Dudognon, 2014). The activation energy at the glass transition temperature can be obtained using several methods such as Arrhenius and Kissinger analytical plots. The obtained activation energy (E_a) can be applied to determine fragility index (m) which is a measure of the deviation from typical Arrhenius behaviour (Dengale *et al.*, 2015). The value of m obtained can then be used to calculate both the strength parameter (D) and the temperature at which molecular mobility is close to zero (T_0) (Pina *et al.*, 2015). Depending on the values obtained, physical stability of amorphous forms can be predicted. It can therefore be determined whether the API in question can form a strong or fragile glass.

In this study, the effect of nucleation and crystal growth rate of neat amorphous clarithromycin form was quantified using non isothermal crystallization kinetics. Crystallization studies of neat amorphous clarithromycin were done on DSC (paragraph 3.2.4.1) at heating rates (β) of 3, 5, 7, 10, 15 and 20 °C min from 25 °C to 250 °C. An Arrhenius plot was constructed and the apparent activation energy (E_a) obtained from the slope and was used to calculate the values of m , D and T_0 . Crystallization studies of neat amorphous clarithromycin both in the absence and presence of crystalline clarithromycin seed were also determined. With each sample that was tested with seed crystals being present, 3 mg seed crystals were accurately weighed into the DSC sample pan. A Kissinger plot was constructed and the activation energy required for recrystallisation was determined from the slope.

3.2.4.2 Hot-stage microscopy (HSM)

HSM or thermo-microscopy is an analytical technique in which an optical property of the solid form is monitored versus time or temperature (Vitez *et al.*, 1998). The analysis can capture images from digital video cameras enabling the morphology of different solid-state forms to be compared by observing differences in thermal behaviour, characteristic points and the

corresponding temperatures (Brittain, 2009; Gilchrist *et al.*, 2012). In HSM, visual observation of crystallisation and other thermal phase changes are very clear and helpful in supporting DSC results in the investigation of different solid-state forms (Brittain, 2009; Chadha *et al.*, 2012). The amorphous solid-state form of a compound is typically characterised by lack of interference colours (Brittain, 2009; Qi *et al.*, 2008; Chadha *et al.*, 2012; Storey & Ymen, 2011). By analysing images collected from HSM, prediction of crystallisation and physical instability can therefore be achieved (Seefeldt *et al.*, 2007).

HSM analyses were performed with a Nikon Eclipse E4000 microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan) and a Linkam THMS600 heating stage equipped with a T95 LinkPad temperature controller (Surrey, England).

3.2.4.3 Fourier-Transform Infrared spectroscopy (FTIR)

Infrared (IR) refers to the electromagnetic spectrum which is the part between the visible and microwave regions consisting of a diverse collection of radiant energy from cosmic rays to X-rays, to visible light, to microwaves (Colthup *et al.*, 1990; Smith, 2011; Smith, 1998). These waves differ from each other in the length and frequency such that the IR region is divided into three regions namely near IR ($12\,500 - 4\,000\text{ cm}^{-1}$), mid IR ($4000 - 400\text{ cm}^{-1}$), and far IR ($400 - 10\text{ cm}^{-1}$) (Storey & Ymen, 2011). Absorption occurs when the radiant energy matches the energy of a specific molecular vibration (Smith, 2011). Each region is a representative of certain bonds e.g. the region ($3700\text{ to }3200\text{ cm}^{-1}$) is used to locate the alcohol (O-H), the terminal alkyne (C-H), the amine or amide (N-H), and the alkyne (C-H) bonds (Storey & Ymen, 2011). The peak positions corresponds with, i) molecular structure of the solid; ii) the presence and strength of specific interactions e.g. hydrogen bonds and iii) the type of functional group (Marsac *et al.*, 2006; Smith, 2011; Van den Mooter *et al.*, 2001).

By analysing hydrogen bonding patterns, the structure of a drug can be determined. In an amorphous solid dispersion, the drug and polymer may interact by a number of mechanisms, including hydrogen bonds. These intermolecular interactions may also cause changes in the orientation of the molecules, angles in functional groups or the number of available hydrogen bond donors and acceptors (Reed *et al.*, 1988; Taylor *et al.*, 2001; Punčochová *et al.*, 2014). Solvates are often observed in the IR region of $2000 - 4000\text{ cm}^{-1}$, but when water is included, the -OH stretching region is observed in the region of $3100 - 3600\text{ cm}^{-1}$ (Brittain & Grant, 1999).

The spectrum peaks of a pure amorphous solid in the absence of a polymer can be compared to the spectrum of the amorphous dispersion (Wegiel *et al.*, 2014). Thus the impact of different polymers in ASD's can also be studied using IR. The determination and selection of polymers with optimal stability to crystallisation can therefore be achieved (Brittain, 2009; Wegiel *et al.*,

2014). More so, it is well known that maximum stabilisation of the amorphous form can only be realised if the drug and the polymer are intimately mixed at the molecular level (Konno *et al.*, 2008; Qian *et al.*, 2010). IR can therefore be used to study specific interactions in ASD's so as to determine miscibility between the API and the polymer molecules (Rumondor *et al.*, 2009).

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

3.2.4.4 X-Ray Powder Diffraction (XRPD)

XRPD is an analytical technique mainly used for phase identification and characterisation of amorphous or crystalline forms (Brittain, 2009; Brittain & Grant, 1999; Newman & Byrn, 2003). In XRPD analysis, X-rays are generated in a cathode ray tube and are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded by a detector. A plot of intensity versus the diffraction angle (2θ) is then used to determine the crystallographic similarity of samples by pattern comparison (Brittain & Grant, 1999; Newman & Byrn, 2003). Every solid form will exhibit its own unique powder pattern due to the unique crystallography of its structure (Brittain & Grant, 1999). Crystalline forms will show Bragg diffraction of X-rays whilst amorphous forms will exhibit no Bragg diffraction peaks but rather a diffuse scattering halo (Brittain & Grant, 1999; Storey & Ymen, 2011; Newman & Byrn, 2003). Quantitative analysis can also be done to calculate the amount of each phase e.g. anhydrate and dihydrate phases present in a mixture of samples (Brittain & Grant, 1999). The extent of crystallinity in a solid can also be quantified. Thus, the XRPD peak positions, cell parameters, and patterns can therefore be used to identify different solid-state forms of a given drug (Chieng *et al.*, 2011).

XRPD data was obtained using a PANalytical Empyrean diffractometer equipped with a PIXcel^{3D} (PANalytical, Almelo, Netherlands) detector. The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; antiscatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, $2^\circ/\text{min}$ (step size, 0.025° ; step time, 1.0 sec).

3.2.4.5 Scanning electron microscopy (SEM)

SEM is used to generate high-resolution images that show surface detail in a sample (Storey & Ymen, 2011; Suga *et al.*, 2014). These images can reveal shapes, sizes and surface

features of APIs hence allowing interpretation, investigation and comparison of surface characteristics of different API solid forms.

Since SEM provides additional visual characterisation that cannot be acquired from other techniques, it should therefore be used in conjunction with X-ray crystallographic studies and other characterisation techniques (Brittain & Grant, 1999; Storey & Ymen, 2011). Different solid forms will show different observable habits such as surface roughness, cracks, crystal faces as well as dimensions, areas and angles. This makes it possible for visual characterisation of API solid-state forms.

During this study, the samples were adhered to a small piece of carbon tape, mounted onto a metal stub and coated with a gold-palladium film. An Eiko Engineering ion Coater IB-2, USA was used to coat the samples with a gold-palladium film. The coated samples were placed into the FEI Quanta 200 ESEM & Oxford INCA 400 EDS system (FEI, USA) and photomicrographs were taken of each sample.

3.2.4.6 High-performance liquid chromatography (HPLC)

HPLC is a separation technique that is used to separate, identify and quantify the individual components in a solution. It is used for quality control of pharmaceutical products including the analysis of macrolide antibiotics (Bekele & Gebeyehu, 2012; Lahane *et al.*, 2014; Gonzalez de la Huebra & Vincent, 2005). HPLC is characterised by five main components, i.e. pump, injector, column, detector and computer display. A small volume of liquid sample is injected into a column packed with stationary phase. The individual components of the sample are then moved down the packed column with the mobile phase from the pump being forced through the column at high pressure. As the solvent moves through the column, molecules from the sample will stick to the column and detach at different times. It is the differences in interaction with the column that help separate and distinguish these components from each other (Dong, 2006; Kazakevich & Lobrutto, 2007; Kromidas, 2008; Kupiec, 2004; McMaster, 2007).

For this specific study, a suitable HPLC method was developed and validated for analysis of the macrolide antibiotics (clarithromycin and spiramycin). API concentrations ranging from 0.2 - 5.0 mg/mL were used to prepare the standard solutions. Samples of 1.0 mg/ml were prepared for the commercially available macrolides. For spiramycin, which is not commercially available in SA, a solution to test method specificity was prepared by adding excipients: PVP K30, microcrystalline cellulose, lactose, magnesium stearate and talc. Different columns and mobile phases were tested and the following method was identified and subsequently validated for the quantification of clarithromycin and spiramycin: A Shimadzu (Kyoto, Japan) UFLC (LC-20AD) chromatographic system was used. A Phenomenex® Luna C8 (5µm) 250 ×

4.6 mm column and mobile phase consisting of 0.1 M phosphate buffer (pH 6.0) : acetonitrile (50 : 50) gave the best separation results. A flow rate of 0.5 mL/min with an injection volume of 10 μ L and 2 μ L for clarithromycin and spiramycin respectively at wavelengths of 205 and 232 nm was used. This HPLC method was accepted as a peer-reviewed manuscript – see Appendix A.

3.2.4.7 Solubility studies

Solubility is defined as the amount of substance that passes into the solution to achieve saturation at a constant temperature and pressure (Aulton, 2007). It is expressed as maximum volume or mass of the solute that dissolves in a given volume or mass of a solvent (Brittain, 2009).

Unless specified, the term "solubility" refers to the "equilibrium solubility" of the most stable crystal form in equilibrium with the solvent. Equilibrium (or thermodynamic) solubility is defined as the concentration of a compound in saturated solution when an excess of solid is present, such that the solution and solid are at equilibrium (Aulton, 2007; Brittain, 2009).

Different crystal forms are characterised by different lattice energies giving rise to different solubilities and dissolution rates. Additionally, transformation of metastable forms into more stable forms gives rise to solubility differences. Examples of such conversions include: transformation of one polymorphic phase into the other, hydration of an anhydrous form, desolvation of a solvate and transformation of an amorphous phase into a crystalline form (Brittain, 2009). It has been shown that the presence of a polymer could lower the surface tension of a drug, thereby improving the wettability thereof which might further lead to the enhancement of the solubility of the drug (Punčochová *et al.*, 2014).

Clarithromycin or spiramycin powder were individually weighed and transferred into 20 mL amber glass tubes with screw caps. The respective solvent (different buffer solutions ranging from pH 1-10) (10 mL) were added to each glass tube. Each tube was sealed with a small piece of Parafilm®, prior to attaching the cap tightly to avoid any leakage from the tubes. Six replicates of each solid-state form were prepared in the aqueous media. The glass tubes with the solutions were placed on a fixed rotating axis (54 rpm) that is submerged in a water bath. The temperature of the water bath was set at 25°C $\pm$ 2°C and maintained throughout the time period of rotation to obtain over saturation. Sufficient time for the solution to reach equilibrium was investigated and 24 hours were deemed sufficient for the solution to reach equilibrium. To remove any remaining solid particles, the solutions were filtered individually through 0.45 μ m polyvinylidene difluoride (PVDF) filters into HPLC vials. The samples were subsequently analysed using HPLC.

3.2.4.8 Dissolution studies

It is a well-known fact that differences in solubility may give rise to difference in dissolution rates which in turn could affect bioavailability (Brittain, 2009). By definition, dissolution is a process by which a solid substance goes into solution in two consecutive stages i.e. i) an interfacial reaction between solid and solvent breaks up the solid crystal for crystalline substances and opens the amorphous lattice for amorphous substances. The phase changes from molecules of solid to molecules of solute creating cavities in the solvent and ii) the solute molecules are transported away from the interface through a boundary layer by means of diffusion or convection (Aulton, 2007).

A VanKel700 dissolution bath was used for dissolution testing. USP apparatus 2 (paddle) was set up at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, with a rotational speed of 75 rpm, 900 ml dissolution medium (distilled water) was added to each dissolution vessel. Of each solid-state form or prepared ASD, approximately 500 mg was weighed into 10 ml test tubes, to which approximately 250 mg glass beads, $\leq 106\ \mu\text{m}$ (Sigma–Aldrich, South Africa) were added. An aliquot (5 ml) of dissolution medium, maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, were 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. For each withdrawal, 5 mL of solution was withdrawn from each dissolution vessel at predetermined time intervals. The samples were subsequently filtered through a $0.45\ \mu\text{m}$ PVDF filter into an HPLC vial. The filtered solutions were then be analysed by HPLC.

3.2.4.9 Vapour sorption analysis

Dynamic vapour sorption (DVS) analysis is a technique used to provide information indicating the relationship between sorbed vapour concentration or pressure and sorption with the solid (Storey & Ymen, 2011). It provides a technique for evaluating the stability of pharmaceutical active ingredients, excipients and formulations during processing as well as under a variety of temperature / humidity combinations (Burnett *et al.*, 2004; Punčochová *et al.*, 2014). It has been shown that amorphous solids often absorb relatively large amounts of water vapour compared to their corresponding crystalline phases (Burnett *et al.*, 2004). More water absorbs *via* hydrogen bonding due to the disordered amorphous structure. Unfavourably, sorbed water can act as a plasticising agent, thus lowering the glass transition temperature (T_g) below storage / room temperature. Moreover, additional water sorption may also lead to crystallisation below the glass transition temperature (Burnett *et al.*, 2004; Roos & Karel, 1991). Furthermore, low molecular weight amorphous materials may revert to their crystalline state over a certain temperature range. Thus it remains very important to determine the

threshold temperature and humidity at which amorphous drugs can be stored optimally (Ambarkhane *et al.*, 2005; Burnett *et al.*, 2004; Hunter *et al.*, 2010).

When using the isotherm experiment, the sample is exposed to a series of humidity step changes at constant temperature. The continuous weight change is recorded over time at any desired relative humidity (RH) ranging from 0 to 95% (Zaihan *et al.*, 2009). The sample is then staged at each humidity level until no further weight change is detected. The rate at which the sample equilibrates at each humidity level, as well as the overall shape of the resulting adsorption / desorption profile provides information about the sample's structure and long-term stability (Burnett *et al.*, 2004; Hogan & Buckton, 2001; Young *et al.*, 2007; Yu *et al.*, 2008). Vapour sorption analysis can therefore be used for evaluation of amorphous structure, hydrate formation and characterisation of morphological stability (Mackin *et al.*, 2002).

Vapour sorption studies were performed using a VTI-SA sorption analyser from TA Instruments (TA Instruments, USA), with a nitrogen gas flow rate of 20 ml/min. The microbalance was calibrated prior to each vapour sorption run with a standard weight range of 25 - 100 mg standard weights. The microbalance was set to zero prior to weighing of the sample into the quartz sample container. The sample was carefully placed into the sample holder and care was taken to evenly distribute the sample. The % RH / temperature program was set using TA Instruments Isotherm software. The % RH ramp was set from 5 to 95% RH, followed by a decrease in % RH from 95 to 5%. The last absorption phase was set to also ramp from 5 to 95% RH. 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 to the % RH ramp program. The temperature was held at a constant 25°C throughout the % RH ramp. The program criteria was set to 0.0001% weight change or 2 min stability of weight gained or lost before the program would continue to the next set parameter.

3.3 Conclusion

The various techniques used allow pharmaceutical solid-state scientists to have a better understanding of the diverse characteristics in crystalline and amorphous solid-state forms. A comprehensive solid-state characterisation of the APIs, spiramycin and clarithromycin, was achieved. The differences in the physico-chemical properties of amorphous solid-state forms as well as ASDs of each API were successfully explored.

References

- Ambarkhane, A.V., Pincott, K. & Buckton, G.** 2005. The use of inverse gas chromatography and gravimetric vapour sorption to study transitions in amorphous lactose. *International journal of pharmaceutics*, 294:129-135.
- An, S.G. & Sohn, Y.T.** 2009. Crystal forms of atorvastatin. *Archives of pharmacal research*, 32:933-936.
- Aulton, M.E.** 2007. Aulton's Pharmaceutics: The design and manufacture of medicines. 3rd ed. New York: Churchill Livingstone.
- Baird, J.A. & Taylor, L.S.** 2012. Evaluation of amorphous solid dispersion properties using thermal analysis techniques. *Advanced drug delivery reviews*, 64:396-421.
- Bekele, L.K. & Gebeyehu, G.G.** 2012. Application of different analytical techniques and microbiological assays for the analysis of macrolide antibiotics from pharmaceutical dosage forms and biological matrices. *International scholarly research network analytical chemistry*, <http://www.hindawi.com/journals/isrn/2012/859473/cta/> Date of access: 14 Aug. 2014.
- Bikiaris, D., Papageorgiou, G.Z., Stergiou, A., Pavlidou, E., Karavas, E., Kanaze, F. & Georgarakis, M.** 2005. Physicochemical studies on solid dispersions of poorly water-soluble drugs: evaluation of capabilities and limitations of thermal analysis techniques. *Thermochimica acta*, 439:58-67.
- Brittain, H.** 2009. Polymorphism in pharmaceutical solids. 2nd ed. New York: Informa healthcare.
- Brittain, H.G. & Grant, D.J.W.** 1999. Effects of polymorphism and solid-state solvation on solubility and dissolution rate. (In Brittain, H.G., ed. Polymorphism in pharmaceutical solids. New York: Marcel Dekker: p. 279-330).
- Burnett, D., Thielmann, F. & Booth, J.** 2004. Determining the critical relative humidity for moisture-induced phase transitions. *International journal of pharmaceutics*, 287:123-133.
- Chadha, R., Arora, P., Bhandari, S. & Bala, M.** 2012. Thermomicroscopy and its pharmaceuticals applications. *Current microscopy contributions to advances in science and technology*. <http://www.formatex.info/microscopy5/book/1013-1024.pdf> Date of access: 10 Aug. 2014
- Chieng, N., Rades, T. & Aaltonen, J.** 2011. An overview of recent studies on the analysis of pharmaceutical polymorphs. *Journal of pharmaceutical and biomedical analysis*, 55:618-644.

Colthup, N.B., Daly, L.H. & Wiberley, S.E. 1990. 3rd ed. Introduction to infrared and raman spectroscopy. London: Elsevier Science.

Dong, M.W. 2006. Modern HPLC for practicing scientists. 1st ed. New Jersey: Wiley

Gilchrist, S.E., Letchford, K. & Burt, H.M. 2012. The solid-state characterization of fusidic acid. *International journal of pharmaceutics*, 422:245-253.

Gill, P.S., Sauerbrunn, S.R. & Reading, M. 1993. Modulated differential scanning calorimetry. *Journal of thermal analysis and calorimetry*, 40:931-939.

Gill, P., Moghadam, T.T. & Ranjbar, B. 2010. Differential scanning calorimetry techniques: applications in biology and nanoscience. *Journal of biomolecular techniques*, 21:167-193.

Gonzalez de la Huebra, M.J. & Vincent, U. 2005. Analysis of macrolide antibiotics by liquid chromatography. *Journal of pharmaceutical and biomedical analysis*, 39:376-398.

Hogan, S.E. & Buckton, G. 2001. The application of near infrared spectroscopy and dynamic vapor sorption to quantify low amorphous contents of crystalline lactose. *Pharmaceutical research*, 18:112-116.

Höhne, G., Hemminger, W. & Flammersheim, H. 2003. Differential scanning calorimetry. 2nd ed. NewYork: Springer.

Hunter, N.E., Frampton, C.S., Craig, D.Q. & Belton, P.S. 2010. The use of dynamic vapour sorption methods for the characterisation of water uptake in amorphous trehalose. *Carbohydrate research*, 345:1938-1944.

Ivanisevic, I. 2010. Physical stability studies of miscible amorphous solid dispersions. *Journal of pharmaceutical sciences*, 99:4005-4012.

Kazakevich, Y.V. & Lobrutto, R., eds. 2007. HPLC for pharmaceutical scientists. New Jersey:Wiley.

Konno, H., Handa, T., Alonzo, D.E. & Taylor, L.S. 2008. Effect of polymer type on the dissolution profile of amorphous solid dispersions containing felodipine. *European journal of pharmaceutics and biopharmaceutics*, 70:493-499.

Kratochvíl, B. 2011. Solid forms of pharmaceutical molecules. (*In Šesták, J., Mareš, J.J. & Hubík, P., ed. Glassy, amorphous and nano-crystalline materials: Thermal physics, analysis, structure and properties. Netherlands: Springer. p. 129-140.*)

Kromidas, S., ed. 2008. HPLC made to measure: a practical handbook for optimization. Saarbrücken: Wiley.

- Kupiec, T.** 2004. Quality-control analytical methods: high-performance liquid chromatography. *International journal of pharmaceutical compounding*, 8:223-227.
- Lahane, S.B., Deokate, U. & Ahire, S.** 2014. Available analytical method for macrolide antibiotic. *International journal of pharmaceutical sciences review & research*, 26: 256-261
- McMaster, M.** 2007. HPLC: a practical user's guide. 2nd ed. Hoboken, New Jersey: Wiley.
- Mackin, L., Zanon, R., Park, J. M., Foster, K., Opalenik, H. & Demonte, M.** 2002. Quantification of low levels (< 10%) of amorphous content in micronised active batches using dynamic vapour sorption and isothermal microcalorimetry. *International journal of pharmaceutics*, 231:227-236.
- Marsac, P.J., Konno, H. & Taylor, L.S.** 2006. A comparison of the physical stability of amorphous felodipine and nifedipine systems. *Pharmaceutical research*, 23:2306-2316.
- Marsac, P. J., Li, T. & Taylor, L.S.** 2009. Estimation of drug–polymer miscibility and solubility in amorphous solid dispersions using experimentally determined interaction parameters. *Pharmaceutical research*, 26:139-151.
- Newman A.** 2012. Using thermal techniques for amorphous materials. http://www.icdd.com/ppxrd/11/presentations/tuesday/ann_newman_thermal_analysis_of_amorphous_materials.pdf. Date of access: 20 Aug. 2014.
- Newman, A.W. & Byrn, S.R.** 2003. Solid-state analysis of the active pharmaceutical ingredient in drug products. *Drug discovery today*, 8:898-905.
- Prime, R.B., Bair, H.E., Vyazovkin, S., Gallagher, P.K. & Riga, A.** 2009. Thermogravimetric analysis (TGA). (In Prime, R. B & Menczel J.D., ed. Thermal analysis of polymers: fundamentals and applications. New Jersey:Wiley. p. 241-317).
- Punčochová, K., Heng, J.Y., Beránek, J. & Štěpánek, F.** 2014. Investigation of drug–polymer interaction in solid dispersions by vapour sorption methods. *International journal of pharmaceutics*, 469:159-167.
- Qi, S., Avalle, P., Saklatvala, R. & Craig, D.Q.** 2008. An investigation into the effects of thermal history on the crystallisation behaviour of amorphous paracetamol. *European journal of pharmaceutics and biopharmaceutics*, 69:364-371.
- Qian, F., Huang, J. & Hussain, M.A.** 2010. Drug–polymer solubility and miscibility: stability consideration and practical challenges in amorphous solid dispersion development. *Journal of pharmaceutical sciences*, 99:2941-2947.
- Reed, A.E., Curtiss, L.A. & Weingold, F.** 1988. Intermolecular interactions from a natural bond orbital, donoracceptor viewpoint. *American chemical society*, 88:899-926.

Roos, Y. & Karel, M. 1991. Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. *Journal of food science*, 56: 38-43.

Rumondor, A.C., Ivanisevic, I., Bates, S., Alonzo, D.E. & Taylor, L.S. 2009. Evaluation of drug-polymer miscibility in amorphous solid dispersion systems. *Pharmaceutical research*, 26:2523-2534.

Seefeldt, K., Miller, J., Alvarez-Núñez, F. & Rodríguez-Hornedo, N. 2007. Crystallization pathways and kinetics of carbamazepine–nicotinamide cocrystals from the amorphous state by in situ thermomicroscopy, spectroscopy, and calorimetry studies. *Journal of pharmaceutical sciences*, 96:1147-1158.

Shamblin, S.L. & Zografi, G. 1998. Enthalpy relaxation in binary amorphous mixtures containing sucrose. *Pharmaceutical research*, 15:1828-1834.

Smith, B.C. 1998. Infrared spectral interpretation: a systematic approach. 1st ed. Florida: CRC press.

Smith, B.C. 2011. Fundamentals of Fourier transform infrared spectroscopy. 2nd ed. Parkway: CRC press.

Storey, R.A. & Ymen, I., eds. 2011. Solid state characterization of pharmaceuticals. UK: Wiley.

Suga, M., Asahina, S., Sakuda, Y., Kazumori, H., Nishiyama, H., Nokuo, T., Alfredsson, V., Kjellman, T., Stevens, M.S., Cho, H.S., Cho, M., Han, L., Che, S., Anderson, W.M., Schüth, F., Deng, H., Yaghi, O.M, Liu, Z., Jeong, Y.H., Stein, A., Sakamoto, K., Ryoo, R. & Terasaki, O. 2014. Recent progress in scanning electron microscopy for the characterization of fine structural details of nano materials. *Progress in solid state chemistry*, 42:1-21.

Surana, R., Pyne, A. & Suryanarayanan, R. 2004. Effect of preparation method on physical properties of amorphous trehalose. *Pharmaceutical research*, 21:1167-1176.

Taylor, L.S., Langkilde, F.W. & Zografi, G. 2001. Fourier transform raman spectroscopic study of the interaction of water vapour with amorphous polymers. *Journal of pharmaceutical sciences*, 90:888-901.

Van den Mooter, G., Wuyts, M., Blaton, N., Busson, R., Grobet, P., Augustijns, P. & Kinget, R. 2001. Physical stabilisation of amorphous ketoconazole in solid dispersions with polyvinylpyrrolidone K25. *European journal of pharmaceutical Sciences*, 12:261-269.

Vitez, I. M., Newman, A.W., Davidovich, M. & Kiesnowski, C. 1998. The evolution of hot-stage microscopy to aid solid-state characterizations of pharmaceutical solids. *Thermochimica acta*, 324:187-196.

Wegiel, L.A., Mauer, L.J., Edgar, K.J. & Taylor, L.S. 2014. Mid-infrared spectroscopy as a polymer selection tool for formulating amorphous solid dispersions. *Journal of pharmacy and pharmacology*, 66:244-255.

Young, P.M., Chiou, H., Tee, T., Traini, D., Chan, H., Thielmann, F. & Burnett, D. 2007. The use of organic vapor sorption to determine low levels of amorphous content in processed pharmaceutical powders. *Drug development and industrial pharmacy*, 33:91-97.

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

Yu, X., Kappes, S., Bello-Perez, L. & Schmidt, S. 2008. Investigating the moisture sorption behavior of amorphous sucrose using a dynamic humidity generating instrument. *Journal of food science*, 73:25-35.

Zaihan, J., Hill, C., Curling, S., Hashim, W. & Hamdan, H. 2009. Moisture adsorption isotherms of acacia mangium and endospermum malaccense using dynamic vapour sorption. *Journal of tropical forest science*, 21:277-285.

CHAPTER 4

THE PHYSICO-CHEMICAL AND THERMOKINETIC PROPERTIES OF CRYSTALLINE AND AMORPHOUS CLARITHROMYCIN

4.1 Introduction

Several studies have revealed that clarithromycin in its crystalline form exhibits very poor aqueous solubility (Esfandi *et al.*, 2014; Mansour *et al.*, 2010; Shahbazi Niaz *et al.*, 2014). This often leads to inadequate rates of dissolution and subsequent oral bioavailability concentrations. In turn, low bioavailability after oral administration can influence the treatment outcome of the drug detrimentally (Derakhshandeh *et al.*, 2014). Clarithromycin has been associated with poor treatment outcomes in *Mycobacterium Avium Complex* (MAC) infections. This is confirmed in a recent study by Kadota *et al.* (2016) who concluded that treatment with the macrolide antibiotic (clarithromycin) should not continue. They recommended that future studies should evaluate the precise clinical efficacy and effectiveness of the drug. Lately, the use of ASDs has been employed as a major strategy in obtaining good physical stability of the amorphous forms of drugs as well as enhanced dissolution and oral bioavailability thereof (Van den Mooter, 2012). A systematic review by Newman *et al.* (2012), assessing the performance of ASDs indicated that they can improve bioavailability by 82%. This implies that treatment outcomes and drug efficacy will consequently improve. In this study thorough physico-chemical characterisation of clarithromycin raw material and neat amorphous form was done. A combination of characterisation techniques was preferred in order to achieve a comprehensive understanding of the solid form properties of clarithromycin. The determined characteristics were used to successfully prepare ASDs of the antibiotic so as to improve the aqueous solubility, dissolution rate and physical stability of the amorphous form of clarithromycin.

4.2 Preparation of clarithromycin neat amorphous form

During this study, several methods were employed in an effort to prepare clarithromycin into an amorphous solid-state form. These methods involved: grinding (milling), heating by using hot air, rapid evaporation of several organic solvents, spray drying and lastly quench cooling of the melt. The quench cooling of the melt method (described in chapter 3) proved to be the best method to prepare an amorphous form of clarithromycin. This method was therefore employed throughout this study. The neat amorphous form will be referred to as (CLAM), since it only consists of the drug without the addition of polymers. Thorough physico-chemical characterisation was done in comparison to the raw material in order to validate if an amorphous form was indeed obtained.

4.3 Physico-chemical characterisation of crystalline clarithromycin and the neat amorphous form

Literature informs that clarithromycin can exist in several crystalline solid-state forms. The certificate of analysis of the clarithromycin used in this study did not specify which solid-state form was purchased. Therefore it was necessary to characterise the initial solid-state form by DSC, SEM, XRPD, FT-IR and HSM. Studies on i) physical and chemical stability, ii) equilibrium solubility in aqueous media at various pH levels and iii) dissolution in water were performed. Results were compared to those obtained for the prepared neat amorphous form (CLAM).

4.3.1 Differential scanning calorimetry

The DSC thermogram obtained for purchased clarithromycin raw material showed a sharp endothermic peak (melting point) at 228.4°C, (Figure 4.1(a)) which resembles that of crystalline clarithromycin form II reported in literature (Kuncham *et al.*, 2014; Lui & Riley, 1998; Nozawa *et al.*, 2015).

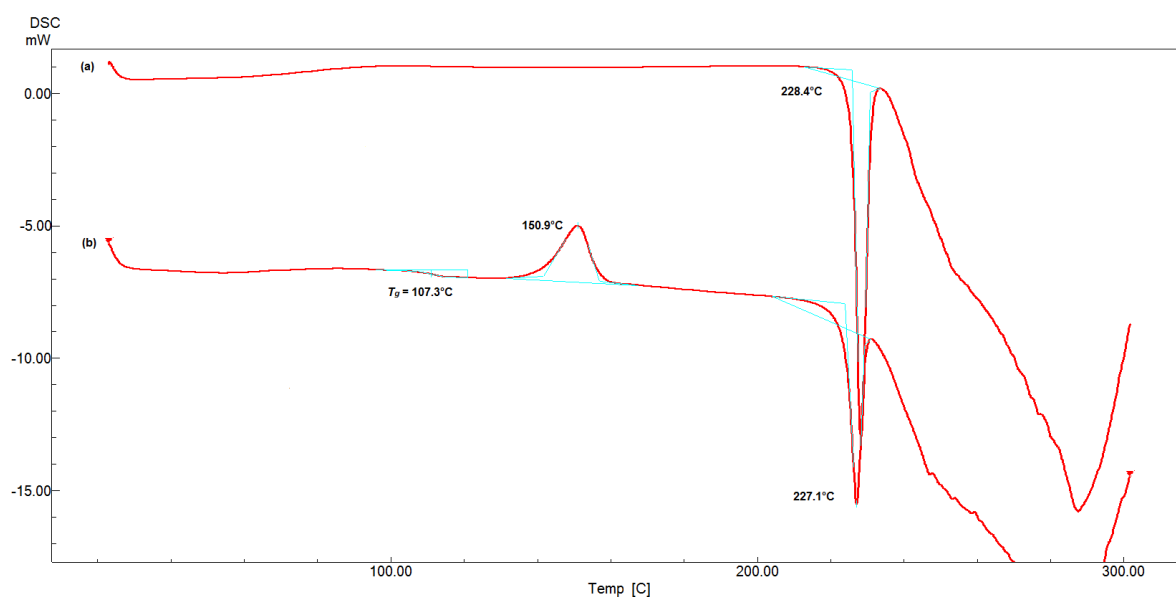


Figure 4.1: Overlay of the DSC thermograms obtained for (a) crystalline clarithromycin versus (b) clarithromycin neat amorphous form.

It is a well-known phenomenon that during heating, amorphous forms become sufficiently mobile above the T_g such that reorganisation of the molecules occur, leading to subsequent crystallisation of the amorphous form. As dictated by literature, DSC results showed this behaviour for the prepared CLAM. Figure 4.1(b) shows a T_g at $\approx 107.3^\circ\text{C}$ accompanied by an exothermic phase transition (recrystallisation) at 150.9°C. Subsequently, upon further heating, an endothermic melting transition (melting of the recrystallised clarithromycin)

occurred at 227.1°C, confirming the physical instability of the neat amorphous form upon heating. At this point, it was quite an interesting observation due to the fact that amorphous forms of two other macrolide antibiotics, namely; azithromycin and roxithromycin, did not show any recrystallisation behaviour upon heating thereof (Aucamp *et al.*, 2012; Aucamp *et al.*, 2015b; Milne *et al.*, 2016). To obtain more information on these two materials, and to investigate whether the amorphous form recrystallised to clarithromycin Form I or II further characterisation was explored by XRPD.

4.3.2 X-ray power diffraction

The purchased clarithromycin raw material showed Bragg diffraction peaks (Figure 4.2 (a)) which serves as evidence that it exist in a crystalline form. On a closer look, the XRPD diffraction pattern resembles that of clarithromycin form II reported in literature (Kuncham *et al.*, 2014; Liu & Riley, 1998; Nozawa *et al.*, 2015). Results correlate well with the obtained DSC thermogram (Figure 4.1 (a)) implying that the purchased clarithromycin exist in its crystalline Form II.

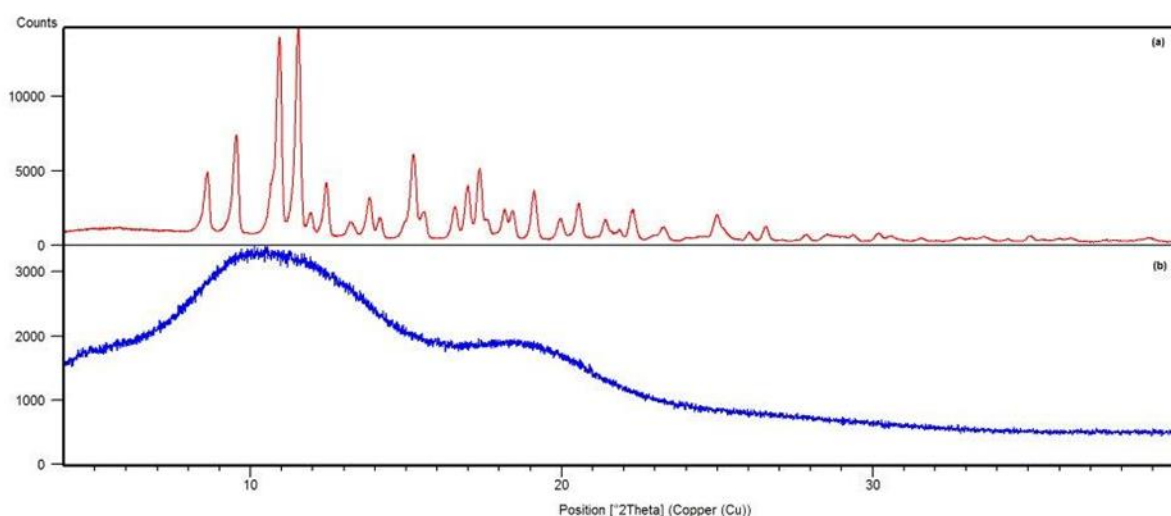


Figure 4.2: Overlay of the XRPD patterns of (a) crystalline clarithromycin vs (b) clarithromycin neat amorphous form.

For the prepared CLAM form, the resulting diffractogram showed a diffuse scattering halo. These results are in correspondence with those obtained by DSC, interpreting that an amorphous form of clarithromycin was successfully prepared from its crystalline counterpart. Since CLAM shows recrystallisation behaviour upon heating (Figure 4.1 (b)), it was imperative to determine whether the amorphous form crystallises to Form I or Form II. Figure 4.3 depicts an overlay of XRPD diffractograms of the purchased clarithromycin Form II and the crystallised amorphous form.

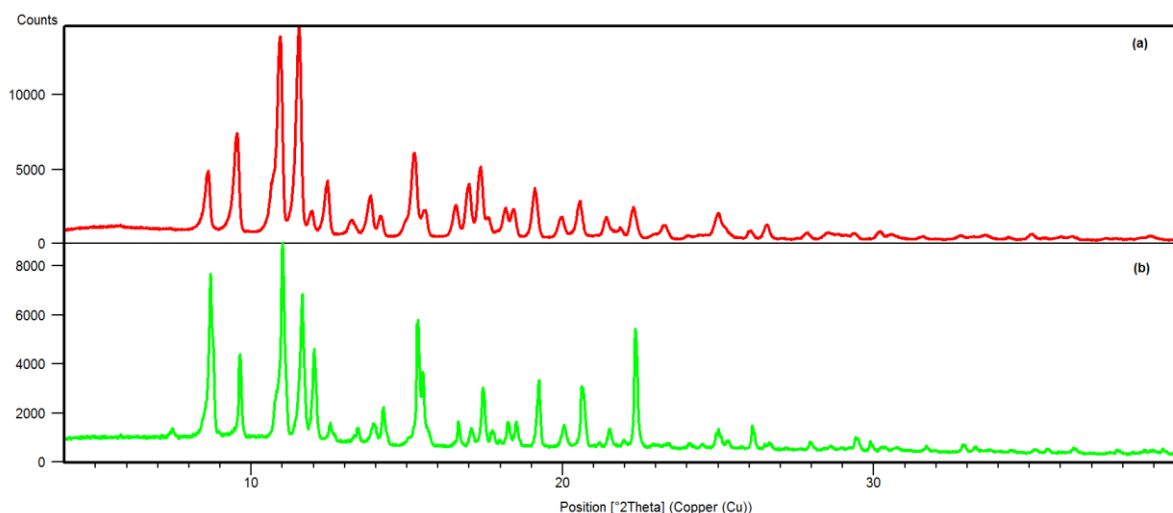


Figure 4.3: Overlay of XRPD diffractograms of (a) clarithromycin Form II and (b) the crystallised solid-state form obtained from the recrystallised amorphous solid-state form upon heating.

The Bragg peaks of clarithromycin Form II and the crystalline form obtained from the crystallisation process of CLAM were tabulated in Table 4.1 for comparison purposes. Some differences in relative peak intensities were identified between the diffraction peaks of the two samples; however both samples indicated peak relative intensity of 100.0% at $^{\circ}2\theta$ position of 10.9 – 11.0. Interestingly, one characteristic peak for clarithromycin form I at a $^{\circ}2\theta$ position of 15.4 (Liu *et al.*, 1999) was only noted in the Bragg peaks of CLAM at a relative peak intensity of 62.9%. This may imply a degree of crystallisation to Form I but to a very negligible extent. Thereby it was deduced that, CLAM crystallised to the thermodynamically most stable Form II. In order to distinguish the differences between CLAM and clarithromycin Form II, further characterisation by FT-IR was explored.

Table 4.1: XRPD Bragg peak listing obtained for purchased crystalline clarithromycin, clarithromycin Form II as discussed in literature and recrystallised amorphous clarithromycin

Purchased clarithromycin		Clarithromycin Form II as described in literature (Liu & Riley, 1998)		Amorphous clarithromycin recrystallised upon heating	
°2θ	Relative intensity (%)	°2θ	Relative intensity (%)	°2θ	Relative intensity (%)
8.7	27.1	8.5	None provided	8.7	86.1
-		-		8.8	43.7
9.6	45.1	9.5		9.6	42.1
10.9	100.0	10.8		11.0	100.0
11.6	97.9	11.5		11.6	76.0
12.0	10.6	11.9		12.0	45.6
12.4	25.4	12.4		-	-
13.9	16.8	13.7		-	-
14.2	8.0	14.1		14.2	16.6
15.3	39.3	15.2		15.3	60.8
-	-	-		15.4	62.9
16.6	14.6	16.5		-	-
17.0	26.2	16.9		-	-
17.4	34.8	17.3		17.4	28.1
18.2	14.3	18.1		18.2	11.6
18.5	13.6	18.4		18.5	11.9
19.1	23.7	19.0		19.3	34.1
19.9	10.0	19.9		-	-
20.6	17.9	20.5		20.7	30.1
-		-		22.3	59.1
-		-		22.4	43.0

4.3.3 Fourier-transform infrared spectroscopy (FT-IR)

The obtained FT-IR spectra for clarithromycin Form II and CLAM are depicted in Figure 4.4.

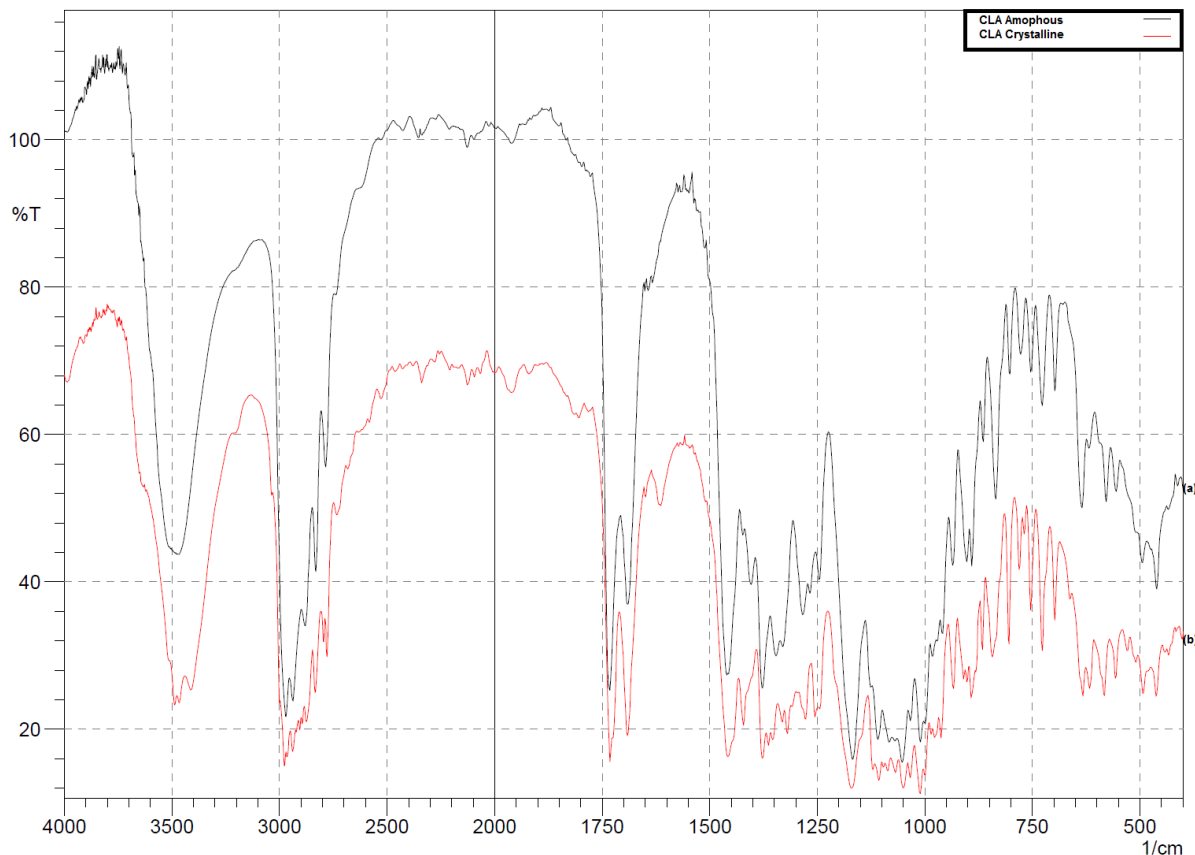


Figure 4.4: Overlay of FT-IR spectra obtained for (a) CLAM (the neat clarithromycin amorphous form) (b) and clarithromycin Form II.

The vibrational frequencies of both solid-state forms of clarithromycin were investigated by IR spectroscopy from 400 to 4000 cm^{-1} and characteristic major peaks of crystalline clarithromycin were found in the region of 1691.57 cm^{-1} (ketone carbonyl), 1732.08 cm^{-1} (lactone carbonyl), 1421.54 cm^{-1} (N-CH₃) and 3500 cm^{-1} (hydrogen bonding between -OH functional groups), as shown in Table 4.2.

Table 4.2: Summary of the most characteristic absorbance bands detected for crystalline clarithromycin over a scanned range of 400 – 4 000 cm⁻¹

Energy (Wavenumber) cm ⁻¹	Functional group
1000 - 1200	-C-O-C-Stretch
1170	Aliphatic –CH stretch.
1340 - 1400	CH ₂ group
1421.54	N-CH ₃
1691.57	C=O ketone carbonyl
1732.08	Lactone carbonyl
2780 - 3000	Alkane C-H stretch
3466 - 3500	Tertiary N-H stretch
3500	Hydroxyl -OH stretch

In correspondence to the DSC and XRPD results, the FT-IR absorbance peaks of the crystalline clarithromycin raw material further confirm that it existed in its Form II polymorph as reported in literature (Liu & Riley, 1998). The FT-IR peak positions of CLAM (Figure 4.4 (a)) correspond with the molecular structure of the raw material but with some broader and lower intensity peaks. In comparison, major spectra differences noted were:

- i) decrease in intensity for N-CH₃ absorbance peak in the amorphous form;
- ii) decrease or absence of the peak at 1614 cm⁻¹ with the amorphous form. This is because formation of an amorphous form causes reduction/weakening of interactions that are a function of crystal lattice packing (Andrews *et al.*, 2010).
- iii) saturated alkane C-H stretch with crystalline Form II and;
- iv) absence or broadening and a shift to a higher wavenumber of N-H stretch in the amorphous form. This implies weakening of the hydrogen bonds thus loss of ability to form hydrogen bonds interactions which can cause instability of amorphous forms (Andrews *et al.*, 2010; Konno & Taylor, 2006).

At this point, it was interesting to note that the FT-IR spectra obtained for CLAM closely resembles that obtained for clarithromycin Form I (Figure 4.5) reported in literature (Liu & Riley, 1998).

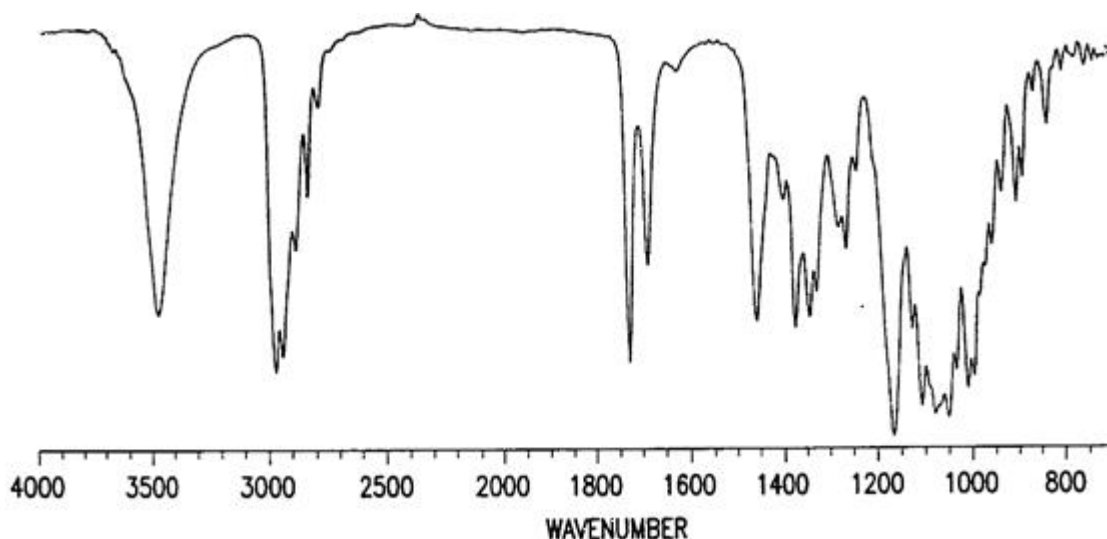


Figure 4.5: FT-IR spectra obtained for clarithromycin Form I (Liu & Riley, 1998).

This could imply molecular relaxation of the amorphous form towards Form I (Graeser *et al.*, 2010). On this note, DSC in this study (Figure 4.1 (b)) recorded recrystallisation of CLAM to Form II at 150.9°C which closely resembles the polymorphic phase transition of clarithromycin Form I to Form II at 151°C in literature (Kuncham *et al.*, 2014).

4.3.4 Scanning electron microscopy

The next step was the visual characterisation of the samples which was done using SEM to obtain particle surface or morphology detail differences between crystalline clarithromycin Form II and CLAM as shown in Figures 4.6 and 4.7, respectively.



Figure 4.6: SEM micrograph obtained for crystalline clarithromycin Form II.

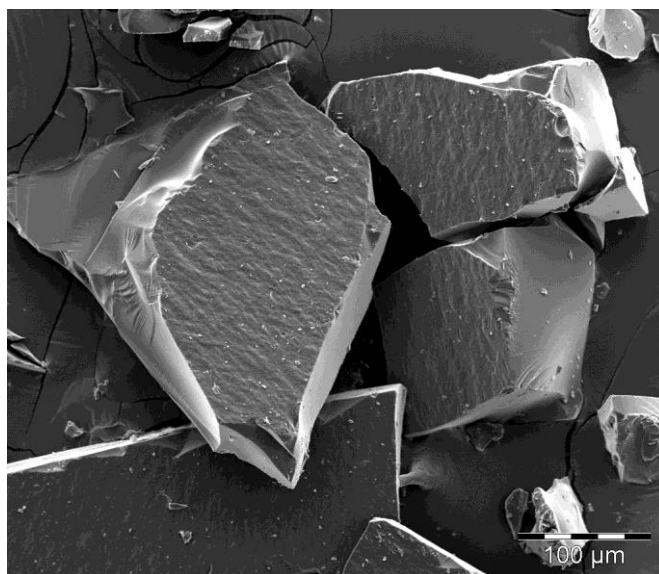


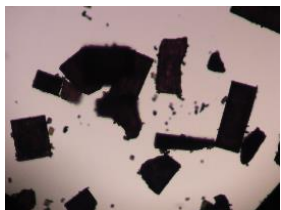
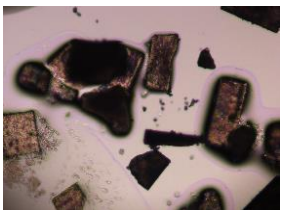
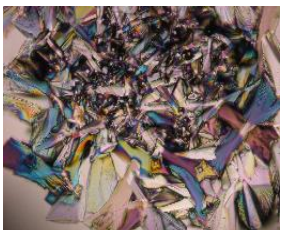
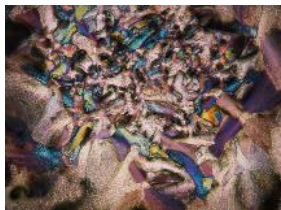
Figure 4.7: SEM micrograph obtained for CLAM.

Clarithromycin Form II exhibits a columnar crystal habit with a more fractured surface whilst CLAM exhibits a smoother surface due to the glassy nature as well as a result of the disorderly molecular structure. In order to enable an in-depth visual observation of the two solid-state forms, further characterisation was then done using hot-stage microscopy.

4.3.5 Hot-stage microscopy (HSM)

Crystalline clarithromycin Form II and CLAM were individually placed on clean glass slides and heated on a heating stage at a heating rate of 1°C/min. Samples were observed through a polarizer and the visual observation of the phase changes were captured as shown in Tables 4.3 and 4.4, respectively.

Table 4.3: Temperature dependent phase changes for crystalline clarithromycin Form II visually observed by HSM

			
a) Clarithromycin Form II (25°C).	b) Start of melting (≈ 231°C).	c) Melting (≈ 233°C).	d) Completely melted (235°C).
			
e) Crystallisation upon cooling (≈ 86°C).	f) Crystal growth (56°C).	g) Continual crystal growth (33°C).	h) Completely recrystallised (27°C).
			
i) Melting of the recrystallised form (225°C).	j) Melting (228°C).	k) Melting (231°C).	l) Completely melted (233°C).

In contrast to quench cooling of the melt, slow cooling allows molecular mobility and sufficient re-arrangement of clarithromycin molecules such that well-defined crystals with birefringence became visible under polarised light during subsequent cooling of the molten clarithromycin Form II (Table 4.3). XRPD analysis of the formed crystals proved that clarithromycin Form I was formed during the cooling process, with resulting Bragg peaks from the XRPD analysis (Figure 4.8) correlating very well with that reported in literature for clarithromycin Form I (Liu & Riley, 1998).

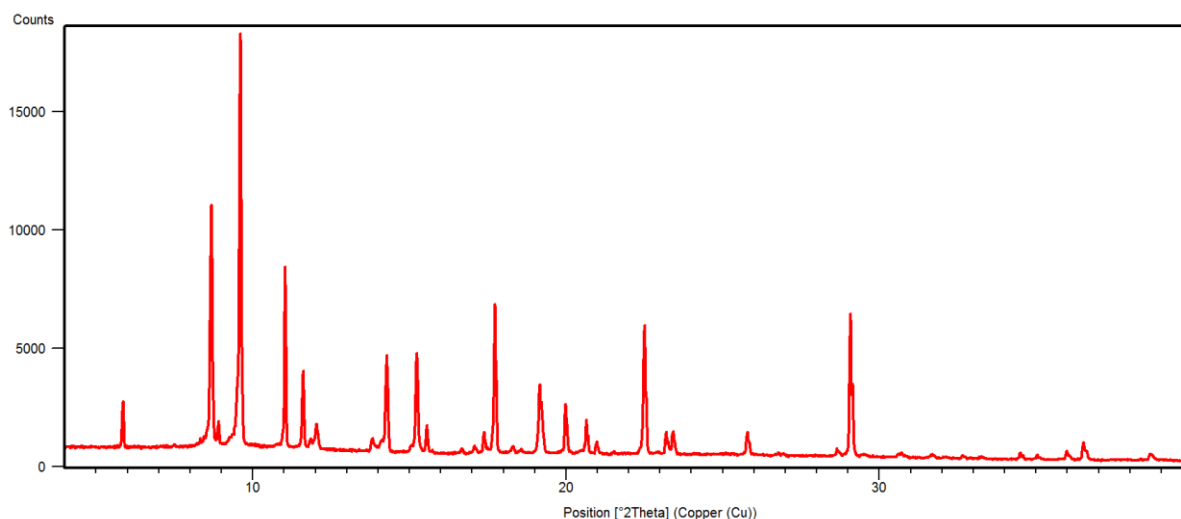
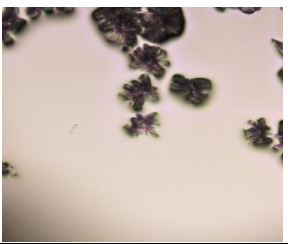
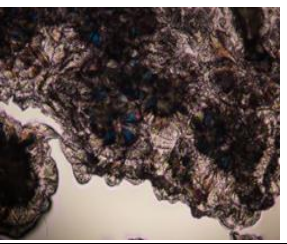


Figure 4.8: XRPD pattern obtained during the slow cooling of molten clarithromycin Form II.

Clarithromycin Form II therefore did not recrystallise upon heating but only upon slow cooling of the melt to form Form I. HSM analysis on phase changes upon heating of CLAM was also captured as shown in Table 4.4

Table 4.4. HSM temperature dependent phase changes for CLAM

			
a) CLAM – showing brittleness (25°C).	b) Glass Transition onset (128°C).	c) CLAM - rubbery phase (133°C).	d) CLAM - liquid phase (136°C).
			
e) Nuclei emerging (149°C).	f) Crystal growth from nuclei (152°C).	g) Crystal growth continues (157°C).	h) Completely recrystallised (170°C).

HSM captured the recrystallisation tendency of CLAM during heating. When an amorphous form is heated above T_g , it transforms from a brittle, less viscous state to a rubbery / fluid like state (Table 4.4(a-e)) (Donth, 2013). The HSM micrographs was in good correlation with the

DSC results obtained (Figure 4.1 (b)). Upon heating (Table 4.4), CLAM underwent T_g at 128°C (b) thereafter molecules were sufficiently mobile at 136°C (d) such that they reorganised to clarithromycin Form II (h) at 170°C. Nucleation could be clearly observed at (e) until complete recrystallisation (h). The crystallisation kinetics of clarithromycin neat amorphous form was therefore deemed necessary to investigate by non-isothermal crystallisation studies and will be discussed in the subsequent section.

4.3.6 Non-isothermal crystallisation kinetics of amorphous clarithromycin

It is well known that nucleation and crystal growth can be influenced by T_g , molecular mobility and viscosity (Descamps & Dudognon, 2014; Sun *et al.*, 2012). The effect of nucleation and crystal growth rate as observed in Table 4.4 can be quantified by either isothermal or non-isothermal methods (Yu, 2001). In this chapter, crystallisation kinetics of CLAM was investigated through non-isothermal heating of the amorphous form (Chapter 3). This method closely resembles non-ambient thermal conditions that were used during preparation of CLAM (Baghel *et al.*, 2016b).

It has been well explained that transformation from the melt (liquid) to amorphous (glassy) state occurs at T_g , where molecular mobility slows down such that below T_g , equilibrium cannot be reached (Baghel *et al.*, 2016a). On this note, amorphous forms show improved stability at temperatures below T_g (ideally at $T_g - 50$ K) as well as at zero mobility temperature (T_0) (temperature at which molecular mobility is close to zero) (Capen *et al.*, 2012; Shamblin *et al.*, 1999). However, in most cases storage or processing temperatures of $T_g - 50$ K is impractical, especially in terms of standard pharmaceutical manufacturing process. Moreover, it should be noted that reduction of molecular mobility does not always lead to improved stability of amorphous forms (Bhardwaj & Suryanarayanan, 2012; Schammé *et al.*, 2015). A useful method that can be applied to determine the physical stability of amorphous forms is the calculation of the fragility index (m), which is a measure of the deviation from typical Arrhenius behaviour (Sippel *et al.*, 2016). It measures how relaxation time (τ) decreases with an increase in temperature around T_g (Arnoult *et al.*, 2007). Strong glass formers categorised by values of $m < 40$ are considered more stable than fragile glass formers which are categorised by values $m > 75$. However, some fragile glassy-forms do not always correlate with this phenomenon (Adrianowicz *et al.*, 2012; Pina *et al.*, 2015). Connected to fragility is the strength parameter (D) whereby strong glass formers are categorised by values of $D > 30$ and fragile glass formers by values of $D < 10$ (Pina *et al.*, 2015).

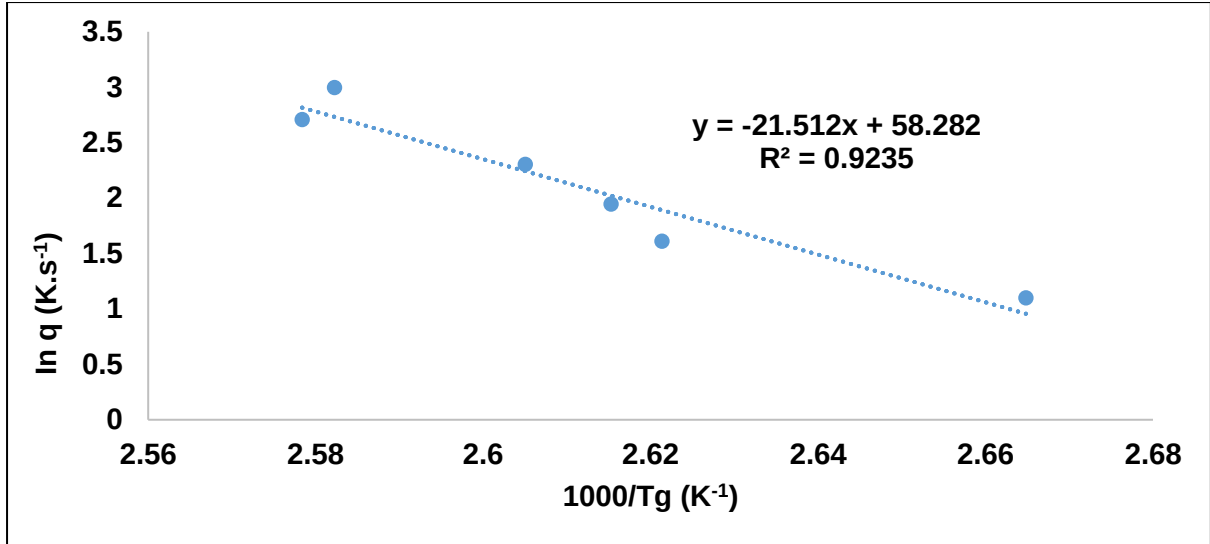


Figure 4.9: Arrhenius plot for the glass transition temperature ($1000/T_g$) affected by varying heating rates (β).

In this study, an Arrhenius plot, Figure 4.9 was constructed as described in Chapter 3. The apparent activation energy (E_a) was obtained from the slope ($\ln(\beta)$ versus $1000/T_g = -E_a/R$) (Baghel *et al.*, 2016a). An E_a -value of 178.85 kJ/mol was obtained and applied to calculate fragility index (m), strength parameter (D) and the Kauzmann temperature (T_0) using the following equations:

Fragility index (Kawakami *et al.*, 2015):

$$m = \left[\frac{d \log \tau}{d \left(\frac{T_g}{T} \right)} \right] = \frac{\Delta H^*(T_g)}{\ln 10 RT_g} \quad (1)$$

thus, equation (2) can be deduced as (Kawakami *et al.*, 2015):

$$m = \frac{1}{2.303} \left[\frac{E_a(T_g)}{RT_g} \right] \quad (2)$$

Strength parameter (D) (Graeser *et al.*, 2009):

$$D = \frac{\ln(10) m^2 \min}{m - m_{\min}} \quad (3)$$

thus

$$D = \frac{2.303 \times 17^2}{m - 17} \quad (4)$$

Whereby 17 is the order of magnitude in the viscosity change from T_g to η_0 . (Baghel *et al.*, 2016b; Aucamp *et al.*, 2015a).

Zero mobility temperature (T_0) (Yu, 2001):

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

Fragility index (m) obtained from equation 2 was substituted in equation 4 to obtain the strength parameter (D), which was in turn substituted in equation 5 to obtain zero mobility temperature (T_0). From the calculation, the prepared neat amorphous clarithromycin by quench cooling is a strong glass with fragility index of (m) = 9.34 and strength parameter (D) of 86.96. Its T_g of $\cong 107.3^\circ\text{C}$ is significantly higher than room temperature, by $> 80^\circ\text{C}$, suggesting that at ambient conditions, mobility should be negligible. This is further supported by the obtained T_0 of 49.2°C which is also above room temperature i.e. a probability of long term stability at room temperature. Although physically stable at room temperature, the preceding discussed results resolved that CLAM crystallise to a more thermodynamically stable Form II upon heating at 150.9°C . It was therefore worth investigating the amount of activation energy required for the recrystallisation process to occur.

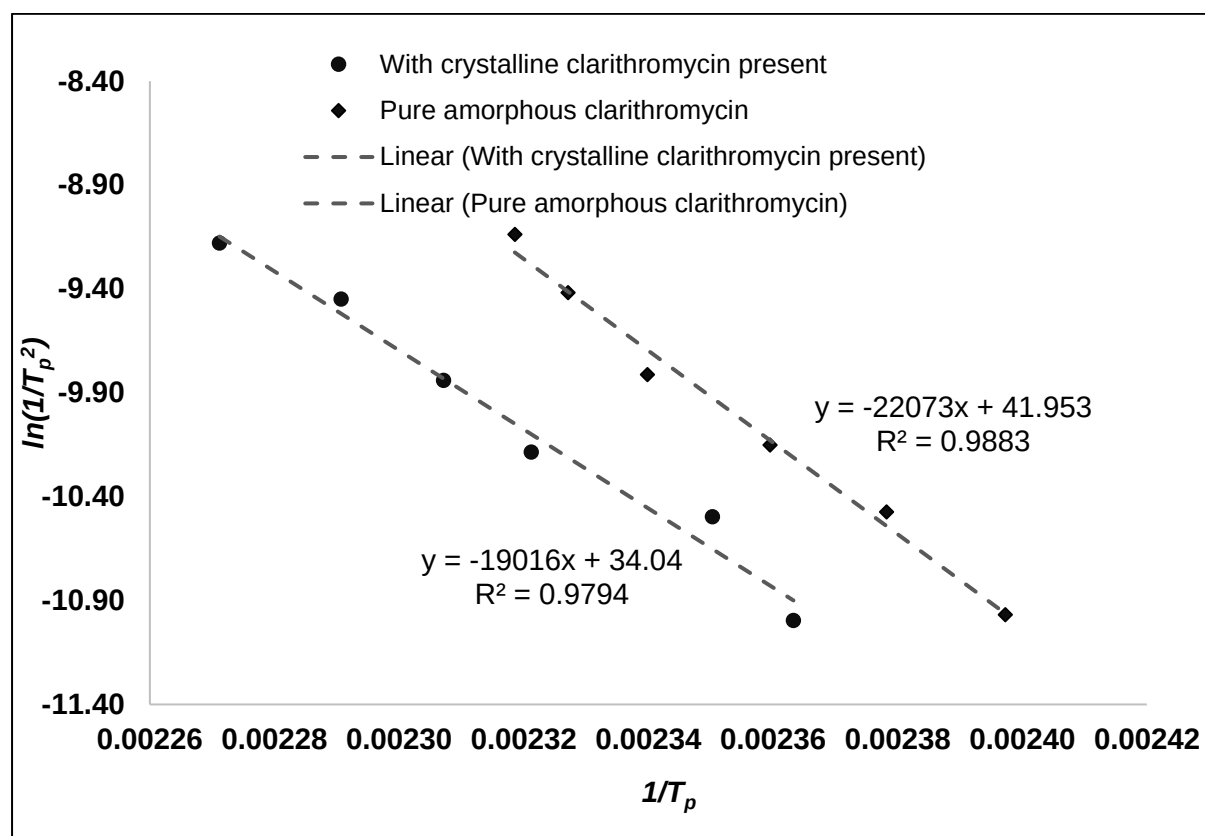


Figure 4.10: A Kissinger plot of the non-isothermal crystallisation of amorphous clarithromycin.

Kissinger's analysis (Chapter 3) was applied to calculate the activation energy required for the recrystallisation of amorphous clarithromycin to occur. Figure 4.10 depicts the Kissinger plots of $\ln(\beta/T_p^2)$ versus $1/T_p$ obtained from DSC analyses of CLAM in the presence and absence of crystalline clarithromycin Form II seeds. The slope of the Kissinger plot is $-E_a/R$. Non-

isothermal activation energy required for CLAM to crystallise without the presence of seed crystals was determined to be $183.51 \text{ kJ.mol}^{-1}$. When seed crystals of clarithromycin Form II were present, the activation energy for recrystallisation was determined to be $158.10 \text{ kJ.mol}^{-1}$. Results concluded that non-isothermal crystallisation of CLAM *via* nucleation (without seed crystals) requires more energy than when seed crystals are present in the sample. The rate of nucleation and crystal growth can be influenced by moisture hence it was necessary to do vapour sorption analysis.

4.3.7 Vapour sorption analyses

It is a well-known fact that moisture can affect the physical stability of an amorphous solid-state form owing to the ability of these solid-state forms to absorb relative large quantities of water vapour. This attribute is linked to the greater void space available due to the lack of a well-arranged molecular order. The physical stability of amorphous clarithromycin was therefore investigated by means of vapour sorption analyses. Figures 4.11 and 4.12 depict the vapour sorption isotherms obtained for CLAM and Form II clarithromycin respectively.

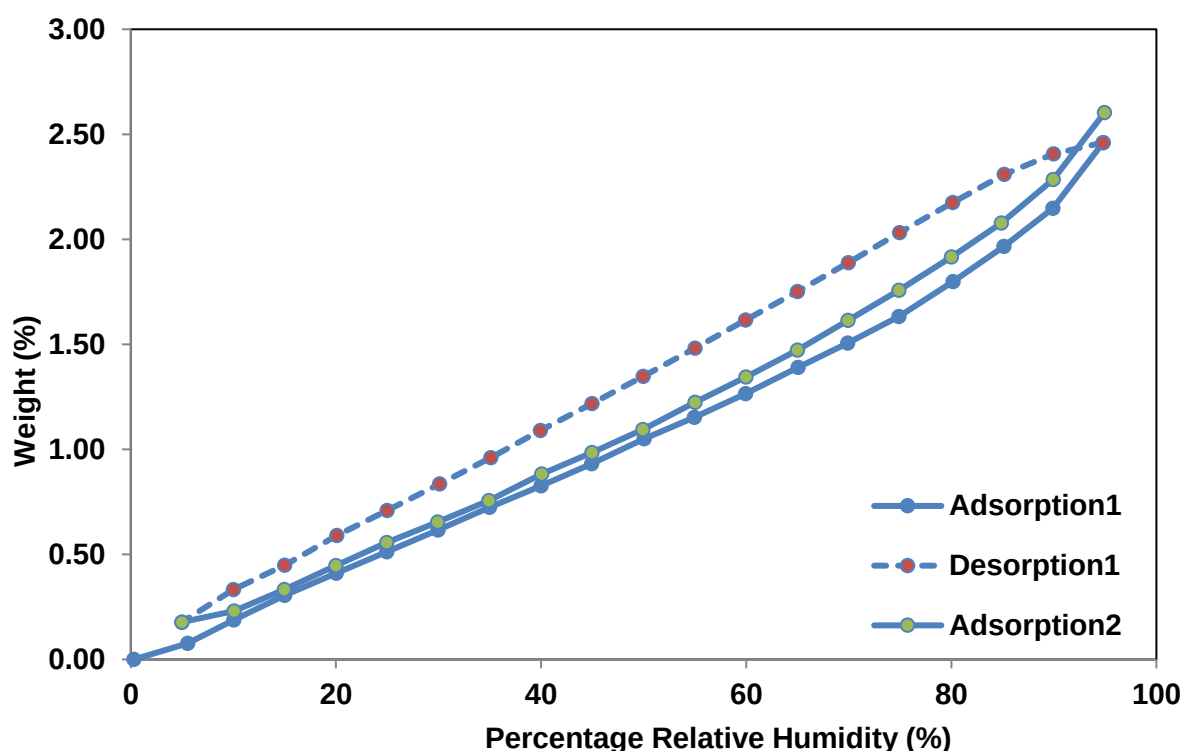


Figure 4.11: Vapour sorption isotherm obtained for amorphous clarithromycin at 25°C and the relative humidity percentage ramped from 0 – 95%, followed by a downwards ramp of 95 – 5% and then a final up-ramping from 5 – 95% RH.

From the vapour sorption data, it is clear that CLAM (Figure 4.11) absorbs approximately 0.8 % more water than crystalline clarithromycin Form II (Figure 4.12). It is also clear that no

molecular rearrangement occurred. If crystallisation of the amorphous form would have occurred, a sharp drop in the measured weight would have been visible during any step of the experiment. Such a drop in weight would be due to excess water which is expelled from the rearrange molecular structure. Such behaviour was not observed during the moisture sorption experiment, also XRPD analysis that was performed after the completion of the vapour sorption experiment, confirm the amorphous habit.

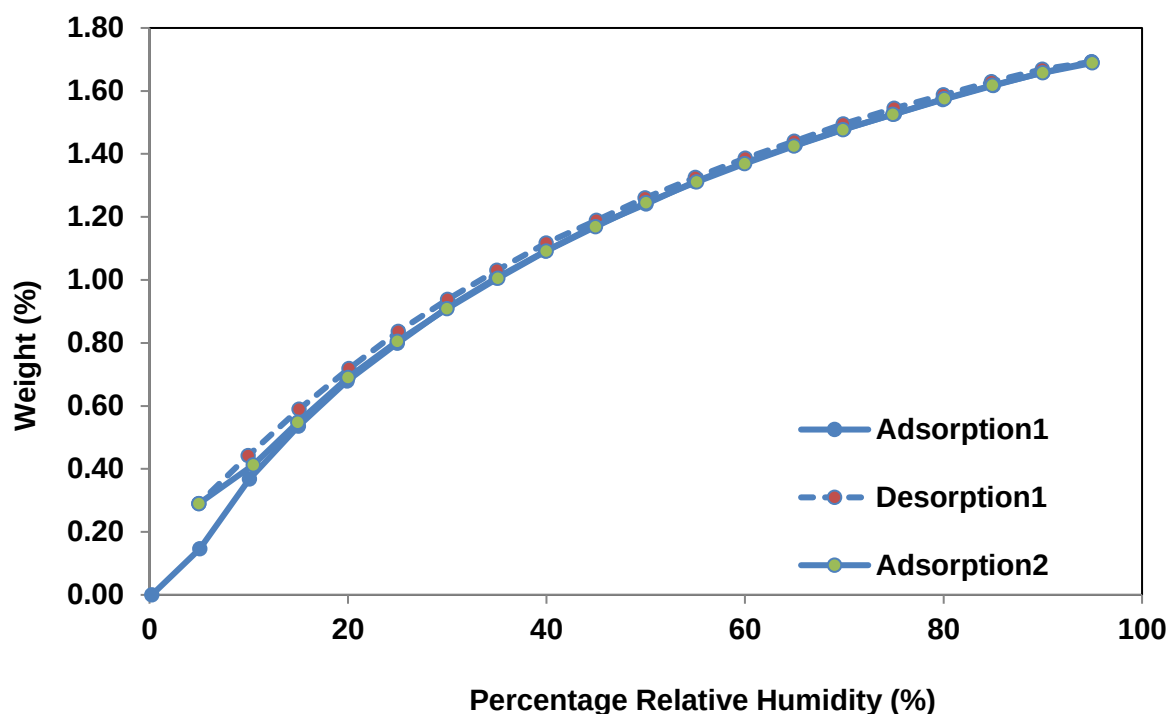


Figure 4.12: Vapour sorption isotherm obtained for crystalline clarithromycin Form II at 25°C and the relative humidity percentage ramped from 0 – 95%, followed by a downwards ramp of 95 – 5% and then a final up-ramping from 5 – 95% RH.

Results showed that amorphous clarithromycin is not sensitive to exposure to high moisture conditions. This is considered an advantage within the pharmaceutical environment. It was then worthwhile to study and compare dissolution and solubility differences between the crystalline Form II and CLAM using HPLC.

4.3.8 Solubility studies

Solubility of crystalline clarithromycin in distilled water was long found to be lower than that of erythromycin and decreases with an increase in temperature (Nagawa *et al.*, 1992). In this study, solubility of clarithromycin Form II at a lower temperature of $25 \pm 2^\circ\text{C}$ in different pH media (pH 2, 4, 5, 6, 7, 8, 9 and 10) was determined (Figure 4.13). The clarithromycin solubility studies were carried out as described in Chapter 3.

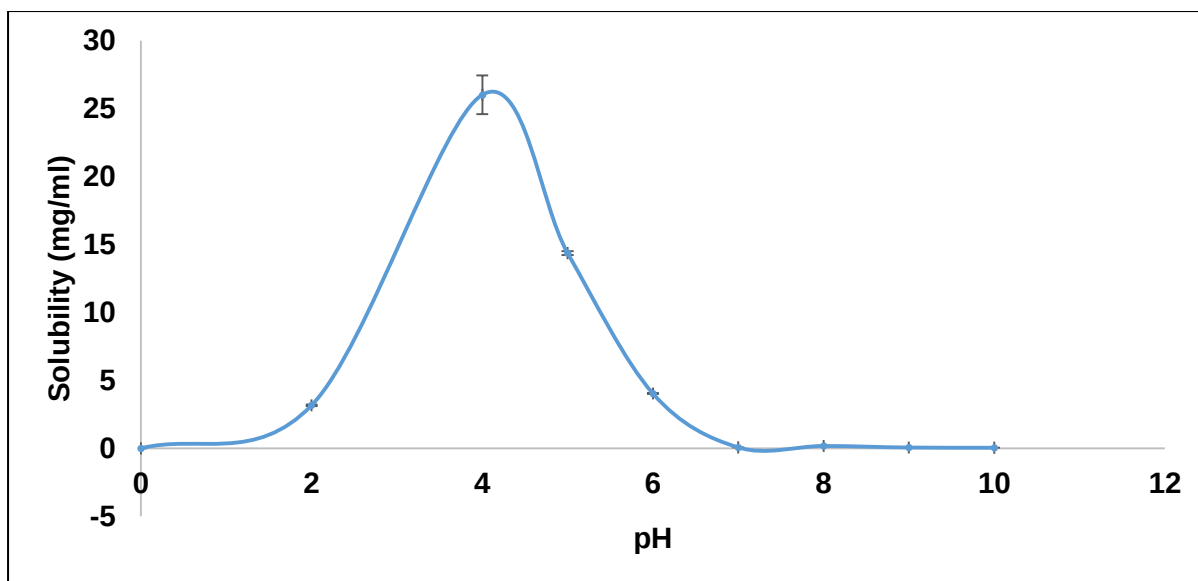


Figure 4.13: Solubility profile of crystalline clarithromycin Form II in different pH media at $25 \pm 2.0^\circ\text{C}$

From the obtained pH solubility profile, clarithromycin Form II shows better solubility in an acidic environment of pH 4.0. Poor aqueous solubility was noted in distilled water (pH ≈ 7.0), just as expected, as well as in basic environments pH 8.0 – 10.0. The aqueous solubility obtained 0.07 ± 0.003 mg/ml at $25.0 \pm 2.0^\circ\text{C}$ closely relates to literature reports (Adrianowicz *et al.*, 2012). Unfortunately, it is not possible to report on true equilibrium solubility of amorphous forms due to their far from equilibrium nature (Hancock & Parks, 2000). Amorphous forms crystallise to the most stable solid-state form through the process of solution-mediated phase transformation. Instead, one may apply dissolution data as an indication of apparent solubility for the amorphous form.

4.3.9 Dissolution studies

Dissolution studies of both clarithromycin Form II and CLAM were carried out in water (pH ≈ 7.0) to determine the dissolution rate difference between these two solid-state forms.

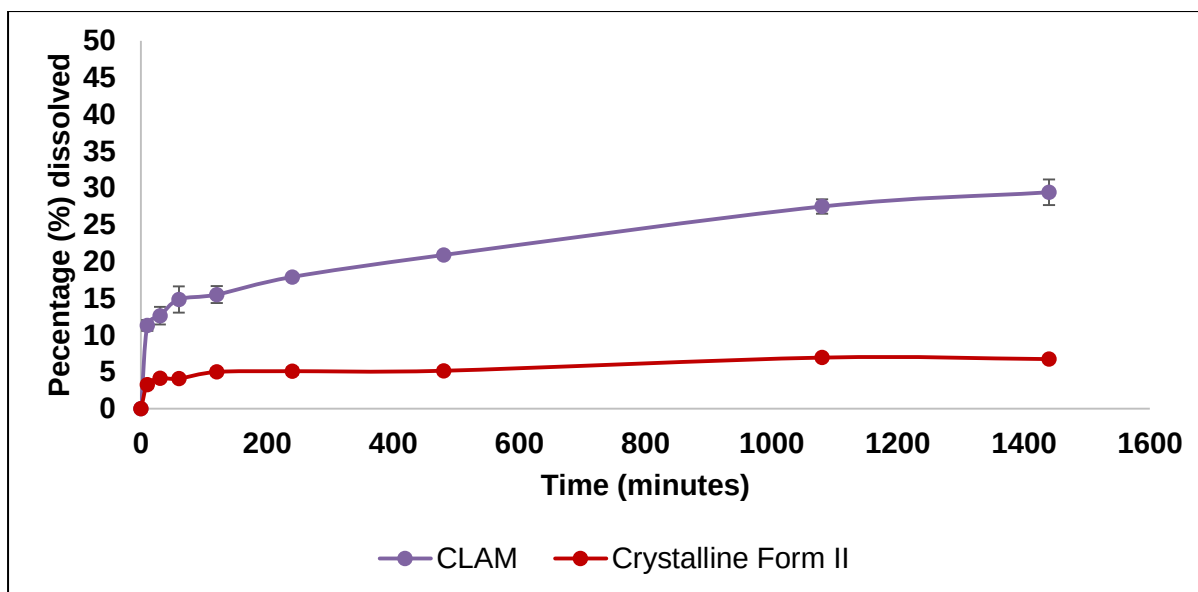


Figure 4.14: Dissolution profile of clarithromycin Form II and CLAM in distilled water at $37 \pm 0.5^\circ\text{C}$.

As depicted by Figure 4.14, the dissolution rate of the amorphous form was clearly more rapid than that of the crystalline Form II yielding an overall improved dissolution concentration. CLAM displayed approximately a 4.5 times improvement in dissolution rate in comparison with crystalline clarithromycin Form II. Interestingly, the dissolution profile obtained for CLAM didn't show any indication of a sudden decrease in the dissolved concentration due to recrystallisation of the less soluble but most stable Form II. This might be due to the fact that super-saturation was not achieved during the dissolution investigation. Furthermore, it is evident that although CLAM is approximately 5 times more soluble than Form II, the overall percentage of dissolved drug is still very low.

4.3.10 Discussion

From abovementioned characterisation results, clarithromycin raw material was confirmed to be the crystalline Form II polymorph. Its successful conversion to a neat amorphous form by quench cooling was also confirmed. On further investigation of the physical stability of amorphous clarithromycin, it was found that it forms a strong glass thus its molecular mobility should be negligible at ambient conditions. However, when non-isothermal heat is applied, CLAM recrystallises back to Form II. The recrystallisation process requires more energy ($\approx 25 \text{ kJ.mol}^{-1}$) when CLAM is free from seed crystals than when seed crystals are present. Vapour sorption analysis showed that CLAM is also physically stable when exposed to high moisture conditions. However, all these advantages may only be for short periods of time and may not apply at supersaturation of solution during dissolution. More so, recrystallisation may be induced by mechanical stress applied during preparation of the solid dosage form, for

example during milling, mixing or even tablet compression. Recrystallisation may also occur when the amorphous form is exposed to elevated temperatures. Incorporating a polymer therefore remains favourable in stabilising the amorphous form over long periods of time and under a variety of such stress conditions. The polymer and clarithromycin should be miscible and able to form a chemically homogeneous phase at molecular level so as to achieve optimised amorphous solid dispersions (ASDs) (Baird & Taylor, 2012; Ivanisevic, 2010; Marsac *et al.*, 2009). Miscibility of clarithromycin / polymer physical mixtures was therefore studied in detail in order to select the most suitable polymers as well as to obtain the best API / polymer ratio to be used.

4.4 Preparation of clarithromycin physical mixtures

All clarithromycin / polymer physical mixtures (PMs) were prepared by mixing crystalline clarithromycin Form II and the corresponding polymer using a mortar and pestle at different drug / polymer w/w ratios. Miscibility for each mixture was investigated by determining the melting point depression of the crystalline form of clarithromycin.

4.5 Miscibility studies of clarithromycin-polymer physical mixtures

Various levels of miscibility may be influenced by factors such as the drug : polymer ratio, temperature, relative humidity, preparation method and analysis conditions (Pham *et al.*, 2010; Qian *et al.*, 2010; Rumondor *et al.*, 2009). Polymers with higher molecular weights, higher melting points and / or higher T_g than that of the drug are expected to form more stable ASDs (Khougaz & Clas, 2000). On the other hand, polymers with low melting points may be more advantageous since they are already in a molten state before the drug melts or dissolves in the molten polymer, hence promoting good interaction. The stronger the interaction e.g. dipole-dipole interactions and hydrogen bonds between the polymer and the drug, the more stable the ASD formed. In this study, miscibility of clarithromycin with various polymers (Table 4.6) was studied in detail. All polymers have molecular weights greater than that of clarithromycin and were therefore expected to form stable ASDs. The physical mixtures of clarithromycin and the polymer were prepared in different w/w ratios namely, clarithromycin : polymer (1:1, 1:2, 1:3, 2:1, 2:3, 3:1, 3:2) and an additional 1:4 for clarithromycin : PEG 8000.

Table 4.6: Properties of selected polymers adapted from Surikutchi et al., 2013

Polymer	Physical state	Molecular weight	Melting point (°C)	Glass transition temperature (T_g) (°C)	H-bond donors(HD) / H- bond acceptors (HA) per monomer unit
Polyethylene glycol 8000 (PEG 8000)	Crystalline	7,000 - 9,000	60 - 63	-	HA = 1 HD = 0
Pluronic® F127 (PF-127)	Crystalline	9,840 - 14,600	52 - 57	-	HA = 1 HD = 0
Vinylpyrrolidone-vinyl acetate copolymer (Kollidon® VA-64)	Amorphous	45,000 - 70,000	> 140	~ 106	HA = 2 HD = 0
Polyvinylpyrrolidone K25 (PVP K25)	Amorphous	30000	-	~ 160	HA = 2 HD = 0
Polyvinylpyrrolidone K30 (PVP K30)	Amorphous	50000	-	~ 160	HA = 2 HD = 0

4.5.1 Differential scanning calorimetry

The observation of the melting point depression of clarithromycin / polymer PMs was done using DSC data. From literature, miscible mixtures should show melting point depression whilst immiscible or partially miscible mixtures show little or no depression of the drug melting point (Bikiaris *et al.*, 2005; Marsac *et al.*, 2009). DSC thermograms obtained for clarithromycin / polymer PMs at different ratios are shown in Figures 4.15 - 4.20.

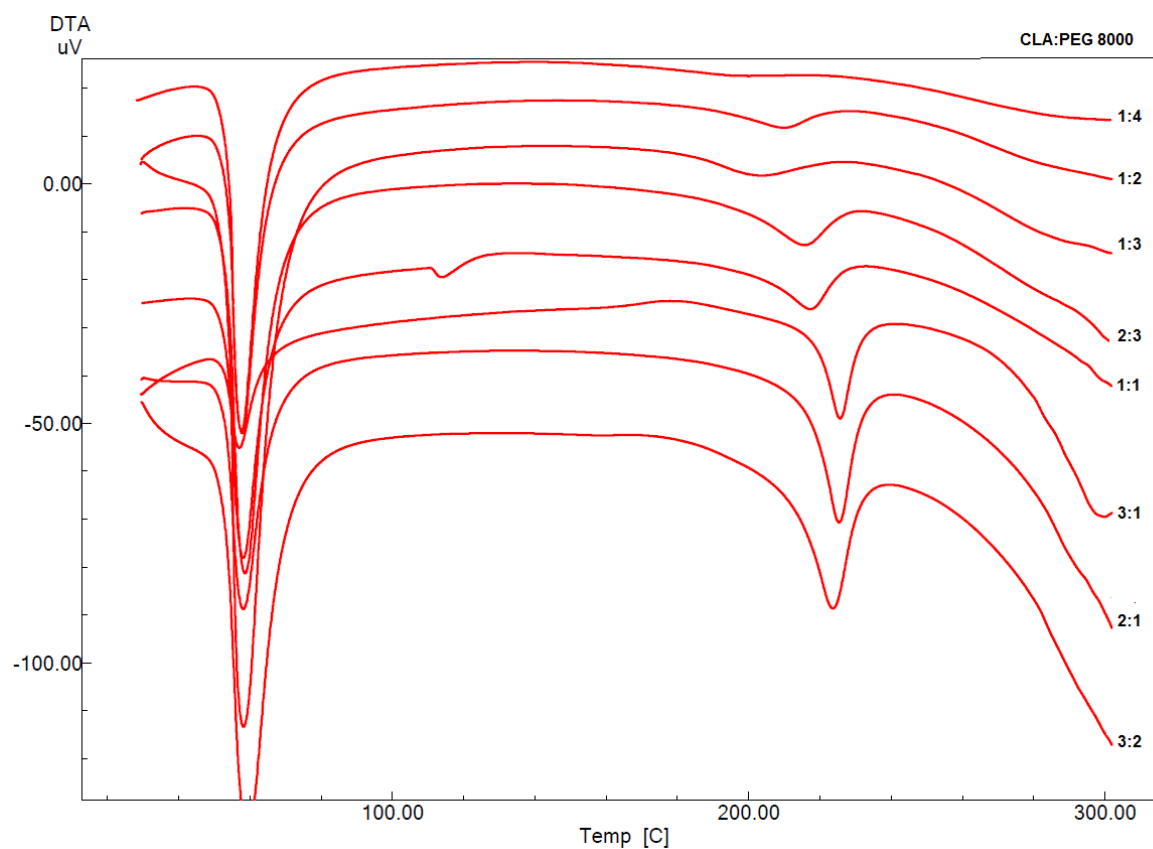


Figure 4.15: Clarithromycin / PEG 8000 PMs.

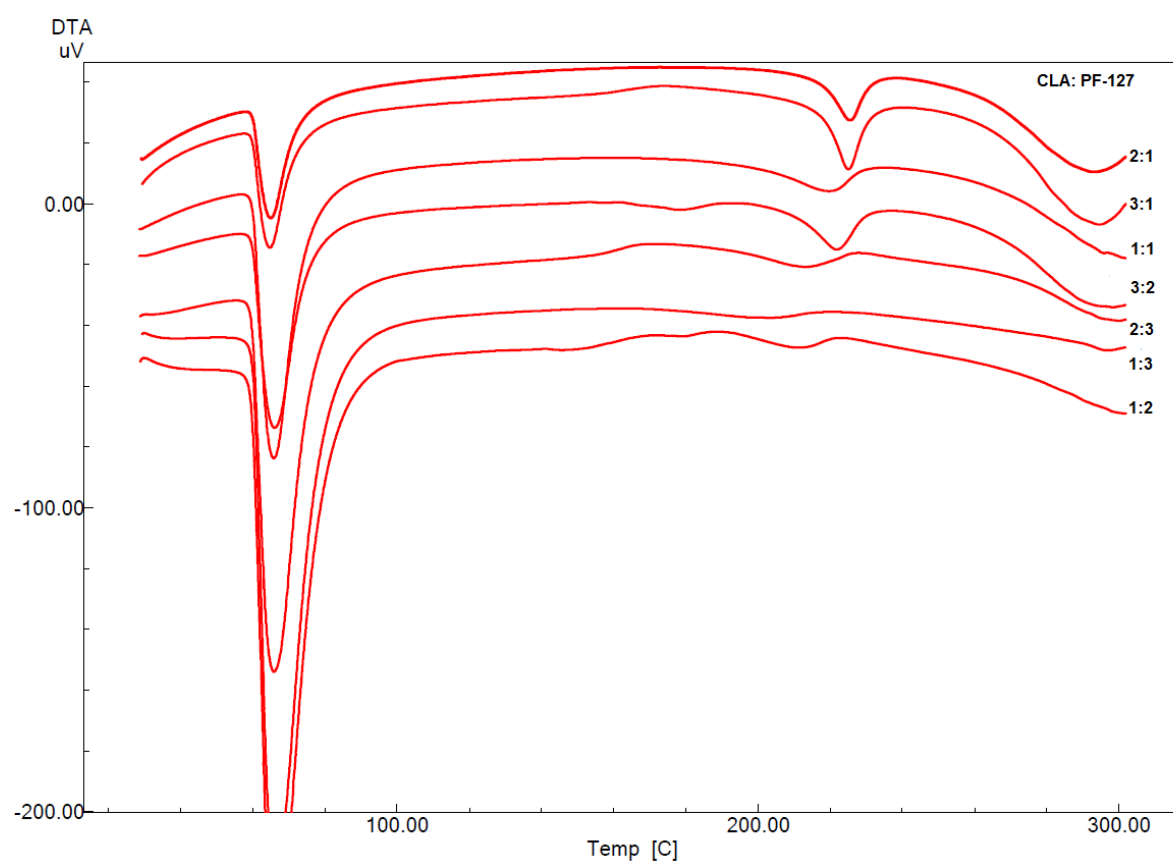


Figure 4.16: Clarithromycin / PF - 127 PMs.

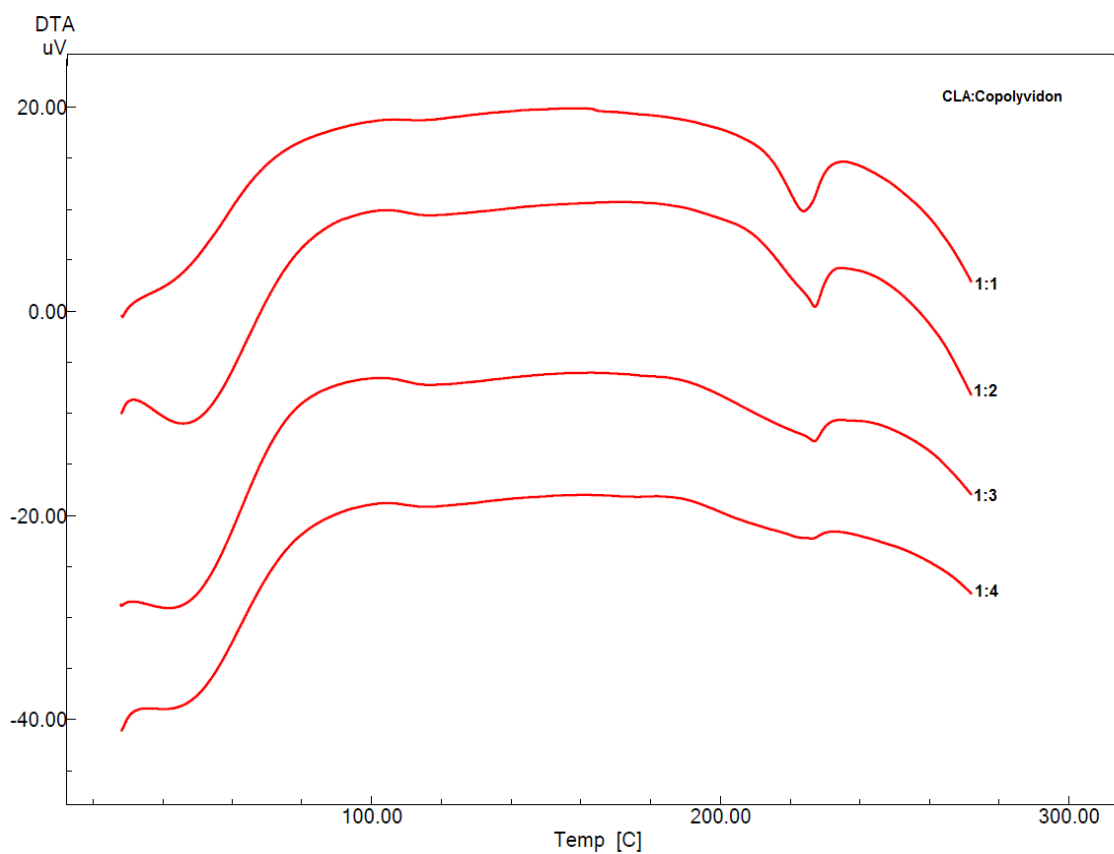


Figure 4.17: Clarithromycin / Kollidon® VA-64 PMs..

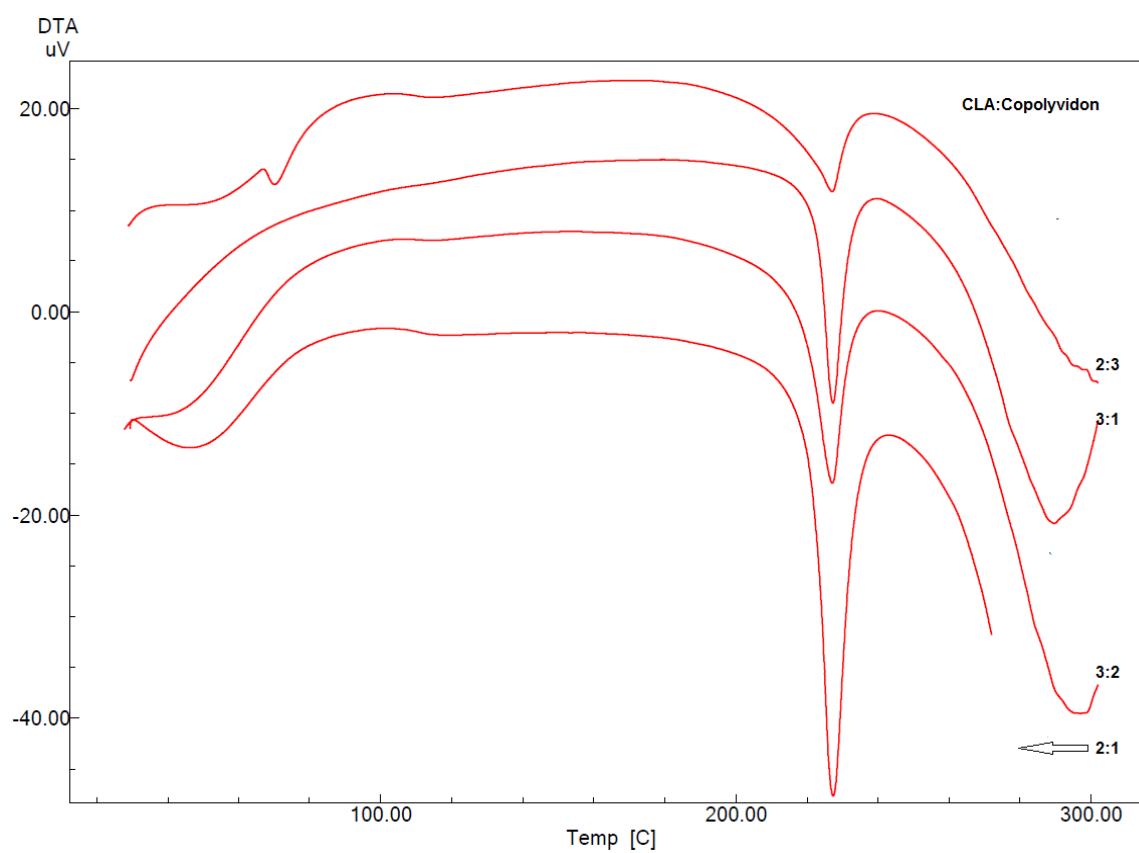


Figure 4.18: Clarithromycin / Kollidon® VA-64 PMs.

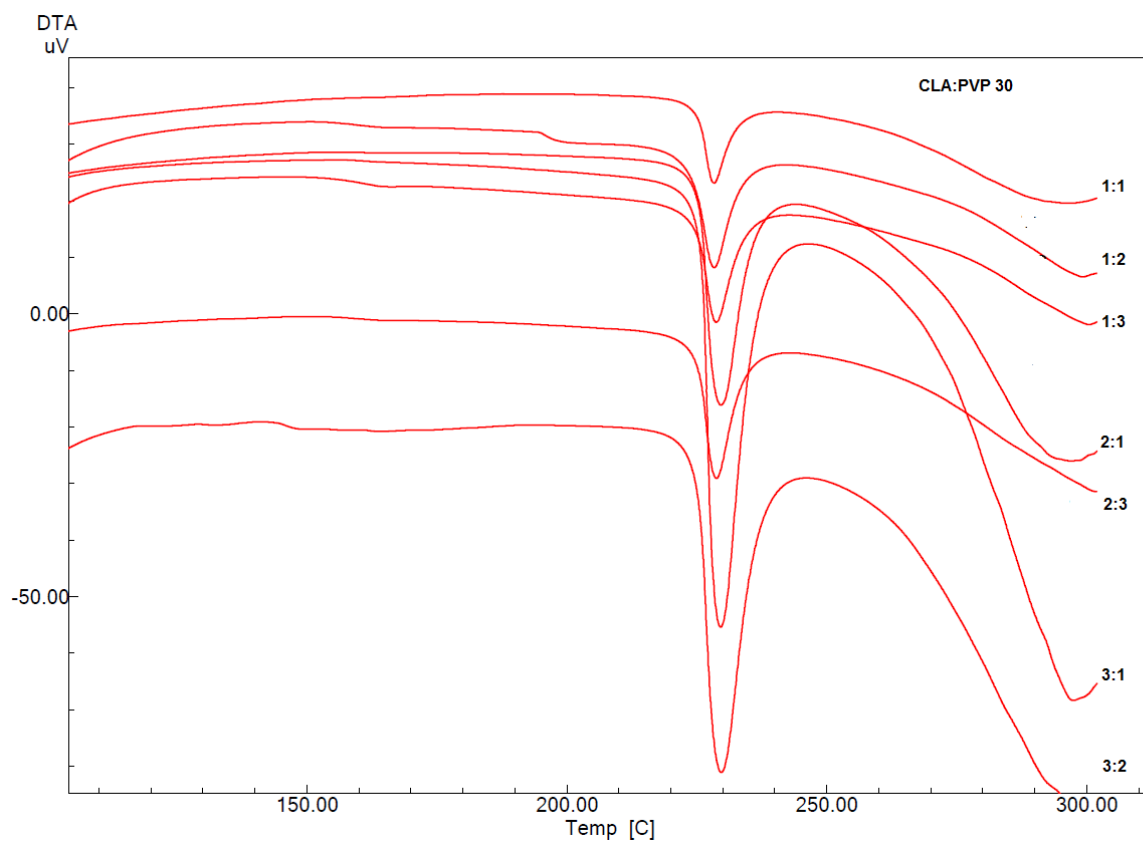


Figure 4.19: Clarithromycin / PVP K30 PMs.

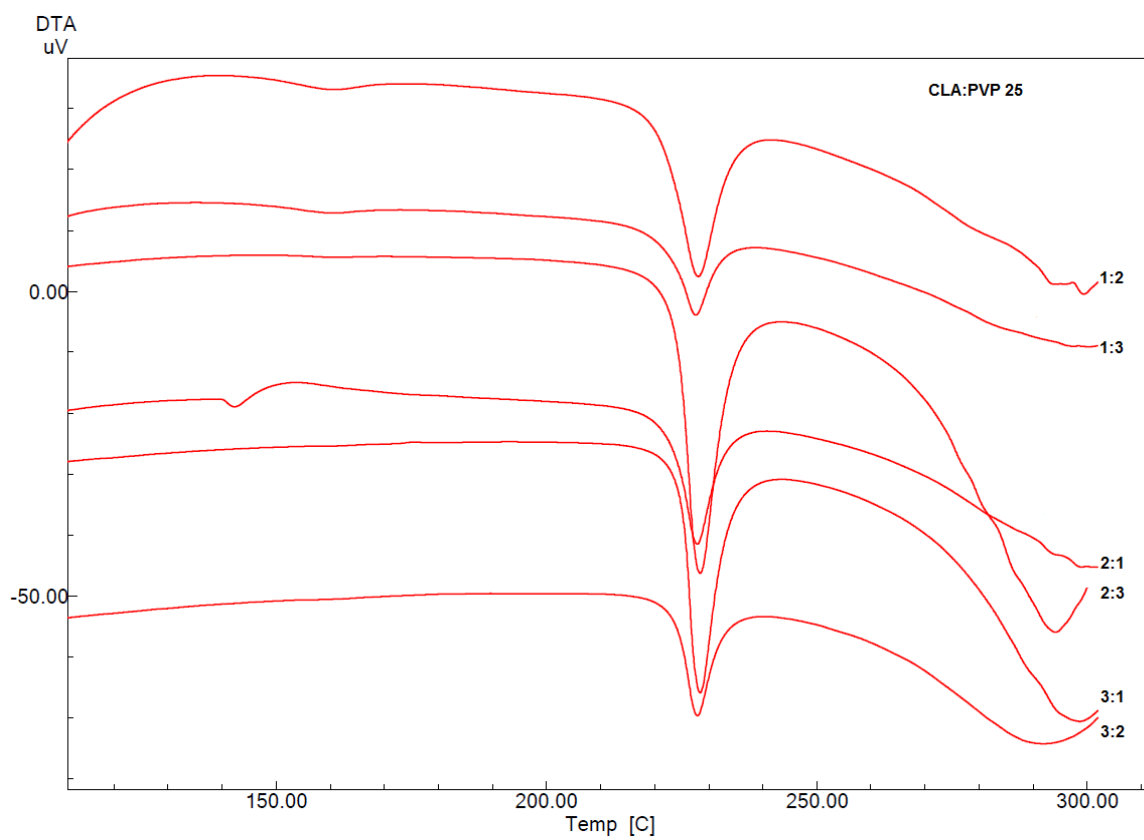


Figure 4.20: Clarithromycin / PVP K25 PMs.

Two separate melting endotherms for mixtures containing crystalline polymers and only one clarithromycin endotherm for mixtures containing amorphous polymers were observed during DSC analyses. In the instances of PEG 8000 and PF-127, the first peak corresponds to the melting point of crystalline polymer whilst the second peak, corresponds to the melting point of crystalline clarithromycin (Figures 4.15 and 4.16). The melting point of clarithromycin in the PMs was observed at temperatures slightly lower than the melting point of clarithromycin alone, thus showing a degree of miscibility. On a closer look, melting point depression for mixtures containing PEG 8000, PF-127 and Kollidon® VA-64 (Figures 4.15 - 4.18), indicate miscibility whilst mixtures containing PVP K25 and PVP K30 are partially miscible (Figures 4.19 - 4.20). The onset of the melting point of clarithromycin decreases with an increase in volume fraction of polymers, PEG 8000, PF-127 and PVP K30 but slightly increases with an increase in Kollidon® VA-64 (Figure 4.15- 4.19). There was hardly any change of onset of melting point of clarithromycin / PVP K25 PM. This means there was an insignificant degree of mixing at the melting temperature which indicates immiscibility. Results were aided by visual HSM characterisation.

4.5.2 Hot-stage microscopy (HSM)

Visual thermal phase changes for clarithromycin Form II in combination with the polymers were captured using HSM. The 1:3 clarithromycin / polymer w/w ratios gave the best visual miscibility when compared to all other prepared ratios as shown in Tables 4.7 - 4.11 and therefore only the 1:3 clarithromycin / polymer mixtures will be discussed further.

The clarithromycin / PEG 8000 PM (Table 4.7) shows melting of PEG 8000 at 60 - 64°C, whilst clarithromycin remains crystalline at that temperature. This correlates with the DSC thermograms obtained for the PMs (Figure 4.15). PEG 8000 remains in its molten state until clarithromycin melts or dissolves in the molten product at 203°C (Table 4.7 (g-j)). Melting point of clarithromycin in the PM (Table 4.7 (j)) was lower than its melting point without being in combination with a polymer ($\cong 228^\circ\text{C}$) indicating good miscibility of the mixture.

Table 4.7: HSM images for PEG 8000 (a –e) and clarithromycin / PEG 8000 (1:3 w/w) PM during heating

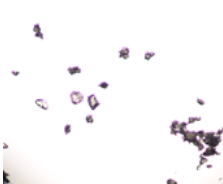
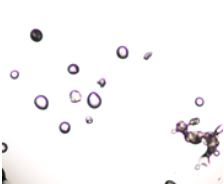
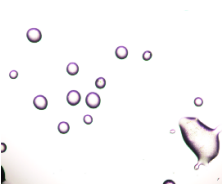
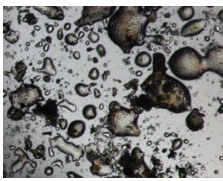
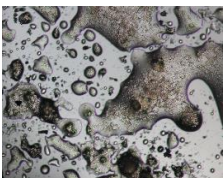
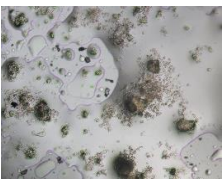
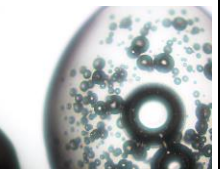
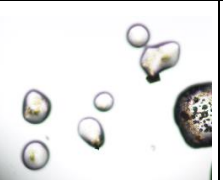
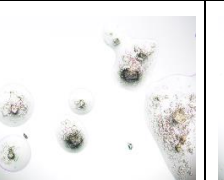
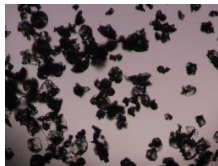
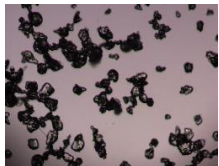
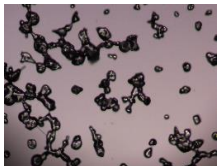
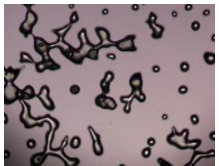
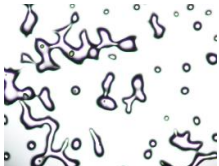
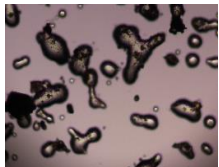
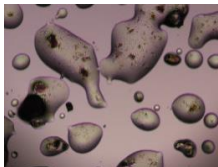
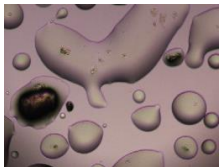
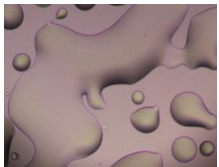
PEG 8000				
				
a) 25°C	b) 58°C	c) 61°C	d) 62°C	e) 64°C
Clarithromycin / PEG 8000 (1:3 w/w)				
				
f) 25°C	g) 64°C	h) 128°C	i) 157°C	j) 203°C

Table 4.8: HSM temperature dependent phase changes for clarithromycin / PF-127 (1:3 w/w) PM during heating

Pluronic F-127				
				
a) 25°C	b) 56°C	c) 120°C	d) 168°C	e) 173°C
Clarithromycin / PF-127 (1:3 w/w)				
				
f) 25°C	g) 58°C	h) 71°C	i) 158°C	j) 204°C

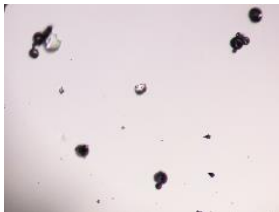
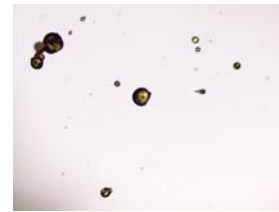
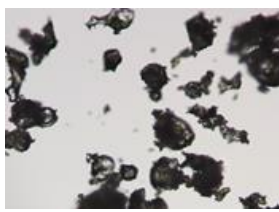
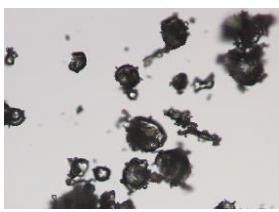
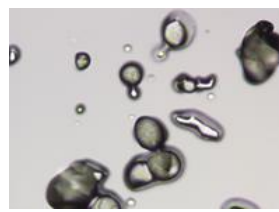
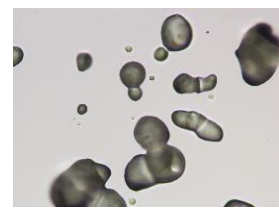
The same trend was observed for clarithromycin / PF-127 PM (Table 4.8). PF-127 melted at 54°C and remained in a molten state whilst emitting gas bubbles (b). The origin of the evolved gas is still unclear, since TGA analysis didn't show any loss of moisture from a PF-127 sample upon heating, furthermore upon heating at high temperatures (120 - 180°C), no discolouration of the liquefied polymer were observed. The absence of discolouration is however not proof that no degradation of the sample is possible. Clarithromycin subsequently melts slowly until completion at 204°C (j). The melting point of clarithromycin in the PM was again lower than that of clarithromycin alone indicating good miscibility. The miscibility was clearly observed by the clarithromycin actually dissolving in the molten PF-127 during continuous heating.

Table 4.9: HSM photos obtained for clarithromycin / Kollidon® VA-64 (1:3 w/w) PM during heating

Kollidon® VA-64				
				
a) 25°C	b) 142°C	c) 148°C	d) 154°C	e) 155°C
Clarithromycin / Kollidon® VA-64 (1:3 w/w ratio)				
				
f) 25°C	g) 185°C	h) 210°C	i) 226°C	j) 230°C

For clarithromycin / Kollidon® VA-64 PM (Table 4.9), DSC recorded variable T_g – values, depending on API / polymer ratios. Again, the polymer, Kollidon® VA-64 showed a lower melting point or T_g (142 - 155°C) than clarithromycin. In this liquid state, Kollidon® VA-64 had sufficient time to interact with clarithromycin before it completely melted at 230°C. In this case, the melting point of clarithromycin in the PM was just slightly lower than that of clarithromycin alone (Table 4.3 (d)), indicating partial miscibility.

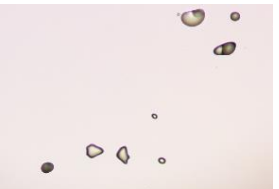
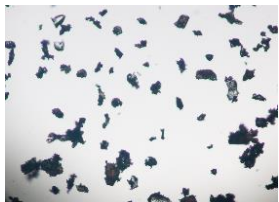
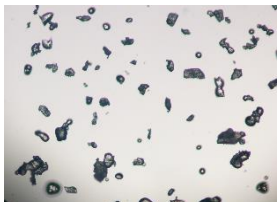
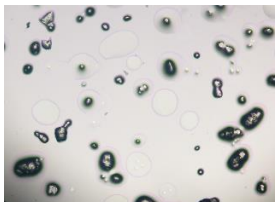
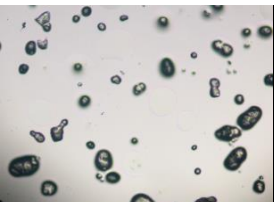
Table 4.10: HSM temperature dependent phase changes for clarithromycin / PVP K30 (1:3 w/w ratio) PM during heating

PVP K30			
			
a) 25°C	b) 240°C	c) 250°C	d) 270°C
Clarithromycin / PVP K30 (1:3 w/w ratio)			
			
e) 25°C	f) 218°C	g) 240°C	h) 249°C

DSC recorded a higher T_g value for PVP K30 than CLAM hence most likely to form an unstable ASD. In correlation to DSC results obtained, PVP K30 does not show a distinct melting point on HSM (Table 4.10) until degradation occurred at 270°C (d). Likewise, the mixture did not show a distinct melting point and the two components could not be distinguished from one another until no further mixing and or liquification occurred beyond 249°C.

A similar trend was observed for PVP K25 PM (Table 4.11) which does not have a distinct melting point due to its amorphous nature. Even though its T_g is higher than that of CLAM, two separate phases could be easily distinguished in the PM at 236°C. Beyond 240°C, no further mixing was observed, showing clarithromycin in the molten state whilst PVP K25 remains a solid, indicating poor miscibility.

Table 4.11: HSM temperature dependent phase changes for clarithromycin / PVP 25 (1:3 weight ratio) PM during heating

PVP K25			
			
a) 25°C	b) 185°C	c) 216°C	d) 230°C
Clarithromycin / PVP K25 (1:3 weight ratio)			
			
e) 25°C	f) 233°C	g) 236°C	h) 240°C

4.5.3 Discussion

Due to immiscibility, clarithromycin / PVP K25 PM was omitted from the rest of the study since it is unlikely to form a stable ASD. Clarithromycin / polymer PMs containing PEG 8000 and PF-127 were miscible, whilst PMs containing Kollidon® VA-64, PVP K25 and K30 were partially miscible. The miscible polymers i.e PF-127 and PEG 8000 were identified to be the most favourable for ASD preparation. However, ASD sample containing PF-127 was sticky and waxy, subsequently being very difficult to do even basic characterisation. Basing on this reason as well as for investigation purposes, 2 polymers i.e. one miscible and one partially miscible (PF-127 and PVP K30) were selected for preparation of clarithromycin PMs. Likewise, the other two i.e PEG 8000 and Kollidon® VA-64 (one showing miscibility and the other showing partial miscibility), were selected for the preparation of clarithromycin ASDs (Table 4.12).

Table 4.12: Summary of miscibility investigations of clarithromycin / polymer PMs

Clarithromycin / Polymer w/w ratio	Physical Mixture	Miscibility	Preparation
1:3	Clarithromycin / PEG 8000	Miscible	ASD
1:3	Clarithromycin / PF-127	Miscible	PM
1:3	Clarithromycin / Kollidon® VA-64	Partially miscible	ASD
1:3	Clarithromycin / PVP K30	Partially miscible	PM
1:3	Clarithromycin / PVP K25	Immiscible	-

4.6 Physico-chemical characterization of solid dispersions of clarithromycin

Clarithromycin ASDs were prepared according to the quench cooling method discussed in Chapter 3. Properties of ASDs were explored using different characterisation methods and compared to those obtained for PMs.

4.6.1 Differential scanning calorimetry

DSC results for clarithromycin ASDs are presented in Figures 4.21 - 4.22 below. The clarithromycin / PEG 8000 ASD present only one melting endotherm for the polymer. The absence of the clarithromycin endotherm in the ASD confirms a successful ASD conversion by quench cooling method. The DSC data obtained for two clarithromycin / polymer mixtures that were used to prepare mere PMs, will not be discussed, since it will be the same as that reported in Figures 4.16 and 4.19.

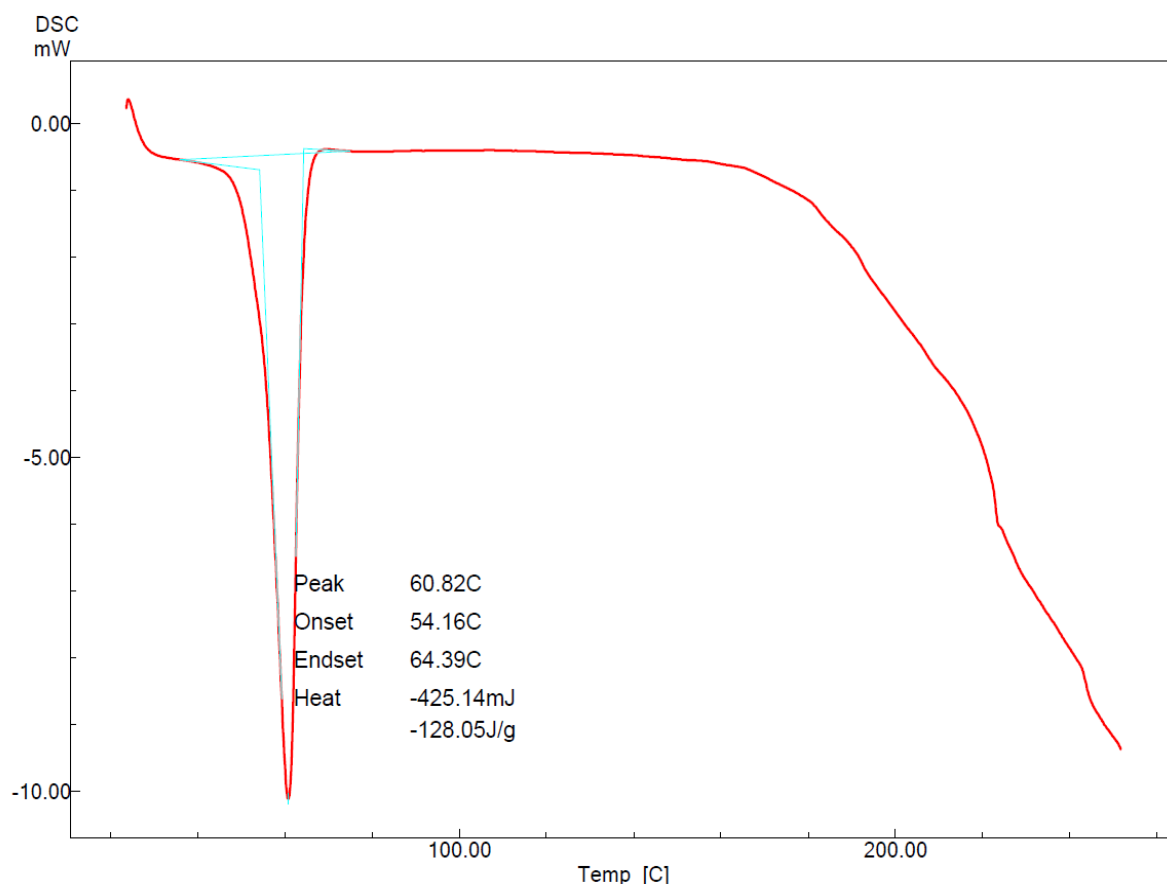


Figure 4.21: Clarithromycin / PEG 8000 ASD (1:3 weight ratio).

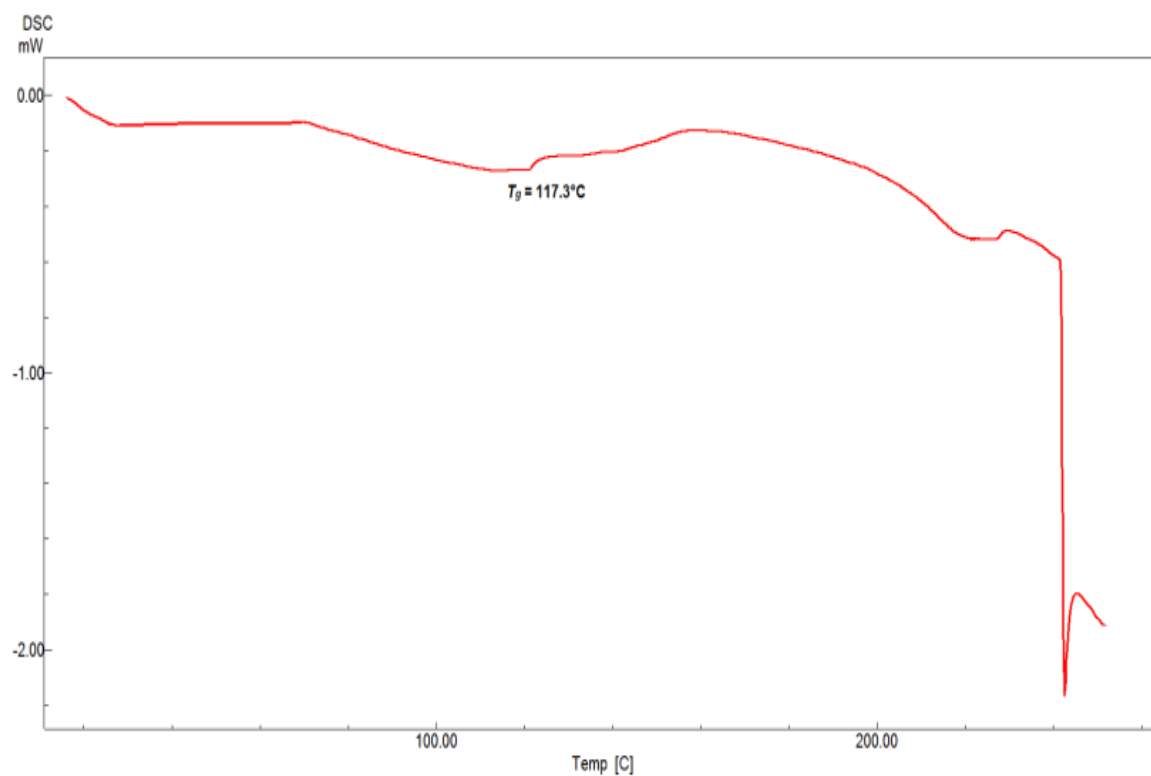


Figure 4.22: Clarithromycin / Kollidon® VA-64 ASD (1:3 weight ratio).

The dispersion prepared from clarithromycin / Kollidon® VA-64, only indicated a T_g ($\cong 117.3^\circ\text{C}$) and no melting of clarithromycin Form II during thermal analysis. This indicated that a homogeneously mixed drug - polymer dispersion at a molecular level formed. To confirm these results, further exploration was done using XRPD (Figure 4.23).

4.6.2 X-ray powder diffraction

The mentioned ASDs and PMs were investigated through XRPD analyses and were compared to diffractograms obtained for the single compounds.

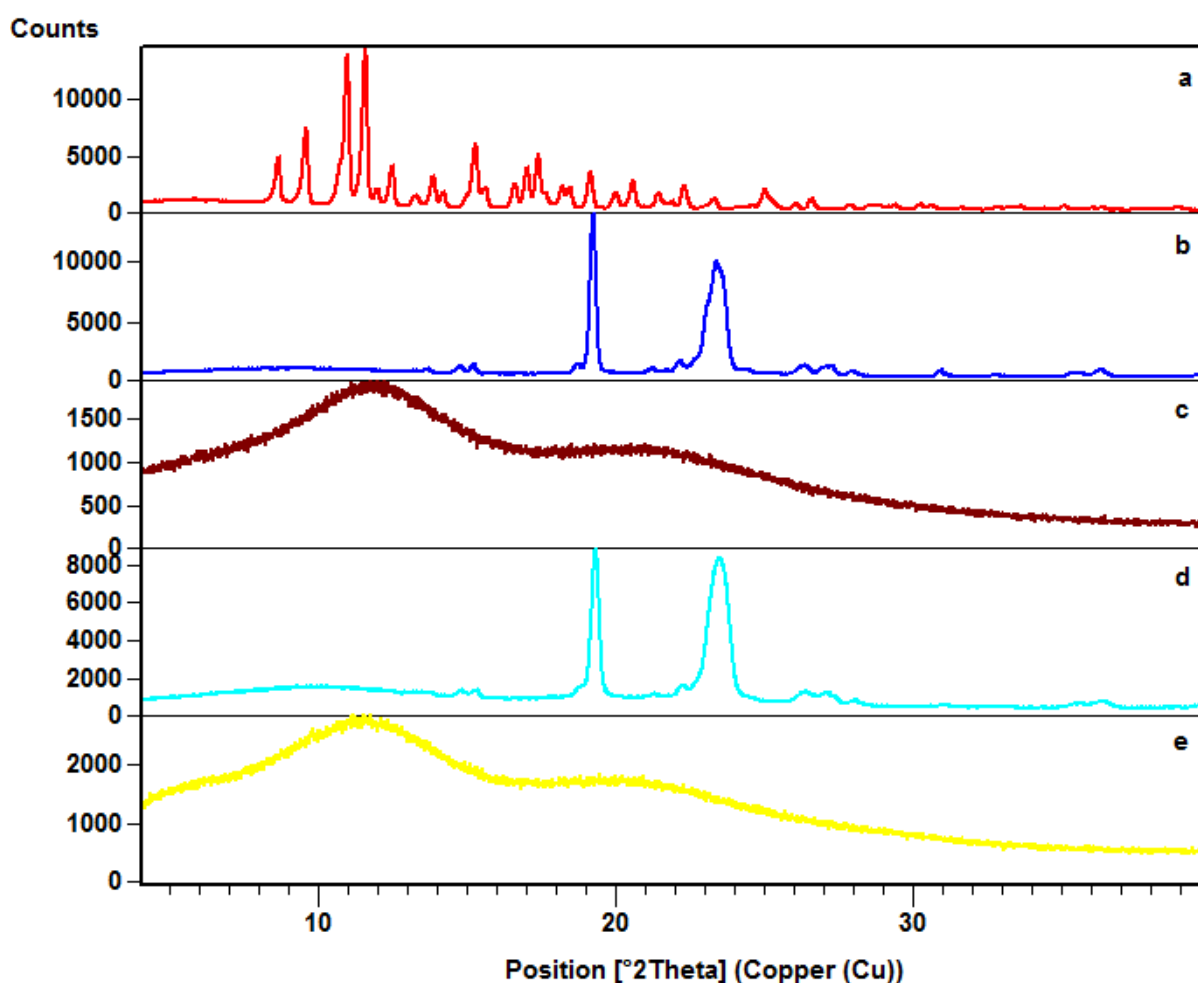


Figure 4.23: Overlay of XRPD diffractograms obtained for (a) clarithromycin Form II, (b) PEG 8000, (c) Kollidon® VA-64, (d) clarithromycin / PEG 8000 ASD and (e) clarithromycin / Kollidon® VA-64 ASD.

PEG 8000 (Figure 4.23 (b)) showed Bragg diffraction peaks and therefore serves as evidence that this polymer exists in a crystalline form. Although the clarithromycin / PEG ASD (d) exhibited Bragg diffraction very similar to that of PEG 8000 alone, there were no traces of crystallinity from crystalline clarithromycin (a). This serves as indication that the polymer didn't transform into an amorphous state during preparation of an ASD, however clarithromycin were

rendered into an amorphous state. As for the ASD containing Kollidon® VA-64 (e), XRPD recorded a diffuse scattering halo showing a successful preparation of a homogeneous ASD without any traces of crystallinity.

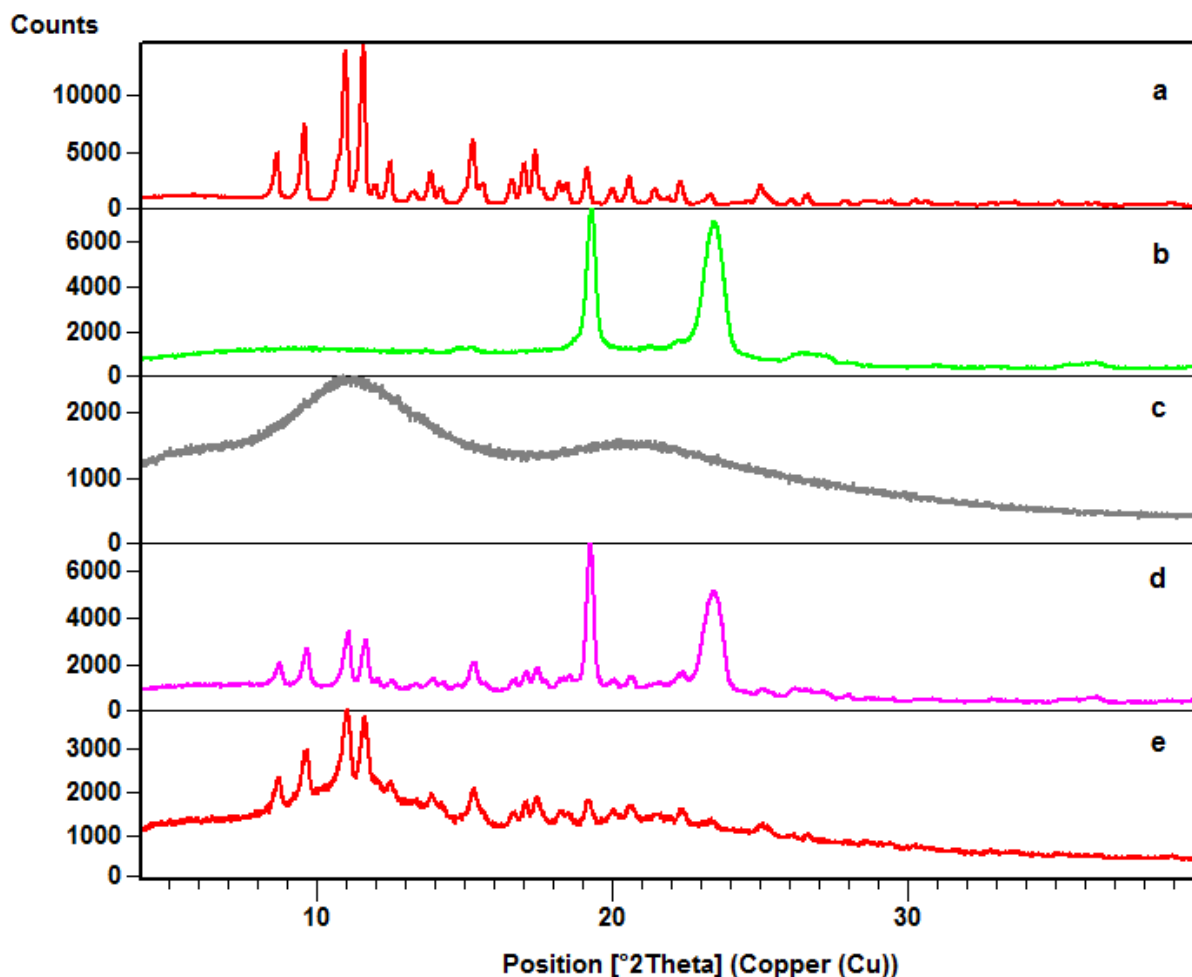


Figure 4.24: Overlay of XRPD diffractograms obtained for (a) clarithromycin Form II, (b) Pluronic PF-127, (c) PVP K30, (d) clarithromycin / PF-127 PM and (e) clarithromycin / PVP K30 PM.

Figure 4.24 depicts an overlay of the individual compounds as well as the PMs. PF-127 (b) showed Bragg diffraction peaks as evidence that it exists in a crystalline form. The subsequent PM (d) also indicated diffraction peaks representing a mixture of both clarithromycin and the polymer. However, the XRPD diffractogram obtained for PVP K30 (c) presented a diffuse scattering halo as evidence that it is an amorphous polymer and this positively lowered the degree of crystallinity of clarithromycin in the PM thereof (e).

In this study, no traces of crystalline clarithromycin were observed in the prepared ASDs, whilst the opposite was true for the PMs. This serves as confirmation of successful formation of clarithromycin ASDs by quench cooling method. Amongst these and other indications for

successful ASD formation, drug–polymer hydrogen bonding and ionic interactions are also of outermost importance (Mistry *et al.*, 2015). In this study, the ability of both the polymer and clarithromycin to form hydrogen bonds and ionic interactions can greatly enhance crystallisation inhibition of the prepared ASDs (Kapoor *et al.*, 2012; Vo *et al.*, 2013; Zhaojie *et al.*, 2014). It has been reported that the strength of interaction between drug and polymer can determine the degree of ASD physical stability (Mistry *et al.*, 2015). In this study, FT-IR was therefore employed to identify any hydrogen bond interactions between clarithromycin and the corresponding polymer.

4.6.3 Infrared spectroscopy

API -polymer interactions for both the ASDs and the PMs were examined and evaluated as depicted in Figures 4.26-4.28.

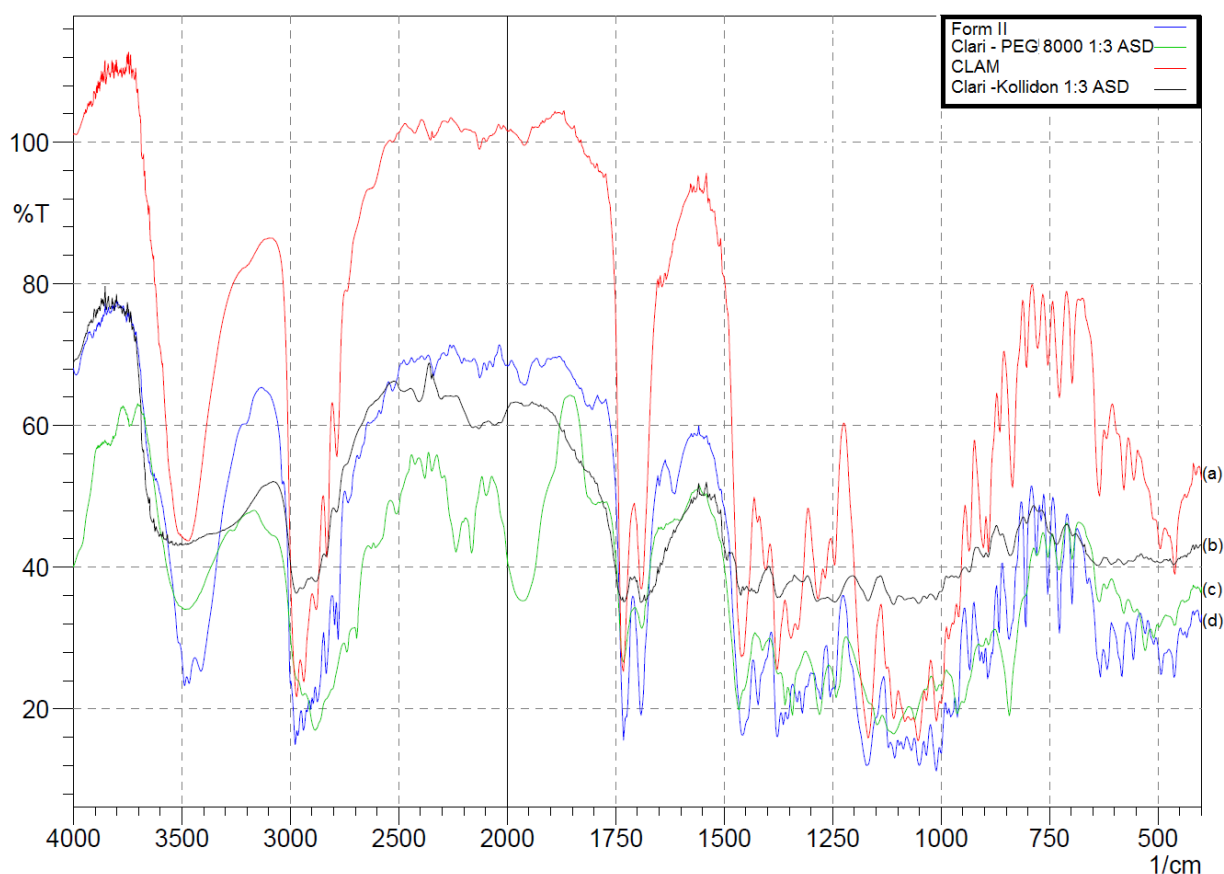


Figure 4.25: Overlay of IR diffractograms of (a) amorphous clarithromycin, (b) 1:3 clarithromycin / Kollidon® VA-64 ASD, (c) 1:3 clarithromycin / PEG 8000 ASD and (d) crystalline clarithromycin.

In comparison to the amorphous form (Figure 4.25 (a)) which shows a sharp non-hydrogen bonded O-H stretch in the region 3500 cm^{-1} , band broadening of the -OH stretch was observed for the ASDs (b) and (c) due to hydrogen bond formation. More so, the -CH stretch in the

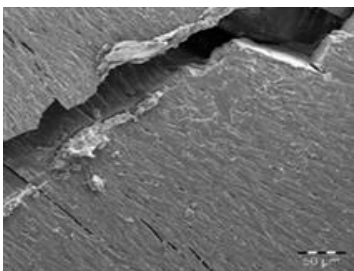
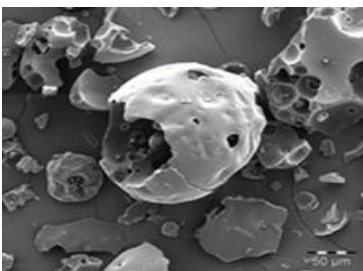
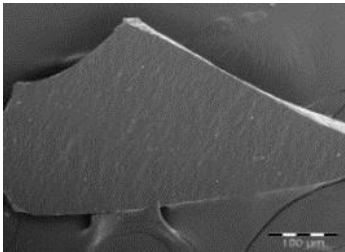
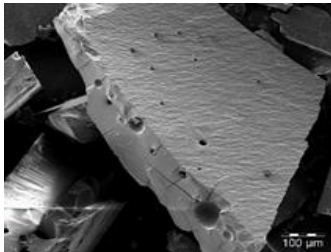
region 3000 cm^{-1} was shifted, broadened, with some peaks showing lower intensity for the ASDs in comparison to CLAM. This was especially significant for the 1:3 clarithromycin / PEG 8000 ASD, an indicative of greater API-polymer interactions. The band behaviour remains true for the ketone and lactone carbonyl peaks in the region of 1691 and 1732 cm^{-1} respectively which were broader and of lower intensity. The obtained results therefore revealed a level of API / polymer interaction and a successful preparation of clarithromycin ASDs. On the other hand, in contrary to the ASDs, very little to no interaction between clarithromycin and the polymers were observed in the PMs through FTIR analysis.

The clarithromycin / polymer interactions observed in the ASDs are an added advantage towards their physical stability. The prepared ASDs were then characterised by SEM for any visual differences in morphology between the two.

4.6.4 Scanning electron microscopy

Surface detail of the prepared ASDs was captured as shown by SEM images in Table 4.12. No surface crystallisation was observed on both ASDs, thus showing a good sign of physical stability. It is a well-known fact that surface area can be influenced by the degree of porosity (Lowell & Shields, 2013). In comparison to the clarithromycin / PEG 8000 ASDs, the clarithromycin / Kollidon® VA-64 ASDs show a larger surface area as a result of its porous nature that could be beneficial during solubility and dissolution.

Table 4.12: SEM images of the individual compounds and the ASDs formed

Single compounds		
		
Form II	PEG 8000	Kollidon® VA-64
ASD preparations		
		
1:3 clarithromycin / PEG 8000 ASD	1:3 clarithromycin / Kollidon® VA-64 ASD	

Differences in morphology of the prepared ASDs (Table 4.12) can be compared to morphology of pure crystalline API and CLAM in Figures 4.6 and 4.7, respectively. From the images, it is clear that both ASDs do not show any surface crystallisation. It was then imperative to determine dissolution rate improvements if any, of clarithromycin due to the inclusion of polymers in ASDs.

4.6.5 Dissolution studies

Dissolution studies of clarithromycin ASDs in distilled water were carried out as explained in Chapter 3. From the preceding studies, clarithromycin Form II shows to be very poorly soluble in water (Figure 4.13), hence only water was investigated as the dissolution medium. The choice of water as a dissolution medium was also governed by the fact that solution-mediated phase transformation needs to be investigated with amorphous solid-state forms of drugs. Dissolution rates of CLAM and PMs were similarly studied and included for comparison purposes.

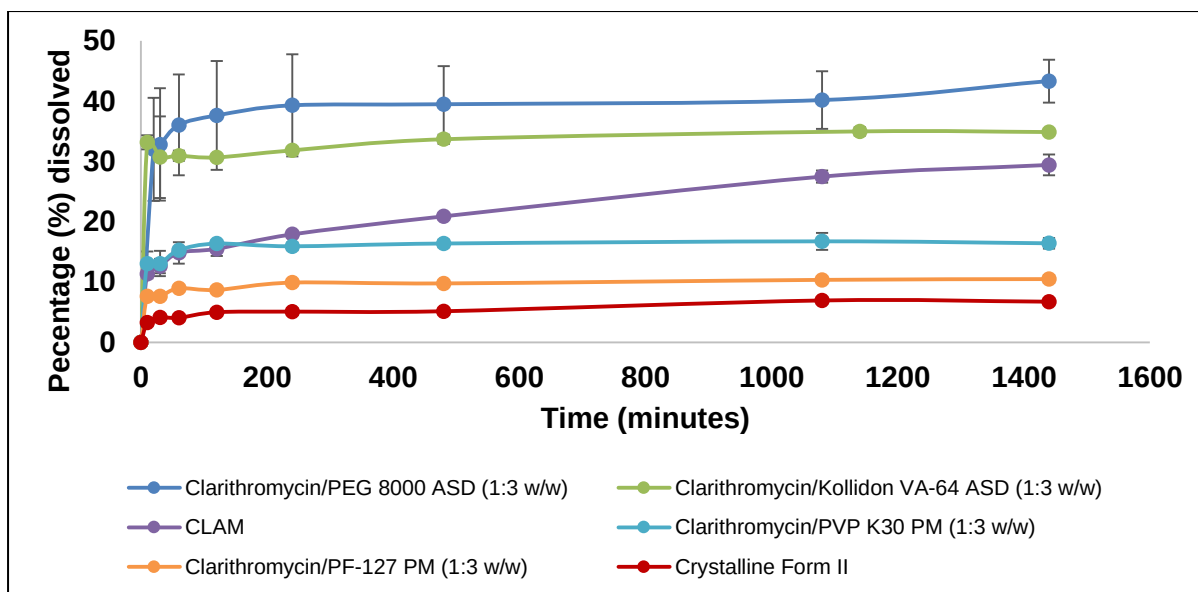


Figure 4.26: Dissolution studies of clarithromycin Form II, PMs and ASDs in water at $37 \pm 0.5^\circ\text{C}$.

Dissolution rate of clarithromycin in PMs was slightly increased due to the presence of polymers in the mixture, with an improved dissolution rate for clarithromycin / PVP 30 PM $\approx 16.5\%$ and clarithromycin / PF-127 PM $\approx 10.5\%$. This could be attributed to the ability of amorphous polymeric carriers offering better dispersibility and wettability than crystalline polymeric carriers (Zecevic *et al.*, 2014). On this note, solubility of the amorphous fraction was more rapid than that of the crystalline fraction present (Sun *et al.*, 2016), yielding an overall improved dissolution $\approx 29.4\%$ in comparison with the crystalline counterpart $\approx 6.7\%$. Dissolution was more enhanced in the ASD containing PEG 8000 than with the one containing Kollidon® VA-64. This could be attributed to the API-polymer miscibility status (Table 4.12) whereby clarithromycin / PEG 8000 proved to be more miscible whilst clarithromycin / Kollidon® VA-64 showed to be only partially miscible.

Usually, good solubility is related to good dissolution characteristics (Hecq *et al.*, 2005). Dissolution data was therefore applied as an indication of apparent solubility for CLAM since true equilibrium solubility of amorphous forms cannot be reported (Hancock & Parks, 2000). From the obtained dissolution profile (Figure 4.26), it is expected that the ASDs should show improved solubility values in the same order when compared to crystalline Form II, PMs and CLAM as depicted in Figure 4.30.

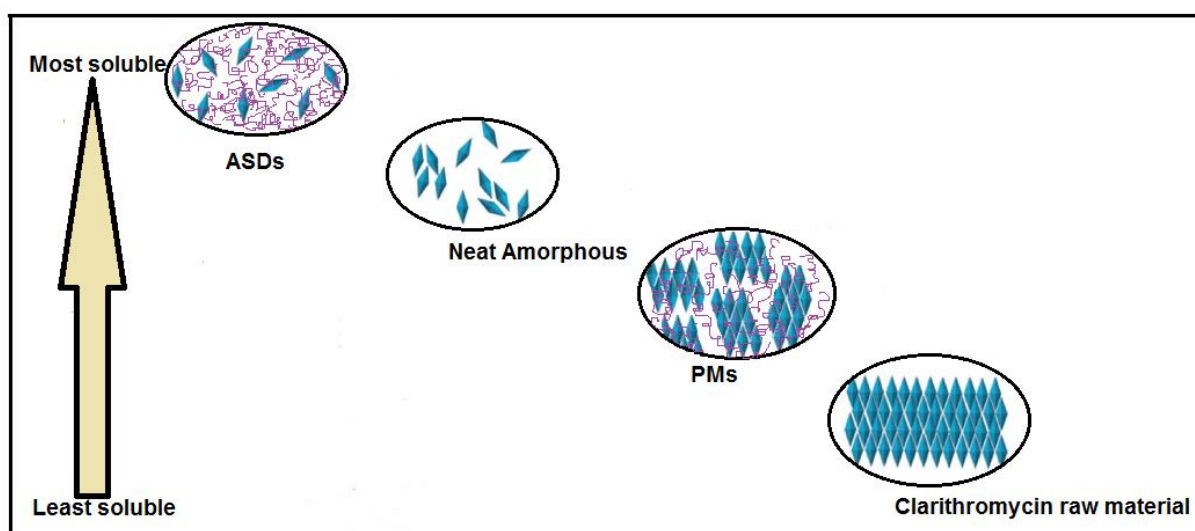


Figure 4.27: Improvement of aqueous solubility for clarithromycin (diagram not drawn to scale).

The aqueous solubility and stability advantages of ASDs may still be negatively influenced by factors such as temperature and water. Thus it remains crucial to retain stability of ASDs under such stress conditions so as to preserve the solubility and dissolution advantages thereof.

4.7 Conclusion

Thorough physico-chemical characterisation of crystalline clarithromycin Form II, CLAM, resulting PMs and ASDs were done using a combination of characterisation techniques. During this study it was proved that an amorphous form of clarithromycin (CLAM) can be prepared. The characterisation of CLAM showed it to be a strong amorphous form, remaining stable during exposure to high relative humidity conditions. However, very high processing temperatures could trigger the crystallisation of the most thermodynamically stable Form II. It was further confirmed that clarithromycin in its crystalline Form II exhibits very poor aqueous solubility $\approx 0.07\text{mg/ml}$ at 25°C . When its dissolution rate was tested and compared to that of its amorphous form, CLAM presented $\approx 22\%$ improvement in dissolution rate.

Through a very thorough pre-formulation study it was found that it is possible to successfully prepare amorphous solid dispersion (ASDs) of clarithromycin. It was found that not all types of pharmaceutical acceptable polymers would render an ASD. From the data gathered it was clearly demonstrated that for clarithromycin only PEG 8000 and Kollidon® VA-64 can be used to successfully prepare ASDs containing clarithromycin. The ASDs successfully countered the recrystallisation tendency of CLAM during sample heating. Due to the fact that the other polymers, PVP K30 and Pluronic PF-127 did not form useable clarithromycin ASDs, these drug / polymer mixtures were tested to establish their effect on the dissolution behaviour of

clarithromycin. When dissolution performance of the ASDs was tested, an improvement of dissolution rate by approximately $\approx 36\%$ in comparison to crystalline Form II was noted. It can therefore be concluded that clarithromycin ASDs can lead to options that could greatly improve the dissolution rate of this poorly soluble API. The API can therefore be delivered with desirable and predictable properties. This is indeed a practical option to solubilise the poorly soluble macrolide and to ultimately improve its treatment outcomes. The results are also confirmed by a recent systematic review by Baghel *et al.* (2016a) which reported that ASDs can ultimately improve bioavailability of an API.

References

- 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.
- Andrews, G. P., AbuDiak, O. A. & Jones, D. S.** 2010. Physicochemical characterization of hot melt extruded bicalutamide–polyvinylpyrrolidone solid dispersions. *Journal of pharmaceutical sciences*, 99:1322-1335.
- Arnoult, M., Dargent, E. & Mano, J. F.** 2007. Mobile amorphous phase fragility in semi-crystalline polymers: comparison of PET and PLLA. *Polymer*, 48:1012-1019.
- Aucamp, M., Liebenberg, W., Strydom, S., Van Tonder, E. & De Villiers, M.M.** 2012. Physicochemical properties of amorphous roxithromycin prepared by quench cooling of the melt or desolvation of a chloroform solvate. *AAPS PharmSciTech*, 13:467-476.
- Aucamp, M., Milne, M. & Liebenberg, W.** 2015a. Amorphous sulfadoxine: a physical stability and crystallization kinetics study. *AAPS PharmSciTech*, 3:1-10.
- Aucamp, M.E., Odendaal, R., Liebenberg, W. & Hamman, J.H.** 2015b. Amorphous azithromycin with improved aqueous solubility and intestinal membrane permeability. *Drug development and industrial pharmacy*, 41:1100-1108.
- Baghel, S., Cathcart, H. & O'Reilly, N.J.** 2016a. Polymeric amorphous solid dispersions: a review of amorphization, crystallization, stabilization, solid-state characterization, and aqueous solubilization of biopharmaceutical classification system class II drugs. *Journal of pharmaceutical sciences*, 105: 2527-2544.
- Baghel, S., Cathcart, H., Redington, W. & O'Reilly, N. J.** 2016b. An investigation into the crystallization tendency/kinetics of amorphous active pharmaceutical ingredients: a case study with dipyridamole and cinnarizine. *European journal of pharmaceutics and biopharmaceutics*, 104:59-71.
- Baird, J.A. & Taylor, L.S.** 2012. Evaluation of amorphous solid dispersion properties using thermal analysis techniques. *Advanced drug delivery reviews*, 64:396-421.
- Bhardwaj, S.P. & Suryanarayanan, R.** 2012. Molecular mobility as an effective predictor of the physical stability of amorphous trehalose. *Molecular pharmaceutics*, 9: 3209-3217.
- Bikiaris, D., Papageorgiou, G. Z., Stergiou, A., Pavlidou, E., Karavas, E., Kanaze, F. & Georgarakis, M.** 2005. Physicochemical studies on solid dispersions of poorly water-soluble

drugs: evaluation of capabilities and limitations of thermal analysis techniques. *Thermochimica acta*, 439:58-67.

Capen, R., Christopher, D., Forenzo, P., Ireland, C., Liu, O., Lyapustina, S., O'Neill, J., Patterson, N., Quinlan, M., Sandell, D. & Schwenke, J. 2012. On the shelf life of pharmaceutical products. *AAPS PharmSciTech*, 13:911-918.

Derakhshandeh, K., Bahrami, G., Mohammadi, B. & Alizadeh, E. 2014. Oral bioavailability and pharmacokinetic study of clarithromycin in different dosage forms in Iranian healthy volunteers. *Journal of bioequivalence & bioavailability*, 6: 206-211.

Descamps M. & Dudognon, E. 2014. Crystallization from the amorphous state: nucleation-growth decoupling, polymorphism interplay, and the role of interfaces. *Journal of pharmaceutical sciences*, 103:2615-2628.

Donnelly, C., Tian, Y., Potter, C., Jones, D.S. & Andrews, G.P. 2015. Probing the effects of experimental conditions on the character of drug-polymer phase diagrams constructed using Flory-Huggins theory. *Pharmaceutical research*, 32:67-179.

Donth, E.J. 2013. The glass transition: relaxation dynamics in liquids and disordered materials. Heidelberg: Springer.

Esfandi, E.H., Ramezani, V., Vatanara, A., Najafabadi, A.R. & Hadipour Moghaddam, S.P. 2014. Clarithromycin dissolution enhancement by preparation of aqueous nanosuspensions using sonoprecipitation technique. *Iranian journal of pharmaceutical research*, 13:809-818.

Flory, P.J. 1953. Principles of polymer chemistry. New York: Cornell University Press.

Graeser, K.A., Patterson, J.E., Zeitler, J.A., Gordon, K.C. & Rades, T. 2009. Correlating thermodynamic and kinetic parameters with amorphous stability. *European journal of pharmaceutical sciences*, 37:492-498.

Graeser, K.A., Patterson, J.E., Zeitler, J.A. & Rades, T. 2010. The role of configurational entropy in amorphous systems. *Pharmaceutics*, 2:224-244.

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

Hecq, J., Deleers, M., Fanara, D., Vranckx, H. & Amighi, K. 2005. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine. *International journal of pharmaceutics*, 299:167-177.

Ivanisevic, I. 2010. Physical stability studies of miscible amorphous solid dispersions. *Journal of pharmaceutical sciences*, 99:4005-4012.

- Kadota, T., Matsui, H., Hirose, T., Suzuki, J., Saito, M., Akaba, T., Kobayashi, K., Akashi, S., Kawashima, M., Tamura, A. & Nagai, H.** 2016. Analysis of drug treatment outcome in clarithromycin-resistant *Mycobacterium avium* complex lung disease. *BMC infectious diseases*, 16:1.
- Kapoor, B., Kaur, R., Kour, S., Behl, H. & Kour, S.** 2012. Solid dispersion: an evolutionary approach for solubility enhancement of poorly water soluble drugs. *International journal of recent advances in pharmaceutical research*, 2:1-16.
- Kawakami, K., Harada, T., Yoshihashi, Y., Yonemochi, E., Terada, K. & Moriyama, H.** 2015. Correlation between glass-forming ability and fragility of pharmaceutical compounds. *The journal of physical chemistry b*, 119: 4873-4880.
- Khougaz, K. & Clas, S.D.** 2000. Crystallization inhibition in solid dispersions of MK-0591 and poly (vinylpyrrolidone) polymers. *Journal of pharmaceutical sciences*, 89:1325-1334.
- Konno, H. & Taylor, L.S.** 2006. Influence of different polymers on the crystallization tendency of molecularly dispersed amorphous felodipine. *Journal of pharmaceutical sciences*, 95:2692-2705.
- Kuncham, S., Shete, G. & Bansal, A.K.** 2014. Quantification of clarithromycin polymorphs in presence of tablet excipients. *Journal of excipients and food chemicals*, 5:65-78.
- Lowell, S. & Shields, J.E.** 2013. Powder surface area and porosity. 3rd ed. Netherlands: Springer.
- Liu, J.H., Riley, D.A. & Spanton, S.G.** 1999. Crystal form I clarithromycin. (Patent: U.S. 5,858,986).
- Liu, J.H. & Riley, D.A.** 1998. Preparation of crystal form II of clarithromycin. (Patent: US 5,844,105).
- Mansouri, M., Pouretedal, H.R. & Ali, M.A.H.** 2010. Preparation and characterization of clarithromycin nanoparticles by antisolvent precipitation. *Drug metabolism reviews*, 42:109-110.
- Marsac, P.J., Li, T. & Taylor, L.S.** 2009. Estimation of drug–polymer miscibility and solubility in amorphous solid dispersions using experimentally determined interaction parameters. *Pharmaceutical research*, 26:139-151.
- Milne, M., Liebenberg, W. & Aucamp, M.E.** 2015. The stabilization of amorphous zopiclone in an amorphous solid dispersion. *AAPS PharmSciTech*, 16: 1190-1202.

- 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.
- Mistry, P., Mohapatra, S., Gopinath, T., Vogt, F.G. & Suryanarayanan, R.** 2015. Role of the strength of drug–polymer interactions on the molecular mobility and crystallization inhibition in ketoconazole solid dispersions. *Molecular pharmaceutics*, 12:3339-3350.
- Nakagawa, Y., Itai, S., Yoshida, T. & Nagai, T.** 1992. Physicochemical properties and stability in the acidic solution of a new macrolide antibiotic, clarithromycin, in comparison with erythromycin. *Chemical and pharmaceutical bulletin*, 40:725-728.
- Newman, A., Knipp, G. & Zografi, G.** 2012. Assessing the performance of amorphous solid dispersions. *Journal of pharmaceutical sciences*, 101:1355-1377.
- Nozawa, K., Iwao, Y., Noguchi, S. & Itai, S.** 2015. Effect of surfactants or a water soluble polymer on the crystal transition of clarithromycin during a wet granulation process. *International journal of pharmaceutics*, 495:204-217.
- Pham, T. N., Watson, S. A., Edwards, A. J., Chavda, M., Clawson, J. S., Strohmeier, M. & Vogt, F. G.** 2010. Analysis of amorphous solid dispersions using 2D solid-state NMR and ¹H T1 relaxation measurements, *Molecular pharmaceutics*, 7:1667-1691.
- Pina, M.F., Pinto, J.F., Sousa, J.J., Craig, D.Q. & Zhao, M.** 2015. Generation of hydrate forms of paroxetine HCl from the amorphous state: an evaluation of thermodynamic and experimental predictive approaches. *International journal of pharmaceutics*, 481:114-124.
- Qian, F, Huang, J., Zhu, Q., Haddadin, R., Gawal, J., Garmise, R. & Hussain M.** 2010. Is a distinctive single T_g a reliable indicator for the homogeneity of amorphous solid dispersion?. *International journal of pharmaceutics*, 395:232-235.
- Rumondor, A.C., Ivanesevic, I., Bates, S., Alonzo, D.E. & Taylor, L.S.** 2009. Evaluation of drug-polymer miscibility in amorphous solid dispersion systems. *Pharmaceutical research*, 26:2523-2534.
- Russell, T.H., Edwards, B.J. & Khomami, B.** 2015. Characterization of the flory-huggins interaction parameter of polymer thermodynamics. *Europhysics letters*, 108:66003.
- Schammé, B., Couvrat, N., Malpeli, P., Delbreilh, L., Dupray, V., Dargent, É. & Coquerel, G.** 2015. Crystallization kinetics and molecular mobility of an amorphous active pharmaceutical ingredient: a case study with biclotymol. *International journal of pharmaceutics*, 490:248-257.

- Shahbazi Niaz, M., Traini, D., Young, P. M., Ghadiri, M. & Rohanizadeh, R.** 2014. Investigation into physical–chemical variables affecting the manufacture and dissolution of wet-milled clarithromycin nanoparticles. *Pharmaceutical development and technology*, 19:911-921.
- Shamblin, S.L., Tang, X., Chang, L., Hancock, B.C. & Pikal, M.J.** 1999. Characterization of the time scales of molecular motion in pharmaceutically important glasses. *The journal of physical chemistry b*, 103:4113-4121.
- Sippel, P., Dietrich, V., Reuter, D., Aumüller, M., Lunkenheimer, P., Loidl, A. & Krohns, S.** 2016. Impact of water on the charge transport of a glass-forming ionic liquid. *arXiv preprint arXiv* (In press).
- Sun, Y., Zhu, L., Wu, T., Cai, T., Gunn, E. M. & Yu, L.** 2012. Stability of amorphous pharmaceutical solids: crystal growth mechanisms and effect of polymer additives. *The AAPS journal*, 14: 380-388.
- Sun, D.D., Wen, H. & Taylor, L.S.** 2016. Non-sink dissolution conditions for predicting product quality and in vivo performance of supersaturating drug delivery systems. *Journal of pharmaceutical sciences*, 105:2477-2488.
- Surikutchi, B.T., Patil, S.P., Shete, G., Patel, S. & Bansal, A.K.** 2013. Drug-excipient behavior in polymeric amorphous solid dispersions. *Journal of excipients and food chemicals*, 4:70-94.
- Templeton, A.C., Byrn, S.R., Haskell, R.J. & Prisinzano, T.E., eds.** 2015. Discovering and developing molecules with optimal drug-like properties. New York: Springer
- Van den Mooter, G.** 2012. The use of amorphous solid dispersions: a formulation strategy to overcome poor solubility and dissolution rate. *Drug discovery today: technologies*, 9:79-85.
- Vo, C.L.N., Park, C. & Lee, B.J.** 2013. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. *European journal of pharmaceuticals and biopharmaceutics*, 85:799-813.
- Yu, L.** 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Advanced drug delivery reviews*, 48:27-42.
- Zecevic, D.E., Meier, R., Daniels, R. & Wagner, K.** 2014. Site specific solubility improvement using solid dispersions of HPMC-AS/HPC SSL–mixtures. *European journal of pharmaceuticals and biopharmaceutics*, 87:264-270.
- Zhaojie, M., Ming, Z., Shengnan, W., Xiaojia, B., Hatch, G. M., Jingkai, G. & Li, C.** 2014. Amorphous solid dispersion of berberine with absorption enhancer demonstrates a

remarkable hypoglycemic effect via improving its bioavailability. *International journal of pharmaceutics*, 467:50-59.

CHAPTER 5

AMORPHOUS SOLID DISPERSIONS OF SPIRAMYCIN

5.1 Introduction

One of the major challenges that has hitherto perplexed, the pharmaceutical sector is poor solubility of a number of drugs (Baghel *et al.*, 2016). As previously mentioned, ASDs have been considered as the major development in improving aqueous solubility, bioavailability and ultimately treatment outcomes (Sareen *et al.*, 2012). Spiramycin (macrolide antibiotic) is the cornerstone drug in the treatment of the OI, Cryptosporidiosis in PLHIV (Huang *et al.*, 2015; Wang & Zhang, 2014). Nevertheless spiramycin, in its different forms, i.e. spiramycin I, spiramycin II and spiramycin III, has shown poor solubility in water and a relative low stability (Crew *et al.*, 2004; Fujimoto & Nakano, 1974; Huang *et al.*, 2015). Over the years structural modification of spiramycin has been employed to enhance aqueous solubility and stability of the drug with little to no success in the treatment of cryptosporidiosis. Alteration of the aldehyde group of the macrolide ring of spiramycin has been done to produce stable hydrazones and more soluble salts (Fujimoto & Nakano, 1974, Gallo *et al.*, 2013). On the other hand, acetylspiramycin, an acetylated derivative of spiramycin, is more stable and soluble than spiramycin (Huang *et al.*, 2015), yet this derivative is not specific for cryptosporidiosis (Maeda, 2003; Jiang *et al.*, 2000). In a solid-state screening study by Van Eeden (2012), an attempt to recrystallise spiramycin in different solvents did not yield any crystalline form or polymorphs of the drug; neither did it yield any solvates nor hydrates of spiramycin. More so, the desolvated spiramycin samples resulted in a further reduction of aqueous solubility of the API (Van Eeden, 2012). Therefore preparation of spiramycin ASDs was the most favourable approach to improve the drug's poor aqueous solubility which is associated with poor treatment outcomes. Thorough physico-chemical characterisation of spiramycin raw material, preparation and characterisation of ASDs was done and evaluated for any improvements in API dissolution rate.

5.2 Physico-chemical characterisation of spiramycin raw material

Limited literature reports spiramycin to exist as an amorphous solid-state form (Abou-Zeid *et al.*, 1980; Leon & Jean, 1961; Van Eeden, 2012) but this is incongruent with other discourse which report poor aqueous solubility of spiramycin (Calza *et al.*, 2010; Klich *et al.*, 2016; Krayz *et al.*, 2016; Murari *et al.*, 2001). Ideally an API in its amorphous form should possess good solubility properties due to its higher energy state and increased mobility. This is however not true for spiramycin and the phenomenon is not explained in literature. It was therefore imperative to consider that any pure phase (crystalline or amorphous solids) may exhibit

mesophase behaviour which further affects physico-chemical properties of the API (Atassi & Byrn, 2006). It is known that a pure amorphous drug will eventually transform to a crystalline state over time (Elder *et al.*, 2015). On the other hand, mesophases may remain stable and not convert back to the crystalline state (Atassi *et al.*, 2013). It was against this background that different characterisation techniques (DSC, SEM, XRPD, IR, HSM and vapour sorption analysis) were used to obtain the physico-chemical properties of spiramycin raw material used in this study. The results are shown in Figures 5.1 - 5.5.

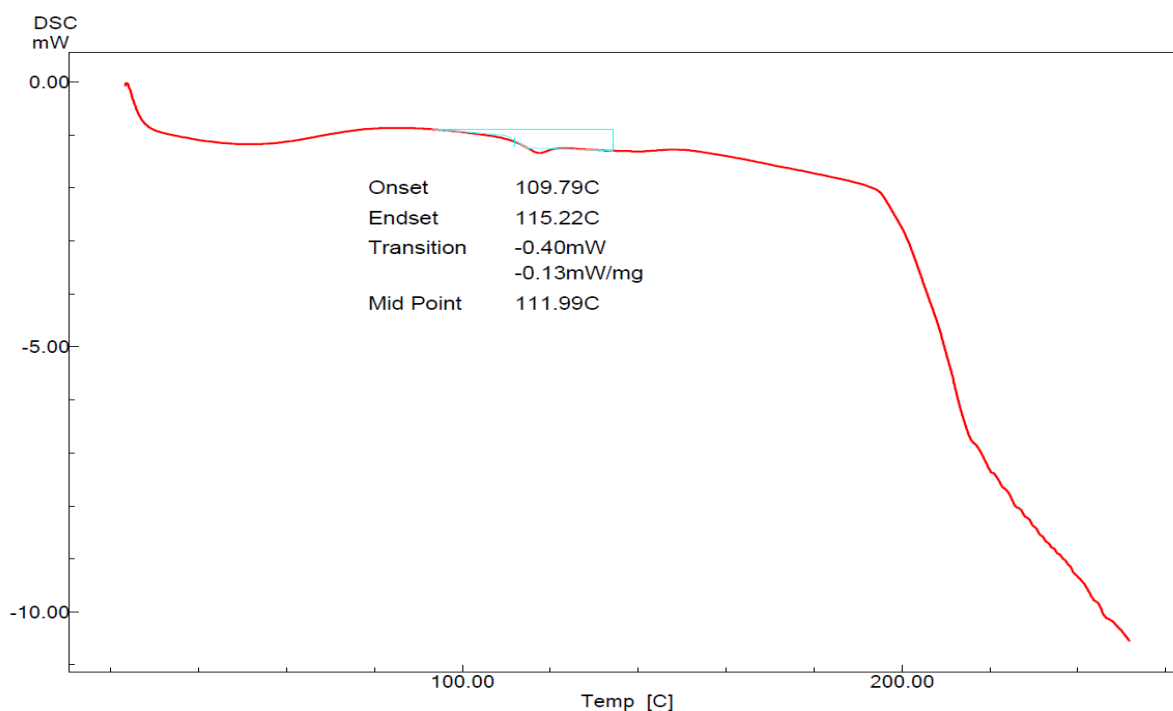


Figure 5.1: DSC thermogram of spiramycin raw material.

During DSC analysis (Figure 5.1), a T_g was detected at 109.79°C, which can be a characteristic of either a mesophase or an amorphous form (Shalaev *et al.*, 2016). XRPD of spiramycin bulk material revealed a diffuse scattering halo without any crystalline peaks, as depicted in Figure 5.2. However, some mesophases due to lack of long range order, can also be characterised by a diffuse scattering halo exhibiting some to no crystalline peaks (Chakravarty *et al.*, 2013; Stevenson *et al.*, 2005). It was therefore imperative to further characterise spiramycin so as to assign whether it is a mesophase or an amorphous form.

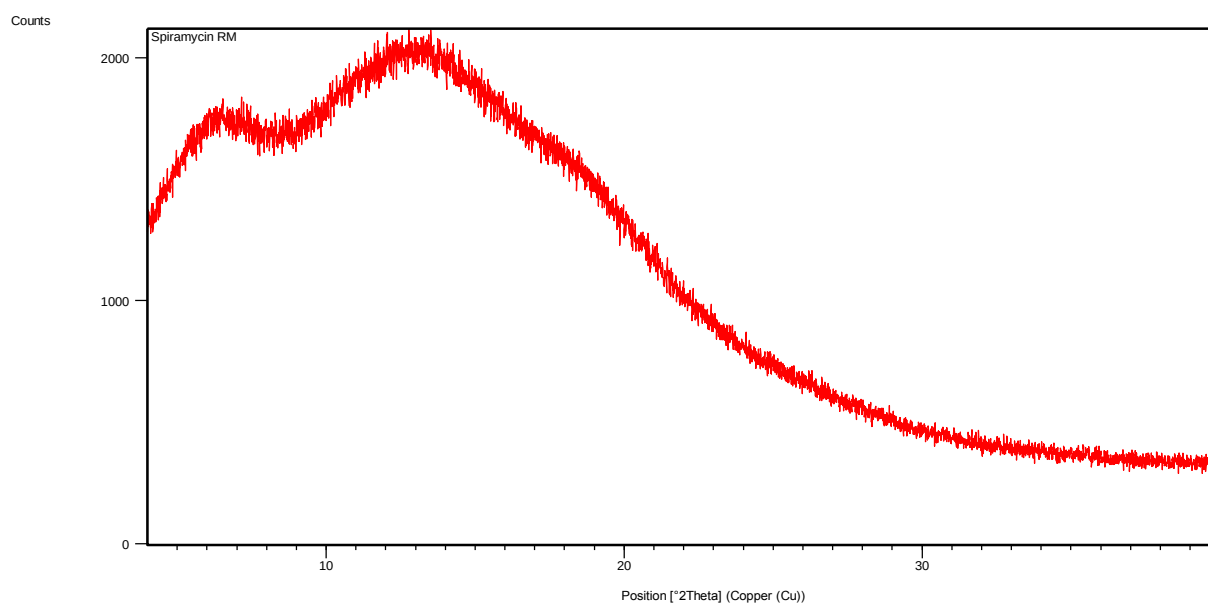
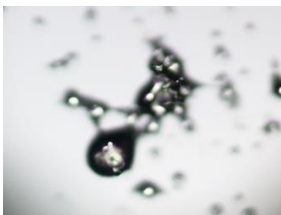


Figure 5.2: XRPD diffractogram of spiramycin raw material.

To enable an in-depth visual observation during thermal analysis of spiramycin, further characterisation was done using HSM (Table 5.1). Spiramycin raw material was observed under non-polarised and polarised modes and the visual observation of thermal phase changes were captured as shown in Table 5.1 (a - d). At this point, the possibility of a mesophase was ruled out due to the absence of birefringence under polarised light.

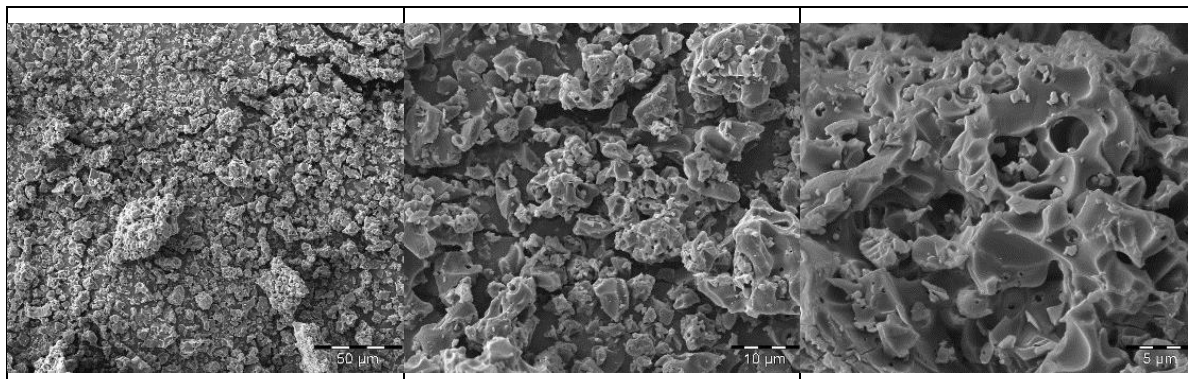
Table 5.1: HSM images depicting the glass transition of spiramycin raw material and glass forming upon cooling

			
a) Spiramycin raw material (25°C).	b) Glass transition (± 114°C).	c) Spiramycin liquid state (±118°C).	d) Glass forming on cooling (50°C).

The T_g of spiramycin raw material was captured at $\approx 114^\circ\text{C}$ (Table 5.1(b)). Above T_g , molecular mobility increased as the sample becomes liquid but no recrystallisation was observed. These findings corresponds to DSC results obtained (Figure 5.1). On slow cooling of the melt, spiramycin remained in its amorphous nature by forming a brittle glass (Table 5.1 (d)). In this case, it is clear that molecular mobility and rearrangement of spiramycin did not lead to any

formation of a crystalline solid-state form which correlates very well with literature (Van Eeden, 2012). This serves as a confirmation that spiramycin raw material already exist in an amorphous form. To obtain surface detail of this drug, SEM micrographs were also collected (Table 5.2).

Table 5.2: SEM micrographs depicting the morphology of spiramycin raw material



Spiramycin raw material exhibits a porous sponge-like surface due to crystal lattice disruption, as depicted in Table 5.2. Results further confirm the amorphous nature of spiramycin raw material, leaving the paradox of “stable amorphous states” to be explained (Stevenson *et al.*, 2005). Structure verification of spiramycin raw material was thereafter done by FT-IR analysis and the resulting spectrum is depicted in Figure 5.3.



Figure 5.3: FT-IR spectrum of commercially obtained spiramycin raw material.

The major peaks of the IR spectrum of the functional groups of spiramycin raw material (Figure 5.3) was compared to the IR spectrum of its individual components i.e. spiramycin I, II and III as reported in literature (Table 5.4) (Leon & Jean, 1961).

Table 5.3: Spiramycin raw material obtained major IR wavenumbers (cm^{-1}) and corresponding functional groups

Wavenumber (cm^{-1})	Functional group
1724.36	Aldehyde carbonyl
2785 - 2970	Alkane C-H stretch
3479.68	Hydroxyl OH stretch

Results indicate that the wavenumbers (cm^{-1}) of the major peaks of spiramycin raw material (Table 5.3) remain more or less the same for its three major components (Table 5.4). The rest of the IR spectrum peaks of spiramycin raw material closely resemble the chemical structures of its 3 major components reported in literature (Leon & Jean, 1961).

Table 5.4: IR peak listing (cm⁻¹) obtained for purchased spiramycin in comparison to its three major components (Leon & Jean, 1961).

Spiramycin raw material	Spiramycin I	Spiramycin II	Spiramycin III
3479.58	3470	3460	3470
2970.38	2970	2970	2970
2933.73	2940	2940	2940
1724.36	1735	1740	1740
1664.57	-	-	-
1452.40	1455	1457	1460
1409.96	1378	-	1380
1371.39		1372	1370
-	1317	1300	1300
1276.88	1275	1275	1280
-	1237	1232	1240
-	-	-	1185
1161.15	1160	1160	1162
1120.64	1122	1122	1122
-	1090	1085	1085
1053.13	1052	1052	1052
-	1015	1015	1015
993.34	993	993	995
-	-	940	-
904.61	905	905	906
-	865	860	865
840.86	840	840	842
-	810	810	810
783.10	782	782	782

It is well known that physical stability of amorphous forms further decrease when exposed to moisture. On this note, vapour sorption data was therefore obtained for spiramycin raw material (Figure 5.4).

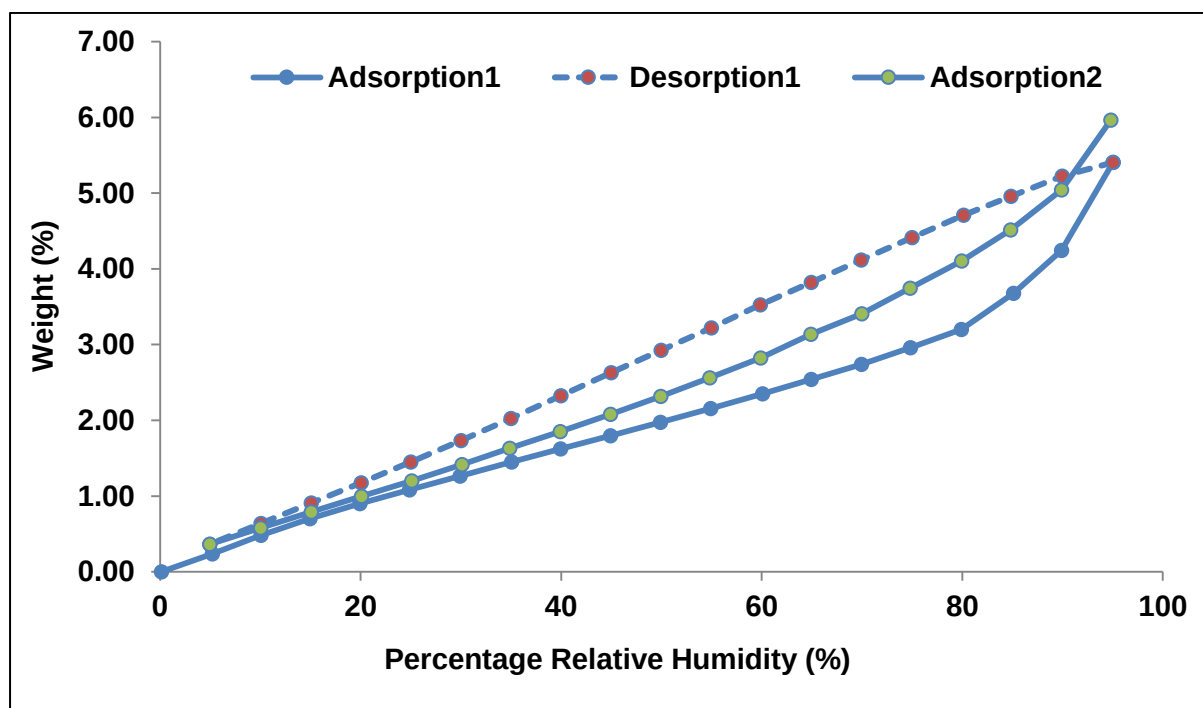


Figure 5.4: Vapour sorption isotherms obtained with spiramycin raw material at 25°C. A sorption ramp of 0 – 95 %RH, followed by a desorption phase from 95 – 5 %RH and then finally an absorption phase of 5 – 95 %RH.

Temperature was ambient for all the steps used to obtain the vapour sorption isotherms of spiramycin bulk material. An increase in measured weight was noted as a result of excess water absorbed, this being due to the highly porous morphology of the spiramycin powder. In correlation with the preceding results, no molecular rearrangement occurred and the amorphous habit of the sample was confirmed by XRPD analysis. It was then worthwhile to determine aqueous equilibrium solubility of spiramycin raw material using the method as described in Chapter 3.

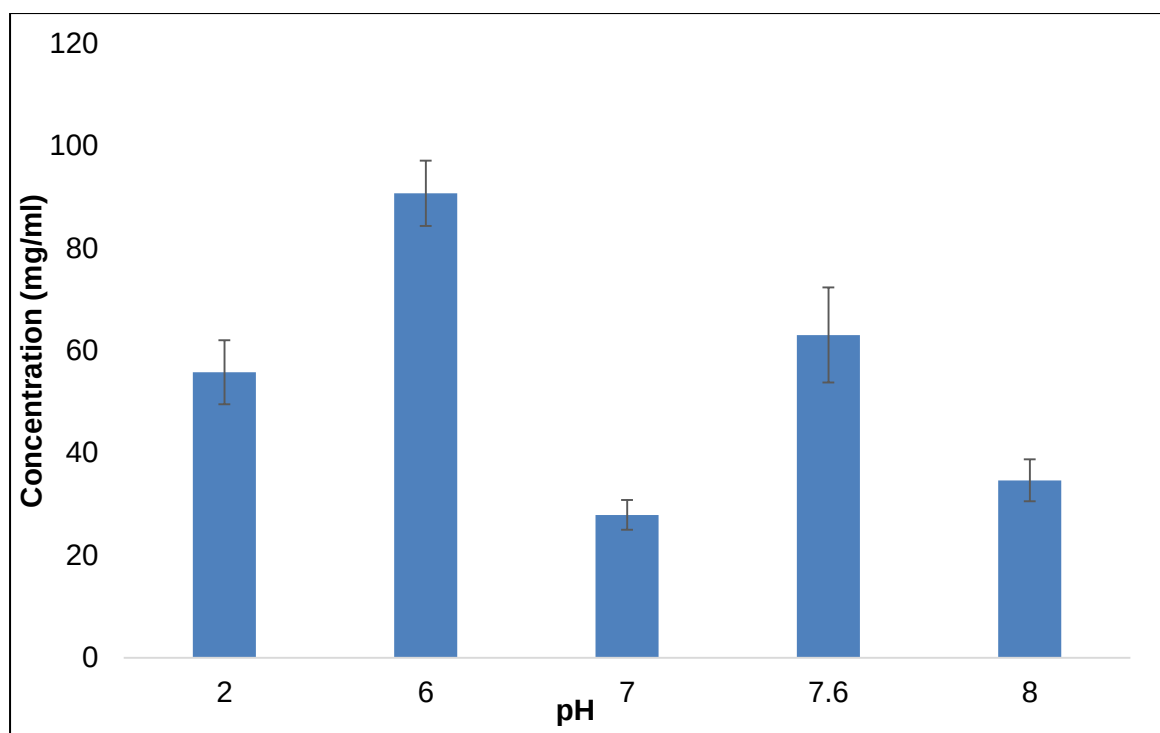


Figure 5.5: Equilibrium solubility profile of spiramycin raw material in different media (different pH-values) at $25 \pm 2.0^\circ\text{C}$.

It has been reported that spiramycin remains stable in aqueous environments between pH 4.0 ~ 10.0 and degradation thereof to occur at pH < 4.0 and pH > 10.0 (Feng *et al.*, 1997). In this study, solubility of spiramycin raw material was assessed in different pH buffer media of (pH 2, 6, 7, 7.6 and 8) at a temperature of 25°C . From the obtained pH solubility profile, spiramycin demonstrates better solubility in a slightly acidic but almost neutral environment of pH 6 whereas the poorest solubility was noted in distilled water pH 7 (Figure 5.5) as was reported in literature. The water solubility noted in this study 27.89 ± 2.9 mg/ml may be attributed to the already amorphous nature of the drug. It is important to note that the values of aqueous solubility of spiramycin are not consistent in literature. Most researchers characterise spiramycin as barely or poorly soluble in water whilst others characterise it as slightly or freely water soluble (Sharma *et al.*, 2014; Veiga & de Eulate, 1994; Vervaeke *et al.*, 1979). The inconsistent values could be influenced by relatively low stability of spiramycin, different experimental conditions as well as significant variation in the amounts of spiramycin components (purity) present in the API mixture. On the other hand, the varying percentage components of spiramycin may lead to different amorphous forms of the same API resulting in different physico-chemical properties. Nevertheless, in their inconsistency, the aqueous solubility values of spiramycin remain inadequate for optimum treatment outcomes of cryosporidiosis (Fujimoto & Nakano, 1974; Huang *et al.*, 2015).

5.2.1 Discussion

The physico-chemical characterisation of spiramycin raw material revealed that spiramycin exists in an amorphous state which interestingly does not crystallise to a more stable crystalline form. Thereby it was concluded that spiramycin exists as a stable amorphous solid-state form. Results obtained correlates with literature reports on the inability of spiramycin to crystallise or to form any polymorphs, solvates or hydrates (Van Eeden, 2012). From the solubility results it was concluded that spiramycin is very slightly soluble in water. Since literature reports that the poor solubility and slow dissolution rate of spiramycin is the fundamental problem for the poor treatment outcomes of this drug, further investigation towards improving the solubility was pursued.

Incorporating a hydrophilic polymer therefore remained a favourable option for improving aqueous solubility of spiramycin. A significant improvement in spiramycin dissolution rate was observed by the mere inclusion of hydrophilic carriers in physical mixtures (PMs) (Veiga & de Eulate, 1994). However, ASDs remain the best option for they can also improve the relatively low stability of spiramycin (Fujimoto & Nakano, 1974). Miscibility of spiramycin / polymer PMs were studied in detail in order to obtain the best drug / polymer ratio for the preparation of optimised ASDs.

5.3 Spiramycin / polymer miscibility studies

All spiramycin / polymer PMs were prepared into different drug / polymer (w/w) ratios of 1:1, 1:2, 1:3, 2:1, 2:3, 3:1, 3:2. Polymers used were: Polyvinylpyrrolidone K25 (PVP K25), Polyvinylpyrrolidone K30 (PVP K30), Kollidon® VA-64 (PVP/VA), polyethylene glycol 8000 (PEG 8000) and Pluronic® F127 (PF-127) (Chapter 4, Table 4.6). Miscibility for each PM was studied using DSC analysis (Figure 5.6 - 5.10).

5.3.1 Differential scanning calorimetry

When compared to the already amorphous raw material, it was difficult to prove drug / polymer miscibility using the melting point depression method. This is due to the absence of a melting point of the amorphous material, thus the Flory-Huggins theory could not be applied in this study. The best applicable method was therefore the presumption of drug / polymer miscibility detected by the presence of a single T_g which may increase as a function of drug / polymer (w/w) composition (Zhang *et al.*, 2003).

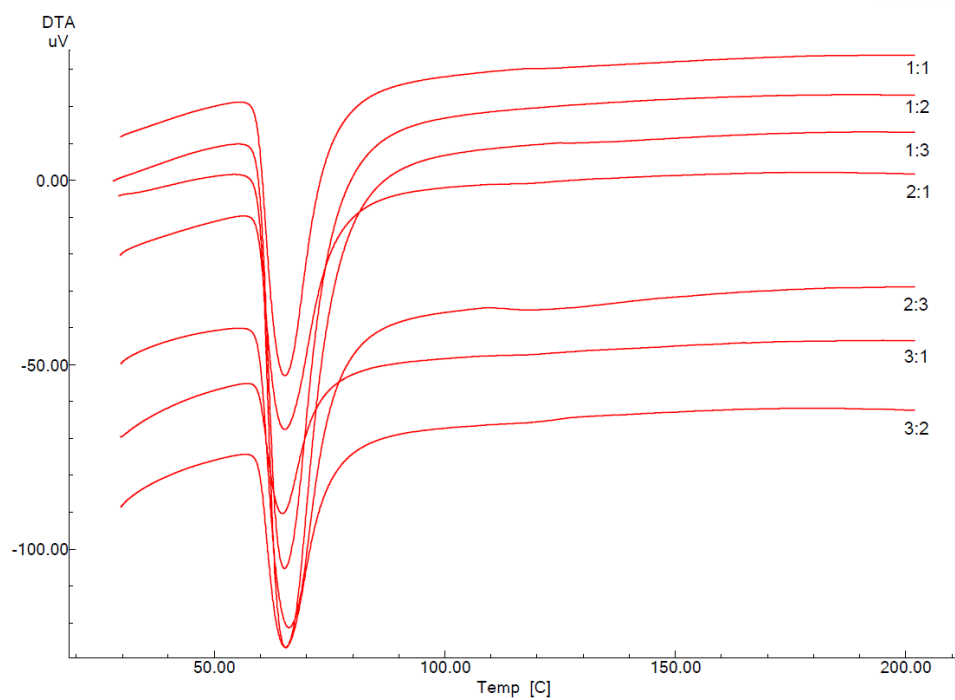


Figure 5.6: An overlay of DSC thermograms obtained with different w/w ratios of spiramycin / PEG 8000 PMs.

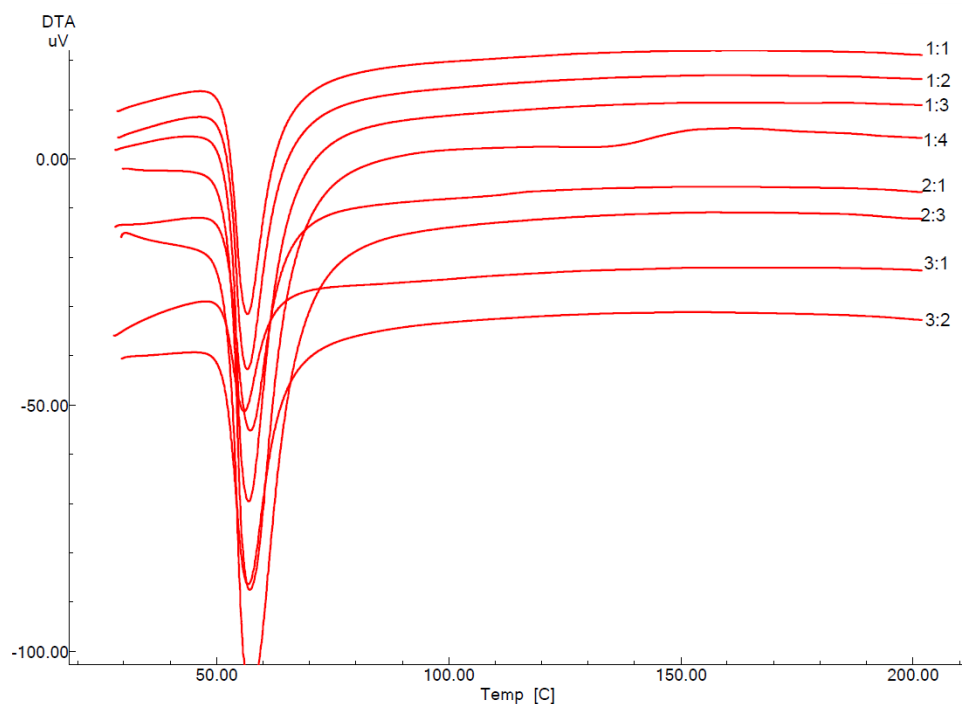


Figure 5.7: An overlay of DSC thermograms obtained with different w/w ratios of spiramycin / PF-127 PMs.

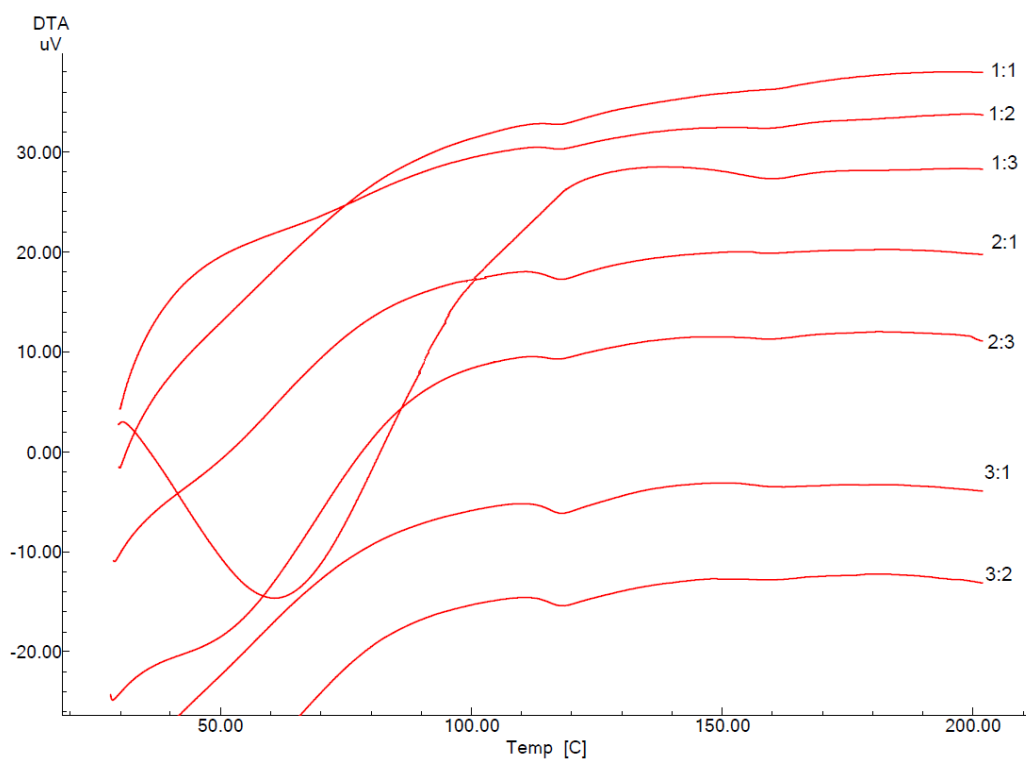


Figure 5.8: An overlay of DSC thermograms obtained with different w/w ratios of spiramycin / PVP K25 PMs.

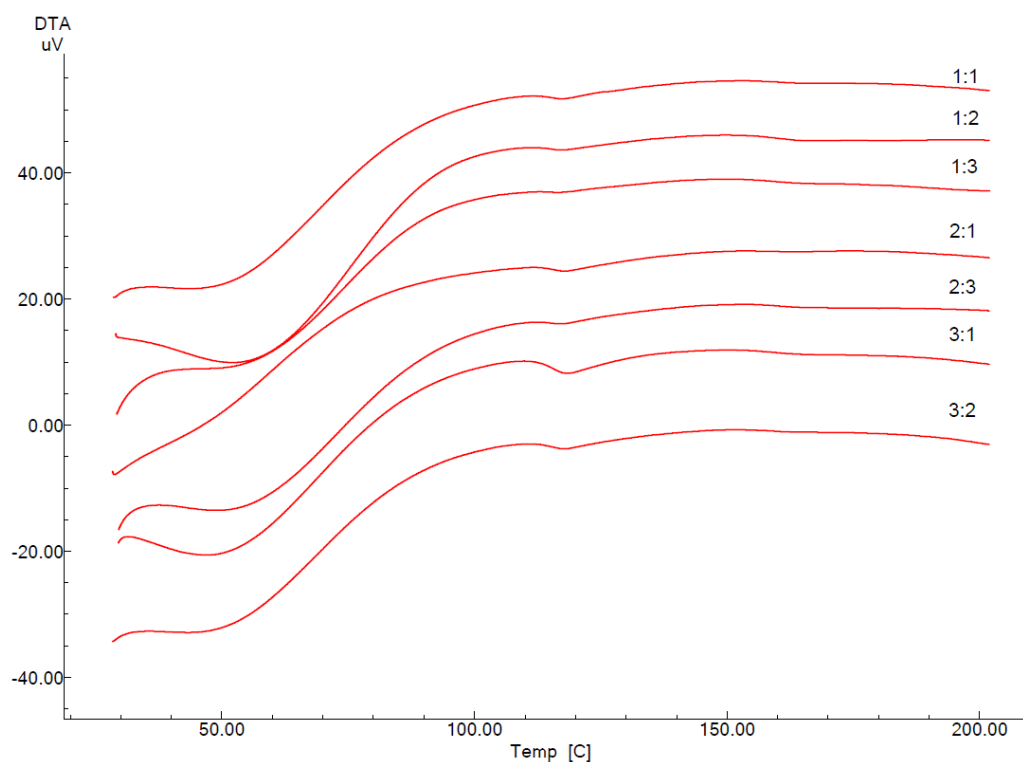


Figure 5.9: An overlay of DSC thermograms obtained with different w/w ratios of spiramycin / PVP K30 PMs.

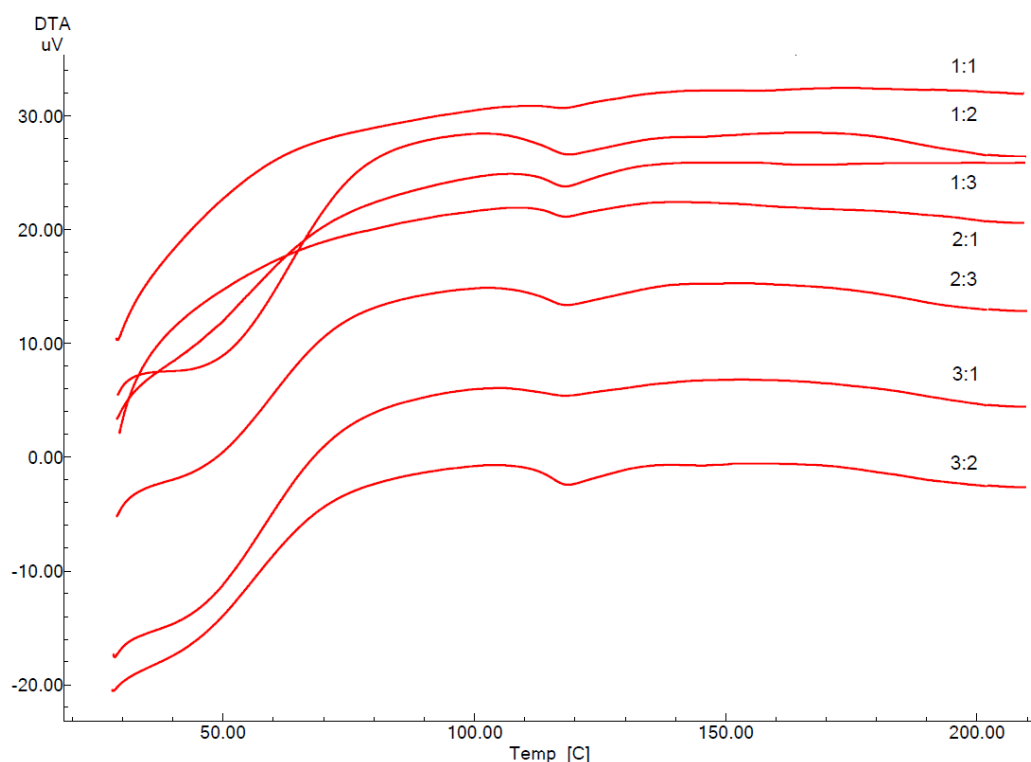


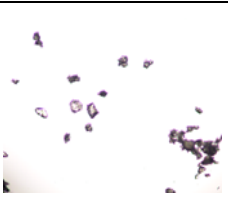
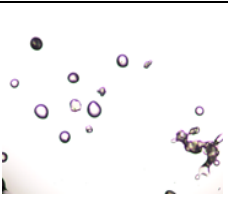
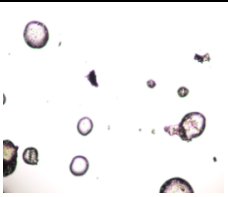
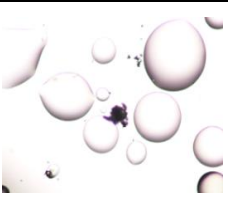
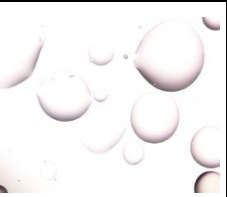
Figure 5.10: An overlay of DSC thermograms obtained with different w/w ratios of spiramycin / Kollidon® VA-64 PMs.

It was difficult to clearly point out a single T_g of spiramycin for PMs containing crystalline polymers i.e. PEG 8000 and PF-127. Nevertheless, a single endotherm corresponding to the melting point of the polymer could clearly be observed (Figure 5.6 - 5.7). On the other hand, PMs containing amorphous polymers presented T_g values greatly representing spiramycin and in some instances the T_g points of the polymers were observable. In addition, there was hardly any shift observed in the T_g of spiramycin in all the blends hence further miscibility studies were done by HSM in order to conclude better on the miscibility as well as the thermal behaviour of the drug / polymer PMs.

5.3.2 Hot-stage microscopy

Phase changes for spiramycin / polymer PM (w/w) ratios of 1:1, 1:2, 1:3, 2:1, 2:3, 3:1, 3:2 were captured using HSM. The 1:1 spiramycin / polymer (w/w) ratios (Table 5.5 – 5.9) gave the best visual miscibility when compared to all other ratios prepared. HSM results obtained for the thermal behaviour of spiramycin raw material (Table 5.1) can be referred to for comparison purposes.

Table 5.5: HSM images obtained during heating of either PEG 8000 and spiramycin / PEG 8000 PM

PEG 8000				
				
a) 25°C	b) 58°C	c) 61°C	d) 62°C	e) 64°C
Spiramycin / PEG 8000 (1:1 w/w ratio)				
				
f) 25°C	g) 64°C	h) 100°C	i) 130°C	j) 136°C

Spiramycin / PEG 8000 PM (Table 5.5 (a - e)) reveals the melting point of PEG 8000 at a lower temperature of 60 - 64°C than the T_g of spiramycin which remains solid at that temperature (Table 5.5 (g)). Thereafter, spiramycin transforms to liquid until completion at 136°C (Table 5.5 (j)). Good miscibility of this blend (Table 5.5 (j)) could be visually observed on HSM implying the ability of spiramycin and PEG 8000 to form a potentially optimised ASD.

The same trend was observed for spiramycin / PF-127 PM (Table 5.6). The HSM micrographs clearly show melting of the polymer starting at $\cong 55^\circ\text{C}$ (Table 5.6 (g)) whilst spiramycin remains encapsulated at that temperature. Spiramycin then subsequently transforms to liquid until completion at 145°C (Table 5.6 (j)). Thereby, indicating good miscibility of the polymer and the drug.

For the spiramycin / Kollidon® VA-64 physical mixture (Table 5.7), it was difficult to detect a distinct melting transition of the blend by HSM. In this case, the blend becomes liquid-like (Table 5.7 (i)) characterised by a rough-like texture of the melt. Although the rough-like texture does not show under non-polarised light (Table 5.7 (j)), it does not disappear with an increase in temperature indicating partial miscibility of the two compounds.

Table 5.6: HSM images obtained during heating of PF-127 or a 1:1 w/w ratio of spiramycin / PF-127 PM

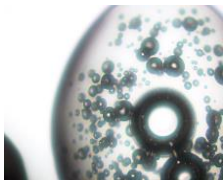
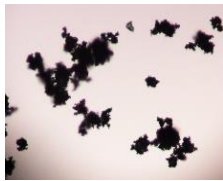
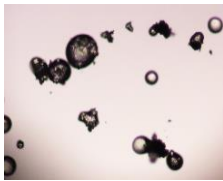
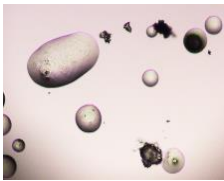
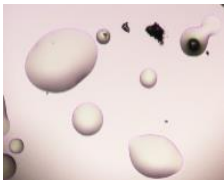
PF-127				
				
a) 25°C	b) 56°C	c) 120°C	d) 168°C	e) 173°C
Spiramycin / PF-127 (1:1 w/w ratio)				
				
f) 25°C	g) 55°C	h) 68°C	i) 108°C	j) 145°C

Table 5.7: HSM images obtained during heating of Kollidon® VA-64 or a 1:1 w/w ratio of spiramycin / Kollidon® VA-64 PM

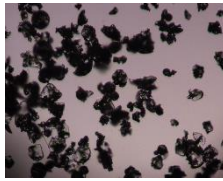
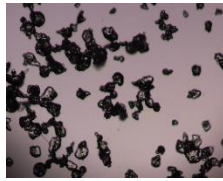
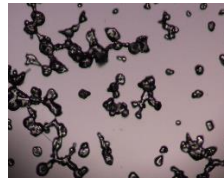
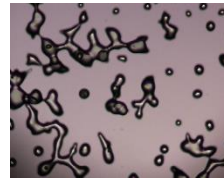
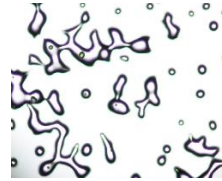
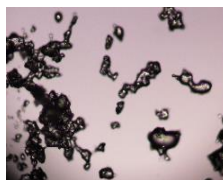
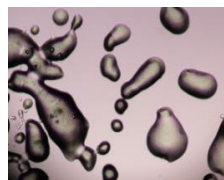
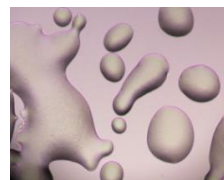
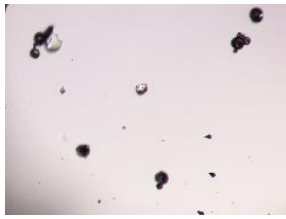
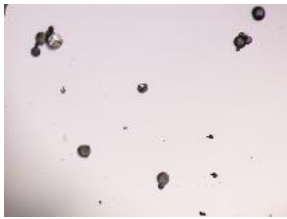
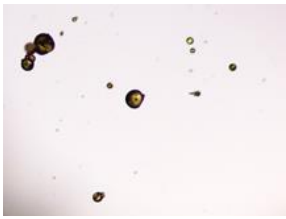
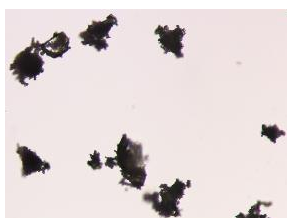
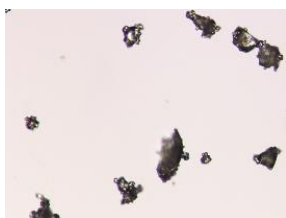
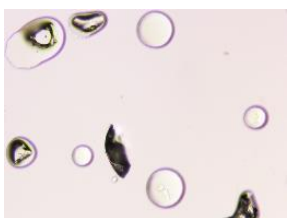
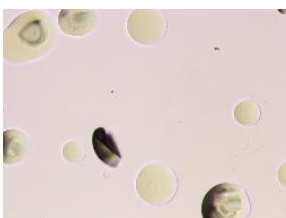
Kollidon® VA-64				
				
a) 25°C	b) 142°C	c) 148°C	d) 154°C	e) 155°C
Spiramycin / Kollidon® VA-64 (1:1 w/w ratio)				
				
f) 25°C	g) 148°C	h) 172°C	i) 179°C	j) 200°C

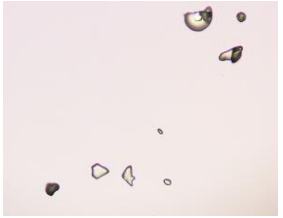
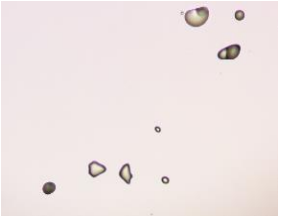
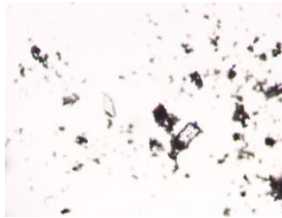
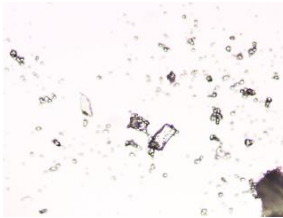
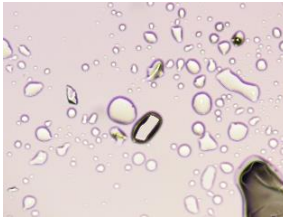
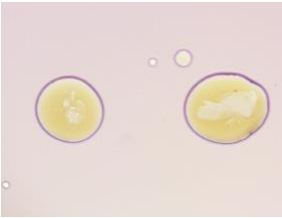
Table 5.8: HSM images obtained during heating of PVP K30 or spiramycin / PVP K30 physical mixture

PVP K30			
			
a) 25°C	b) 240°C	c) 250°C	d) 270°C
Spiramycin / PVP K30 (1:1 w/w ratio)			
			
e) 25°C	f) 136°C	g) 165°C	h) 230°C

PVP K30 did not show a distinct melting point on the HSM and only degradation was observed at $\cong 270^{\circ}\text{C}$ (Table 5.8 (a-d)). Spiramycin can be clearly observed in its transition state (Table 5.8 (f)) and in its liquid state (Table 5.8 (g)). However, PVP did not show any distinct melting or liquefaction point such that separate phases of the mixture could be clearly observed until spiramycin eventually starts to discolour due to degradation (Table 5.8 (h)). This visual observation clearly indicates poor miscibility of the mixture due to the fact that the PVP K30 did not dissolve in the liquefied spiramycin.

A similar trend was observed in the PM containing PVP K25 (Table 5.9). Transition of spiramycin could be clearly observed at 133°C (Table 5.9 (f)), thereafter two separate phases could be easily distinguished in the physical mixture (Table 5.9 (g)) until degradation occurs (Table 5.9 (h)) indicating poor miscibility of the two components of the PM.

Table 5.9: HSM images obtained during heating of PVP K25 and spiramycin / PVP K25 physical mixture

PVP K25			
			
a) 25°C	b) 185°C	c) 216°C	d) 230°C
Spiramycin / PVP K25 (1:1 w/w ratio)			
			
e) 25°C	f) 133°C	g) 200°C	h) 220°C

5.3.3 Discussion

From the spiramycin / polymer miscibility results obtained, it was concluded that spiramycin PMs containing PEG 8000 or Pluronic® F-127 were miscible, whilst PMs containing Kollidon® VA-64 were partially miscible. On the other hand, PMs containing PVP K25 or PVP K30 were poorly miscible as indicated by the clear distinction of separate phases observed during HSM analysis. These results showed that multiple techniques should always be used when testing for drug / polymer miscibility, especially in instances where the drug is already in an amorphous solid-state. At this stage of the study a few questions came to mind, namely: (i) in the light of spiramycin already existing in an amorphous state and since it doesn't exhibit a melting phase but only a glass transition phase, will it be possible to successfully combine the API with polymer as an ASD? (ii) given the fact that spiramycin is not that poorly soluble, due to its amorphous habit, will the preparation of an ASD be of any advantage? and (iii) what will be the best option, to prepare an ASD or to use a mere PM of spiramycin and a polymer to improve solubility and dissolution rate? It was against this background that spiramycin ASDs formed with PEG 8000 and Kollidon® VA-64 were further characterised and evaluated in comparison to PMs containing Pluronic® F-127 and PVP K30 (Table 5.10).

Table 5.10: A summary of the results obtained during miscibility studies of spiramycin / polymer physical mixtures and the subsequent decision of further steps

Spiramycin / Polymer w/w ratio	Physical Mixture	Miscibility	Prepared
1:1	Spiramycin / PEG 8000	Miscible	ASD
1:1	Spiramycin / PF-127	Miscible	PM
1:1	Spiramycin / PVP/VA	Partially miscible	ASD
1:1	Spiramycin / PVP K30	Poorly miscible	PM
1:1	Spiramycin / PVP K25	Poorly miscible	-

5.4 Physico-chemical characterisation of amorphous solid dispersions of spiramycin

During preliminary studies, slow as well as rapid solvent evaporation techniques were investigated for the preparation of spiramycin ASDs. These studies showed that the solvent-based techniques did not result in acceptable ASDs, since polymer and API could clearly be distinguished from one another after the solvent has evaporated. In other instances, solvent still remained trapped in the spiramycin / polymer matrix, which was seen as a disadvantage since solvents could influence further processing, solubility as well as dissolution characteristics. The presence of solvents in such a matrix is also seen as detrimental due to possible toxicity thereof. The quench cooling of the melt method (described in Chapter 3) was therefore employed for preparation of spiramycin ASDs. Properties of ASDs were explored using a number of different characterisation methods and compared to those obtained for PMs (Figure 5.11 – 5.14).

5.4.1 Differential scanning calorimetry

The interpretation of the DSC thermogram of the prepared ASD of spiramycin using PEG 8000 as polymer, Figure 5.11, only showed the melting point of the crystalline PEG 8000. No clearly distinguishable T_g was observed. This made it impossible to determine whether a true ASD of spiramycin did indeed form.

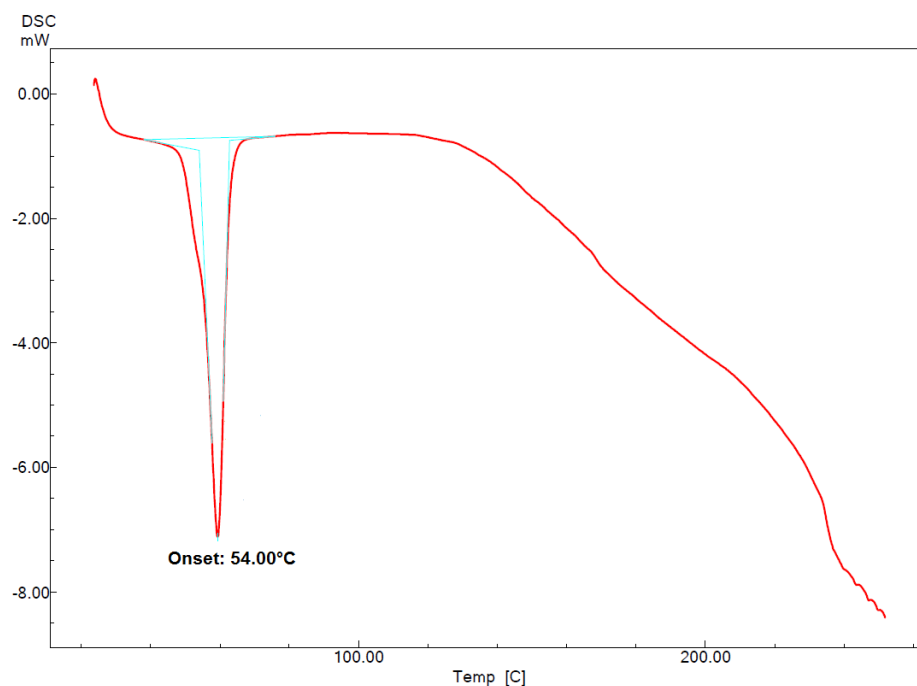


Figure 5.11: DSC thermogram obtained for the prepared spiramycin / PEG 8000 ASD (1:1 weight ratio).

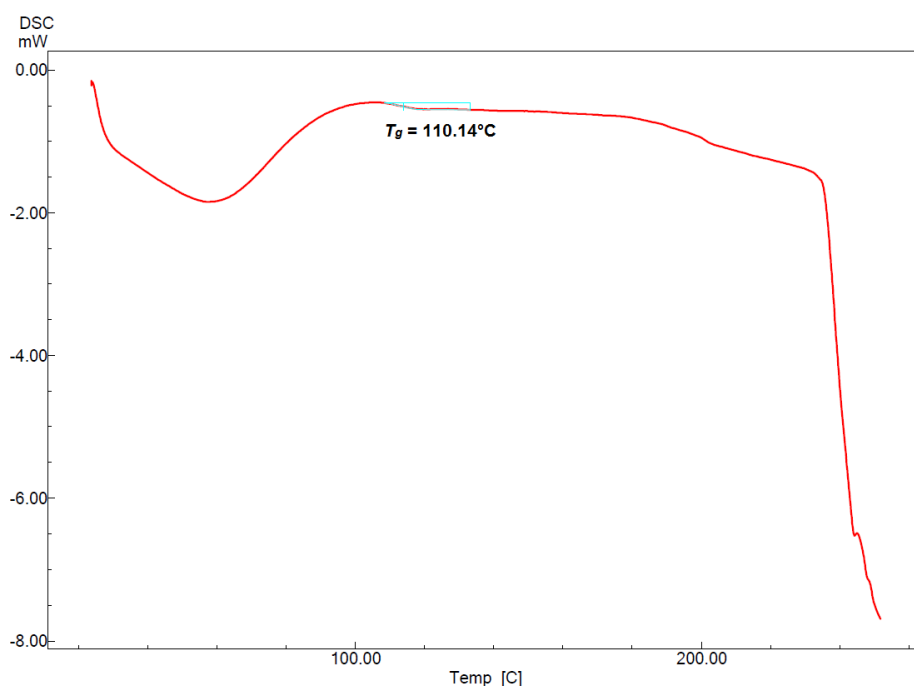


Figure 5.12: DSC thermogram obtained for the prepared spiramycin / Kollidon® VA-64 ASD (1:1 weight ratio).

In the instance of the DSC results obtained for the spiramycin / Kollidon® VA-64 ASD, the same applied (Figure 5.12). Only a single T_g at 110.14°C was observed, this correlating with the T_g of spiramycin (Figure 5.1). This also lead to questioning the fact wether a true ASD of spiramycin did form. Since only PMs of spiramycin / PF-127 (1:1 w/w ratio) and

spiramycin / PVP K30 (1:1 w/w ratio) were used further, the DSC results will not be presented again, since it correlate with that depicted in Figures 5.7 and 5.9. In order to obtain more results so that a better conclusion can be made towards the successful preparation of spiramycin ASDs, XRPD was used as an investigative technique.

5.4.2 X-ray powder diffraction

PEG 8000 polymer (Figure 5.13 (b)) exhibited Bragg diffraction peaks, this being due to the crystalline habit of PEG 8000, as already discussed in Chapter 4. The prepared spiramycin / PEG 8000 ASD also showed the same diffraction peaks, thereby indicating that the preparation technique did not render the polymer in an amorphous state. This made a conclusion as to if an ASD was successfully prepared even more challenging.

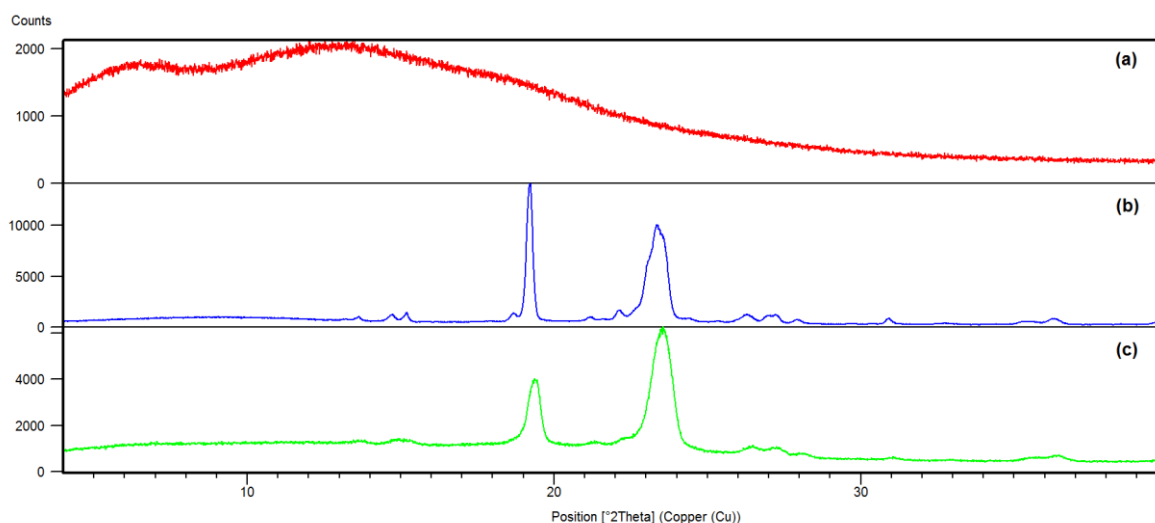


Figure 5.13: XRPD for (a) spiramycin raw material, (b) PEG 8000 and (c) spiramycin / PEG 8000 ASD (1:1 weight ratio).

It was considered an added advantage that the ASD containing Kollidon® VA-64 (Figure 5.14 (c)) was prepared using both the API and the polymer in their amorphous state. XRPD recorded a diffuse scattering halo suggesting that amorphicity was maintained, but if it resulted in a true ASD or just a mere mixture of the two compounds remained a question.

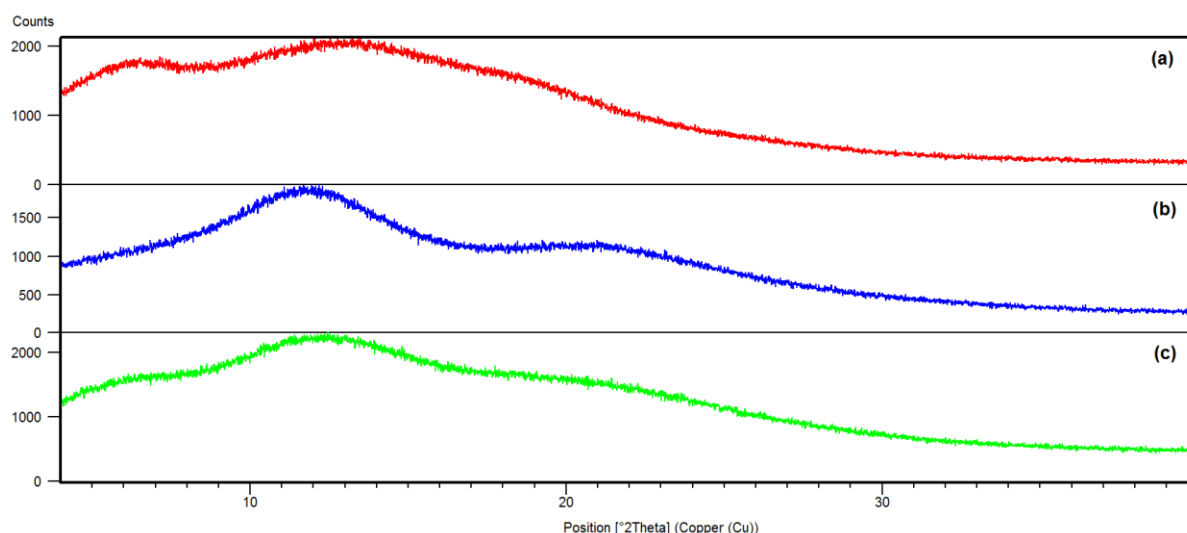


Figure 5.14: XRPD for (a) spiramycin raw material, (b) Kollidon® VA-64 and (c) spiramycin / Kollidon® VA-64 ASD (1:1 weight ratio).

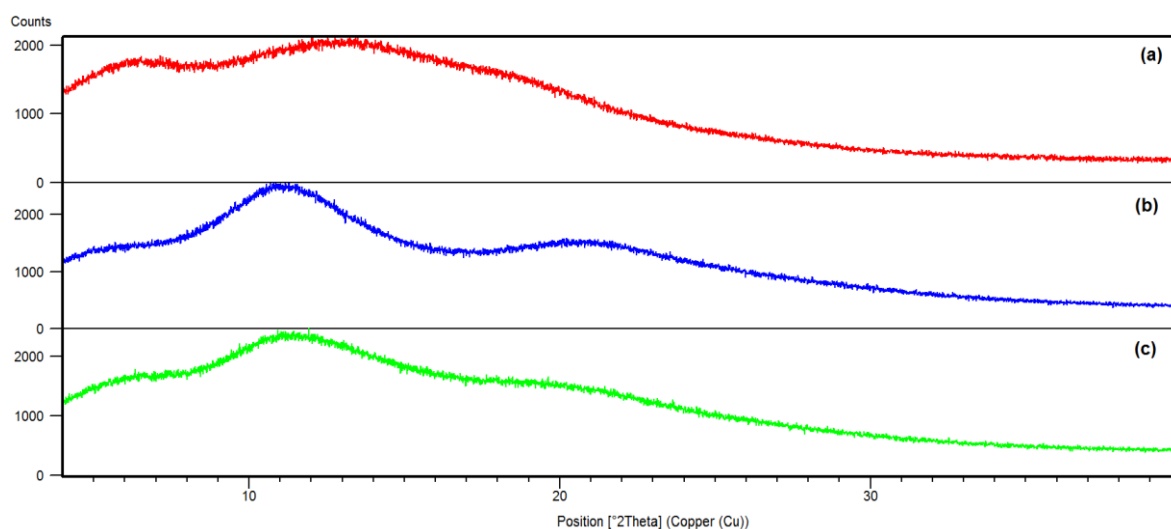


Figure 5.15: XRPD for a) spiramycin raw material, b) PVP 30 and c) spiramycin / PVP K30 PM (1:1 weight ratio).

XRPD for the PM containing PVP K30 (Figure 5.15(c)) also showed a diffuse scattering halo due to the combination of an amorphous API and an amorphous polymer. Figure 5.16 a – c depicts the XRPD data obtained with the spiramycin / PF-127 PM in a 1:1 w/w ratio. Once again the diffraction peaks due to the crystalline PF-127 was observed, which makes sense since this was only a PM of spiramycin and the polymer.

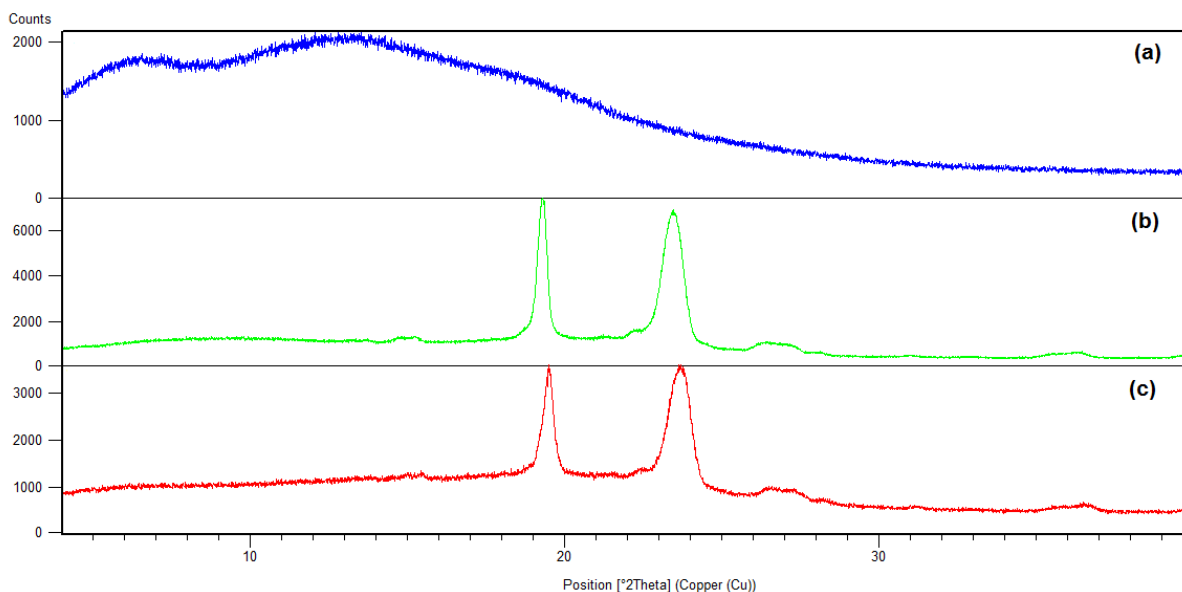


Figure 5.16: XRPD for a) spiramycin raw material, b) PF-127 and c) spiramycin / PF-127 (1:1 weight ratio) as a PM.

In this study, traces of crystallinity were only observed in the ASD and PM prepared using crystalline polymers. Here, the degree of crystallinity of polymers was lowered due to the amorphous influence of spiramycin. Spiramycin remained in its amorphous state in both the PMs and the ASDs. From the XRPD data it was however impossible to conclude unequivocally whether ASDs were successfully prepared, due to the already amorphous nature of spiramycin.

Thus, the ability of spiramycin to form hydrogen bonds and ionic interactions with the polymer to form a stable ASD was one last aspect that could shed light on the possibility of successful ASD formation (Mistry *et al.*, 2015; Vo *et al.*, 2013; Zhaojie *et al.*, 2014). FT-IR was therefore employed to identify any hydrogen bond interactions between spiramycin and the corresponding polymer in all PMs and ASDs prepared (Figures 5.17 - 5.21).

5.4.3 Infrared spectroscopy

There were no API-polymer interactions observed for prepared PMs whilst the opposite was true for the prepared ASDs. For the spiramycin / PVP 30 PM (Figure 5.17), the -OH and -CH stretch remained exactly at the same position as for spiramycin raw material at 3479.68 cm^{-1} and 2970.38 cm^{-1} respectively (Figure 5.3). This implies that no interaction between spiramycin and the polymer occurred in the PM. The IR spectrum for spiramycin / PF-127 PM (Figure 5.18) followed suit whereby no interaction occurred between spiramycin and the polymer. This is expected due to the fact that it is only a physical mixture.

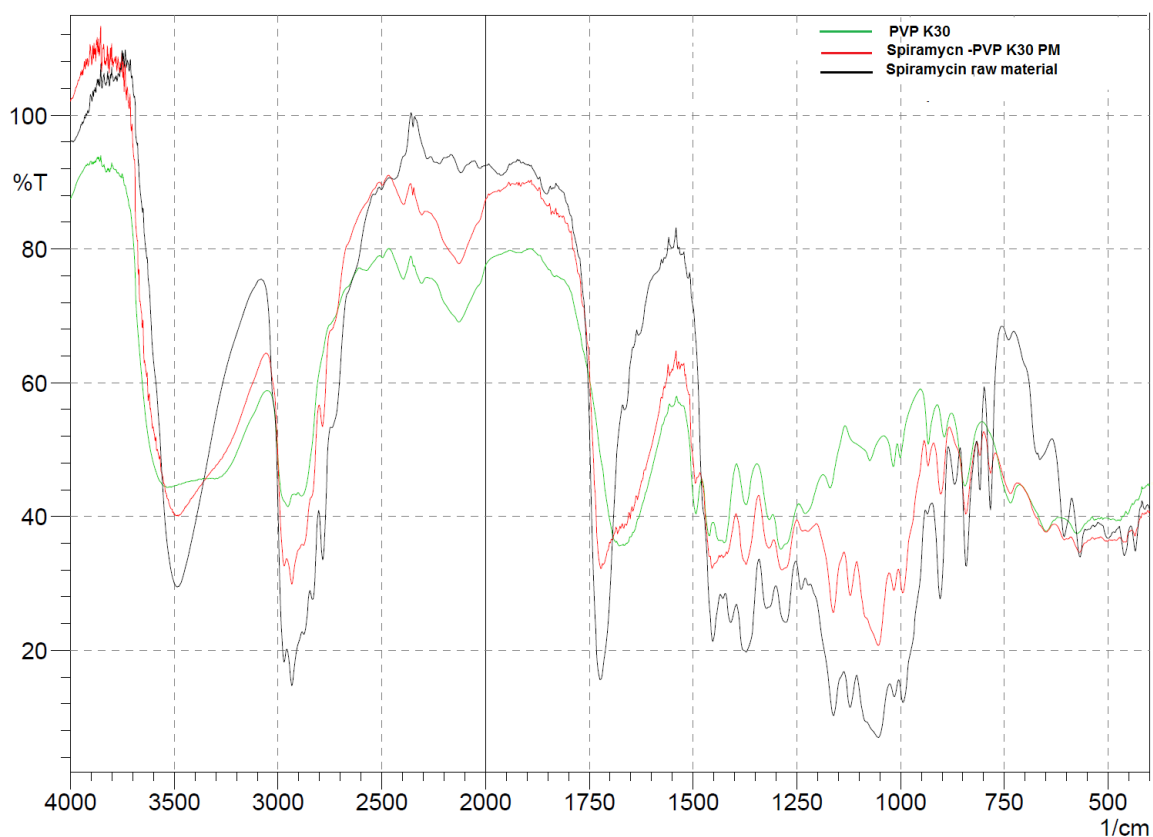


Figure 5.17: Overlay of the IR spectra obtained for spiramycin raw material, PVP K30 and spiramycin / PVP K30 (1:1 w/w ratio) as a PM.

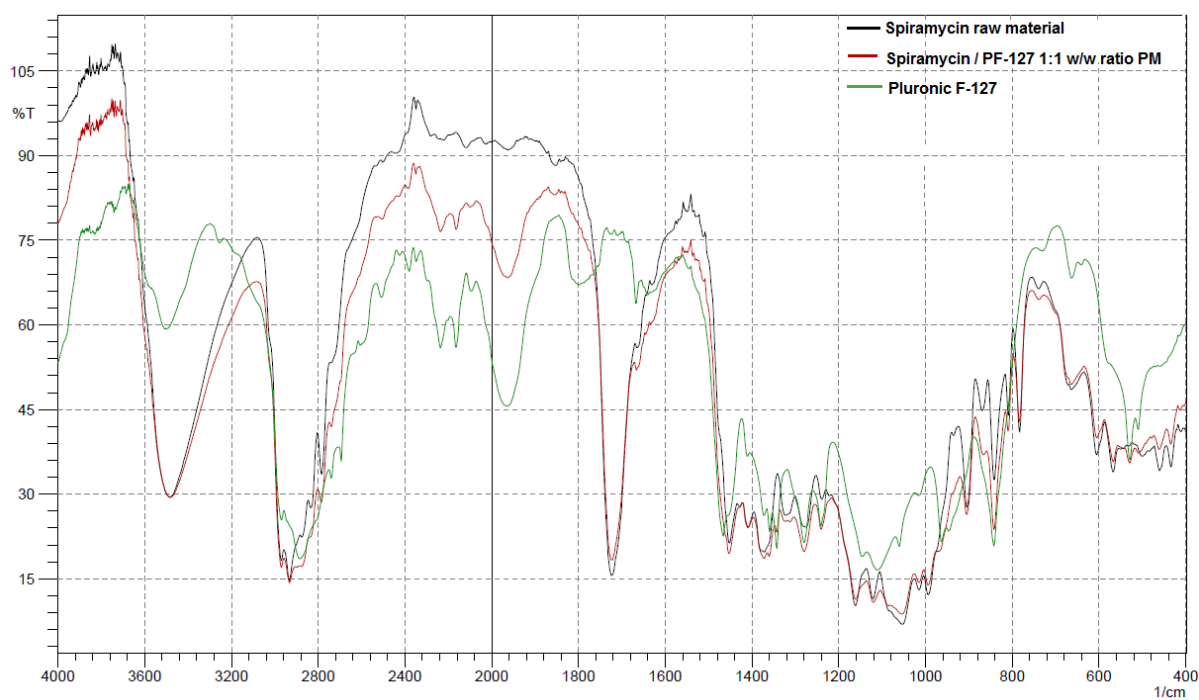


Figure: 5.18: Overlay of the IR spectra obtained for spiramycin raw material, PF-127 and spiramycin / PF-127 (1:1 w/w ratio) as a PM.

On the other hand, band broadening of the -OH stretch was observed in ASDs in comparison to a sharp non-hydrogen bonded -OH stretch observed in spiramycin amorphous raw material at 3479.68 cm^{-1} (Figure 5.19). The band broadening of the -OH stretch observed in ASDs is a sign of hydrogen bond formation. A shift in the -CH stretch (2970 cm^{-1}) was also clearly observed in the spiramycin / PEG 8000 ASD. In general, all ASDs peaks were of lower intensity and broadened in comparison to amorphous spiramycin. This confirms a level of API / polymer interaction which is a prerequisite for optimised ASDs. The interaction between spiramycin and the polymer observed in both ASDs prepared could lead to an improved stability of spiramycin. Visual morphology differences in the prepared ASDs were then detected by SEM.

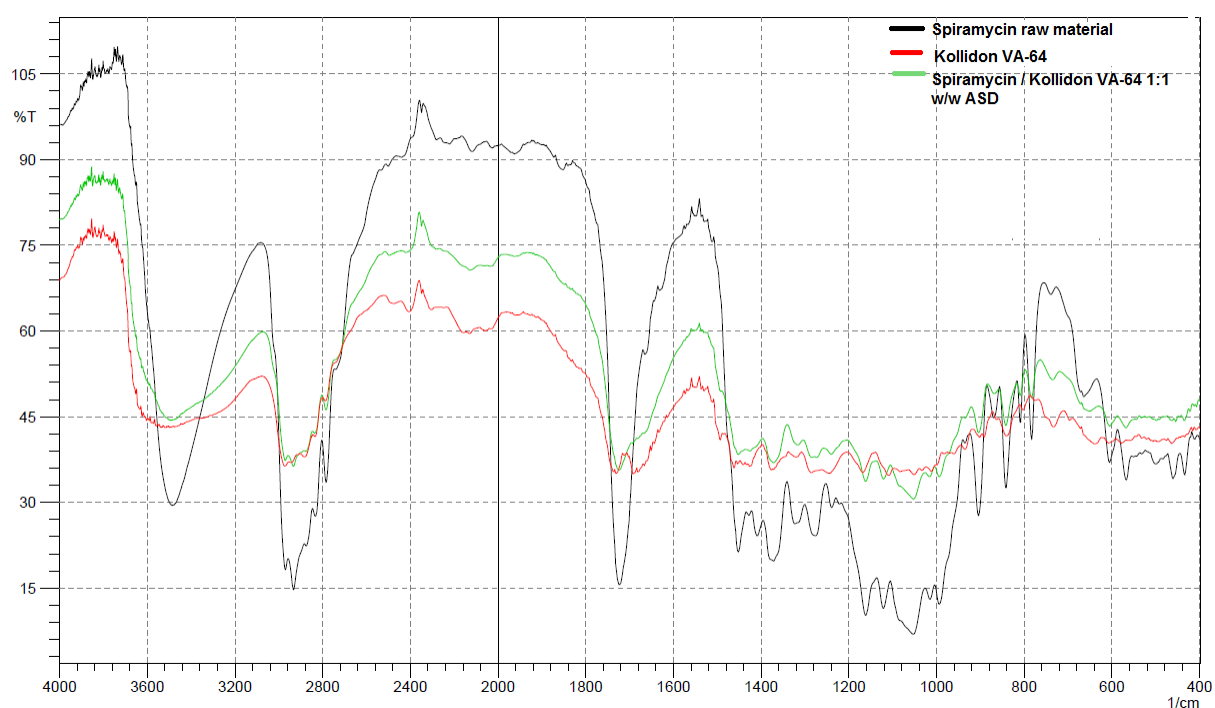


Figure 5.19: Overlay of the IR spectra obtained for spiramycin raw material, Kollidon[®] VA-64 and the 1:1 w/w ratio ASD.

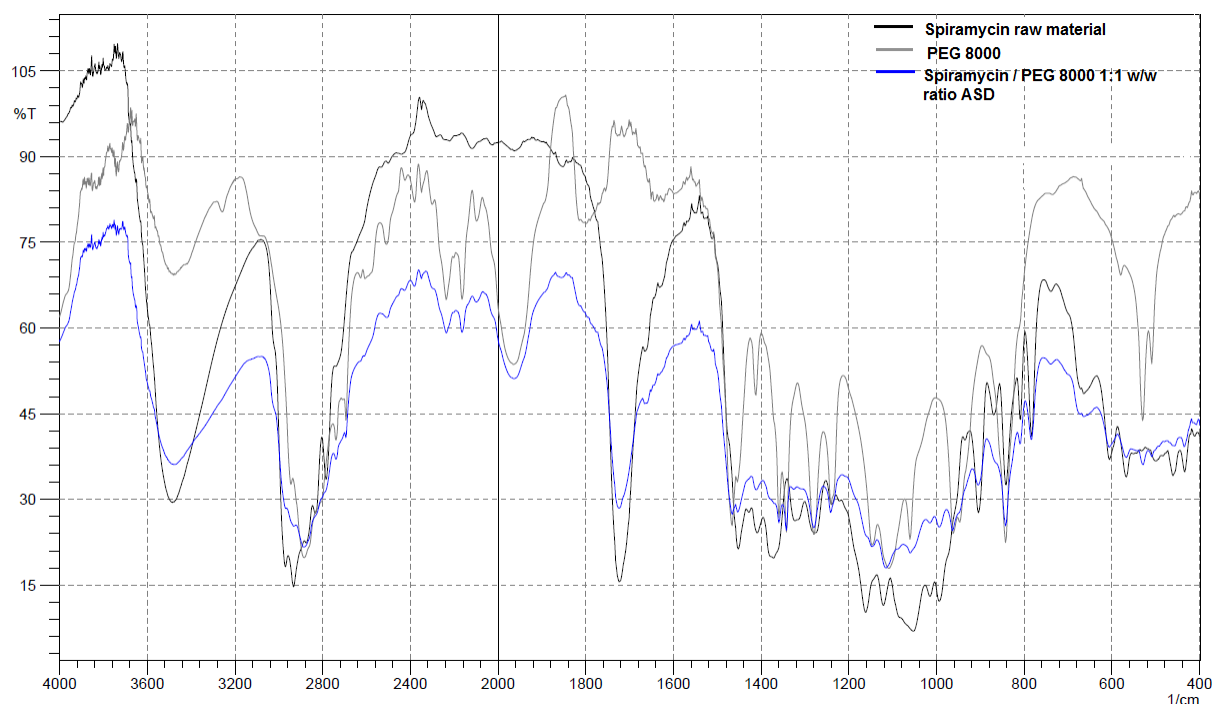
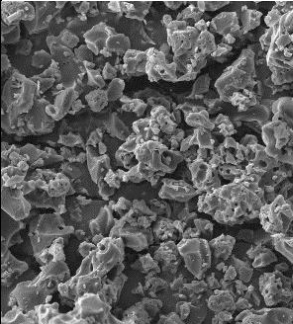
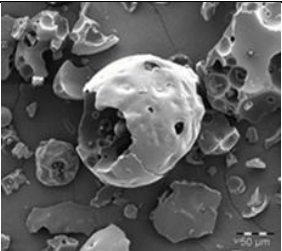
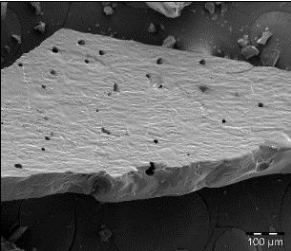
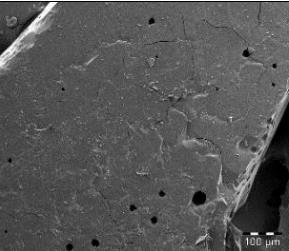
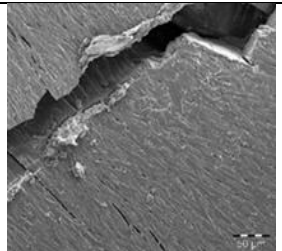
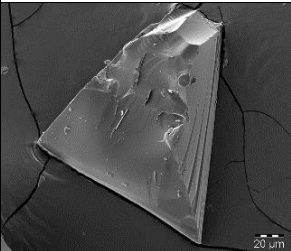
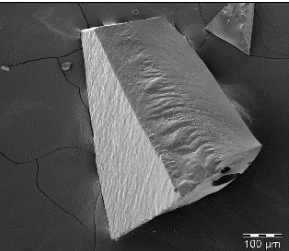


Figure 5.20: Overlay of the IR spectra obtained for spiramycin raw material, PEG 8000 and the 1:1 w/w ratio ASD.

5.4.4 Scanning electron microscopy

Differences in morphology of the prepared ASDs (Table 5.11) can be compared to morphology of spiramycin raw material (Table 5.3). ASDs containing PEG 8000 and Kollidon® VA-64 show some level of porosity that could lead to improved solubility and dissolution rate of the API. From the SEM micrographs one can also clearly see that an amorphous mass formed during the preparation process, since the individual components cannot be distinguished. The appearance of both ASDs looked fused therefore being indicative that it is not a mere mixture of the drug and the polymer. The spiramycin / Kollidon® VA-64 ASD exhibited even a larger surface area in comparison to spiramycin / PEG 8000 ASD (Table 5.11), due to the more porous structure being visible. It was then imperative to determine dissolution rate improvements of spiramycin due to the inclusion of polymers in ASDs.

Table 5.11: SEM images of polymers and the spiramycin ASDs formed

 Spiramycin raw material	 Kollidon® VA-64	 1:1 ASD	 1:1 ASD
	 PEG 8000	 1:1 ASD	 1:1 ASD

5.4.5 Dissolution studies

Dissolution studies of spiramycin ASDs in distilled water were carried out as outlined in Chapter 3. From the preceding studies, spiramycin showed the poorest solubility in water (Figure 5.5). Its amorphous nature made it imperative to investigate any solution-mediated phase transformation in water, although this was considered as unlikely due to the fact that no thermal- or moisture-induced crystallisation of this amorphous drug was identified during the initial physico-chemical characterisation of spiramycin. Only water was therefore investigated as the dissolution medium. Dissolution studies of spiramycin / polymer PMs and ASDs in distilled water were done and evaluated to determine the influence of polymer addition on the amorphous API (Figure 5.20).

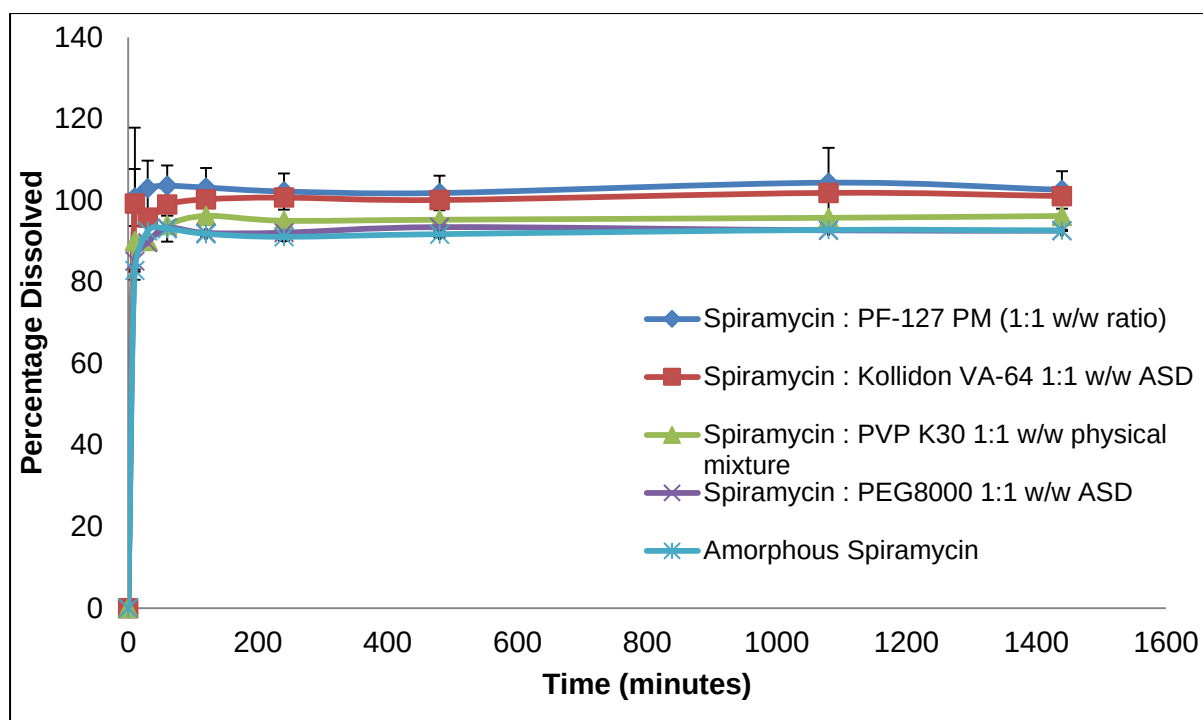


Figure 5.21: Dissolution studies of spiramycin raw material (amorphous), PMs and ASDs in water at $37 \pm 0.5^\circ\text{C}$.

The dissolution results showed to be very interesting. In a sense the dissolution data and the solubility results contradict one another. Since the equilibrium solubility of spiramycin raw material was determined to be much lower in distilled water than in other media, one would've expected a much slower dissolution rate as well. This was however not the case, with more than 90% dissolved after 30 minutes. It can therefore be argued that the poor treatment outcomes obtained with spiramycin can actually not be ascribed to poor dissolution.

As expected, the dissolution of spiramycin was improved to 100% by merely incorporating a hydrophilic polymer either in the form of a PM or an ASD (Figure 5.20). The advantages of polymers (solubilisation) either in PMs or ASDs were clearly noticed implying that addition of a polymer can successfully enhance the dissolution rate of spiramycin. This could be attributed to hydrophilic carriers acting as disaggregants whereby they diminish the existing electrostatic forces between drug particles (Veiga & de Eulate, 1994).

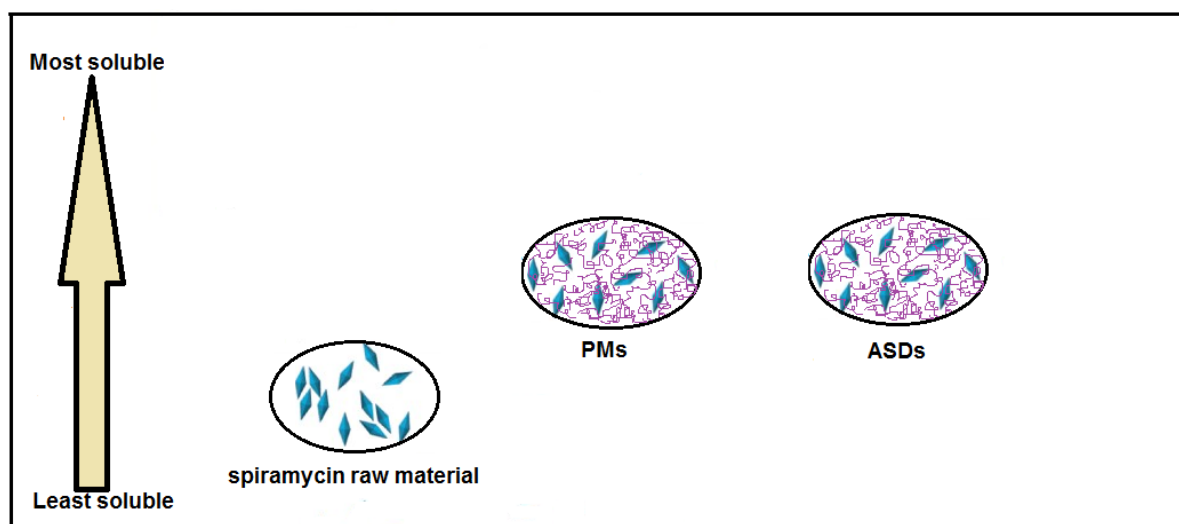


Figure 5.22: Improvement of aqueous solubility for spiramycin (diagram not drawn to scale).

Usually, improvement in dissolution is related to improvement in solubility (Hecq *et al.*, 2005). Dissolution data was therefore applied as an indication of apparent solubility for spiramycin when incorporated into an ASD (Figure 5.21). From the dissolution profile (Figure 5.20), both PMs and ASDs equally managed to improve dissolution rate of spiramycin. It is expected that they should equally show improved solubility of the drug. The noted aqueous dissolution and solubility advantages of spiramycin in the presence of a polymer may be better preserved by ASDs rather than PMs. This is because the preceding IR results revealed some API-polymer interactions in the ASDs whereas such interactions were obviously absent in the PMs.

5.4.6 Discussion

Thorough physico-chemical characterisation of spiramycin raw material enabled successful preparation of spiramycin ASDs by the quench cooling method. IR and SEM characterisation proved to be the only methods that could be used to establish whether ASD preparation was successful. Dissolution rate was equally improved by the addition of a polymer in either ASDs or PMs in the order of PEG 8000 < PVP K30 < Kollidon® VA-64 ≤ Pluronic F-127, owing to the already amorphous nature of the drug.

5.5 Conclusion

In view of its existence as an amorphous form, spiramycin lacks the long range order of a crystalline solid-state. Literature dictates that the existence of a pure amorphous drug alone is highly unlikely due to its high degree of free energy which will eventually change to a lower energy state, in other words a crystalline state over time (Elder *et al.*, 2015). Interestingly, spiramycin in its amorphous state remains stable and does not recrystallise upon heating or

cooling of the melt. This study proved that it can be quite a challenge to determine if a successful ASD of an amorphous API and a polymer was prepared. This being due to the fact that most characterisation techniques rely on the amorphisation of a crystalline solid-state form of an API during the preparation of an ASD. During the dissolution process of an amorphous form, no energy is required to break the bonds such as in the case of a crystalline state leading to a higher dissolution. Normally, a high dissolution rate is associated with good biological performance of a formulation (Newman *et al.*, 2012), however this is not always true with spiramycin which has a high dissolution rate (due to its amorphous nature), but exhibits poor / slight aqueous solubility and an oral bioavailability ranging between 30 to 40% (Brook, 1998). There is paucity of literature regarding this phenomenon. In an attempt to improve aqueous solubility and stability of the amorphous drug, neat ASDs were prepared. In this study, ASDs improved dissolution rate of spiramycin from approximately 90% to 100%. The 10% increase in dissolution implies that ASDs will in turn lead to an enhancement of aqueous solubility of spiramycin to some significant extent.

Further studies on the ASDs of spiramycin is however necessary, especially in terms of the chemical stability of this macrolide antibiotic. The possibility that an ASD could positively affect the chemical stability of spiramycin is worth investigation. In essence this study proved that spiramycin is a poorly soluble antibiotic but that the poor treatment outcomes thereof cannot be attributed to a slow dissolution rate. Future studies will be necessary to clarify the discrepancies between current literature sources and data on the successful treatment of cryptosporidiosis.

References

- Abou-Zeid, A. Z. A., Abd-El-Galil, M. K. & Rabei, M.** 1980. Spiramycin, a macrolide antibiotic. *Zentralblatt für bakteriologie, parasitenkunde, infektionskrankheiten und hygiene. zweite naturwissenschaftliche abteilung: mikrobiologie der landwirtschaft, der technologie und des umweltschutzes*, 135:443-453.
- Atassi, F. & Byrn, S. R.** 2006. General trends in the desolvation behavior of calcium salts. *Pharmaceutical research*, 23:2405-2412.
- Atassi, F., Behme, R. J. & Patel, P. J.** 2013. Mesomorphous versus traces of crystallinity: the itraconazole example. *Thermochimica acta*, 574:133-139.
- Baghel, S., Cathcart, H. & O'Reilly, N. J.** 2016. Polymeric amorphous solid dispersions: A review of amorphization, crystallization, stabilization, solid-state characterization, and aqueous solubilization of biopharmaceutical classification system class II drugs. *Journal of pharmaceutical sciences*, 105:2527-2544
- Brook, I.** 1998. Pharmacodynamics and pharmacokinetics of spiramycin and their clinical significance. *Clinical pharmacokinetics*, 34:303-310.
- Calza, P., Marchisio, S., Medana, C. & Baiocchi, C.** 2010. Fate of antibacterial spiramycin in river waters. *Analytical and bioanalytical chemistry*, 396:1539-1550.
- Chakravarty, P., Bates, S. & Thomas, L.** 2013. Identification of a potential conformationally disordered mesophase in a small molecule: experimental and computational approaches. *Molecular pharmaceuticals*, 10:2809-2822.
- Crew, M., Friesen, D., Miller, W., Shanker, R. & Smithey, D.** 2004. Stabilized pharmaceutical solid compositions of low-solubility drugs and poloxamers and stabilizing polymers. (Patent: U.S. 10/596,885).
- Elder, D. P., Patterson, J. E. & Holm, R.** 2015. The solid-state continuum: a perspective on the interrelationships between different solid-state forms in drug substance and drug product. *Journal of pharmacy and pharmacology*, 67:757-772.
- Feng, W. Z., Qi, P. Y., Zhou, T., Miao, Y. & Duan, X. B.** 1997. Study on the degradation kinetics of spiramycin in acid and alkaline solutions. *Yao xue xue bao= Acta pharmaceutica sinica*, 32: 934-937.
- Fujimoto, Y. & Nakano, K.** 1974. Derivatives of spiramycin and a process for producing thereof. (Patent: US 3,795,669).
- Gallo, G. G., Lancini, G. & Parenti, F.** 2013. Antibiotics: a multidisciplinary approach. 2nd ed. New York: Plenum Press.

- Hecq, J., Deleers, M., Fanara, D., Vranckx, H. & Amighi, K.** 2005. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine. *International journal of pharmaceutics*, 299:167-177.
- Huang, M. Z., Li, J., Guan, L., Li, D. Q., Nie, X. M., Gui, R. & Chen, X.** 2015. Therapeutic effects of acetylspiramycin and garlicin on cryptosporidiosis among drug users. *International journal for parasitology: drugs and drug resistance*, 5:185-190.
- Jiang, H., Wen, L. & Ling, X.** 2000. The clinical evaluation of curative effect of acetylspiramycin on toxoplasmosis infection during pregnancy. *Chinese journal of practical gynecology and obstetric*, 2:107-108.
- Klich, K., Pyta, K., Kubicka, M. M., Ruszkowski, P., Celewicz, L., Gajeka, M. & Przybylski, P.** 2016. Synthesis, antibacterial, and anticancer evaluation of novel spiramycin-like conjugates containing C (5) Triazole Arm. *Journal of medicinal chemistry*, 59:7963-7973.
- Krayz, G. T., Averbuch, M., Zelkind, I. & Gitis, L.** 2016. Compositions comprising lipophilic active compounds and method for their preparation. (Patent: US 9,254,268).
- Leon, N. & Jean, P. H.** 1961. Spiramycins and their production. (Patent: US 3,000,785).
- Maeda, T.** 2003. Toxoplasmosis. *Japanese journal of clinical medicine*. 61:603-607
- Mistry, P., Mohapatra, S., Gopinath, T., Vogt, F. G. & Suryanarayanan, R.** 2015. Role of the strength of drug–polymer interactions on the molecular mobility and crystallization inhibition in ketoconazole solid dispersions. *Molecular pharmaceutics*, 12:3339-3350.
- Murari, R., Chrai, S., Chen, J. C., Kadare, A., Parry, G., & Karetny, M.** 2001. Solid pharmaceutical dosage formulation of hydrophobic drugs. (Patent: U.S. 09/925,348).
- Newman, A., Knipp, G. & Zografi, G.** 2012. Assessing the performance of amorphous solid dispersions. *Journal of pharmaceutical sciences*, 101:1355-1377.
- Sareen, S., Mathew, G. & Joseph, L.** 2012 . Improvement in solubility of poor water-soluble drugs by solid dispersion. *International journal of pharmaceutical investigation*, 2:12.
- Shalaev, E., Wu, K., Shamblin, S., Krzyzaniak, J. F., & Descamps, M.** 2016. Crystalline mesophases: structure, mobility, and pharmaceutical properties. *Advanced drug delivery reviews*, 100:194-211.
- Sharma, A., Sharma, J., Kaur, R. & Saini, V.** 2014. Development and characterization of in situ oral gel of spiramycin. *BioMed research international*, 2014:876182-876182.

Stevenson, C. L., Bennett, D. B. & Lechuga-Ballesteros, D. 2005. Pharmaceutical liquid crystals: the relevance of partially ordered systems. *Journal of pharmaceutical sciences*, 94:1861-1880.

Van Eeden, R. 2012. The physico-chemical properties of spiramycin and clarithromycin. (Potchefstroom: North-West University). (Dissertation- Doctoral).

Veiga, M. D. & de Eulate, J. A. 1994. Dissolution study of spiramycin: influence of agitation intensity and addition of several substances to the dissolution medium. *International journal of pharmaceutics*, 110:223-229.

Vervaeke, I. J., Decuypere, J. A., Dierick, N. A. & Henderickx, H. K. 1979. Quantitative evaluation of the energy metabolism influenced by virginiamycin and spiramycin used as growth promoters in pig nutrition. *Journal of animal science*, 49:846-856.

Vo, C. L. N., Park, C. & Lee, B. J. 2013. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. *European journal of pharmaceutics and biopharmaceutics*, 85:799-813.

Wang, D. & Zhang, Y. Y. 2014. Intestinal pathological changes of Kunming mice infected by *Cryptosporidium* and the therapeutic efficacy of spiramycin on infected mice. *Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi= Chinese journal of parasitology & parasitic diseases*, 32:225-228.

Zhang, G., Zhang, J., Wang, S. & Shen, D. 2003. Miscibility and phase structure of binary blends of polylactide and poly (methyl methacrylate). *Journal of polymer science part b: polymer physics*, 41: 23-30.

Zhaojie, M., Ming, Z., Shengnan, W., Xiaojia, B., Hatch, G. M., Jingkai, G. & Li, C. 2014. Amorphous solid dispersion of berberine with absorption enhancer demonstrates a remarkable hypoglycemic effect via improving its bioavailability. *International journal of pharmaceutics*, 467:50-59.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Physico-chemical properties of a drug either in solution or solid state play a significant role in the formulation of drugs (Wang *et al.*, 2016). Stability and solubility are the most important physico-chemical properties that must be considered for a successful drug formulation. The solid state form of the drug is normally desired because it is more stable and easier to process and administer when compared to the liquid formulations. However, the solid state has to dissolve first for it to become therapeutically active; therefore it must also be soluble, chemically and physically stable (Wang *et al.*, 2016). The greatest challenge amongst pharmaceutical scientists has been to formulate a drug that is stable and does not degrade during shelf life or during dissolution after the drug has been administered (Blessy *et al.*, 2014). Solubility and stability among other factors affects bioavailability and ultimately treatment outcomes. Several factors that affect solubility and stability are reported in literature. For example polymorphism, crystal habits, hygroscopicity, temperature etc. According to Fridgeirsdottir *et al.* (2016) the number of poorly soluble drugs on the market has been exponentially increasing. It is reported that approximately 40-70% of the new drug entities suffer from poor aqueous solubility (Lu & Park, 2013; Yasmin *et al.*, 2016). Solubility and stability enhancement strategies therefore remain indispensable for optimised drug formulations. Recently the use of ASDs has been employed to address this problem and the number of solid dispersion formulations on the market is growing.

Physico-chemical properties of the crystalline and/or amorphous forms of the macrolide antibiotics clarithromycin and spiramycin were determined by various techniques: FTIR, XRPD, DSC, SEM, HSM, HPLC, vapour sorption analysis, solubility studies, and dissolution studies as discussed in Chapters 4 and 5. The two are the cornerstone drugs in the treatment of MAC and cryptosporidiosis respectively in PLHIV. However several studies have reported poor treatment outcomes of the selected OIs. A comprehensive understanding of the solid state properties of the two macrolide antibiotics was gained. Thereafter, ASDs of the two antibiotics were prepared in an attempt to improve the aqueous solubility and physical stability thereof.

From all the characterisation results, clarithromycin raw material was confirmed to be the crystalline form II polymorph. It was successfully converted to an amorphous form (CLAM) by quench cooling of melt. Upon non-isothermal heating, it recrystallised back to the thermodynamically stable Form II. Figure 6.1 summarises the proposed CLAM-temperature

dependent conversion pathway as obtained by different characterisation methods used in this study. On further investigation of the physical stability of CLAM, it was found that it is physically stable at room temperature, high moisture content and during dissolution. It yielded an overall improved dissolution of ≈ 4 times higher than the crystalline counterpart. Despite all these noted physical stability advantages at room temperature and at high moisture content, CLAM still showed potential to crystallise over time. ASDs have been found to improve physical stability and dissolution rate of an amorphous API. ASDs were therefore prepared to preserve and enhance the noted physical stability and dissolution properties of CLAM. Dissolution of the resulting ASDs was the most improved when compared to that of Form II, PMs and CLAM. A dissolution improvement of ≈ 1.6 times and ≈ 2.6 times higher than Form II was noted for clarithromycin in PMs containing PF-127 and PVP 30 respectively. ASDs containing Kollidon® VA-64 and PEG 8000 gave the most significant improvement in dissolution of ≈ 5.2 times and ≈ 6.5 times respectively higher than crystalline Form II. There was a negligible difference in the dissolution rate between 1:3 w/w and 1:4 w/w ASDs containing PEG 8000, hence it was concluded that a further increase in polymer fraction did not have any additional impact on dissolution rate.

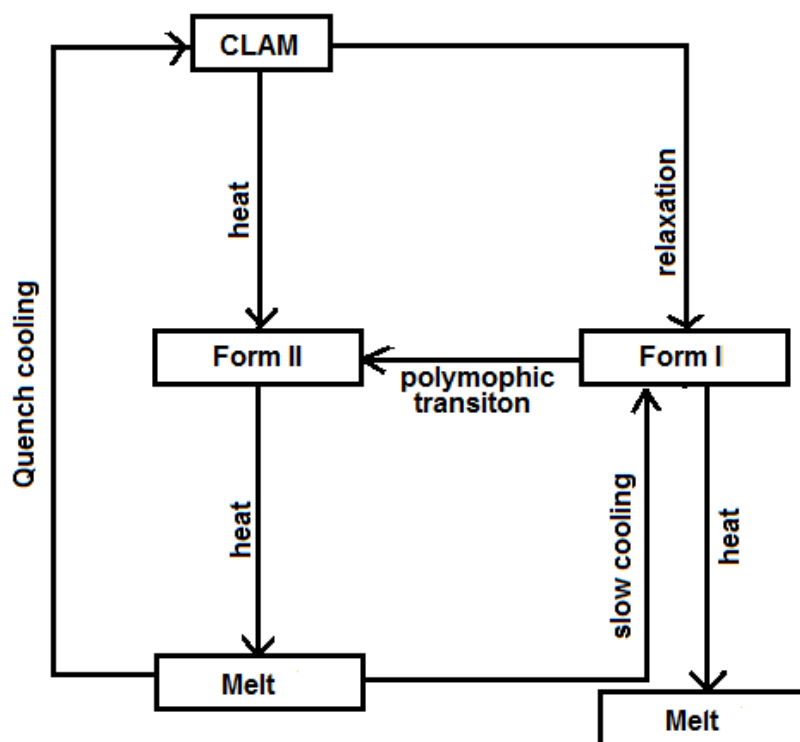


Figure 6.1: Temperature dependent conversions among clarithromycin Form I clarithromycin Form II and amorphous clarithromycin (CLAM).

Overall, the dissolution rate of a BSC II classified drug (clarithromycin) was improved by approximately 6.5 times by ASDs. This will have a significantly positive impact on its aqueous

solubility. Results obtained in this study are consistent with a number of international literature studies on the ability of ASDs in improving dissolution / solubility of poorly soluble drugs. It can therefore be concluded that ASDs successfully enabled better control over the solid-state chemistry of clarithromycin by maintaining the API in a stable amorphous solid-state form and enhancing dissolution / solubility which will ultimately lead to improved treatment outcomes.

Characterisation techniques used on clarithromycin were also used on spiramycin. Results showed that spiramycin exists in an amorphous state which does not recrystallise to a more stable crystalline form. Regarding stability, literature dictates it is more preferable to use an ASD than an amorphous API. However, for spiramycin, it was a challenge to determine whether ASDs were successfully prepared since the drug already exists in an amorphous API. Results show that dissolution of spiramycin improved from approximately 90% up to 100% by the mere incorporation of a polymer with the amorphous API to form either PMs or ASDs. Figure 6.2 suggest the proposed dissolution behaviour of spiramycin in a polymer matrix (PM and/or ASD). The drug and the polymer is gradually released whilst the undissolved drug particles remain amorphous.

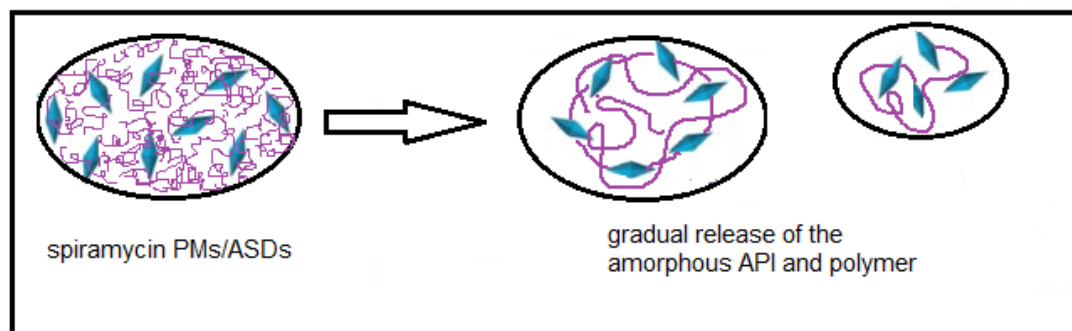


Figure 6.2: Dissolution behaviour of polymeric spiramycin systems.

Due to the fact that amorphous spiramycin is already physically stable, the advantage of adding polymers (solubilisation) was easily noticed in this study. Therefore, adding a polymer might have only enhanced dissolution and aqueous solubility of spiramycin. The study results are consistent with what has been reported in other studies regarding the ability of amorphous drug / polymer miscibility in improving rate of dissolution. Even though ASDs improved dissolution rate of spiramycin to 100%, the drug already had a high dissolution rate of 90%. It is well known that dissolution is the rate limiting step in absorption in many cases of poorly soluble drugs (Florence & Attwood, 2015). However, this is not true for spiramycin which shows a high dissolution rate but with a poor oral absorption rate. It can therefore be concluded that the poor treatment outcomes of spiramycin cannot be attributed to a slow dissolution rate. Future studies on spiramycin will be necessary to clarify the discrepancies between current literature sources and data on the successful treatment of cryptosporidiosis.

6.2 Recommendations

There is no literature which states the BCS classification of spiramycin hence poor intestinal permeability may be a possible defect. Further investigations on spiramycin permeability are therefore worth doing. On the other hand, literature dictates that an amorphous API is more chemically unstable than the crystalline counterpart (Nagapudi & Jona, 2008). Hence, future studies should investigate the chemical stability of this macrolide antibiotic. The possibility of an ASD to enhance chemical stability of spiramycin is also recommended for further investigation. For clarithromycin, CLAM is stable at room temperature and high humidity. However, further physical and chemical stability studies of clarithromycin ASDs are strongly recommended.

References

- Blessy, M.R.D.P., Patel, R.D., Prajapati, P.N. & Agrawal, Y.K.** 2014. Development of forced degradation and stability indicating studies of drugs-A review. *Journal of pharmaceutical analysis*, 4:159-165.
- Florence, A.T. & Attwood, D.** 2015. Physicochemical principles of pharmacy: in manufacture, formulation and clinical use. 6th ed. London: Pharmaceutical Press.
- Fridgeirsdottir, G.A., Harris, R., Fischer, P.M. & Roberts, C.J.** 2016. Support tools in formulation development for poorly soluble drugs. *Journal of pharmaceutical sciences*, 105:2260-2269.
- Lu, Y. & Park, K.** 2013. Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. *International journal of pharmaceutics*, 453:198-214.
- Nagapudi, K. & Jona, J.** 2008. Amorphous active pharmaceutical ingredients in preclinical studies: preparation, characterization, and formulation. *Current bioactive compounds*, 4:213-224.
- Wang, B., Hu, L. & Siahaan, T.J,** eds. 2016. Drug delivery: principles and applications. 2nd ed. New Jersey: John Wiley & Sons.
- Yasmin, R., Rao, S., Bremmell, K. & Prestidge, C.** 2016. Synergistic role of solid lipid and porous silica in improving the oral delivery of weakly basic poorly water soluble drugs. *European journal of pharmaceutical sciences*, 96: 508-514.

ANNEXURE A

Paper submitted and accepted after peer-reviewing for publication in:

International Journal of Pharmacy and Pharmaceutical Sciences.

Current impact factor: 0.49.

INSTRUCTIONS TO AUTHORS

International Journal of Pharmacy and Pharmaceutical Sciences (Int J Pharm Pharm Sci) is peer reviewed, Monthly (Onward April 2014) open access Journal. IJPPS publishes original research work that contributes significantly to further the scientific knowledge in pharmacy and pharmaceutical sciences (Pharmaceutical Technology, Pharmacognosy, Natural Product Research, Pharmaceutics, Novel Drug Delivery, Biopharmaceutics, Pharmacokinetics, Pharmaceutical/Medicinal Chemistry, Computational Chemistry and Molecular Drug Design, Pharmacology, Pharmaceutical Analysis, Pharmacy Practice, Clinical and Hospital Pharmacy, Cell Biology, Genomics and Proteomics, Pharmacogenomics, Bioinformatics, Pharmacoeconomics). Research outcomes from medical sciences/case study and biotechnology of pharmaceutical interest are also considered. IJPPS publishes original research work either as an Original Article or as a Short Communication. Review articles on current topic under mentioned scopes are also considered for publication. In addition, hypothesis are also invited now for the publication onwards March 2016.

Before submission of manuscript, please see "**Minimum standards for manuscripts**" for **consideration of Publication in IJPPS** ([Click Here](#))

*Type-written manuscripts prepared using MS Word should be submitted by online submission system (<http://innovareacademics.in/journals/index.php/ijpps/about/submissions#onlineSubmissions>) or email to editor@ijppsjournal.com

***(Article should be submitted through any one of the mode- email or online submission system).**

Online submission system must be preferred

Manuscripts will be subjected to peer review process to determine their suitability for publication provided they fulfill the requirements of the journal as laid out in the instructions to authors. After the review, manuscripts will be returned for revision along with reviewer's and/or editor's comments. Prepare the manuscript in Times New Roman font using a font size of 12. There shall not be any decorative borders anywhere in the text including the title page.

All the tables and figures should be in the text at suitable place.

Submission of a manuscript to International Journal of Pharmacy and Pharmaceutical Sciences for publication implies that the same work has not been either published or under consideration for publication in another Journal. Authors, in their cover note to the Editor, have to clearly mention whether the manuscript shall be considered as a Research Paper,

Short Communication or Review Article and also confirm that the manuscript has not been submitted to any other Journal for publication. Authors publishing results from in vivo experiments involving animals or humans should state whether due permission for conduction of these experiments was obtained, from the relevant ethics committees, in the Materials and Methods section. In addition, authors wishing to publish research work involving human studies should also send a notary verified letter of approval from the Ethics Committee or the Institutional Review Board.

Manuscript should be divided into Title page, Abstract, Introduction, Materials and Methods, Results, Discussion or Results and Discussion, conclusion, Acknowledgement, References.

Title page- In separate lines, title page should contain the title of the manuscript, the name(s) and affiliation(s) of the author(s), and the mailing address, telephone and fax numbers, and e-mail address of the corresponding author. The title must be specific, concise and informative.

Title - EVALUATION OF -----(12 FONT SIZE, TIMES NEW ROMAN, NORMAL, BOLD) - IN UPPERCASE ONLY

Authors - Aneesh K, Laxmi Kumar --- (12 FONT SIZE, TIMES NEW ROMAN, NORMAL, BOLD) - IN UPPERCASE ONLY

Keywords and abstract- Please include 3-10 keywords for indexing purposes; Full-length manuscript submission should contain an abstract of up to 250 words in a structured form, consisting of: Objective, Methods, Results, and Conclusion.

Headings – **INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION, CONCLUSION**

Subheadings- **Preparation of extracts**

Introduction- It should summarize the rationale, provides a concise research background (not an exhaustive review) and states in single sentence the objective of the study. Please do not include any results or the conclusion of the study.

Materials and methods- All the ethical permission associated in the research work must be specified. It should provide technical information about the study. Published methodological details are not needed to describe that have been published previously. Specifications (including the manufacturer, city, and the country) should be given for the main drugs, chemicals, and instruments. Indicate the statistical methods used and identify statistical significance using superscripts (* and **) following the data (*P<0.05, **P<0.01).

Results- It should reveal the findings of works.

Discussion- It should be with the interpretation of the results and their comparison with those of other studies. No need to repeat the results, review literature, textbook knowledge or cite references that do not have a close relationship with the present result.

Conclusion – conclude the study linking back to the aim of the study.

Abbreviations- At the first appearance in the abstract and the text, abbreviations should be preceded by words for which they stand.

Tables- Tables must be concise and cited consecutively using Arabic numerals in the text (table 1, table 2...etc.). The title of the table should clearly indicate the nature of the contents and sufficient detail should be included in the footnote to facilitate interpretation without reference to the text. Use horizontal rules only.

Table Format – It should be designed using table tools of MS Word and exactly same as below

Table 1: ---(12 font size, times new roman, normal, bold)

Groups	Change in body weight (unit)
Control	
Negative control	
Protective	
Curative	

Figures -Figures (photographs, drawings, diagrams and charts) should be clear, easily legible and cited consecutively using Arabic numerals in the text(fig. 1, fig. 2...etc.). Please supply figures 1.5 to 2 times the size at which they will be finally reproduced. For line work, submit black-ink drawings of professional quality. Micrographs or other glossy photographs must be of the highest quality. Use standard symbols: ○, ●, ×, □, ■, △, ▲. Freehand or typewritten lettering is unacceptable. If a figure comprises more than one glossy photograph, these should be marked A, B, C...etc. Figure legends should be marked clearly with their correspond letters. Legends should contain sufficient detail to permit figure interpretation without reference to the text. Scale markers should be indicated in the photographs. Color plates are also welcome. The choice of cover art illustration will be made by the Editor.

Figure format –

Fig. 1: ---(12 Font size, Times New Roman, Normal, Bold)

Manuscripts that fail to conform to the requirements of the Journal, as specified under 'Instructions to Authors', will be rejected outright.

Tables and figures should be cited in the text in numerical order. Table 2 should not be first cited before Table 1.

Units and Symbols - The use of the International System of Units (SI) is recommended.

Physical quantity	Base unit	SI Symbol
Length	meter	m
Mass	gram	g
	kilogram	kg
	microgram	µg
Time	second	s
	minute	min
	hour	h
	day	d
	week	w
	month	mo
	year	y
Amount of substance	mole	mol
Area	square meter	m ²
Volume	cubic meter	m ³
	liter	l

	milliliter	ml
	microliter	μl
	temperature	°C

Specification	Example	Correct style
Use lowercase for symbols or abbreviations,	Kilogram	kg
Symbols are not followed by a period, exception end of sentence	meter	m
Do not pluralize symbols	kilograms	kg
When numbers are printed symbols are preferred	100 meters	100 m
Space between number and symbol	2mol 10mg 60°C	2 mol 10 mg 60 °C
Place a zero before a decimal		0.01
Decimal numbers are preferable to fractions		0.75
Space used to separate long number exception four-digit numbers		1 500 000 1000

References

References should be numbered consecutively in the order in which they are first mentioned in the text (not in alphabetic order). Identify references in text, tables, and legends by Arabic numerals in parenthesis/bracket like- [1]. References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. Use the style of the examples below. Use complete name of the journal for non-indexed journals. Avoid using abstracts as references. Information from manuscripts submitted but not accepted should be cited in the text as “unpublished

observations” with written permission from the source. Avoid citing a “personal communication” unless it provides essential information not available from a public source, in which case the name of the person and date of communication should be cited in parentheses in the text. For scientific articles, contributors should obtain written permission and confirmation of accuracy from the source of a personal communication.

The commonly cited types of references are shown here, for other types of references such as electronic media, newspaper items, etc. please refer to ICMJE Guidelines (<http://www.icmje.org>).

Articles in Journals

1. Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci 2006;68:1-6. **List the first six contributors followed by et al.**
2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994;102 Suppl 1:275-82.
3. Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women’s psychological reactions to breast cancer. Semin Oncol 1996;23(1, Suppl 2):89-97.

Books and Other Monographs

1. Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers;1996.
2. Editor(s), compiler(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone;1996.
3. Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

Journal abbreviations source

Journal names should be abbreviated according to CAS Source Index (CASSI) Search Tool abbreviations: <http://cassi.cas.org/search.jsp>

Short Communication

The journal publishes exciting findings, preliminary data or studies that did not yield enough information to make a full paper as short communications. These have the same format requirements as full papers but are only up to 5 pages in length. Short Communications should not have subtitles such as Introduction, Materials and Methods, Results and

Discussion - all these have to be merged into the running text. Short Communications preferably should have only 3-4 illustrations.

Review Articles

Should be about 18 pages long, contain up-to-date information, comprehensively cover relevant literature and preferably be written by scientists who have in-depth knowledge on the topic. All format requirements are same as those applicable to full papers. Review articles need not be divided into sections such as Materials and Methods and Results and Discussion, but should definitely have an Abstract and Introduction, if necessary.

Conflict of Interest statement

It must be declared by authors.

Fee

IJPPS is an open access journal, free to access, read and print. There is no pay-per-view, pay-per-print fee for the published articles. There are no editorial/peer review charges being solicited from the authors. However, the journal charges minimum amount towards author registration (on accepted article, after peer review) for publishing and maintaining the content online, outsourcing facilities, tools and resources towards publication of the article. The amount (effective from 1st October 2016) will be as follows:

Manuscript	Indian authors		Foreign authors	
	1 or 2 authors	More than 2 authors	1 or 2 authors	More than 2 authors
Original Research Article, Short Communication, Case Study, Letter to Editor etc.	Rs. 1000 per author	Rs. 750 per author	US\$ 100 or 75 per author	US\$ 50 per author
Review Article	Rs. 1000 per author		US\$ 100 or 75 per author	US\$ 50 per author

A NOVEL RP-HPLC METHOD FOR THE DETECTION AND QUANTIFICATION OF CLARITHROMYCIN OR SPIRAMYCIN IN BULK DRUG SAMPLES AND DOSAGE FORMS

RUNAKO MASLINE KATSIDZIRA^a, ANITA WESSELS^b, MARIQUE AUCAMP^{a*}

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

^bDepartment of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Health Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa

* Corresponding author: Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa. Tel.: +2718 299 4472; Fax: +2787 231 5432. E-mail address: Marique.Aucamp@nwu.ac.za

ABSTRACT

Objective: This study was aimed at developing an HPLC method that would be suitable and sufficiently robust to analyze clarithromycin or spiramycin from bulk materials, amorphous solid dispersions as well as when included into solid dosage forms.

Methods: A C₈ column (250 x 4.6 mm, 5 µm) was used as stationary phase, the mobile phase consisted of 0.1 M di-potassium hydrogen orthophosphate buffer (pH 6.0) and acetonitrile in a 50:50 (% v/v) ratio. The flow rate was set to 0.5 ml/min. UV detection of 210 nm was used for clarithromycin and 232 nm for spiramycin. Ambient column and sample tray temperatures were used.

Results: The method proved to be suitable for the detection of both macrolide antibiotics in bulk samples, as part of amorphous solid dispersions as well as in dosage forms. The isocratic elution was rapid. The method was validated in terms of system suitability, limits of detection (LOD), limit of quantification (LOQ), accuracy, precision, linearity, and specificity. This method showed linearity across the concentration range of 4.0 – 5000.0 µg/ml for both antibiotics.

Conclusion: The developed method showed to be a simple and sufficiently sensitive method for the detection and quantification of either clarithromycin or spiramycin from samples that might contain even very small quantities of the antibiotics.

Keywords: clarithromycin, spiramycin, RP-HPLC

Recent studies have shown that opportunistic infections of patients suffering from HIV are the cause of 90% of patient morbidity and mortality [1, 2]. The most prevalent and life threatening opportunistic infections among people living with HIV in different populations include: *Mycobacterium tuberculosis*, *Pneumocystis jirovecii* pneumonia, *Mycobacterium avium* complex (MAC) and *Cryptosporidiosis* [1, 3]. Azithromycin and clarithromycin are currently the two macrolide antibiotics of choice prescribed for the prophylaxis and treatment of MAC opportunistic infections in HIV patients [4]. *Cryptosporidiosis* is a life-threatening infection that is very difficult to treat since it causes cholera-like diarrhoea with profuse daily fluid loss of about 17 litres [5]. Spiramycin is one of the macrolide antibiotics recommended for treating *cryptosporidiosis* among immuno-suppressed individuals [6, 7]. Considering the global dilemma of antimicrobial resistance there is currently a renewed focus on antibiotics that were not usually the drug of choice for the treatment of certain infections. Spiramycin is one such

drug. During our research on clarithromycin and spiramycin we identified the need for a suitable and single HPLC method that could be used for the identification and analysis of both drugs. Especially, considering that the British Pharmacopoeia (BP) only reports on an HPLC method that is suitable for the analysis of spiramycin from veterinary formulations [8]. No other pharmacopoeia contains an analytical method for spiramycin. Furthermore, the methods included in both the BP and the United States Pharmacopoeia [9] for clarithromycin uses high buffer concentrations and those methods lead to long HPLC run times.

Clarithromycin is classified as a semi-synthetic antibiotic which forms part of the macrolide group. This antibiotic is derived from erythromycin but differs in the fact that the O-methyl group has been substituted for a hydroxyl group at position six of the lactone ring (fig. 1a) [10]. Spiramycin consists of a 16-membered lactone (platenolide), two amino sugars (D-mycaminose and D-forosamine) and one neutral sugar (L-mycarose) (fig.1b). Spiramycin has three major (I, II, III), three minor components (IV, V, VI) and two other additional spiramycins 18-deoxy-18-dihydrospiramycin (DSPM) and 17-methylenespiramycin. Though spiramycin I is shown to be the main component, the quantity of each component varies according to the manufacturer [11 - 13].

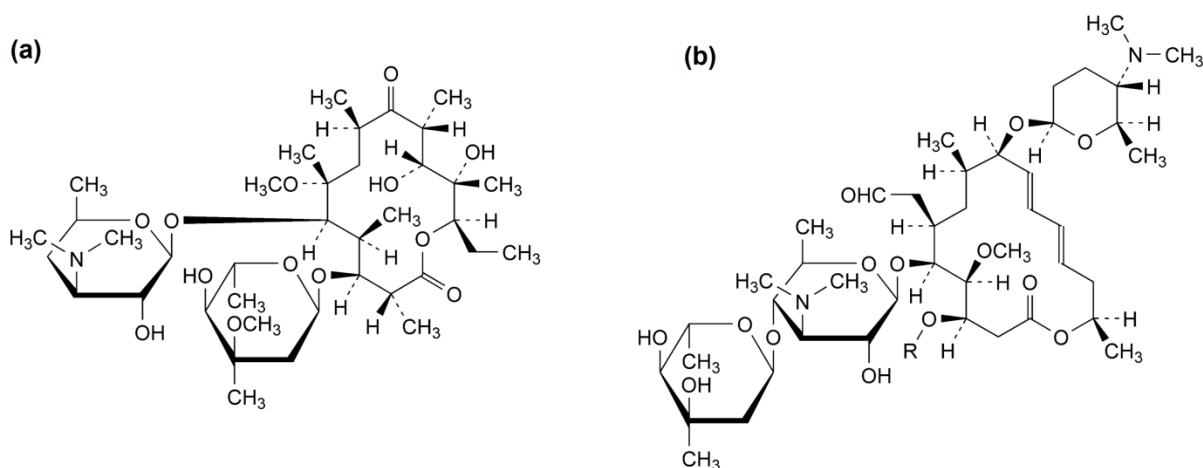


Fig. 1: Molecular structure of (a) clarithromycin and (b) spiramycin [8].

The objective of this study was to develop an easy, cost-effective but sufficiently robust RP-HPLC method for the detection of clarithromycin or spiramycin. The rationale for this was that the current methods, that are available in literature for both drugs, use high concentration buffers as part of the mobile phases and in the instance of clarithromycin gradient elution is being applied. Therefore, this is a simple method with the ability to accurately detect and quantify both drugs, thereby making it very useful for laboratories that need to analyse both macrolide antibiotics.

Clarithromycin working standard (97.7% purity) and spiramycin (purity of 94.9%, only spiramycin I) was purchased from Sigma Aldrich (Johannesburg, South Africa) and DB Fine Chemicals (Johannesburg, South Africa). HPLC grade acetonitrile, potassium di-hydrogen orthophosphate and hydrochloric acid (32%) were purchased from ACE Chemicals (Johannesburg, South Africa). The pharmaceutical dosage form used during specificity testing was Clacee[®] 250 mg tablets (Aspen Pharmacare, South Africa). In the case of spiramycin no commercial product was available for purchase and therefore a mixture of typical pharmaceutical excipients was made. This mixture constituted of microcrystalline cellulose, polyvinylpyrrolidone (PVP) K 30, magnesium stearate, talc and croscarmellose sodium. All the excipients used were a kind donation from the Department of Pharmaceutics of the School of Pharmacy, North-West University, Potchefstroom, South Africa.

A Shimadzu (Kyoto, Japan) UFLC (LC-20AD) chromatographic system, equipped with a SIL-20AC auto sampler fitted with a sample cooler, a UV/VIS PDA detector (SPD-M20) and an LC-20AD solvent delivery module was used during the method development and validation. A Phenomenex® Luna C8 5µm, 250 x 4.6 mm column was used as stationary phase. The mobile phase consisted of 0.1 M phosphate buffer (pH adjusted to 6.0 with 0.1 M HCl) and acetonitrile in a ratio of 50/50 %v/v. Isocratic elution was used with a flow rate of 0.5 ml/min. An injection volume of 10 µl was used for the analysis and validation of clarithromycin while an injection volume of 2 µl was found to be suitable for spiramycin. For the detection of clarithromycin a wavelength of 210 nm was used while spiramycin was detected at 232 nm [9]. For both antibiotics, stock solutions were prepared by separately and accurately weighing 50 mg of drug and diluting it to 10 mL with mobile phase. This resulted in a stock solution of 5000.0 µg/ml, for each drug. Aliquots of these stock solutions were then diluted with mobile phase to obtain standard solutions with concentrations ranging from 4.0 – 5000.0 µg/ml. Each standard solution was filtered using a 0.45 µm PVDF filter into HPLC vials.

During the preparation of product sample for the testing of the specificity of clarithromycin, 10 tablets were weighed and grinded using a mortar and pestle. In the case of spiramycin a sample was prepared as discussed previously described. For each drug sufficient sample powder was weighed, equivalent to result in a final drug concentration of 1000 µg/ml. Each sample was diluted with mobile phase. The flasks were shaken for 10 minutes using an ultrasonic shaker. Thereafter each sample was filtered through a 0.45 µm PVDF filter into HPLC vials.

The method was validated as specified by the ICH guideline on the validation of analytical procedures (ICH) [14]. Linearity, range, precision, accuracy, recovery, specificity, limit of detection (LOD) and limit of quantification (LOQ) were the validation parameters. For each drug, eight standard solutions with concentrations over the stated working range were analysed in duplicate. This was done during two different times by two different analysts. From the obtained data, regression plots of peak area *versus* drug concentration provided linear regression data as stipulated in Table 1.

Table 1: Validation parameters determined during method validation for the detection and quantification of clarithromycin and spiramycin

Validation parameter*	Clarithromycin	Spiramycin
Linearity range (µg/ml)	4.0 – 5000.0	4.0 - 5000
Correlation coefficient (r^2)	0.9999	0.9999
Regression equation	$y = 505.55x - 9083.33$	$y = 14801x - 276251$
Recovery (%) ($\pm$ % RSD)	$99.95 \pm 1.25\%$	$99.65 \pm 1.02\%$
LOD (µg/ml) ($\pm$ % RSD)	$4.0 (\pm 6.6 \%)$	$1.5 (\pm 12.9\%)$
LOQ (µg/ml) ($\pm$ % RSD)	$16.0 (\pm 0.36 \%)$	$8.0 ((\pm 0.13\%))$

*The validation parameters were determined from the analysis of eight standard solutions (n = 8) over a concentration range of 4.0 – 5000.0 µg/ml. %RSD: Percentage relative standard deviation. LOD: Limit of detection, LOQ: Limit of quantification.

The recovery obtained with this method proved to be acceptable for both drugs, due to the fact that the recovery was between the limits of 98.0 – 102.0%. The determined LOD and LOQ limits for this method also proved to be acceptable with very small concentration levels of both drugs being detectable and quantifiable. The accuracy of the proposed method was tested at clarithromycin concentration levels of 250.0 µg/ml and 1000.0 µg/ml. The recovery for clarithromycin was determined to be between 98.32 – 101.64%, with a mean recovery of 99.95% and a percentage relative standard deviation (%RSD) of 1.25%. For spiramycin concentration levels of 250.0 µg/ml and 1030.0 µg/ml was used for accuracy testing. The results showed recovery between 98.21 – 101.10%, with a mean recovery of 99.65% ($\pm$ 1.02%). From these results it can be concluded that the method delivers accurate results. The precision of the method was investigated through determination of repeatability and intermediate precision. The repeatability for both drugs was determined at concentration levels, 200.0, 500.0 and 1025.0 µg/ml, the samples were analysed in triplicate (Table 2).

Table 2: Summary of repeatability data obtained for clarithromycin and spiramycin

Theoretical concentration (µg/ml)	Mean clarithromycin concentration (µg/ml) $\pm$ %RSD*	Mean spiramycin concentration (µg/ml) $\pm$ %RSD*
200.0	210.44 ($\pm$ 0.05)	213.43 ($\pm$ 0.56)
500.0	511.16 ($\pm$ 0.62)	513.88 ($\pm$ 0.18)
1025.0	1030.45 ($\pm$ 0.84)	1020.73 ($\pm$ 0.28)

*Each reported value is the mean $\pm$ %RSD of n observations. For repeatability n is 3. %RSD denotes the percentage standard deviation

The intermediate precision was measured during three different sampling times. Samples were prepared by different analysts as well and analysed in duplicate. The resulting data is presented in table 3. The % RSD for these determinations were not more than 5.0 % and were therefore deemed as sufficient proof of the precision of this method.

Table 3: Summary of intermediate precision data obtained for both drugs. Samples were analysed on different days, by different analysts

Theoretical concentration (µg/ml)	Mean clarithromycin concentration (µg/ml) $\pm$ %RSD	Mean spiramycin concentration (µg/ml) $\pm$ %RSD
1000.0	(1) 999.29 ($\pm$ 0.11%)	(1) 1017.43 ($\pm$ 1.45%)
	(2) 995.53 ($\pm$ 1.91%)	(2) 1018.05 ($\pm$ 0.59%)
	(3) 1018.89 ($\pm$ 0.58%)	(3) 1019.82 ($\pm$ 0.12%)

*Each value is representative of the mean $\pm$ %RSD of n observations. For the determination of intermediate precision n is 3. %RSD denotes the percentage standard deviation

The specificity of the method was tested by analysing the prepared sample solutions which contained typical formulation excipients. Fig.2 depicts the chromatograms obtained for clarithromycin standard and sample solutions while, fig. 3 depicts the chromatograms obtained

for spiramycin standard solution and the sample solution prepared in combination with the mentioned excipients. No peak interference of any of the excipients was observed and therefore the method was deemed suitable for the analysis of formulated dosage forms containing either of the macrolides. The presented results show that the developed method is accurate, precise and specific. A simple HPLC method was developed and validated for the detection and quantification of either clarithromycin or spiramycin. The method proved to be accurate and reproducible and to be suitable for the analysis of either clarithromycin or spiramycin either as bulk drug or when in combination with typical pharmaceutical excipients.

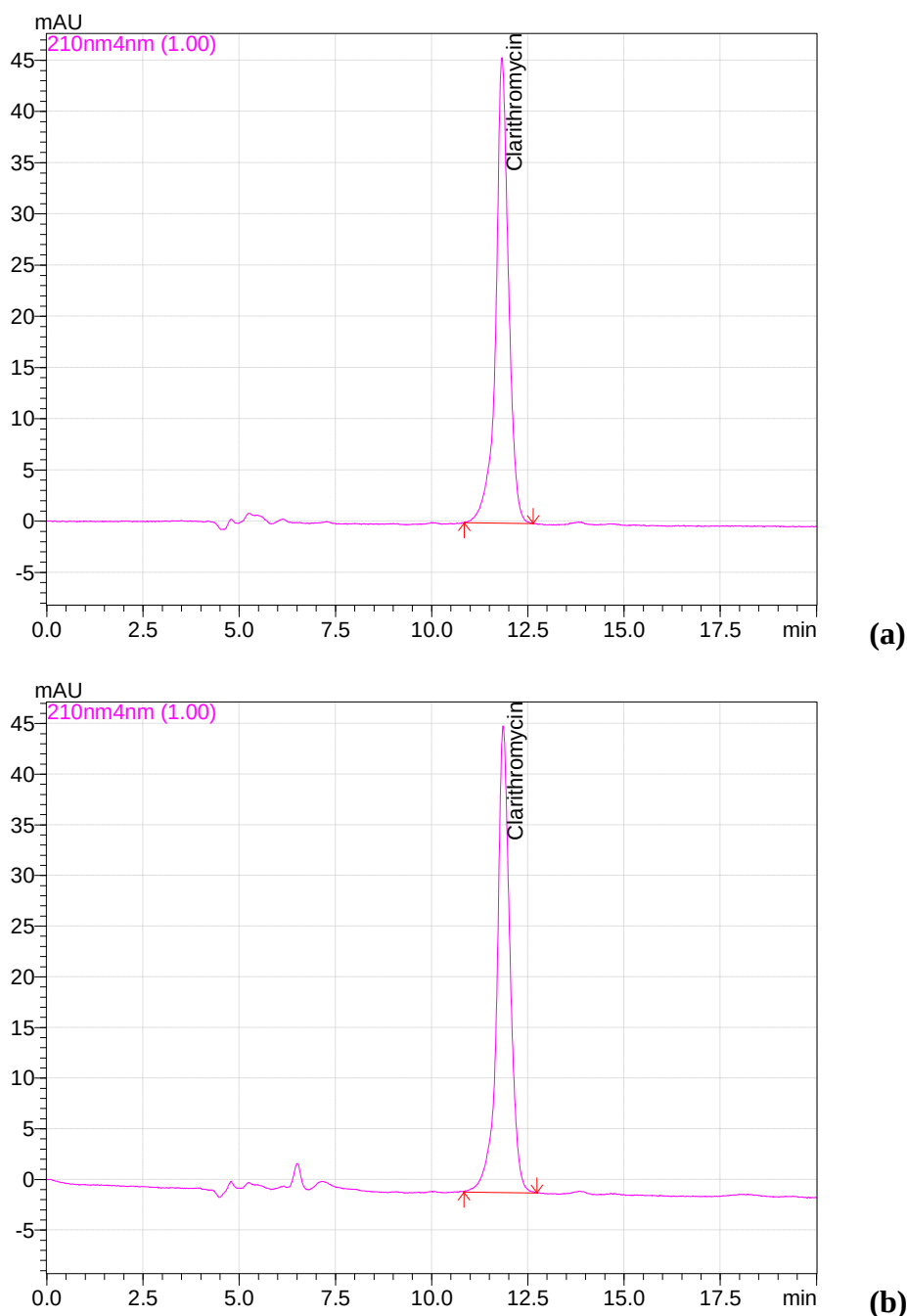


Fig. 2: Chromatograms obtained for clarithromycin during specificity testing with chromatogram (a) showing the peak obtained for clarithromycin from a standard

solution and (b) showing the peak obtained for clarithromycin detected from a commercial sample.

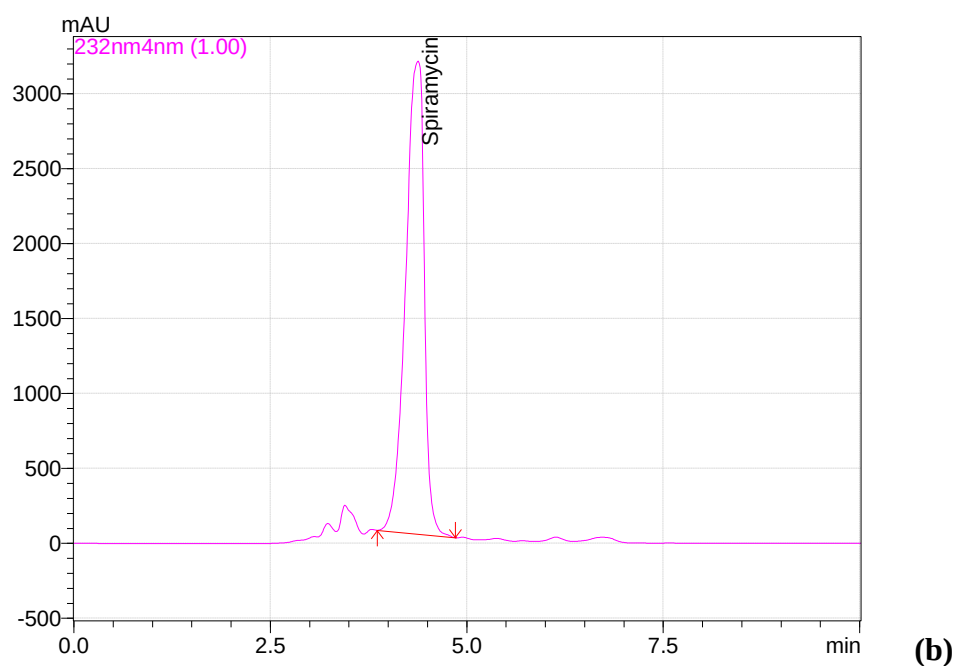
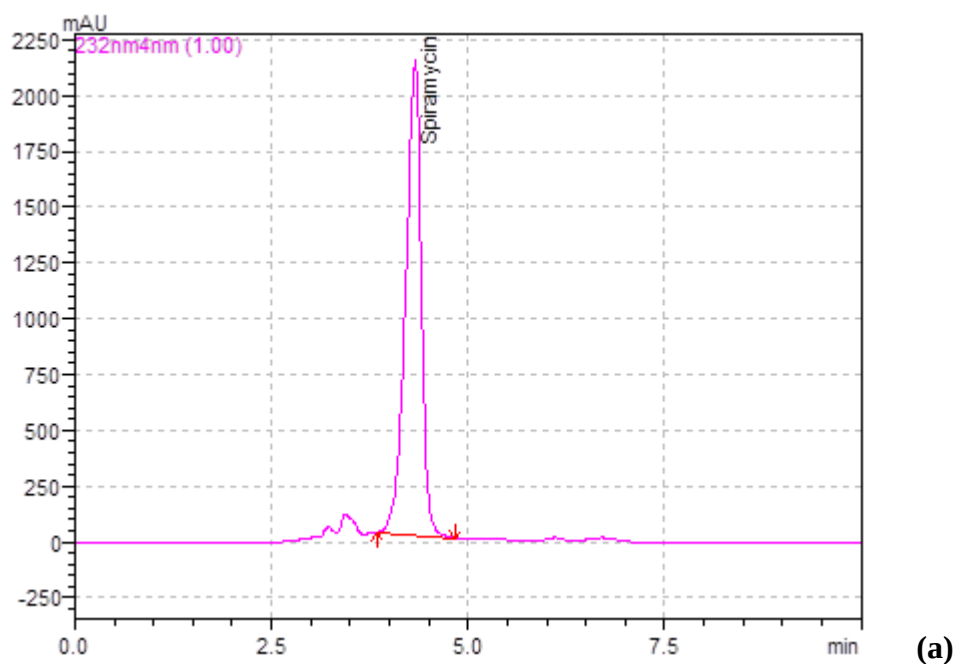


Fig. 3: Chromatograms obtained for spiramycin during specificity testing with chromatogram (a) showing the peak obtained for spiramycin from a standard solution and (b) showing the peak obtained for spiramycin detected from a commercial sample.

CONFLICT OF INTEREST

None to declare.

ACKNOWLEDGEMENTS

This work was carried out with the financial support of the National Research Foundation of South Africa (NRF) (Grant no. TTK13020718661) and the Centre of Excellence for Pharmaceutical Sciences, (Pharmacén) of the North-West University, Potchefstroom Campus, South Africa.

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. Iroezindu MO. Disparities in the magnitude of human immunodeficiency virus-related opportunistic infections between high and low/middle-income countries: Is highly active antiretroviral therapy changing the trend? *Ann Med Health SciRes* 2016;6:4.
2. Rubaihayo J, Tumwesigye NM, Konde-Lule J. Trends in prevalence of selected opportunistic infections associated with HIV/AIDS in Uganda. *BMC Infect Dis* 2015;15:1.
3. Sepkowitz KA. Opportunistic infections in patients with and patients without acquired immunodeficiency syndrome. *Clin Infect Dis* 2002;34:1098-107.
4. Davis S. Macrolides and ketolides: more than just antimicrobials. *SAPJ* 2011;78(5):24-7.
5. Sprinz E, Mallman R, Barcellos S, Silbert S, Schestatsky G, Bem David D. AIDS-related cryptosporidial diarrhoea: an open study with roxithromycin. *J Antimicrob Chemother* 1998;41, Suppl B:85-91.
6. Huang MZ, Li J, Guan L, Li D Q, Nie XM, Gui R. & Chen X. Therapeutic effects of acetylspiramycin and garlicin on cryptosporidiosis among drug users. *Int J Parasitol Drugs Drug Resist* 2015;5:185-90.
7. Wang D, Zhang YY. Intestinal pathological changes of Kunming mice infected by *Cryptosporidium* and the therapeutic efficacy of spiramycin on infected mice. *Zhongguoji sheng chongxueyuji sheng chongbingzazhi*= Chinese journal of parasitology & parasitic diseases, 2014;32:225-8.
8. British Pharmacopoeia. The Department of Health. Volume VI. London: The Stationary office; 2016.
9. United States Pharmacopoeia-39. National Formulary-34; Pharmacopeial forum; 2016. p. 8027.
10. Li W, Jia H, Zhao K. Determination of clarithromycin in rat plasma by HPLC-UV method with pre-column derivatization. *Talanta* 2007;71:385 – 90.
11. Maher HM, Youssef RM, Khalil RH. El-Bahr SM. Simultaneous multiresidue determination of metronidazole and spiramycin in fish muscle using high performance liquid chromatography with UV detection. *J Chromatogr B* 2008;876:175 – 81.
12. Oka H, Harada KI, Suzuki M, Ito Y. Separation of spiramycin components using high-speed counter-current chromatography. *J Chromatogr A* 2000;903:93 – 8.
13. Wang MJ, Pendela M, Hu CQ, Jin SH, Hoogmartens J, Van Schepdael A; Adams E. Impurity profiling of acetylspiramycin by liquid chromatography-ion trap mass spectrometry. *J Chromatogr A* 2010;1217:6531 – 44.
14. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf. Date accessed: 25 August 2016.