FORMULATION AND EVALUATION OF AMORPHOUS CLARITHROMYCIN TABLETS FOR ENHANCED DISSOLUTION

by

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DISSERTATION

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DECLARATION

I Sello Herlot Mongalo declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science (Medical) in Pharmacy has not been previously submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

26/08/2021

………………………………………………… ………………………………..

Signature **Date**

DEDICATION

This Dissertation is dedicated to my sister Moloko Mongalo-Maphasa, who passed away a week before her Masters Degree graduation with the University of Pretoria. Her resilience has since been my source of strength.

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I would like to thank God the almighty for seeing me through my years of studying and helping me to successfully complete this project. I also extend my sincere appreciation to the following people for their enormous contributions to my study:

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TABLE OF CONTENTS

	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENTS	iv
	LIST OF FIGURES	viii
	LIST OF TABLES	xi
	ABBREVIATIONS AND ACRONYMS	xii
	ABSTRACT	xiii
CHAPTER 1	**INTRODUCTION**	**1**
1.1	INTRODUCTION	1
1.2	RESEARCH PROBLEM	3
1.3	AIM	4
1.4	OBJECTIVES	4
CHAPTER 2	**LITERATURE REVIEW**	**5**
2.1	INTRODUCTION	5
2.2	CRYSTALLINE SOLIDS	7
2.2.1	Solvates	8
2.2.2	Hydrates	10
2.2.3	Salts	11
2.2.4	Co-crystals	13
2.3	AMORPHOUS SOLIDS	14
2.3.1	Preparation of Amorphous Pharmaceuticals	17
2.3.1.1	Freeze drying	19
2.3.1.2	Spray drying	19
2.3.1.3	Milling	20
2.3.1.4	Quench cooling	21
2.3.2	Characterization of Amorphous Drugs	21
2.3.2.1	Differential scanning calorimetry (DSC)	22
2.3.2.2	Infrared spectroscopy (IR)	23
2.3.2.3	X-ray powder diffraction (XRPD)	25
2.3.2.4	Hot stage microscopy	26

2.3.2.5	Raman Spectroscopy	27
2.3.3	Stabilisation of Amorphous Solids	29
2.4	CLARITHROMYCIN	30
2.5	MODE OF ACTION OF CLARITHROMYCIN	31
2.6	STRUCTURAL ASPECTS OF CLARITHROMYCIN	32
2.7	PHYSICO-CHEMICAL PROPERTIES	32
2.8	PHARMACOKINETICS OF CLARITHROMYCIN	33
2.8.1	Absorption	33
2.8.2	Bioavailability	33
2.8.3	Distribution	34
2.8.4	Metabolism and Elimination	34
2.9	SPECTRUM OF ACTIVITY	34
2.10	SAFETY OF CLARITHROMYCIN	35
2.11	DOSAGE OF CLARITHROMYCIN	35
2.12	SOLUBILITY AND DISSOLUTION ENHANCEMENT OF CLARITHROMYCIN	36
2.13	CONCLUSION	38
CHAPTER 3	**MATERIALS AND METHODS**	**39**
3.1	INTRODUCTION	39
3.2	MATERIALS	39
3.3	PREPARATION OF AMORPHOUS CLARITHROMYCIN	39
3.4	CHARACTERIZATION OF AMORPHOUS CLARITHROMYCIN	40
3.4.1	Differential Scanning Calorimetry (DSC)	40
3.4.2	Hot-Stage Microscopy (HSM)	40
3.4.3	Infrared Spectroscopy	41
3.4.4	X-Ray Powder Diffraction (XRPD)	41
3.5	ASSAY	42
3.5.1	Mobile phase preparation	42
3.5.2	Preparation of standard solution	42
3.5.3	Preparation of sample solutions	43

3.6	COMPATIBILITY STUDIES	43
3.7	*IN-VITRO* DISSOLUTION STUDIES	44
3.8	STABILITY STUDIES	44
3.9	MANUFACTURING OF CLARITHROMYCIN TABLETS	45
3.10	EVALUATION OF CLARITHROMYCIN FORMULATIONS	45
3.10.1	Appearance	45
3.10.2	Weight variation	46
3.10.3	Thickness and diameter	46
3.10.4	Hardness	46
3.10.5	Friability	47
3.10.6	Disintegration	47
3.11	CONCLUSION	47
CHAPTER 4	**RESULTS AND DISCUSSION**	**48**
4.1	INTRODUCTION	48
4.2	PREPERATION AND PHYSICO-CHEMICAL CHARACTERISATION	48
4.2.1	Differential scanning calorimetry (DSC)	49
4.2.2	X-ray powder diffraction (XRPD)	50
4.2.3	Infrared spectroscopy (IR)	51
4.3	COMPATIBILITY STUDIES	54
4.3.1	X-Ray Powder Diffraction (XRPD)	54
4.3.2	Infrared Spectroscopy	59
4.3.3	Hot-stage microscopy (HSM)	60
4.3.4	Summary of compatibility studies	63
4.4	TABLET FORMULATION	64
4.5	STABILITY STUDIES	69
4.5.1	Physico-chemical Parameters	69
4.5.2	*In-Vitro* Dissolution Studies	72
4.6	SUMMARY	77

CHAPTER 5	**CONCLUSION AND RECOMMENDATIONS**	**78**
5.1	INTRODUCTION	78
5.2	PRE-FORMULATION STUDIES	79
5.3	FORMULATION DEVELOPMENT	79
5.4	REAL TIME AND ACCELERATED STABILITY TRIALS	79
5.5	RECOMMENDATIONS	81
REFERENCES		**82**
Annexure A:	**STANDARD CALIBRATION CURVE**	**90**

LIST OF FIGURES

Figure 2.1:	Classification scheme of single and multi-component solid forms of API	6
Figure 2.2:	Schematic representation of the possible crystalline modifications (polymorphs, solvates, cocrystals, salts, solid solution and eutectic composition) and multi-solid component systems	8
Figure 2.3:	Schematic representation of API solvates/hydrates	9
Figure 2.4:	Example of channel hydrate carbamazepine dihydrate	10
Figure 2.5:	Schematic representation of API Salt former	12
Figure 2.6:	Schematic representation of pharmaceutically significant binary crystal systems: (a) crystal solvate; (b) cocrystal	13
Figure 2.7:	Schematic representation of the amorphous and crystalline solids	14
Figure 2.8:	Schematic description of the variation in enthalpy (or volume) with temperature *T*m, *Tg*,*T*k	15
Figure 2.9:	Figure 2.9: Schematic differential scanning calorimeter thermogram	23
Figure 2.10:	IR spectra of Mefenamic acid, amorphous NaTC and co-amorphous Mefenamic acid/NaTC	25
Figure 2.11:	Typical amorphous and crystalline x-ray diffraction patterns	26
Figure 2.12:	HSM temperature dependent phase changes for amorphous clarithromycin	27
Figure 2.13:	Raman spectra of neat crystalline and amorphous Cimetidine	28
Figure 2.14:	Clarithromycin mechanism of action	31
Figure 2.15	Chemical structure of Clarithromycin and degradation products	32
Figure 4.1:	Overlay of the DSC thermograms obtained for (a) crystalline clarithromycin and (b) clarithromycin neat amorphous form.	49
Figure 4.2	The XRPD of (a) crystalline clarithromycin raw material and (b) quench cooled amorphous clarithromycin raw material.	50

Figure 4.3: IR spectrum of the clarithromycin raw material 52
Figure 4.4: Overlay of FT-IR spectra obtained for (a) Crystalline Clarithromycin Raw material and (b) Amorphous Clarithromycin. 53
Figure 4.5: Overlay XRPD of amorphous clarithromycin with Emcompress® 55
Figure 4.6: Overlay XRPD of amorphous clarithromycin with Lactose 56
Figure: 4.7: Overlay XRPD of amorphous clarithromycin with Avicel® PH200 57
Figure 4.8: Overlay XRPD of amorphous clarithromycin with Ac-Di-Sol® 58
Figure 4.9: Overlay XRPD of amorphous clarithromycin with magnesium stearate 59
Figure 4.10: IR Overlay of Amorphous Clarithromycin with selected excipients 60
Figure 4.11: HSM images of clarithromycin and Emcompress® 1:1 ratio 61
Figure 4.12: HSM images of clarithromycin and Lactose 1:1 ratio 61
Figure 4.13: HSM images of clarithromycin and Avicel PH200 1:1 ratio 62
Figure: 4.14: HSM images obtained during heating of clarithromycin and Ac-Di-Sol 1:1 ratio 62
Figure 4.15: HSM images of clarithromycin and magnesium stearate 1:1 ratio 63
Figure 4.16: Comparative dissolution profile overlay of amorphous clarithromycin tablets from initial to 6 months with commercial product at 25 °C ± 2 °C / 60 ± 5% RH (n=12) 73
Figure 4.17: Comparative dissolution profile overlay of amorphous clarithromycin tablets from initial to 6 months with commercial product at 40 °C ± 2 °C / 75 ± 5% RH (n=12) 73

LIST OF TABLES

Table 2.1: Techniques used to enhance solubility and dissolution of clarithromycin 37

Table 3.1: The measurement conditions of X-ray powder diffraction 41

Table 3.2: Specification for weight variation of solid dosage 46

Table 4.1: Characteristic absorbance bands for crystalline clarithromycin Form II 52

Table 4.2: Summary of compatibility results of clarithromycin / Excipients 63

Table 4.3: Composition of formulations F1 to F4 65

Table 4.4: Pre and post compression parameters of formulations F1 to F4 65

Table 4.5: Composition of formulations F5 to F8 67

Table 4.6: Pre and post compression parameters of formulations F5 to F8 67

Table 4.7: Composition and tablet parameters of the final formulation of amorphous clarithromycin tablets 68

Table 4.8: Stability results of F6 for physico-chemical properties at 25 °C ± 2 °C / 60 ± 5% RH 70

Table 4.9: Stability results of F6 for physico-chemical properties at 40 °C ± 2 °C / 75 ± 5% RH 71

Table 4.10: Stability results for *in-vitro* dissolution of F6 in comparison with commercial tablets from initial to 6 months with commercial product at 25 °C ± 2 °C / 60 ± 5% RH (n=12) 74

Table 4.11: Stability results for *in-vitro* dissolution of T final in phosphate buffer (pH 6.8) in comparison with Niaspan® after 0, 3 and 6 months at 40 °C ± 2 °C / 75 ± 5% RH (n=12) 75

ABBREVIATIONS AND ACRONYMS

ASD	-	Amorphous Solid Dispersion
API	-	Active Pharmaceutical Ingredient
AUC	-	Area Under The Curve
BSC	-	Biopharmaceutical Classification System
CYP	-	Cytochrome P450
DSC	-	Differential Scanning Calorimetry
FT IR	-	Fourier Transform Infrared Spectroscopy
GIT	-	Gastrointestinal Tract
HPLC	-	High Performance Liquid Chromatography
HSM	-	Hot Stage Microscopy
IR	-	Infrared Spectroscopy
NCES	-	New Chemical Entities
NMR	-	Nuclear Magnetic Resonance
PVA	-	Poly Vinyl Alcohol
PVDF	-	Polyvinylidene Difluoride
RH	-	Relative Humidity
RNA	-	Ribonucleic Acid
RS	-	Raman Spectroscopy
SEM	-	Scannin Electron Microscopy
SPADS	-	Polymers For Amorphous Drug Stabilization
Tg	-	Glass Transition Temperature
***TG*A**	-	Thermogravimetric Analysis
XRPD	-	X - Ray Powder Diffraction

ABSTRACT

Introduction:

According to the biopharmaceutical classification system, Clarithromycin is considered a class II molecule with low solubility. Poorly soluble drugs result in low bioavailability. Various techniques have been studied to improve the solubility of drugs and subsequently bioavailability. Of these techniques, preparation of amorphous form is the preferred method because it is a more effortless and convenient way to improve the aqueous solubility and dissolution of poorly water-soluble drugs. The only disadvantage of amorphous materials is that they are less thermodynamically stable and can recrystallize during processing and storage.

Aim:

The aim of this study is to prepare amorphous form of clarithromycin to improve its solubility, dissolution rate, and, subsequently, bioavailability**.**

Methods:

In this study, preparation of amorphous form of clarithromycin was conducted using the quench cooling method in which the purchased anhydrous crystalline clarithromycin was spread on an aluminum foil and heated to a melting point (217˚C - 220˚C) and then rapidly cooled. Various techniques were conducted to characterize the prepared amorphous clarithromycin, and these include Differential Scanning Calorimetry (DSC), Fourier-Transform Infrared Spectroscopy (FTIR), and X-Ray Powder Diffraction (XRPD). In addition, tablets were formulated using the amorphous clarithromycin mixed with selected excipients from compatibility studies, and *in vitro* dissolution and stability studies were conducted over a period of 6 months.

Results:

The DSC thermogram results confirmed that the material prepared using the quench cooling process is an amorphous solid-state. Furthermore, the XRPD confirmed an amorphous solid-state with scattering halo peaks. The FTIR also depicted some broader and lower intensity peaks that indicated a formation of an amorphous material. The dissolution rate of amorphous clarithromycin tablets

improved by more than 30% when compared to commercial crystalline clarithromycin tablets. The study revealed a drop in dissolution rate at months 3 to 6 under accelerated conditions due to recrystallization. The 6 monthly stability study at long term conditions showed no change in the integrity of the tablets and their contents.

Conclusion:

As indicated by the study, it can be concluded that the amorphous clarithromycin remained stable during processing and storage under long-term stability for 6 months. Furthermore, based on dissolution results, it can be concluded that amorphous solids have an improved dissolution rate.

Keywords: Clarithromycin, amorphous, stability, dissolution, solution-mediated phase transformation

CHAPTER 1

INTRODUCTION

1.1 NTRODUCTION

Adequate aqueous solubility of new chemical entities (NCEs) is one of the critical properties required for successful pharmaceutical formulation development. Solubility is generally defined as the concentration of the compound in a solution that is in contact with an excess amount of the solid compound when the concentration and the solid form do not change over time. Thus, solubility is closely related to dissolution, a kinetic process involving the detachment of drug molecules from the solid surface and subsequent diffusion across the diffusion layer surrounding the solid surface (Bosselmann, 2012).

Poor aqueous solubility of new chemical entities presents various challenges in developing effective drug-delivery systems for different delivery routes. Poorly soluble drugs delivered orally commonly result in low bioavailability and are subject to considerable food effects (Bosselmann, 2012). The therapeutic effect of drugs depends on the drug concentration at the site of action. The absorption of the drug into the systemic circulation is a prerequisite to reach the site of action for all drugs, except for those drugs that are applied at the site of action or those that are intravenously injected. After oral administration, many factors determine the bioavailability. Since the only dissolved drug can pass the gastrointestinal membrane, dissolution is one of those factors that determine bioavailability (Saindane *et al*., 2011).

Most pharmaceutical compounds are used in the solid phase and most of these compounds exist in a crystalline state. The advantage that most crystalline solids hold over their amorphous counterparts is that they usually are more stable and therefore impose fewer problems with handling and storage (Odendaal, 2012).

Therefore, the amorphous form of pharmacologically active materials has received considerable attention because, in theory, it represents the most energetic solid-state of material and should thus provide the most significant advantages in terms of dissolution rate and bioavailability. However, the amorphous form also shows various disadvantages, such as lower physical stability than crystal forms (Einfalt *et al*., 2013).

Various techniques have been used to improve the solubility of drugs, which would lead to an increase in vitro drug release. The techniques include preparing an amorphous form, prodrugs, solid dispersions, and complexation with various natural and synthetic cyclodextrins. The preparation of an amorphous form is the easiest and most convenient way to enhance aqueous solubility and dissolution rate of poorly water-soluble drugs. However, materials in the amorphous form are less thermodynamically stable than any crystalline form, leading to a tendency for amorphous materials to transform to a known or potentially unknown crystalline form (Saindane *et al*., 2011).

The development of amorphous pharmaceuticals raises the opportunity of developing dosage forms with a potential increase in bioavailability. It is also essential to understand the challenges it poses due to its metastable nature. Due to this reason, it is essential to study the solid-state characteristics of amorphous forms alone and also in combination with other excipients when exposed to different stress conditions during processing and storage. This is imperative for the sake of patenting, therapeutic and commercial applications and is also a requirement for regulatory authorities (Strachan *et al*., 2005).

Tablets are the most popular oral dosage form existing today because of its convenience of self-administration and compactness. Tablets are also easy and cost-effective to manufacture. However, in many cases, immediate onset of action is required during patient treatment, an option that is not always obtained with

conventional solid-dosage form therapy. Oral administration is the most popular route for systemic effects due to its ease of ingestion, pain avoidance, versatility, and, most importantly adherence thereof. Wankhede *et al* (2010) report that the enhancement in oral bioavailability of amorphous atorvastatin tablets was attributed to a combination of higher apparent solubility and higher dissolution rate due to its amorphous nature (Charan *et al.*, 2015).

1.2 RESEARCH PROBLEM

According to the biopharmaceutical classification system (BCS), clarithromycin is considered a class II molecule with low solubility and high in vivo permeability. Clarithromycin is a macrolide antibiotic with a broad spectrum of activity. It is prescribed for the treatment of respiratory tract infections as well as skin and soft tissue infections. It has poor water solubility of about 0.342 μg/ml. It has been estimated that approximately 60–70% of the drug molecules are insufficiently soluble in aqueous media and have very low permeability to allow for their adequate and reproducible absorption from the gastrointestinal tract (GIT) following oral administration (Gupta, 2013).

In general, it can be stated that the rate of absorption, the onset, and extent of the clinical effect, are determined by the dissolution of the drug and the subsequent transport over the intestinal membrane and passage of the liver (Ambike *et al*, 2005). Low aqueous solubility is the major problem encountered with the formulation development of new chemical entities (Bajaj, 2011). Preparation of amorphous forms is a possible approach to improve the physicochemical behavior of pharmaceutical solids. Compared to a crystalline solid, the amorphous material can have advantages such as enhanced dissolution rate, bioavailability, and tableting properties (Sadeghi *et al.*, 2013).

The research question in this study is, can the preparation of amorphous clarithromycin immediate-release tablet yield an enhanced dissolution rate? The present study focuses on investigating the tablet ability of the amorphous form of clarithromycin and determining whether formulation steps and subsequent storage of the final product will induce re-crystallization of the amorphous form to the stable crystalline form of clarithromycin. Furthermore, to determine whether excipients will influence the solution-mediated phase transformation of the amorphous solid-state form during dissolution testing.

1.3 AIM

The aim of this study is to formulate and prepare amorphous clarithromycin for enhanced dissolution.

1.4 OBJECTIVES

- To prepare amorphous clarithromycin through quench cooling process.
- To conduct Physico-chemical characterization of the amorphous solid-state for validation of an amorphous habit.
- To identify and select potential excipients for developing an immediate release formulation by conducting compatibility studies between the excipients and clarithromycin.
- To conduct in vitro dissolution studies to evaluate the amorphous clarithromycin's dissolution profiles and possible solution-mediated phase transformation.
- To conduct stability studies at elevated temperatures and humidity to determine the effect of temperature and humidity on the excipient / amorphous form.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Most active pharmaceutical ingredients (APIs) are solid and exist in various solid-state forms. Crystalline materials have structural units repeated in a regular manner forming a well-defined lattice. Unlike crystalline compounds, amorphous materials show no long-range order of molecular packing. However, local molecular assemblies characterized by short-range order may occur in the amorphous form (Heinz, 2008). Polymorphs are different crystalline forms of the same pure substance, in which the molecules have different arrangements and conformations of the molecules. Solvates are formed when a solvent is included within the crystal lattice, and when the solvent is water, it is known as a hydrate (Vippagunta, 2001).

The subject of drug polymorphism has received extensive academic and industrial attention since the early pioneering reports of Aguiar and colleagues at Parke-Davis, in which effects of polymorphism on dissolution and bioavailability were highlighted for chloramphenicol palmitate (Singhal, 2003). Many active pharmaceutical ingredients (APIs) exhibit polymorphism which is frequently defined as the ability of a substance to exist as two or more crystalline phases, having different arrangements and conformations of the molecules in the crystalline lattice (Duplessis, 2004). APIs exist in various solid-state forms (Figure 2.1), and they are broadly classified as crystalline or amorphous depending on the degree of long-range arrangement and periodicity of molecules in the crystal lattice (Healy *et al.*, 2017).

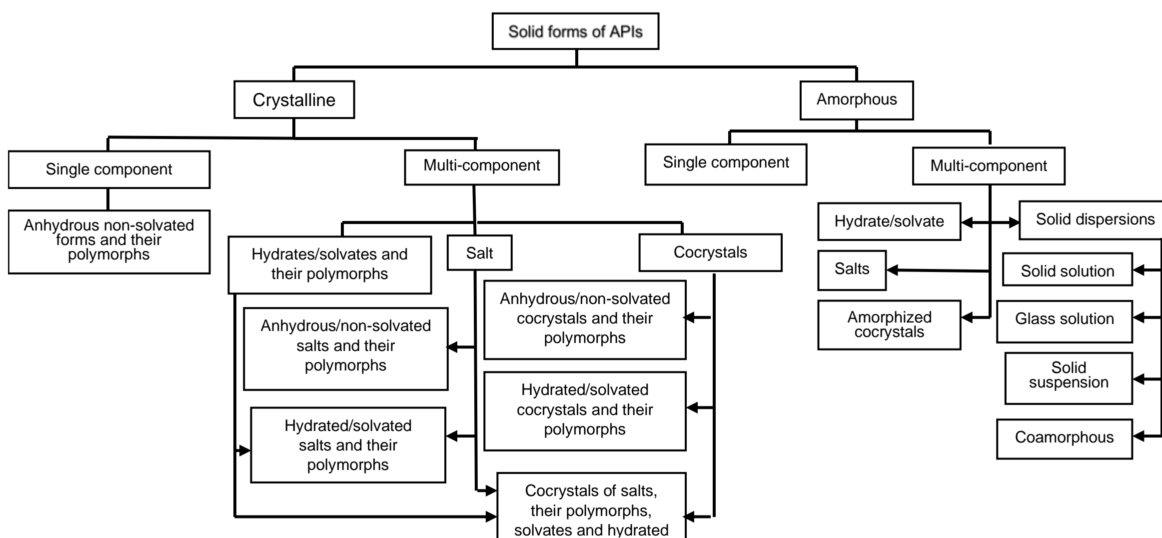


Figure 2.1: Classification scheme of single and multi-component solid forms of API (Healy, 2017).

Different polymorphic forms exhibit different physicochemical properties, and therefore the biopharmaceutical factors may be different for each polymorphic form. Since polymorphism is related to the packing arrangements and conformations of the molecules, this characteristic is only exhibited by a substance in its solid form. Polymorphs may display different chemical and physical properties. Properties that polymorphism might influence include solubility, dissolution rate, hygroscopicity, stability, melting point, crystal shape, and size. Acetaminophen, for example, exhibits polymorphism and can exist as the monoclinic or orthorhombic forms. The selection of the optimum solid-state form of an active pharmaceutical ingredient (API) is critical for the formulation and development of a dosage form (Zhang, 2004).

Hence, solid-state characterization plays a vital role in the development and manufacture of medicinal products because they may influence pharmaceutical products' effectiveness, stability, and processibility. The main reason for the growing interest in amorphous materials is the need to improve the bioavailability of compounds with poor aqueous solubility (Antal, 2008).

2.2 CRYSTALLINE SOLIDS

The crystalline state, is the most preferred solid sub-phase, can be reproduced reliably, and details surrounding its physical properties include physical stability and processibility. The molecules of each crystal are held together via weak inter and intra-molecular forces like hydrogen or van der Waals bonds which are different for every crystal form, displaying different free energies as exemplified by many of its physical properties such as solubility, chemical stability, melting point, density, etc. Thus, a crystalline solid can exist in various interchangeable states, including polymorphs (Form I and Form II), solvates, salts, and hydrates, as indicated in figure 2.2 (Van Eeden, 2012).

Crystalline solids are the backbone of solid dosage forms and are usually considered the starting point for formulation development. They possess particular physical and chemical properties that can be utilized and manipulated to achieve the desired formulation effect. The lowered free energy of the crystal, achieved through stabilizing molecules in a uniform arrangement, sets crystalline materials apart from their liquid, gas, and even amorphous solid counterparts in terms of their properties. Typically, crystalline solids are the most thermodynamically stable phase at room temperature and are thus the preferred form for formulations since phase transformations are not thermodynamically favoured. However, the same lower internal free energy that leads to better solid-state and chemical stability can also yield issues with dissolution and solubility because the most thermodynamically stable form is also the least soluble (Jackson, 2015).

There are various methods for preparing different crystal forms of a substance. The most common method employed by pharmaceutical sciences is to crystallize from different solvents under a range of temperature regimes (Van Eeden *et al*., 2012). Improvements in physicochemical properties can be achieved by altering the

physical forms of a given compound such as polymorphs, solvates, amorphous, salts, co-crystals, and hydrates, etc. (Lee, 2014).

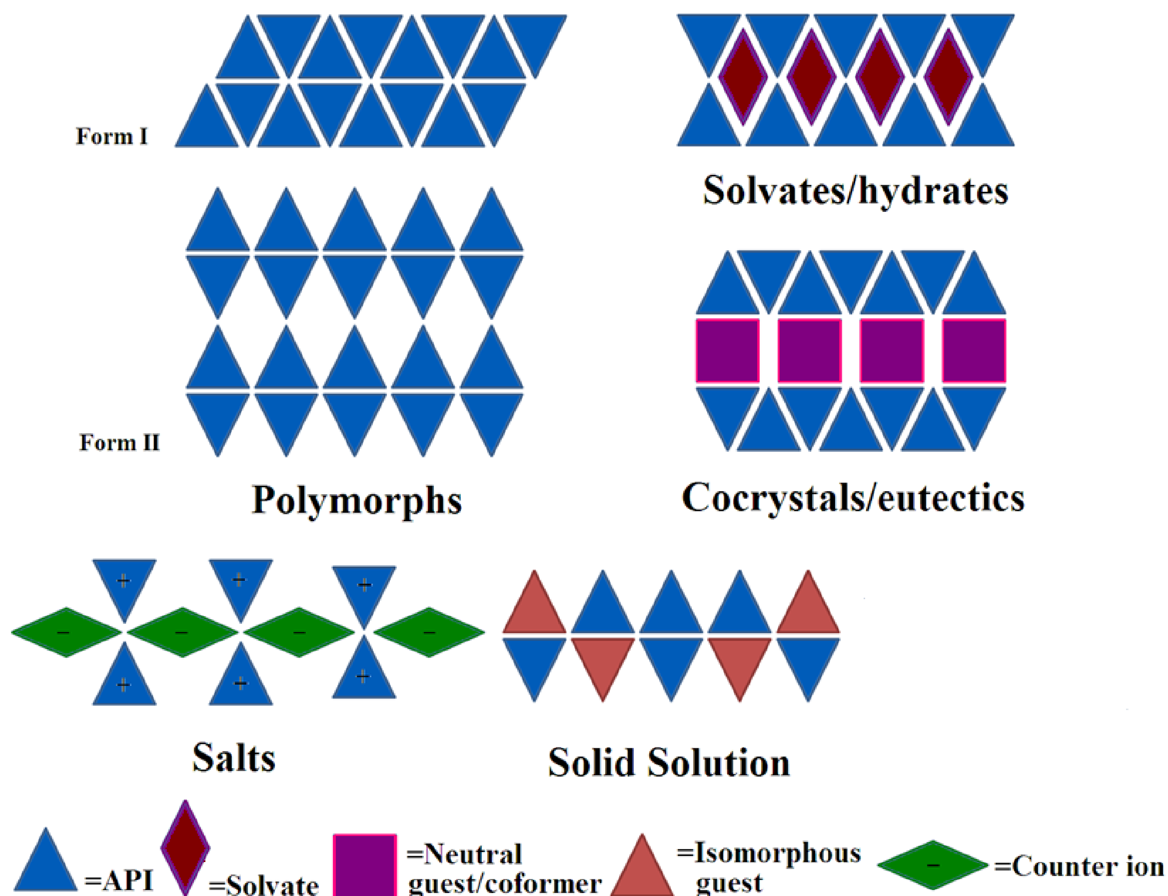


Figure 2.2: Schematic representation of the possible crystalline modifications (polymorphs, solvates, co-crystals, salts, solid solution, and eutectic composition) and multi-solid component systems (Kumar, 2014).

2.2.1 Solvates

Solvates, also inappropriately termed pseudo polymorphs (Vippagunta, 2001), are crystalline solids containing either stoichiometric or nonstoichiometric proportions of solvent within the crystal structure. When the incorporated solvent is water, the solvate is called a hydrate (Seddon, 2004). In general, it is undesirable to use solvates for drugs and pharmaceuticals, as the presence of organic solvent residues

may be toxic (Censi, 2015). Solubility studies conducted by Chadha *et al* between commercial Atorvastatin (Crystalline form) and its different solvates suggested that its Solvate-VIII was most soluble (0.374±0.06 mg/ml) with 2.07 times increase in solubility. During Solubility and Dissolution studies of Rifampicin and its polymorphs by Henwood *et al* results indicated that 2-pyrrolidone solvate was the most soluble crystal form with a solubility of 1.576mg/ml, and the dissolution rates of the 2-pyrrolidone solvate was the fastest in both buffer and water.

Solvents can lead to problems in organic compounds by inducing disorder in the crystal structure, leading to the formation of relatively metastable systems. However, solvent molecules in the crystal lattice can also create strong interactions and hydrogen bonding with APIs and other solvent molecules to form flexible clusters, improving the stability of metastable solid forms. Solvent molecules may also play the role of space fillers in the crystal lattice without forming strong interactions with host molecules (Healy, 2017).

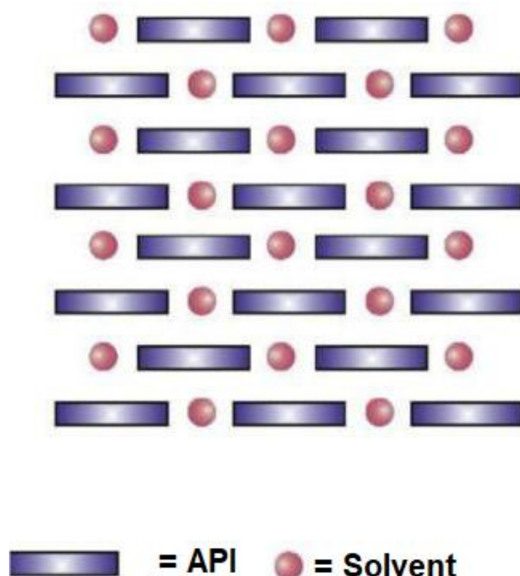


Figure 2.3: Schematic representation of API solvates/hydrates (Korotkova, 2014).

2.2.2 Hydrates

A hydrate is a solid containing both the parent compound (the anhydrate of an API) and water. A hydrate is formed when the solvent is water or when water is present in the organic solvent during crystallization. The water molecules occupy definite positions within the crystal lattice, usually through the formation of coordinate covalent bonds or hydrogen bonds with the API molecules. When the water molecules are incorporated into the crystal lattice, it produces a new cell unit, which is different from the anhydrate. The hydrate may therefore have different physical properties compared to the anhydrate (Joubert, 2016).

Hydrates have been classified according to the energetic state and to the structure. The first category involves isolated-site hydrates, where water molecules are separated from direct contact with one another by intervening drug molecules, as shown in Figure 2.4. The second type comprises channel hydrates, where hydrogen bonds connect water molecules and form a tunnel-like structure through the crystal. An example is carbamazepine dihydrate depicted in Figure 2.4 below.

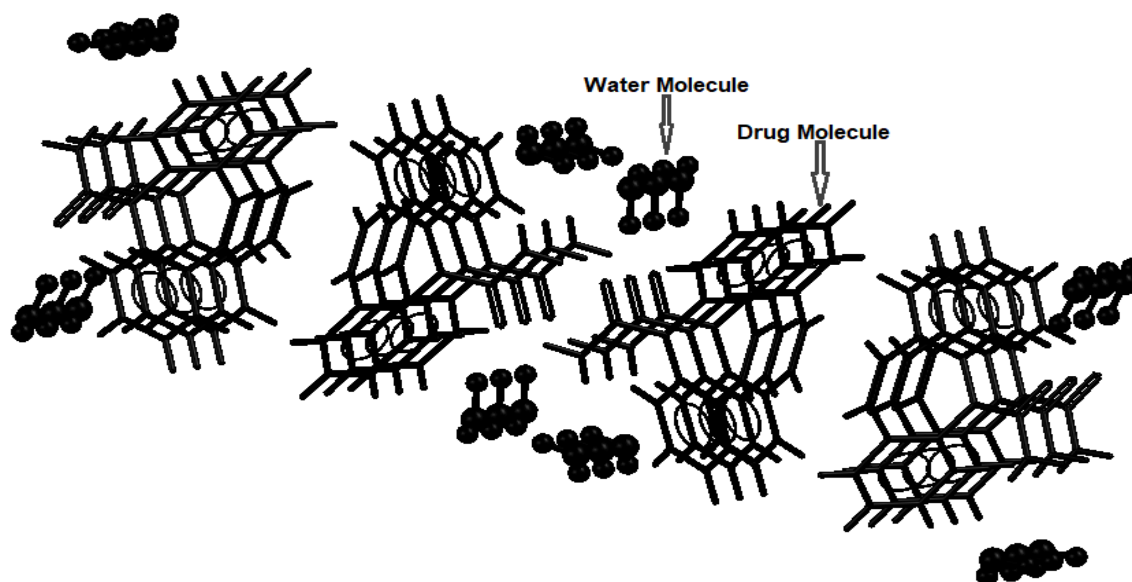


Figure 2.4: Example of channel hydrate carbamazepine dihydrate (Kogermaan, 2008).

Hydrates' stability depends on the relative humidity, water activity in its vapour phase, and partial vapour pressure. This feature of hydrates is essential in the quality control of a hydrate since the conditions for handling and storage depend upon the reaction of the hydrate to a change in relative humidity. Hydrates may be susceptible to recrystallization when the hydration level changes with changes in relative humidity or consequently convert into a different form, which can be a new or pharmaceutically undesirable form. Dehydration of a hydrate to a lower hydrated form (or anhydrous form) will increase the solubility of the hydrate, but at the cost of reduced stability (Odendaal, 2012).

Hydrates are of particular interest among solid APIs solvates for several reasons. First, the unique character of the water molecule—its relatively small size and the possibility to form the interactions as both a donor and acceptor of H-bonding, sometimes simultaneously, make it a necessary “building material” in the field of crystal engineering. Further, from the pharmaceutical point of view, it is a non-toxic substance, in contrast to most of the other organic solvents. Finally, owing to the presence of moisture in the air, spontaneous hydration may occur at any stage of drug production, such as wet granulation and storage, leading to hydrate formation (Jurczak, 2020). Although lowered bioavailability has been reported with hydrate forms of several APIs, hydrates are typically the most stable forms in aqueous solutions and under high humidity conditions (Kogermaan, 2008).

2.2.3 Salts

The formation of salts is invaluable for the preparation of safe and effective dosage forms of many drugs. Whether the drug products are solutions or solids, the use of salt provides a higher concentration in solution than the free acid or free base (nonionized forms). Typically, salts readily undergo crystallization, and the resulting material facilitates subsequent processing. Thus, salt is often the preferred form for isolating and purifying the drug (Wiedmann, 2016).

Salt formation is a common method to tune and change ionizable drug compounds' desired physical and chemical properties. The main drug properties such as solubility, dissolution rate, hygroscopicity, stability, impurity profiles, and crystal habit can be regulated by various pharmaceutically acceptable acids or bases (Kumar, 2015). In addition to solubility and manufacturing, salt is typically a more stable form of the drug. This, too, is an essential advantage for developing a product with a long shelf-life (Lee, 2014).

The salt form is separated into individual entities (i.e., the ionized drug and the counterion) in a liquid medium, and its solubility depends upon the solvation energy in the solvent. The solvent must overcome the crystal lattice energy of the solid salt and create space for the solute, as depicted in Figure 2.3. Thus, the solubility of salt depends on its polarity, lipophilicity, ionization potential, and size. A salt's solubility also depends on the properties of solvent and solid such as the crystal packing and presence of solvates (Kumar, 2008).

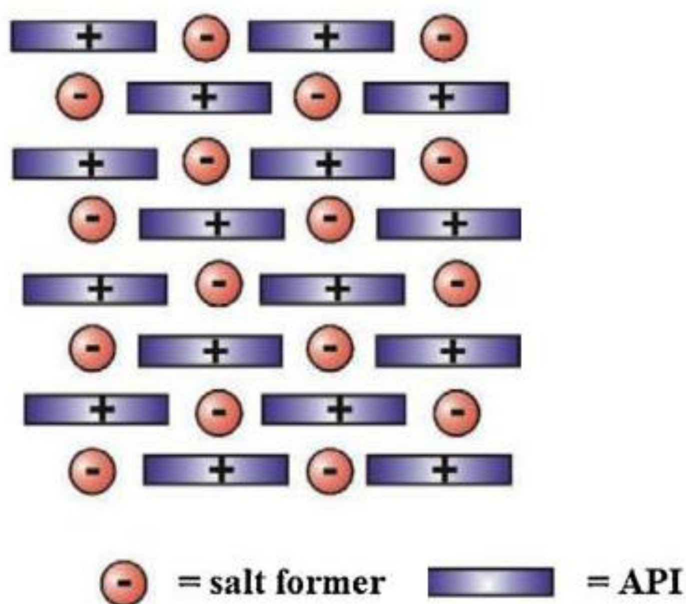


Figure 2.5: Schematic representation of API Salt former (Korotkova, 2014).

2.2.4 Co-crystals

In the literature, researchers define co-crystals as solids that are crystalline single-phase materials composed of two or more different molecular and ionic compounds generally in a stoichiometric ratio which are neither solvates nor simple salts as in Figure 2.5 (Kumar, 2017).

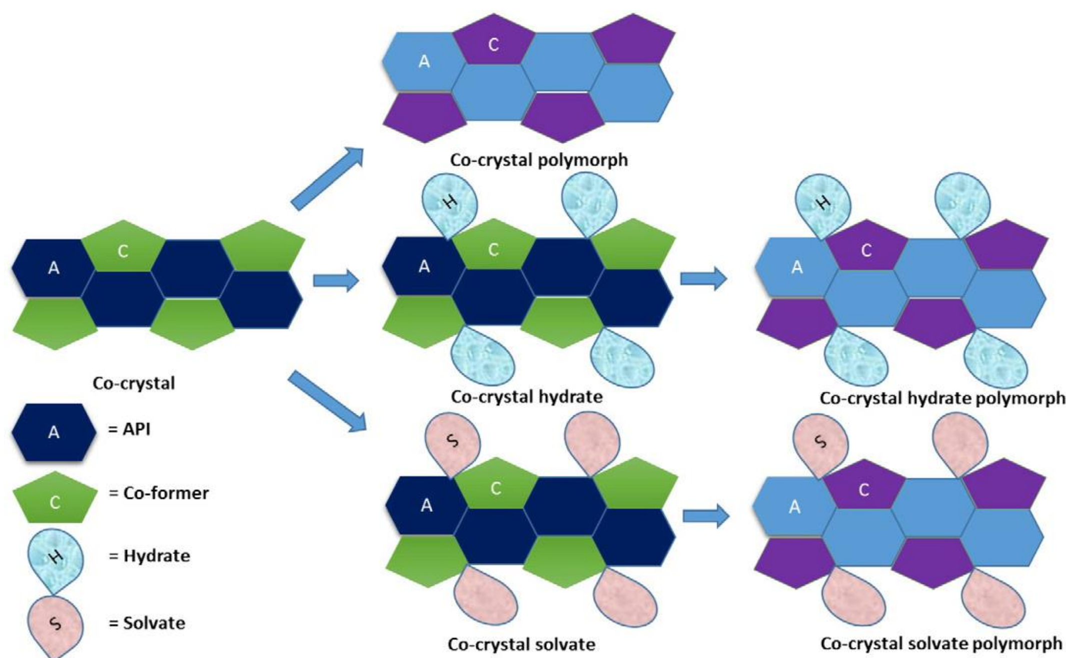


Figure 2.6: Schematic representation of pharmaceutically significant binary crystal systems: (a) crystal solvate; (b) co-crystal (Perlovich, 2012).

Co-crystals are attractive in that they make it possible to prepare new crystalline API forms with enhanced solubility, thermodynamic stability, and improved mechanical properties (Perlovich, 2012). The formation of cocrystals also stabilizes APIs that tend to form amorphous solids and/or unstable crystals by themselves. Superior physical properties and storage stabilities provide opportunities to improve current formulations and to develop dosage forms for new administration routes. Cocrystals present complex structures that substantially affect their clinical Chemical and Pharmaceutical Bulletin Advance Publication performance, highlighting the importance of their physical and chemical stability during processing and storage. Their lattice reduces molecular mobility, enhancing the chemical

stability of some oily and amorphous APIs under various storage conditions (Izutsu, 2016).

2.3 AMORPHOUS SOLIDS

Vrani *et al* (2004) define amorphous forms as non-crystalline materials which possess no long-range order. Their structure can be considered similar to that of a frozen liquid with the thermal fluctuations present in a liquid frozen out, leaving only "static" structural disorder. On the other hand, Saindane *et al* (2011) define amorphous solid (glass) as materials with no long-range order of molecular packing or well-defined molecular conformation if the constituent molecules are conformationally flexible.

As depicted in Figure 2.7, the immediate environment of a molecule (m) in an amorphous solid may not be significantly different from that in a crystal. However, an amorphous solid lack any long-range translational-orientational symmetry that characterizes a crystal (Yu, 2001).

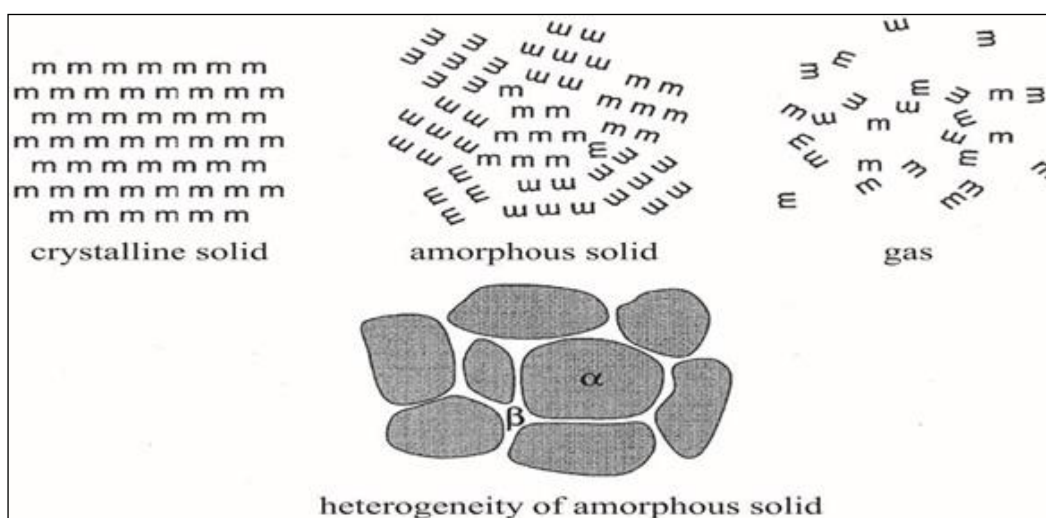


Figure 2.7: Schematic representation of the amorphous and crystalline solids (Einfalt, 2013).

From a thermodynamic point of view, amorphous materials can be delineated as supercooled liquids and glasses separated by the glass transition temperature (*Tg*). Above the *Tg*, the material is a supercooled liquid (Figure 2.8) with high molecular mobility and a heat capacity that is continuous with the heat capacity of the equilibrium liquid. However, supercooled liquids occur below the melting temperature of the compound and are thus metastable relative to the crystal. On the other hand, glasses are amorphous solids below the *Tg*, have lower molecular mobility, and have lower heat capacity than the supercooled liquid (Einfalt, 2013).

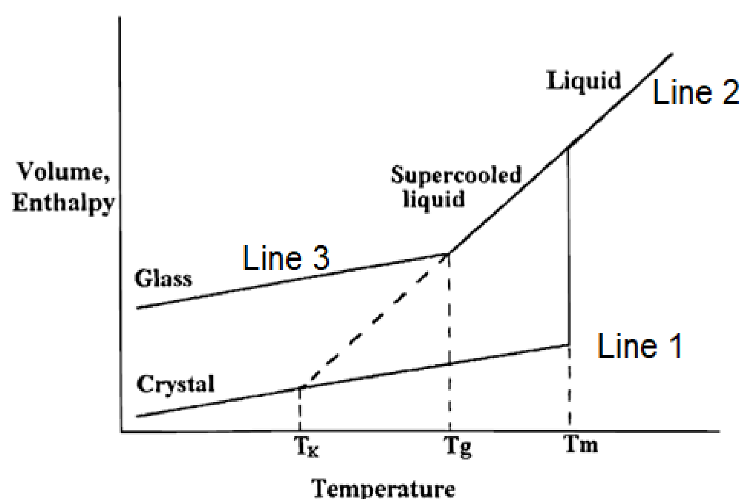


Figure 2.8: Schematic description of the variation in enthalpy (or volume) with temperature Tm, *Tg*, Tk (Einfalt, 2013).

Amorphous solids are thermodynamically metastable compared to the crystalline complement. They have short-range intermolecular interactions such as hydrogen bonding and other non-covalent forces but lack the long-range order and periodicity in crystalline solids (Jackson, 2015). Furthermore, compared to a crystalline state, the positioning of amorphous state molecules is more irregular and possesses high internal energy and specific volume, which improves dissolution and bioavailability (Karolewicz *et al*, 2019).

Amorphous solids below the glass transition temperature have a rigid physical structure but lack the basic molecular packing, defining a point/space group, habit, or shape. Amorphous solids have higher free energy than the crystalline form and consequently are metastable relative to the crystalline form. These properties can be taken advantage of for oral drug delivery, as the higher energy can lead to increased dissolution/solubility and subsequently higher bioavailability. However, amorphous solids are more chemically reactive and tend to crystallize into a more stable form, creating issues with solid oral dosage formulation stability. The use of additives to inhibit crystallization from both the solid dosage form and the aqueous dissolution medium (i.e., GI tract) is common (Jackson, 2015).

According to Van Eeden *et al* (2012), amorphous pharmaceutical solids are of interest because of their apparent higher solubility and faster dissolution rate when compared with their crystalline counterparts. However, decreased physical stability has been reported because of excess properties of enthalpy and Gibbs free energy responsible for the devitrification (crystallization) tendency of the amorphous state, which endows the desirable property of high solubility. Hence, stability is of great concern when developing these drugs. They have shown not to be in thermodynamic equilibrium and depend on several factors, including time, temperature, percentage relative humidity (%RH), and *Tg* of the solid (Van Eeden, 2012). Insoluble drug materials are difficult to work with, and the effect *in vivo* may be limited by dissolution as well. Developing the amorphous form of a drug can offer solubility and bioavailability advantages over the development of a crystalline form, provided the stability problem can be overcome (Calahan, 2011).

Amorphous solids exhibit a higher energy state than crystalline solids, are inherently less stable, and have the potential for converting to the thermodynamically more stable crystalline form over time. In addition, because of their higher molecular mobility, they often show stronger chemical reactivity and hence a faster rate of chemical degradation. As a result, the amorphous solid-state possesses several advantages compared to the crystalline state, such as better compression

characteristics than corresponding crystalline drugs, thus facilitating pharmaceutical processing (Florence, 2016). Also, amorphous drugs have higher solubility, higher dissolution rate, and thus higher bioavailability than the corresponding crystalline drug, providing an opportunity to enhance its bioavailability in the case of poorly water-soluble drugs (Florence, 2016).

2.3.1 Preparation of Amorphous Pharmaceuticals

Amorphous substances may be formed both intentionally and unintentionally during normal pharmaceutical manufacturing operations. Amorphous forms, and especially those of poor glass formers, can be induced deliberately or accidentally, by inhibiting crystallization through processes, like grinding, milling, cryo-milling, compression (mechanical stress), wet granulation (chemical stress), conventional drying, heating, cooling (Yu, 2001).

Various techniques are used to prepare amorphous solids, and these techniques include quenching of melts, rapid precipitation by anti-solvent addition, freeze-drying, spray drying, compression, rapid solvent evaporation, and many others (Katsidzira, 2017). These techniques can be categorized according to two principal transformation mechanisms. In most instances, the crystalline material is first intermediately transformed into a thermodynamically unstable non-crystalline form (a melt or a solution). Moreover, the thermodynamically unstable amorphous solid material is prepared by either quench cooling of the melt or rapid precipitation from solution, e.g., spray drying. A second transformation mechanism involves direct solid conversion from the crystalline to the amorphous form. It has also been established that amorphous forms which are prepared differently exhibit different properties (Vrani, 2004).

For thermodynamic and kinetic reasons, the preparation of amorphous solids is easy for some materials (good glass formers) but difficult for others (poor glass formers). Thermodynamically, glass-forming ability originates from a crystalline

state that is not substantially more stable than the amorphous state, which may be the case for molecules that pack poorly or contain many internal degrees of freedom. Kinetically, a slow crystallization rate allows the material to become a "frozen liquid" or vitrify without crystallization. One general cause for reduced crystallization tendency among organics is conformational flexibility (Vrani, 2004).

It should also be noted that differently prepared amorphous form exhibits different properties. For example, Salvonien *et al* (2008) have found that the cryo-milled simvastatin has lower stability and a reduced recrystallization rate than quench cooled samples of the same substance. In comparison, Karmwar *et al* (2011), found that the cryo-milled amorphous form of indomethacin is the least stable compared to the amorphous form prepared by quench cooling and spray drying (Einfalt *et al.*, 2013).

The term “polymorphism” has been used to describe amorphous states produced by different annealing times or preparative routes. An example is glasses that have been aged below *Tg* for different times and hence developed various degrees of “relaxation enthalpy,” that is, the enthalpy of an aged amorphous substance. The term polymorphism has also been used in literature to describe the existence of distinct amorphous phases separated by first-order phase transitions (Einfalt *et al.*, 2013). Patterson *et al* (2005) have shown that the susceptibility to amorphous conversion by different methods is compound specific.

The dehydration of a crystalline hydrate is probably the gentlest way to create an amorphous form. First, the sample is exposed to heat (below the melting point) to remove the water/solvent. Another process of forming an amorphous state is by introducing impurities which causes a poor glass former to exist in the amorphous state in a multi-component formulation (Joubert *et al.*, 2016).

2.3.1.1 Freeze-drying

Freeze-drying involves desiccating a substance by crystallization of water, followed by sublimation of water vapour from the solid-state at reduced pressure. Depending on the cooling rate, some solutes may crystallize during the freezing stage. Those solutes which do not crystallize are converted to amorphous solids when the temperature drops below the *Tg* of the maximally concentrated solute. At the end of a freeze-drying process, when the solvent is completely removed through sublimation, the freeze-dried formulation exists as an amorphous or partially amorphous system (Einfalt, 2013).

In a study conducted by El-Badry *et al*, Albendazole (ABZ) dissolution rate was enhanced remarkably from microparticles with hydrophilic Hydroxypropyl methylcellulose (HPMC) prepared by free-drying technique. The percent dissolution of the drug from its freeze-dried microparticles with HPMC was 90% and 95% after one hour and two hours, respectively, while that from its physical mixture was 45% after two hours. Thus, the improvement in the dissolution rate of ABZ could be attributed to the amorphization of the drug by freeze-drying with HPMC.

Freeze-dried formulations have the advantage of better stability and provide easy handling (shipping and storage). However, freeze-drying is a time- and energy-intensive process that could take days or even weeks to finish if the freeze-drying cycle is not optimized. Therefore, the stability of the drug during freeze-drying and storage and the duration of the cycle are the two major considerations for freeze-drying process optimization (Tang, 2004).

2.3.1.2 Spray drying

Spray drying can be used to enhance the solubility and dissolution rate of poorly soluble drugs. This usually occurs via the formation of pharmaceutical complexes or via the development of solid dispersions. Ambike *et al* (2005) elude in a comparison study of valdecoxib spray-dried, solid dispersions of valdecoxib and

corresponding physical mixtures found that all spray-dried samples, as well as physical mixtures, suggested increased saturation solubility and dissolution rate immediately after processing, which could be attributed to the formation of amorphous form as confirmed by DSC and XRPD results of the study. In addition, stability was found to be low after 1 month and 15 days.

One advantage of spray drying is how readily excipients can be incorporated into the process. As long as the excipient is soluble in a spray solvent, it can be included in the formulation. For example, suppose the drug is not prone to degradation under acidic conditions. In that case, ionizable cellulosic polymers are often a good excipient choice because of their high glass transition temperature and low hygroscopicity in a solid-state (Broadbent, 2015). However, this process has certain weaknesses, such as poor flowability of the resulting powder making downstream processing challenging and the need for a secondary drying step to remove the residual solvent (Phapale, 2017).

Drugs that have a relatively low *Tg* make it very difficult to obtain a stable amorphous product in the form of free-flowing powder by spray drying. As the outlet temperature rises above the *Tg*, there is always a possibility that the final product is present in the supercooled rubbery state. (Einfalt, 2013).

2.3.1.3 Milling

Milling techniques such as ball milling and cryogrinding are also useful to make small quantities of amorphous materials. Their primary advantage lies in the fact that they are widely available techniques requiring very little training. The final product of milling, among other things, will depend on the milling temperature and its relationship to the glass transition temperature of the API and the strength of the crystal (Nagapudi, 2008). Products prepared by the milling technique will be amorphous if the milling temperature is below the glass transition temperature of the API (Descamps, 2007).

During a study of amorphous Salbutamol Sulphate (SS), which was prepared by milling technique, SS particles were stored in accelerated stability conditions of 40°C, and 75% RH indicated complete recrystallization after 5 hours (Pfeiffer, 2003). However, in a study of SS and PVP (1:5) ratio which were co-milled at 300 rpm, samples that were stored under 22°C/75% RH were able to retain their amorphous character. Furthermore, the results showed that co-milling SS with PVP at a (1:5) ratio was effective in stabilizing the amorphous form of SS (Kiong, 2008).

2.3.1.4. Quench cooling

Quench cooling is a technique in which an amorphous form is prepared by melting the crystalline form and rapidly cooling it below the glass transition temperature *Tg*. The quench-cooling process is fast enough, and the molecules do not have time to rearrange themselves into a regular array. Thus, an amorphous state is formed. Furthermore, the quench-cooling temperature could strongly pharmaceutically influence the relevant properties, which may be related to the physical stability of amorphous drugs (Chang, 2017).

The rapid cooling rate prevents nucleation and crystal growth, thus facilitating the formation of amorphous solids. It has been demonstrated that amorphous solids prepared via melt quenching might exhibit better physical stability than those prepared via milling. One interpretation is that the milling may not completely remove trace amounts of residual crystals, which can serve as seeds to promote crystallization. The additional relaxation process observed in the milled sample is probably connected with its re-crystallization behaviour in comparison with the melt-quenched products (Shi, 2018).

2.3.2 Characterization of Amorphous Drugs

The strategy of characterizing amorphous solids differs from that of crystalline solids. Molecular-level structural elucidation, which is feasible for crystalline solids

by diffraction and spectroscopic methods, is less applicable to amorphous solids, and greater emphasis is placed on structural mobility and changes (Vranic, 2004).

Effective characterization of amorphous pharmaceutical products requires a multidisciplinary approach using complementary analytical methods, such as Raman spectroscopy (RS), Fourier transform infrared spectroscopy (FT-IR), or solid-state nuclear magnetic resonance (ss-NMR), which are primarily intramolecular methods probing the sample at the molecular level. In addition, intermolecular information is gained by directly employing techniques such as differential scanning calorimetry (DSC), thermogravimetric analysis (*TG*A), and XRPD, which analyze the sample on the particulate level (Einfalt *et al.*,2013).

2.3.2.1 Differential scanning calorimetry (DSC)

DSC is a thermal analysis apparatus measuring how the physical properties of a sample change, along with temperature against time. In other words, the device is a thermal analysis instrument that determines the temperature and heat flow associated with material transitions as a function of time and temperature (Gill *et al.*, 2010). Several methodologies based on the calorimeter type (conventional DSC or MTDSC), measured parameters, and experimental conditions are used for monitoring the behaviour of pharmaceutical nanosolids.

The phase transitions that occur upon heating or cooling an amorphous sample can be classified into first and second-order transitions. First-order phase transitions involve latent heat, i.e., release or absorption of energy. Such transitions occur during crystallization (Tc) of an amorphous sample or melting (Tm) of crystalline material. Figure 2.8 DSC thermograms, illustrating the release of energy due to crystallization or absorption of energy due to melting as an exothermic or endothermic peak (Milne *et al.*, 2015). Figure 2.8 also shows that when no physical or chemical change occurs within the sample then there is neither a temperature change nor input energy to maintain an isotherm. However, when phase changes

occur then latent heat suppresses a temperature change and the isothermal energy required registers as an electrical signal generated by thermocouples. Crystalline transitions, fusion, evaporation and sublimation are obvious changes in state which can be quantified (Aulton, 2001).

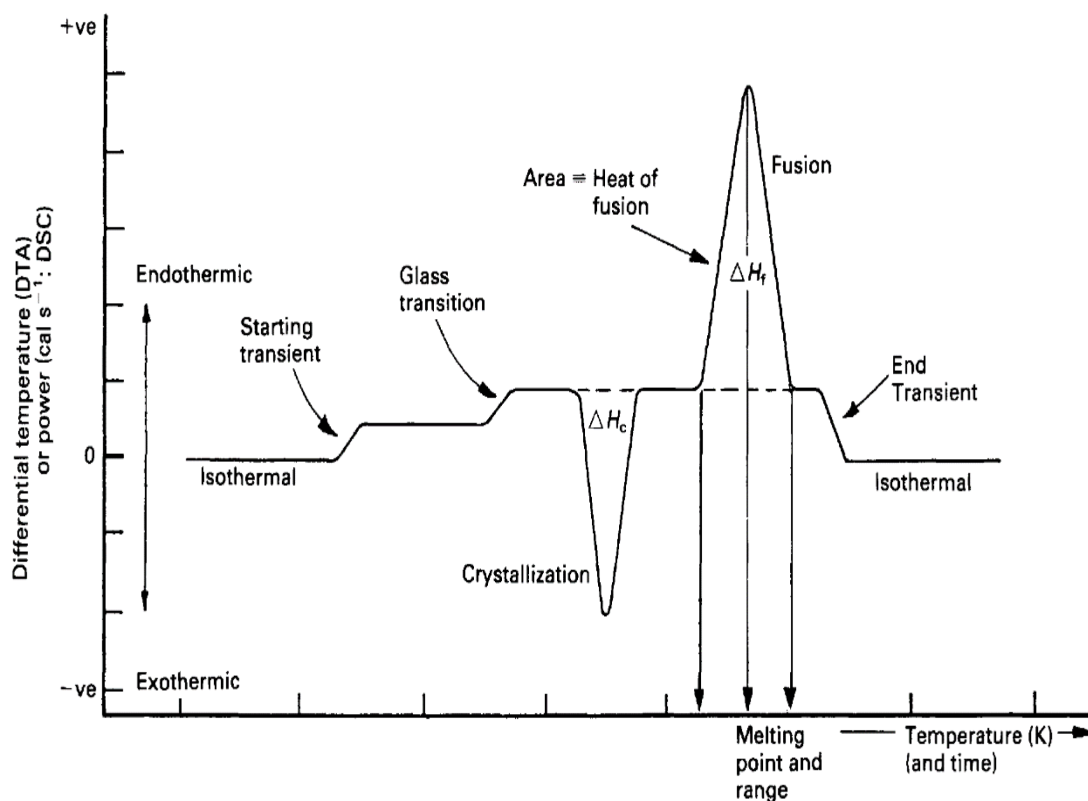


Figure 2.9: Schematic differential scanning calorimeter thermogram (Aulton, 2001)

In second-order phase transitions such as that at the glass transition temperature (*Tg*), there is no release or absorption of energy, but rather represents a step change in heat flow in the DSC thermogram (Milne *et al.*, 2015).

2.3.2.2 Infrared spectroscopy (IR)

Infrared spectroscopy (IR) is used extensively in pharmaceutical analysis for fingerprint identification of a drug molecule and proof of its structure. In addition, IR is known to be the principal method for studying the molecular properties of a compound in its solid-state. The analysis of different polymorphs with IR is

complementary to other techniques being used to characterize polymorphic properties. IR is also becoming increasingly efficient in the quantitative analysis of polymorphs and is therefore utilized to establish polymorphic purity (Gohil *et al.*, 2011).

The basic principle of IR relates to measuring the vibrational modes of the bound atoms of the solid particles, and therefore the molecules' vibrational motions are fully analyzed and measured. The energy coupled with IR can be categorized into three regions, i.e. the near-IR (20,000 – 4,000 cm^{-1}), mid-IR (4,000 - 400 cm^{-1}) and far-IR (400 - 50 cm^{-1}) regions. The absorption is generally measured within the range of 4,000 - 400 cm^{-1}. Absorption bands in the mid-IR region between 3,500-3,000 cm^{-1} (also referred to as the OH stretching region) represent OH-groups, which may result from the presence of water molecules or hydrogen bonds (Odendaal *et al.*, 2012).

Notable spectral differences are observed between the amorphous and the crystalline species The intensity of the peaks is directly proportional to the amount of the species present (less intense peaks suggest more of the amorphous phase is present). IR spectroscopy is not as widely used for quantification purposes as Near Infrared (NIR) spectroscopy due to problematic sample preparation, which can be a major source of error – including, but not limited to: polymorphic transformations at pressure application, small penetration depth, or a chemical reaction with a carrier (sodium 26 bromide powder or nujol), found to cause polymorphic transformations (Katarzyna, 2018). The broadening of the bands indicates the formation of an amorphous form, and loss of long-range order is depicted in Figure 2.10 for amorphous sodium taurocholate (NaTC).

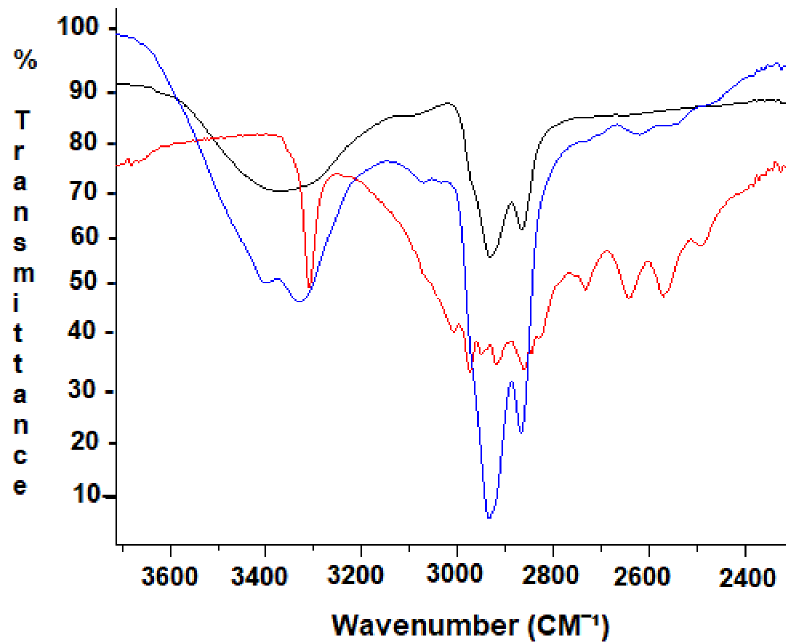


Figure 2.10: IR spectra of Mefenamic acid (commercial form, red), amorphous NaTC (blue), and co-amorphous Mefenamic acid/NaTC (black) (Katarzyna, 2018).

2.3.2.3 X-ray powder diffraction (XRPD)

X-ray powder diffraction (XRPD) is a rapid analytical technique primarily used to identify crystalline substances and can provide information on unit cell dimensions (Brittain *et al.*, 1995). This technique is useful for distinguishing between solid-state forms of a bulk drug substance and characterizing solid-state changes (e.g., distinguishing between polymorphs, hydrates, and solvates and characterizing phase transition between them). It is also used for characterizing changes in the drug substance in a solid-state as it exists in a matrix of a formulation, for example, a change from a crystalline to an amorphous form or hydration, dehydration, etc. (Palermo, 2001).

Typically, the occurrence of a non-crystalline (amorphous) solid form can be determined by observing the loss of the distinct XRPD peaks characteristic of crystalline order and the appearance of a general Bhalo'' pattern (Bates, 2006).

Amorphization generally results in broad, diffuse scattering signals, while the signals for crystal materials are sharp Bragg reflections. For a mixture of amorphous and crystalline materials, the degree of crystallinity is the ratio of integrated crystalline intensity to the total integrated amorphous and crystalline intensity (Lui, 2017). Amorphous powders give powder patterns that are typified by Figure 2.11 (b). The lack of long-range order gives a very broad hump, quite unlike a crystalline powder pattern Figure 2.11 (a) (Gilmore, 2011).

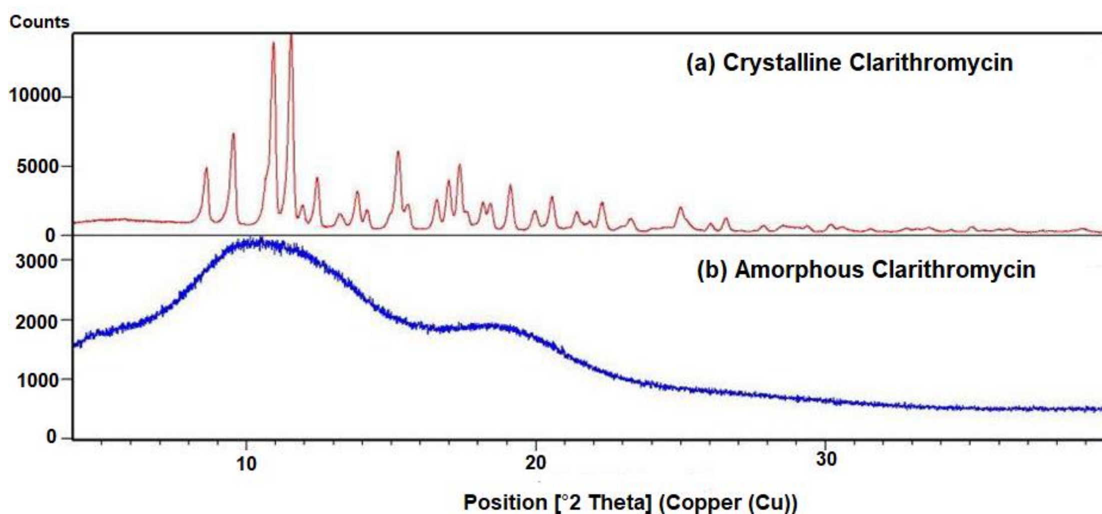


Figure 2.11: Typical amorphous and crystalline x-ray diffraction patterns (Katsidzira, 2017).

2.3.2.4 Hot stage microscopy

Hot stage microscopy (HSM) is the coupling of thermal analysis with microscopy for the solid-state characterization of materials as a function of temperature and time. HSM combines traditional thermo-analytical techniques with the latest technological advancements in imaging, such as an optical microscope, digital camera, and the use of sophisticated software for analysis of the changes in the sample through the images generated during a thermal experiment. In pharmaceuticals, HSM can be used to obtain valuable data on the morphology and solid-state properties of APIs and other pharmaceutically relevant compounds. HSM can be used to observe the crystallization process, desolvation, polymorphism, phase transitions, melting/boiling points, glass transitions, etc. (Kumar, 2020).

The melting point is a significant characteristic of a polymorph since polymorphic forms may exhibit different melting points, although the melting points of various polymorphs may also be similar. The melted products of polymorphic forms all conclude into the same liquid, which would confirm the existence of polymorphic forms. HSM provides insight into the nature of phase transformations. Processes such as nucleation (crystal growth), dehydration, and desolvation can be observed with thermomicroscopy. The desolvation of a solvate can be more accurately observed when the sample is covered with silicone gel before HSM analysis. HSM has also been used to observe crystallization at different temperatures, and samples are prepared on microscopic slides (Odendaal, 2012). HSM captured the recrystallization tendency of amorphous Clarithromycin during heating. When an amorphous form is heated above *Tg*, it transforms from a brittle, less viscous state to a rubbery/fluid-like state, as illustrated in Figure 2.12 below:

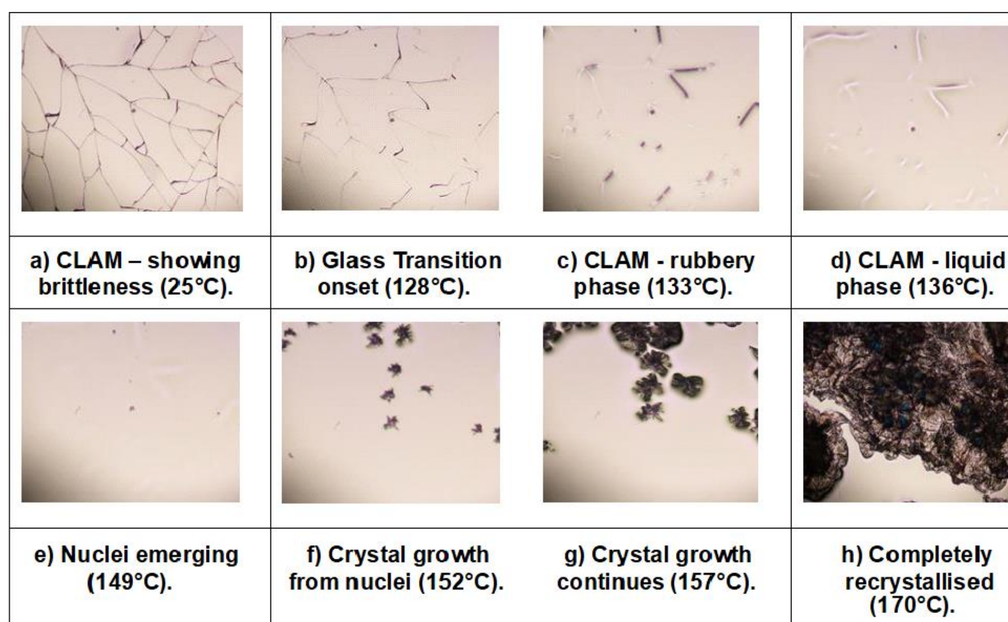


Figure 2.12: HSM temperature-dependent phase changes for amorphous Clarithromycin (Katsidzira, 2017)

2.3.2.5 Raman Spectroscopy

Raman spectroscopy is a powerful light scattering technique used to diagnose the internal structure of molecules and crystals. Also, an insight into the crystal packing

may be obtained by studying low-energy lattice vibration associated with different crystal packing arrangements. This technique has been used for a range of pharmaceutical applications, including polymorph identification, phase transition, recrystallization stability, evaluation of different manufacturing methods for solid dispersions, phase separation, and nature and extent of drug-polymer interaction (Baghel, 2016).

Raman spectroscopy is a complement to IR spectroscopy. Raman spectroscopy depends on changes in the polarizability of a molecule, while IR spectroscopy depends on changes in the dipole moment. In addition, Raman spectroscopy measures the relative frequencies at which a sample scatters radiation. This is unlike IR spectroscopy, which measures the absolute frequencies at which a sample absorbs radiation (Liu, 2017). Figure 2.13 shows two significant differences between the crystalline and amorphous form of Cimetidine whereby the crystalline ha separated Raman peaks, and the amorphous form has one halo peak in the range of 710-810 cm^{-1}.

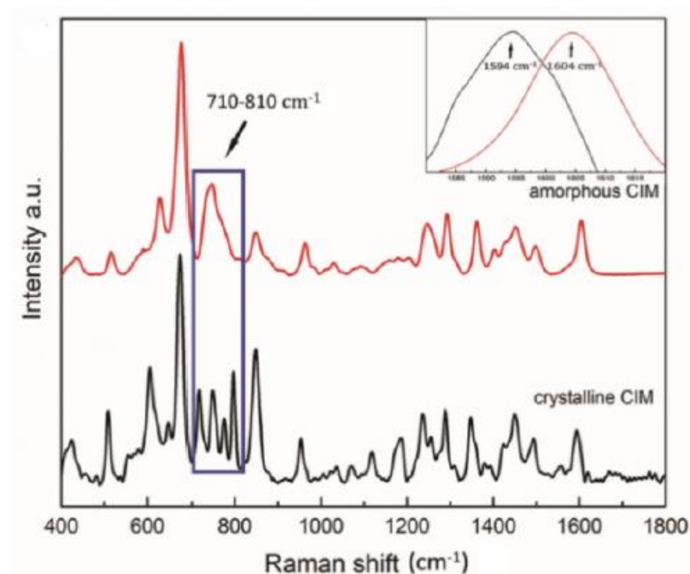


Figure 2.13: Raman spectra of neat crystalline and amorphous Cimetidine (Lui, 2019).

2.3.3 Stabilisation of Amorphous Solids

Given the inherent instability of amorphous solids with respect to crystallization, the current general strategy employs appropriate polymeric matrices to stabilize the amorphous state by inhibiting the crystallization of the drug. Thus, a critically important factor toward the successful development of formulations containing amorphous drugs involves kinetic stabilization of the amorphous state of the drug below the glass transition temperature (*Tg*) (Gao, 2008).

Pure amorphous drugs are rarely used in formulation development because of their inherent physical or chemical instability. However, the solubility advantage of these systems can be retained by devising effective strategies to "kinetically stabilize" amorphous APIs. This resulted in the development of amorphous solid dispersions (ASDs) products (Baghel, 2016).

The aim of stabilization of amorphous solids is multifaceted, including:

(i) The stabilization of labile biomolecules (e.g., proteins and peptides) through additives.

(ii) The prevention of crystallization of excipients that must remain amorphous for their intended functions.

(iii) The specification of appropriate storage temperatures to achieve acceptable shelf life.

(iv) The prevention of chemical degradation and microbial growth through anti-oxidant, pH buffer, preservatives (Yu, 2001).

The chemical and physical stability of amorphous pharmaceutical materials is controlled by the same basic factors for crystalline materials (molecular structure, purity, molecular orientation, and molecular mobility). For any sample of a given molecular structure and purity, there will be more possible molecular orientations occurring in an amorphous sample than in a crystalline sample. Thus many more different types of chemical and physical transformations could potentially take place. At a given temperature, the molecular mobility in an amorphous material will also

be significantly higher than in any of the corresponding crystalline forms, and this can give rise to a greater chemical and physical reactivity in the amorphous sample (Vranic, 2004).

Several methods have been developed to improve the solubility and bioavailability of poorly water-soluble drugs, such as solid dispersion (SD), complexation, lipid-based systems, micronization, nanonization, and co-crystals (Tran, 2019).

2.4 CLARITHROMYCIN

The name “macrolide” covers a family of different antibiotics produced from fungi of the genus Streptomyces and some bacteria such as Arthrobacter spp. Construction of macrolides is based on the large macrocyclic lacton ring, the activity of which is due to the presence of macrolide ring containing one or more deoxy sugar (usually cladinose-neutral sugar and desosamine-amino sugar). Lactone rings usually consist of 14, 15, or 16 members (Kwiatkowska, 2012). They have excellent tissue penetration and antimicrobial activity, mainly against Gram-positive cocci and atypical pathogens (Kanoh, 2010).

The first macrolide, erythromycin, was discovered in the early 1950s. The main structural component of this molecule is a large lactone ring to which glycosidic bonds attach amino and neutral sugars. It consists of a macrocyclic 14-membered lactone ring attached to two sugar moieties (Zuckerman JM, 2004). To address the limitations of erythromycin, like chemical instability, poor absorbance, and bitter taste, newer 14-, 15-, and 16-membered ring macrolides such as Clarithromycin and the azalide, Azithromycin, have been developed (Van Hoek, 2011).

Macrolides include erythromycin and related substances, Azithromycin, Clarithromycin, dirithromycin, roxithromycin, flurithromycin, josamycin, rokitamycin, kitasamycin, mycinamycin, mirosamycin,oleandomycin, rosaramicin, spiramycin,

and tylosin. Macrolides are reported to be produced by Streptomyces Venezuela (Chaudhary, 2013).

Clarithromycin is a semisynthetic erythromycin-derivative, a macrolide antibiotic. It exerts its antibacterial action by binding to the 50S ribosomal subunits of susceptible bacteria with subsequent suppression of protein synthesis. As a result, it is highly potent against a wide variety of aerobic and anaerobic Gram-positive and Gram-negative organisms, including Mycobacteria, Helicobacter, Mycoplasma, Chlamydia, Legionella, Clostridia & Staphylococci (El-Mahmoudy, 2016).

2.5 MODE OF ACTION OF CLARITHROMYCIN

Clarithromycin exerts its antibacterial effects by reversibly binding to the 50S subunit of the bacterial ribosome, as illustrated in Figure 2.14. This interaction inhibits RNA-dependent protein synthesis by preventing transpeptidation and translocation reactions (Zuckerman JM, 2004). It is believed that the antimicrobial activity of clarithromycin appears to be enhanced by the formation in vivo of the micro-biologically active 14-hydroxy metabolite (Peters DH, 1992). At lower concentrations, clarithromycin displays a considerable anti-inflammatory action, inhibiting interleukin-1 (IL-1) production by murine peritoneal macrophages, lymphocyte proliferation, and lymphocyte transformation of mutine spleen cells (Van Eeden, 2012).

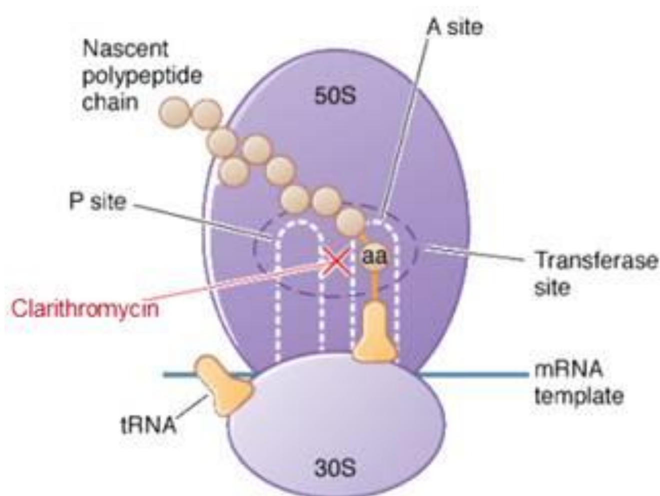
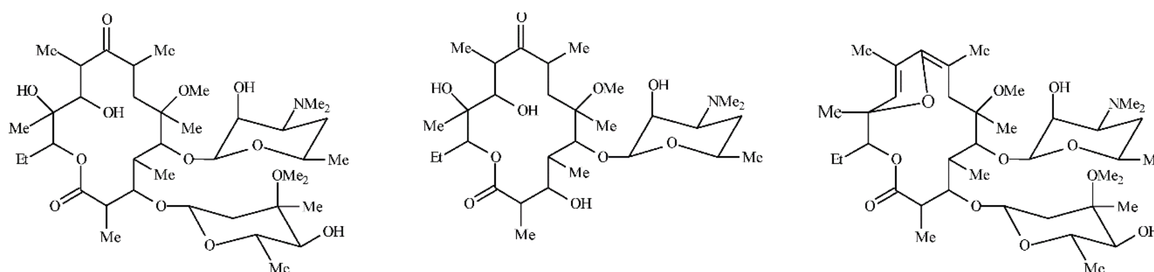


Figure 2.14: Clarithromycin mechanism of action (Nipa, 2004).

2.6 STRUCTURAL ASPECTS OF CLARITHROMYCIN

Clarithromycin (6-O-methylerythromycin) is synthesized by substituting a methoxy group for the C-6 hydroxyl group of erythromycin. This substitution creates a more acid-stable antimicrobial and prevents the degradation of the erythromycin base to the hemiketal intermediate. The increased acid stability of clarithromycin results in improved oral bioavailability and reduced gastrointestinal intolerance (Zuckerman, 2004). Clarithromycin has poor aqueous and pH-dependent solubility with dissolution rate-limited absorption corresponding to Biopharmaceutics Classification System (BCS) Class II. The drug undergoes rapid degradation in strong acid to form decladinosyl clarithromycin and clarithromycin 9,12-hemiketal, whose structures are shown in Figure 2.15 (Manani, 2017).



Clarithromycin Decladinosyl clarithromycin Clarithromycin-9,12-hemiketal

Figure 2.15: Chemical structure of clarithromycin and degradation products (Manani, 2017)

2.7 PHYSICO-CHEMICAL PROPERTIES

Clarithromycin is a white to off-white crystalline, odourless powder (Van Eeden,2012). It has a molecular weight of 747.95g/mol (USP,2011). Practically insoluble in water; slightly soluble in dehydrated alcohol, methyl alcohol, and acetonitrile; soluble in acetone; slightly soluble in phosphate buffer at pH values of 2 to 5. It is stored in airtight containers (Sweetman, 2005). According to the literature, clarithromycin exists in five forms. Form 0 exists as a solvate, form I is a metastable form, form II is the stable form, form III is a solvate of acetonitrile, and

form IV is a hydrate. The stable form II is used in formulations currently on the market (Van Eeden, 2012).

2.8 PHARMACOKINETICS OF CLARITHROMYCIN

The pharmacokinetics of clarithromycin is non-linear and dose-dependent; High doses may produce disproportionate increases in peak concentrations of the parent drug due to saturation of the metabolic pathways (Sweetman, 2005).

2.8.1 Absorption

Clarithromycin is more acid-stable and has greater oral bioavailability (Zuckerman, 2004). It is readily absorbed through the gastrointestinal tract, the meantime for reaching maximum serum levels (tmax), being within the range of 1.8 to 2 hours. The onset of absorption is slightly delayed when administered with food, while a slower formation rate of the active 14-hydroxy metabolite is also noted. Despite this, the bioavailability appears to be increased, which suggests that clarithromycin can be taken orally without regard to the timing of its administration with food (Van Eeden, 2012). Peak concentrations of clarithromycin and its metabolite, 14-hydroxyclarithromycin, are reported to be about 900 and 600 nanograms/ml, respectively, following a single 250 mg dose by mouth; at a steady-state, the same dose given at 12 hours as tablets produce a peak concentration of clarithromycin of about 1microgram/ml (Sweetman, 2005).

2.8.2 Bioavailability

After receiving a single oral dose of 500 mg of clarithromycin, the absolute availability in healthy fasting subjects is approximately 55%. Peak serum concentrations of 2.51 μg/ml are reached within two hours of administration and display a long serum half-life of 4.9 hours (Peters & Clissold, 1992). The active 14-hydroxy metabolite, formed by the rapid first-pass metabolism, takes two hours to

reach the maximum peak serum concentrations (2.1 µg/ml) after administering a single 500 mg dose (Van Eeden, 2012).

2.8.3 Distribution

Clarithromycin and its 14-hydroxy metabolite are well distributed into most body tissue and fluids, having extensive diffusion into saliva, sputum, lung tissue, epithelial lining fluid, tonsils, nasal mucosa, and middle ear fluid. In addition, both compounds can penetrate rapidly human neutrophils and alveolar macrophages. These high intracellular concentrations allow tissue concentrations to exceed simultaneous serum concentrations; i.e., lung tissue and fluids concentrations may be up to 30-fold higher. In adults, the mean apparent volume of distribution (Vβ/F) ranges between 191 and 306 L (Van Eeden, 2012).

2.8.4 Metabolism and Elimination

In vitro studies have shown that the major metabolic pathway for clarithromycin is 14-hydroxylation and N-demethylation by the cytochrome P-450 (CYP) 3A subfamily of microsomes (Van Eeden R, 2012). Clarithromycin is metabolized in the liver and the stomach. Approximately 22% of an oral dose is recovered as the parent compound, 18% in the urine, and 4% in feces. Clearance of clarithromycin decreases with increasing dose, possibly because of saturable hepatic metabolism (Shalf, 2015).

2.9 SPECTRUM ACTIVITY

Clarithromycin is highly potent against a wide variety of aerobic and anaerobic Gram-positive and Gram-negative organisms, including Mycobacteria, Helicobacter, Mycoplasma, Chlamydia, Legionella, and Clostrid-ia (El-Mahmoudy A, 2016). Azithromycin and clarithromycin are generally inactive against methicillin-resistant staphylococci. Staphylococci and streptococci that are resistant to erythromycin are also resistant to azithromycin and clarithromycin (Zuckerman JM,

2004), although Sweetman *et al* (2005) suggest that clarithromycin is more active than erythromycin against susceptible streptococci and staphylococci in vitro. Clarithromycin is also reported as more active than azithromycin and erythromycin against some mycobacteria, including Mycobacterium avium complex and against mycobacterium leprae. It is reported to have some *in vitro* activity against the protozoan toxoplasma gondii and may have some activity against cyptosporidia (Sweetman, 2005). Azithromycin and clarithromycin have similar or increased in vitro activity against genital pathogens compared with erythromycin. Neisseria gonorrhoeae, Haemophilus ducreyi, and Ureaplasma urealyticum are susceptible to both antibiotics, with azithromycin demonstrating better activity as evidenced by a lower minimum inhibitory concentrations (Zuckerman, 2004).

2.10 SAFETY OF CLARITHROMYCIN

Macrolide antibiotics are regarded as safe, especially for treating children. Azithromycin and Clarithromycin are used in the treatment of common paediatric infections (Abu-Gharbeih, 2004). Clarithromycin has been FDA-approved for the treatment of children six months of age and older for acute otitis media, community acquired pneumonia, pharyngitis/tonsillitis, skin and skin structure infections, and acute bacterial sinusitis. For the management of mycobacterium avium complex, clarithromycin has been studied in children 20 months of age and older. Clarithromycin should not be used in pregnancy unless the potential benefit justifies the potential risk to the fetus (Macrolides, 2010).

2.11 DOSAGE OF CLARITHROMYCIN

Clarithromycin is given by mouth or by intravenous infusion. Usual doses in adults are 250mg twice daily by mouth, increased to 500mg twice daily if necessary, in severe infections. Modified-release tablets allowing a once-daily use are available in some countries. A course is usually for 7 to 14 days. Children may be given 7.5mg/kg twice daily for 5 to 10 days. Doses may need to be reduced in patients with severe renal impairment (Sweetman, 2005).

2.12 SOLUBILITY AND DISSOLUTION ENHANCEMENT OF CLARITHROMYCIN

The apparent solubility advantage of amorphous substances stems from their large excess of internal energy and is predicted to show a solubility increase of 1.1 to 1000 fold with respect to the crystalline counterpart. Therefore, in principle, all amorphous drugs should be more effective due to enhanced apparent solubility and bioavailability. However, aside from a higher energetic state of the amorphous phase, other attributes contribute to the higher bioavailability, and these are reduced particle size and increased surface area, and hygroscopicity of the amorphous phase. In solution, the amorphous phase, hydrates rapidly and acquires solubility in an aqueous medium. However, unwanted recrystallization may occur as water is a known plasticizer and thus accelerates the process (Katarzyna, 2018).

Differences in solubility may give rise to a difference in dissolution rates, which could affect bioavailability. 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. As a result, the phase changes from molecules of solid to molecules of a 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 (Katsidzira, 2017).

Over the years, different techniques have been studied to enhance the solubility and dissolution of Clarithromycin. Techniques like co-grinding, Preparation of Nanosuspensions using Sonoprecipitation Technique, Ternary Ground Mixtures: Nanocrystal Formulation and Solid Dispersion and Effervescence assisted fusion technique has been proven to increase dissolution and solubility of clarithromycin, See table 2.1.

Table 2.1: Techniques used to enhance solubility and dissolution of Clarithromycin

Technique used	Solubility	Dissolution	Stability	Reference	Remarks
Co-grinding	0.678mg/cm^{2}min	An increase of 267.11% was observed by co–grinding with hydrophilic carriers in a 1:2 ratio.	N/A	Khan *et al,* 2018	Increases amorphousness; leading to enhanced dissolution rate
Preparation of Nanosuspensions using Sonoprecipitation Technique	N/A	Almost 100% of nanoparticles were dissolved instantly. In contrast, it took 15 minutes for 70% and 1 hour for 100% of the corase drug particles.	Formulation with HPMC stabilized for 14 days at room temperature.	Esfandi *et al*, 2014	Increased dissolution rate due to greater surface area. Pharmaceutical contaminations and degradations during grinding processes.
Ternary Ground Mixtures: Nanocrystal Formulation	found significantly higher than the ubtreated drug.	Solubility of various mixtures ranged from 3 to 5 folds higher than the untreated drug.	Dissolution remained the same after 3 months of accelerated stability.	Shahbazinaz *et al*, 2013	Micronisation is not sufficient to lead to a high bioavailability. It is suitable for nanocrystals with mean particle size below 1 µm.
Solid Dispersion and Effervescence assited fusion technique	2.3mg/ml	N/A	Stability studies indicated that the optimized formulation is stable	Mishra *et al*, 2016	Decreased particle size

2.13 CONCLUSION

Clarithromycin belongs to class II of the BCS, having low aqueous solubility and high lipid permeability, resulting in a dissolution rate-limited absorption and low bioavailability. Pharmaceutical materials exist in two solid forms, namely the crystalline solid-state and the amorphous solid state. Consequently, there is a growing interest in amorphous materials, which improve the solubility, dissolution, and subsequently bioavailability of compounds with poor aqueous solubility.

For drugs with poor solubility and high *in vivo* permeability like clarithromycin, it is important to develop a formulation in the amorphous form that would improve their poor bioavailability and subsequently improve efficacy. Of various ways to improve solubility and bioavailability, the development of formulations in amorphous form has been a preferred method. Quench cooling technique of preparing amorphous solids is the most preferred method as it yields amorphous solid that is more stable, and the process is fast. Although amorphous solids are metastable, several studies have proved that when formulated with proper excipients, they become more stable in stress conditions during handling and storage. Therefore, it is imperative to study the physicochemical properties of amorphous raw materials when mixed with different excipients in the quest to improve solubility and bioavailability. Hence the current study seeks to formulate and prepare a solid dosage form of amorphous clarithromycin for enhanced dissolution and bioavailability.

CHAPTER 3
MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter deals with the materials, apparatus, reagents, methodology, and analytical techniques used to prepare and characterize amorphous clarithromycin. The methodology also includes a description of the analytical techniques used to evaluate the drug-excipient compatibilities, manufacturing method used for producing clarithromycin tablets, Physico-chemical properties, *in vitro* dissolution profiles, and stability studies of the developed formulations.

3.2 MATERIALS

Clarithromycin raw material was supplied by DB Fine chemicals (Johannesburg, South Africa). In addition, the following materials were donated by Ascendis Health Supply Chain: Microcrystalline cellulose (Avicel® PH200), (JRS Pharma LP, Rosenberg Germany), Lactose (Wassenburg, Germany), Magnesium Stearate (Kirsch Pharma GmbH, Salzgitter, Germany), and Talc (Imerys Talc, Italy).

3.3 PREPARATION OF AMORPHOUS CLARITHROMYCIN

Various methods used to prepare amorphous forms include: quenching of melts, rapid precipitation by anti-solvent addition, freeze-drying, spray drying, compression, rapid solvent evaporation, lyophilization, and many others. In this study, the quench cooling of the molten drug was used as a preparation method for the amorphous clarithromycin (Katsidzira, 2017).

Clarithromycin powder was evenly sprinkled on an aluminium foil pan in a thin layer, where after it was placed in a conventional hot plate that had been pre-heated at 240˚C. This set temperature was higher than the determined melting point (217˚C - 220˚C) of clarithromycin. Therefore, the crystalline clarithromycin powder was allowed sufficient time to dehydrate and melt. The melt was then removed from the

oven and allowed to cool to room temperature. During cooling, the melt transformed into a solid (then characterized as a supercooled solid) that was presented as a glass. Finally, the amorphous clarithromycin was removed from the foil paper and characterized to ensure the amorphous habit thereof.

3.4 CHARACTERIZATION OF AMORPHOUS CLARITHROMYCIN

Several methods were employed in order to characterize the amorphous clarithromycin prepared. These techniques included in this study are; Differential Scanning Calorimetry (DSC), Hot-Stage Microscopy (HSM), Infrared Spectroscopy (IR), and X-Ray Powder Diffraction (XRPD).

3.4.1 Differential Scanning Calorimetry (DSC)

In this study, a Shimadzu (Kyoto, Japan) DSC-60 instrument was used to record the DSC thermograms. The instrument was calibrated using an ultra-pure indium standard, having a melting point of 156.4°C. Samples (3 - 5 mg) were weighed and sealed in aluminium crimp cells with pierced lids. The samples were heated from 25°C to 300°C with a heating rate of 10°C/min and 35 ml/min nitrogen gas purge. The onset temperatures of the thermal events were reported. 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.

3.4.2 Hot-Stage Microscopy (HSM)

Hot stage microscopy was used to compare the morphology of different solid forms of clarithromycin. HSM analyses for this study were performed using a Nikon Eclipse E4000 40 microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan). A Metratherm 1200d temperature regulator (Metratherm, Germany), coupled with a Leitz regulator (Leitz, Germany), was connected to the microscope to enable heating of the hot stage. Samples on the microscopic slide were coated with silicone oil before HSM analysis. Examination of samples was done at 10x magnification.

Images were taken at increasing temperatures, starting at 25˚C up to 300˚C, or even higher if necessary. Each prepared sample was individually observed and screened under the above conditions, while taking images and recording the corresponding temperatures.

3.4.3 Infrared Spectroscopy

IR-spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer (Shimadzu, Japan) over a range of 400 - 4000 cm-1. Potassium bromide (KBr) was used as background for sample measurements. Each sample was disseminated in a powdered KBr matrix, and the IR spectrum was measured in a reflectance cell using DRIFTS. The transmittance (percent) is represented on the vertical axis, while the wavenumber is represented on the horizontal axis (cm-1).

3.4.4 X-Ray Powder Diffraction (XRPD)

During the analysis of x-ray powder diffraction, samples were evenly distributed on a zero background sample holder, and then placed into the sample holder. X-ray powder diffraction patterns were obtained using a Malven PANalytical Empyrean diffractometer (Malven, United Kingdom). The measurement conditions are represented in Table 3.1.

Table 3.1: The measurement conditions of X-ray powder diffraction

Measurement Conditions	
Target	Cu
Voltage	40Kv
Current	30mA
Divergence Slit	2mm
Antiscatter Slit	0.6mm
Detector Slit	0.2mm monochromator
Scanning Speed	2° /min (step size, 0.025°; step time, 1.0 sec).

3.5 ASSAY

A Shimadzu (Kyoto, Japan) UFLC (LC-20AD) chromatographic system consisting of a SIL- 20AC auto-sampler fitted with a sampler cooler, a UV/VIS Photodiode Array detector (SPDM20A), and an LC-20AD solvent delivery module was used to determine the assay of crystalline and amorphous clarithromycin tablets. The method used in this study is pre-validated and accepted in a peer-reviewed journal as published by Katsidzira *et al* (2016).

3.5.1 Mobile phase preparation

A mobile phase consisting of 0.1 M potassium dihydrogen orthophosphate (Batch number: MD0M980421, Saarchem, Johannesburg), pH adjusted to 6.0 with 0.1M HCl, mixed with acetonitrile in a ratio of 50:50 % v/v was used. A Luna C18, 150 × 4.6 mm column was used, with an injection volume of 10 µl and flow rate set to 0.5 mL/min. The wavelength was set to 210 nm.

3.5.2 Preparation of standard solution

Stock solution for clarithromycin was prepared by accurately weighing 100 mg of the raw material and diluting it to 100 mL with the mobile phase. This resulted in a stock solution of 1000 µg/ml. Aliquots of these stock solutions were then diluted with mobile phase to obtain standard solutions with concentrations ranging from 50 to 1000.0 µg/ml. Each standard solution was filtered using a 0.45 µm PVDF filter into HPLC vials. These standard solutions were injected, and a calibration curve was constructed from the area under the curve (Annexure A). A regression (r2) of 0.9991 was obtained for clarithromycin. This HPLC method was used for the analysis of all samples during solubility studies of amorphous clarithromycin.

3.5.3 Preparation of sample solutions

Ten amorphous and crystalline clarithromycin tablets were selected randomly, weighed, and crushed in a mortar and pestle. A quantity of powder equivalent to the 500 mg of the clarithromycin was weighed, and the powder was transferred into a 100 ml volumetric flask and dissolved in about 50 ml of mobile phase and placed in a Sonorex® Digital 10 Pm sonicator bath (Bandelin Electronics, Berlin, Germany) for 20 minutes. The flask was shaken regularly to avoid the formation of lumps and diluted to volume with the mobile phase. Ten ml of the above solution was transferred into an A-grade 100 ml volumetric flask (Merck, Johannesburg, South Africa) using a pipette, diluted to volume with mobile, and transferred into HPLC vials by filtering through 0.45 µm membrane filters.

3.6 COMPATIBILITY STUDIES

Compatibility studies of a drug with various excipients are important in the pre-formulation phase of all dosage forms. Potential physical and chemical interactions between a drug and excipient can affect the stability and bioavailability of the specific drug (Corvi, 2006).

Compatibility studies were done by preparing physical mixtures of amorphous clarithromycin and the chosen excipients. The mixtures were prepared by mixing the drug and individual excipients in a mortar and pestle to obtain a 1:1 w/w ratio. The mixtures and the single compounds were analyzed by DSC, IR spectroscopy, and XRPD.

The results of the above techniques of the mixtures were compared to determine if any incompatibilities exist. In this study, a change in peak temperature or peak broadening of the clarithromycin melting peak was considered as a low degree of incompatibility, whereas disappearance of melting peaks for both the drug and excipients or presence of new peaks were hypothesized as a high degree of

incompatibility. APIs Diffraction and spectrum patterns from the XRPD and IR were compared with the patterns of the sample (API + excipients).

3.7 *IN VITRO* DISSOLUTION STUDIES

A dissolution bath (VanKel700®, USA) was used for dissolution testing. USP apparatus 2 (paddle) was set at 37°C ± 0.5°C, with a rotational speed of 100 rpm; a 900 ml dissolution medium (distilled water) was added to each dissolution vessel. Of each solid-state form, amorphous clarithromycin and commercial clarithromycin tablets (Crystalline form) were used. Aliquots of dissolution media (5 ml) were withdrawn at 5, 10, 15, 30, 60, 120, 240, 360, and 480 minutes and filtered using 0.45 µm PVDF filters. Since these dissolution studies are designed to evaluate the solution-mediated phase transformation of amorphous clarithromycin tablet formulations, no dissolution media replacement was done. The samples were then analyzed using the same HPLC method as for the assay (3.5)±. The data was analyzed by calculating the amount of drug released and the cumulative percentage of drug released at different time intervals.

3.8 STABILITY STUDIES

Stability tests are significant as tablet formulations containing the active pharmaceutical ingredient (API) in any high-energy amorphous form are expected to enhance solubility, dissolution rate, and consequently, oral bioavailability of poorly water-soluble drugs. However, the amorphous solid formulations are thermodynamically unstable and are therefore susceptible to recrystallization upon storage (Surampalli, 2014).

Amorphous clarithromycin tablets inside HDPE containers were stored in a Binder™ climatic chamber (Accelerated Stability Tester) while exposed to elevated temperatures and humidity of ±40°C and ±75% RH, respectively. Other amorphous clarithromycin tablets were stored in a Binder climatic chamber (Normal Stability Tester) exposed to temperature and humidity of 25°C and 60% RH.

Samples were taken every month for six months. The samples were subsequently analyzed to determine the effects of temperature and relative humidity on the stability of amorphous clarithromycin tablets. In addition, the compressed tablets were evaluated in terms of appearance, weight variation, thickness, diameter, hardness, friability, disintegration, *in vitro* drug release, and assay, using the specifications as stated in the United States Pharmacopoeia (USP, 2014) and compared with initial results to determine the changes.

3.9 MANUFACTURING OF CLARITHROMYCIN TABLETS

A total weight of 500g amorphous clarithromycin was prepared through the quench cooling process by heating crystalline clarithromycin raw material to the melting point and subsequently cooling it. The cooled crystals were made fine by crushing the crystals with a pestle in a mortar. The fine powder of amorphous clarithromycin was weighed and mixed with chosen excipients. Dry granulation and direct compression are the methods of choice for this study. The tablets were compressed using a 12 mm punch on a Modul™D-tablet compression machine (Frankfurt, Germany). Excipients were included in the formulation to improve the flowability and stability of amorphous clarithromycin for effective tableting.

3.10 EVALUATION OF CLARITHROMYCIN FORMULATIONS

During the development of amorphous clarithromycin tablets, all the formulations were tested for both pre and post-compression parameters such as appearance, weight variation, thickness, diameter, hardness, friability, *in vitro* drug release, and assay using the methods described below, sections 3.5 and 3.8 respectively (USP, 2019).

3.10.1 Appearance

Tablets were evaluated for organoleptic properties such as colour and shape and the presence of lamination, cracking, capping, sticking, picking and chipping.

3.10.2 Weight variation

Twenty tablets of each batch were selected randomly and weighed individually on an analytical balance. The tablets were dusted with a brush before weighing. First, the average tablet weight and standard deviation (SD) were calculated. Then, the individual weights of the tablets were compared to the average weight. Tablets pass the test if not more than two tablets fall outside the percentage limit, and if no tablet differs by more than two times the percentage limit (USP, 2019:). Table 3.2 was used as guide for acceptable percentage weight variation. 5% weight variation will be used as the desired weight of the formulated tablet is more than 324mg.

Table 3.2: Specification for weight variation of solid dosage (USP, 2019)

Average Weight Of Tablet	Percentage Weight Variation
130 mg or less	10 %
More than 130 mg and less than 324 mg	7.5 %
324 mg or more	5 %

3.10.3 Thickness and diameter

Ten tablets were randomly selected, and their thickness and diameter were measured individually using an ERWEKA® TBH 425 hardness and thickness tester (Langen, Germany). The average and relative standard deviations (%RSD) were calculated.

3.10.4 Hardness

The resistance of tablets to capping, abrasion, or breakage under conditions of storage, transportation, and handling before usage depends on its hardness. Hardness, which is now more appropriately called crushing strength determinations, are made during tablet production and are used to determine the need for pressure adjustment on a tablet machine. If the tablet is too hard, it may not disintegrate in the required period to meet the dissolution specifications; if it is too soft, it may not

withstand the handling during subsequent processing such as coating or packaging and shipping operations (Bhadange *et al.*, 2015). The hardness of 10 randomly selected tablets was determined using an ERWEKA® TBH 425 hardness tester (Langen, Germany).

3.10.5 Friability

The friability of tablets was determined using a Erweka® TA 100 friability tester (Langen, Germany). Ten tablets were dusted before weighing (initial weight) and transferred into the friabilator. The friabilator is operated at 25 rpm for 4 minutes. Then, the tablets were dusted and weighed again (final weight). The percentage friability, the percentage difference between the two readings, was calculated using equation 2. A percentage friability of less than 1% is considered acceptable (USP, 2014: 1735).

$$\% \text{ Friability} = \frac{Initial\ weight - Final\ weight}{Final\ weight} \times 100 \qquad \text{(Equation 3.)}$$

3.10.6 Disintegration

The disintegration times of the tablets were determined in distilled water at 37.0 ± 2°C, using the Erweka ZT 120 disintegration testing apparatus (Langen, Germany). A disintegration test was done using 6 tablets from each batch, and the time taken for all of the six tablets to disintegrate and pass through the mesh wire was recorded. Each test was repeated in triplicate. The BP (USP, 2019) recommends that conventional tablets should be disintegrated within 15 minutes of initializing the test.

3.11 CONCLUSION

This chapter describes the preparation of the amorphous solid-state form of clarithromycin, characterization, and the pre-formulation and formulation processes involved to obtain a successful product.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

The main objective of this study was to prepare amorphous clarithromycin, then formulate it into a stable immediate release tablet dosage form. In order to successfully formulate a drug into a dosage form, it is imperative to complete comprehensive pre-formulation studies. Proper pre-formulation studies will reveal a substantial amount of information in terms of the physical and chemical properties of the drug to be formulated. Since this study focuses on the formulation of amorphous clarithromycin into tablets followed by the stability study of resulting formulations, a fair amount of attention was paid to investigating the drug compatibility in combination with different excipients. This chapter will discuss all pre-formulation tests conducted, emphasizing the stability of amorphous clarithromycin in the dosage form. During this study, a strong focus was placed upon the stability of the amorphous form of clarithromycin. The aim was to investigate whether the amorphous solid-state form of clarithromycin will remain amorphous during pre-formulation, formulation, and stability studies. During the study *in vitro* dissolution behaviour of amorphous clarithromycin was compared with that of crystalline.

4.2 PREPARATION AND PHYSICO-CHEMICAL CHARACTERIZATION

Amorphous clarithromycin was prepared through the method of quench cooling of the melt as described in Chapter 3 under 3.3. Considering the fact that this preparative method involves heating of crystalline clarithromycin until it melts, followed by the rapid cooling of the molten product, it was important to confirm the purity of clarithromycin after the preparation of the amorphous form. The purity of amorphous clarithromycin was confirmed through HPLC analysis for assay as described under 3.5. The purity was calculated to be 99.5 % ± 1.6 %. This was a confirmation that the drug remained intact during the preparation method and that

no degradation of clarithromycin occurred. Furthermore, it was significant to confirm the amorphous habit of the prepared solid-state form. This was done using DSC, IR, HSM, and XRPD analyses.

4.2.1 Differential scanning calorimetry (DSC)

The DSC thermograms were recorded on a Shimadzu DSC-50 instrument (Shimadzu, Kyoto, Japan). Samples weighing 3-5 mg were heated in sealed aluminium crimp cells as per the method described under 3.4.1. Samples were heated to a maximum temperature of 300°C. The DSC results were used for the identification of amorphous and crystalline materials. The Merck Index (2001:408) reports a melting point of 222-225°C for clarithromycin recrystallized from ethanol and a melting point of 217-220°C for needles being recrystallized from chloroform and diisopropyl ether. As shown in figure 4.1 (a), the melting point of the clarithromycin raw material was recorded as 230°C. This thermogram showed a melting endotherm at 228.6°C, with an onset at 225.57°C and an endset at 236°C. The decomposition of clarithromycin was represented by another endothermic peak at 288°C yielding a black substance in the DSC sample pan after the heating cycle is completed.

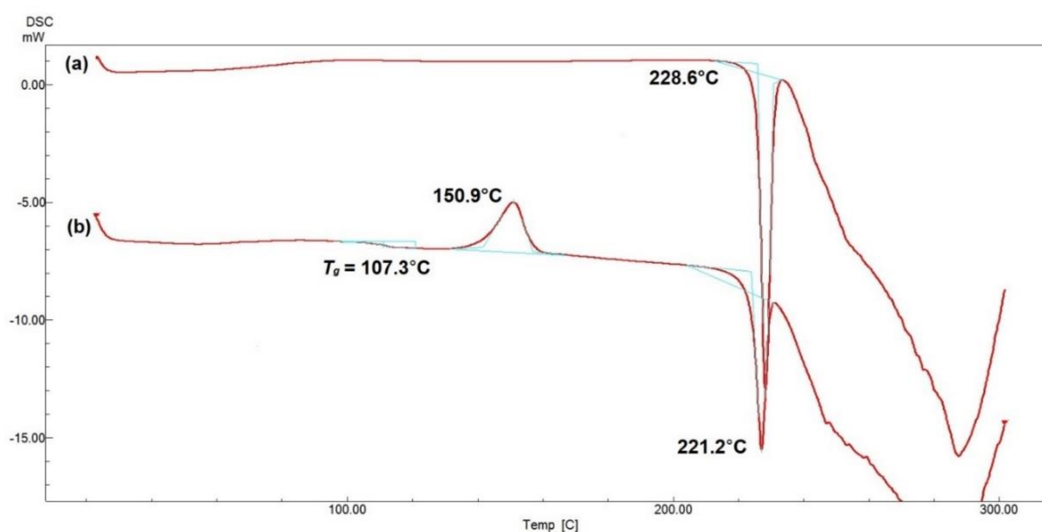


Figure 4.1: Overlay of the DSC thermograms obtained for (a) crystalline clarithromycin and (b) clarithromycin neat amorphous form.

As depicted in 4.1 (b), the thermogram of amorphous clarithromycin shows a glass transition phase (*Tg*) at a temperature of 107.3 °C, which is a characteristic feature of the amorphous materials. This is followed by a phase of recrystallization peak at 150.9 °C, followed by the melting peak at 221.2 °C. These findings are well in agreement with the reports presented in the literature chapter (figure 2.11) to confirm the formation of amorphous clarithromycin through the quench cooling process.

4.2.2 X-ray powder diffraction (XRPD)

The X-ray powder diffraction profiles were obtained at room temperature on a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2OImin (step size, 0.025": step time, 1.0 sec). Approximately 200 mg of sample was loaded into an aluminium sample holder, taking care not to introduce a preferential orientation to the crystals. The XRPD pattern of crystalline clarithromycin raw material and prepared amorphous clarithromycin raw material are shown in figure 4.2 (a) and (b) respectively.

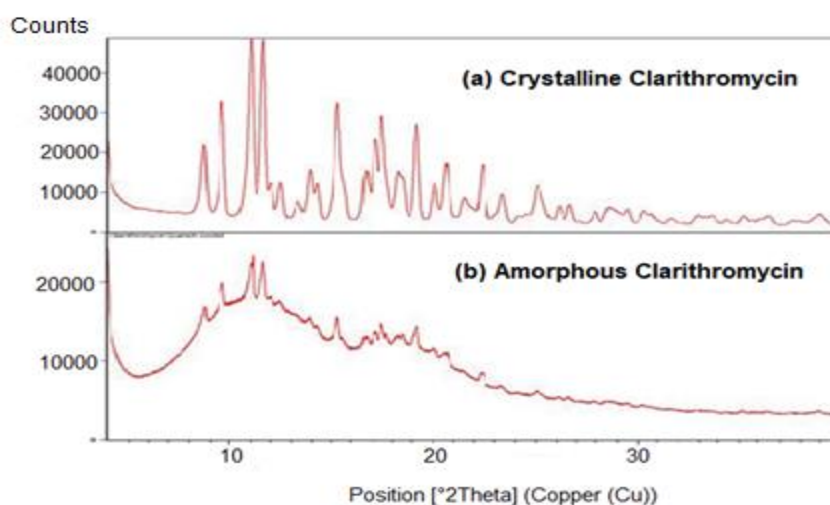


Figure 4.2 The XRPD of (a) crystalline clarithromycin raw material and (b) quench cooled amorphous clarithromycin raw material.

Crystalline clarithromycin raw material showed Bragg diffraction peaks (figure 4.2 (a), indicating proof that it exists in a crystalline form. Upon closer analysis, the XRPD diffraction pattern resembles that of clarithromycin form II reported in the literature (Katsidzira, 2017). Results correspond with the obtained DSC thermogram (figure 4.1), implying that the purchased clarithromycin exists in its crystalline Form II. For the prepared amorphous clarithromycin, the results showed a scattering halo. These results indicate that the quench cooling process resulted in an amorphous form of clarithromycin from crystalline clarithromycin raw material.

4.2.3 infrared spectroscopy (IR)

On a Nicolet Nexus 470-FT-IR spectrometer (Madison, Wisconsin, USA), the infrared spectra were recorded over a range of 400-4000 cm-I using the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) method. DRIFTS spectrometry was implemented as the sampling method of choice to eliminate the possibility of polymorphic transformations during conventional mulling, or KBr pellet sampling procedures (Van Eeden R, 2012). Samples were prepared for DRIFT spectrometry by dispersing the raw material and sample in KBr. The vibrational frequencies of both solid-state forms of clarithromycin were investigated by IR spectroscopy from 400 to 4000 cm^{-1}.

From the literature, the characteristic peaks of crystalline clarithromycin were reported in the region of 1691.64 cm^{-1} (ketone carbonyl), 1733.12 cm^{-1} (lactone carbonyl), 1412.92 cm^{-1} (N-CH3), and 3488.41 cm^{-1} (hydrogen bonding between -OH functional groups), as shown in Table 4.2. Furthermore, the FT-IR absorbance peaks of the crystalline clarithromycin raw material (figure 4.4), confirm the presence of these major peaks at the respective wavelengths indicating that the purchased raw material existed in its Form II polymorph.

Table 4.1: Characteristic absorbance bands for crystalline clarithromycin Form II (Katsidzira RM, 2017).

Energy (Wavenumber) cm^{-1}	**Functional group**
1000 - 1200	-C-O-C-Stretch
1174	Aliphatic –CH stretch.
1355 - 1412	CH_2 group
1422.55	$N-CH_3$
1691.64	C=O ketone carbonyl
1733.12	Lactone carbonyl
2781 - 3001	Alkane C-H stretch
3466.23 - 3488	Tertiary N-H stretch
3488.41	Hydroxyl -OH stretch

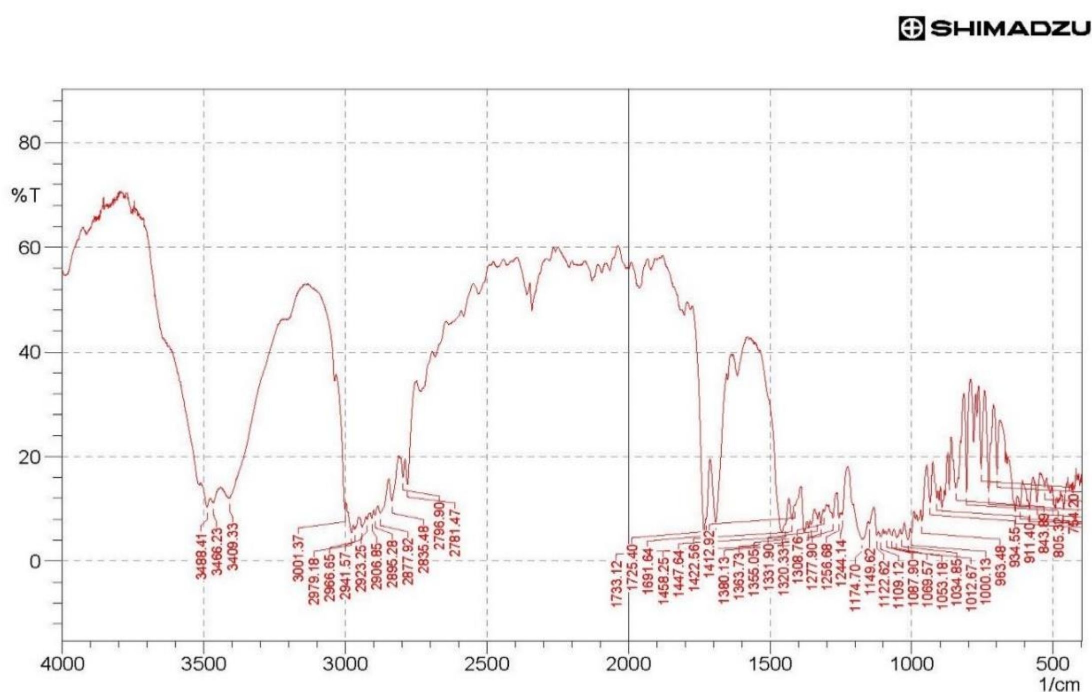


Figure 4.3: IR spectrum of the clarithromycin raw material.

The FT-IR peak positions of amorphous clarithromycin (figure 4.4 (a)) correspond with the molecular structure of the raw material but with some broader and lower intensity peaks. However, when the two are compared, there is a decrease in intensity for the $N\text{-}CH_3$ absorbance peak in the amorphous form, decrease or absence of the peak at 1600 $cm^{-1}$with the amorphous form. This is because the formation of an amorphous form causes reduction/weakening of interactions that are a function of crystal lattice packing. Also, the saturated alkane C-H stretch (in the region of 2781 – 3001 cm^{-1}) was found broadening, and the N-H stretch (3466 – 3488 cm^{-1}) broadening and moved to a higher wavenumber were observed for the amorphous form. This implies the weakening of the hydrogen bonds, thus loss of ability to form hydrogen bonds interactions, which can cause instability of amorphous forms (Fedorov *et al.*, 2001).

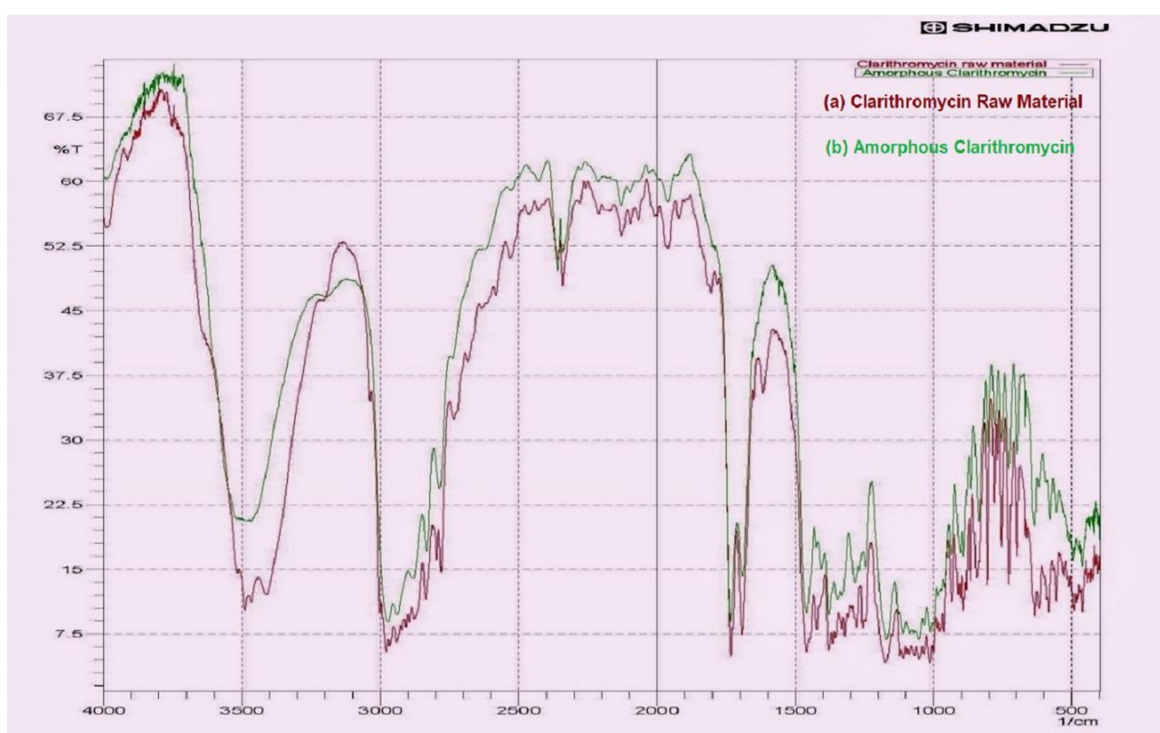


Figure 4.4: Overlay of FT-IR spectra obtained for (a) Crystalline Clarithromycin Raw material and (b) Amorphous Clarithromycin.

4.3 COMPATIBILITY STUDIES

To complete pre-formulation studies, compatibility testing must be conducted between samples of the drug and excipients in order to determine whether any reaction will occur between the different compounds. Most excipients are usually chemically inactive by nature, however, there is still the possibility of some reaction or interaction between a drug and excipients. Therefore, compatibility testing is a prerequisite to manufacturing an acceptable product without the possibility of the individual compounds significantly influencing the product's performance.

Excipients used in the study were chosen based on the previous studies to formulate solid dosage forms of macrolide antibiotics such as azithromycin (Obulapuram, 2014). Excipients were chosen to include the fillers (Emcompress®, Avicel® PH200), disintegrants (Croscarmellose sodium (Ac-Di-Sol)), binders (Lactose – Spray dried), and lubricants (Magnesium Stearate). Therefore, the screening process included excipients that could be used in the design of an appropriate formulation.

All clarithromycin/excipients physical mixtures were prepared by mixing quench cooled amorphous clarithromycin and the corresponding excipient using a mortar and pestle at 1:1 w/w ratios. Mixtures were stored at ambient conditions for 3 days prior to analysis to allow sufficient time for compounds to react. This was done to understand the possible compatibility of excipients with the amorphous clarithromycin during storage. This study determined the compatibility between the excipients and the amorphous clarithromycin using XRPD, HSM, and FTIR methods.

4.3.1 X-Ray Powder Diffraction (XRPD)

The 1:1 mixture of amorphous clarithromycin and excipients was investigated through XRPD analyses and compared to diffractograms obtained for the single compounds.

Figure 4.5 shows an overlay diffractogram of a quench cooled amorphous clarithromycin, Emcompress®, and a mixture of amorphous clarithromycin with Emcompress®. The diffractograms of amorphous clarithromycin (a) have halo peaks, indicating that it is in an amorphous state. However, when mixed with Emcompress®, the diffractogram (c) showed the characteristic peaks of the excipient (b), and the halo peaks of amorphous clarithromycin were not visible.

Randal *et al* (2010), reported that it is not uncommon for crystalline excipient peaks to overlap with active peaks in a drug product, and it is concentration-dependent. A mixture of PVP (amorphous form) mixed with a crystalline material by the author showed this phenomenon with crystalline material overlapping the amorphous materials starting from a concentration of 2.5%. For concentrations 10% and above, the diffractograms are completely dominated by the crystalline material peaks. The current mixture is a 1:1 composition of clarithromycin, and the excipient also showed the same phenomenon. Hence, other techniques should be used to conclude the compatibility.

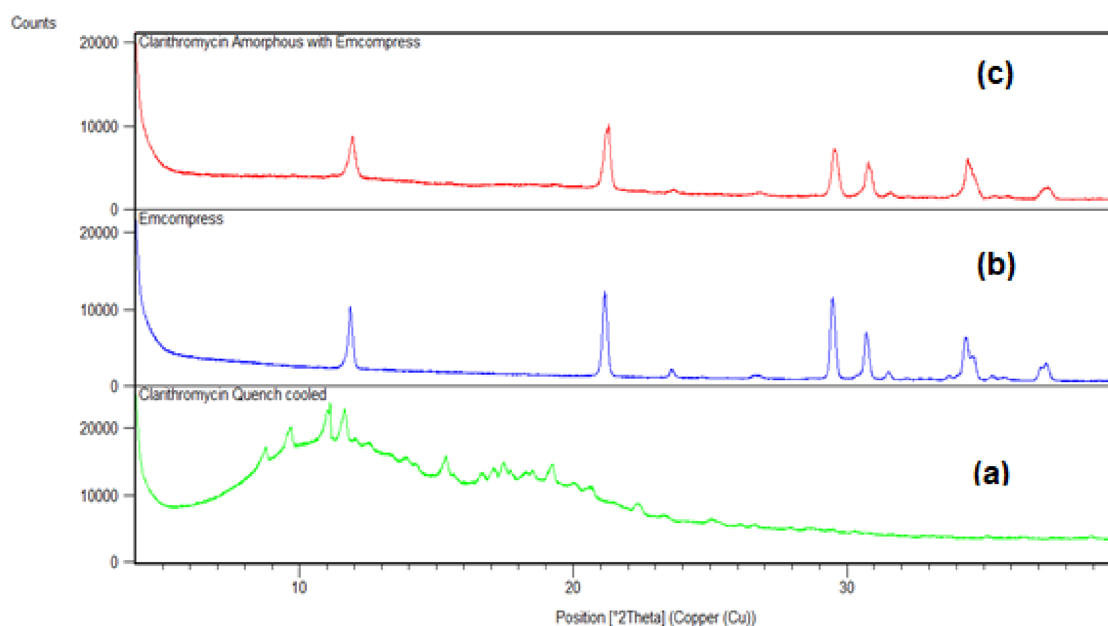


Figure 4.5: Overlay XRPD of amorphous clarithromycin with Emcompress®

As depicted in figure 4.6 (b), the diffractogram of Lactose shows halo peaks indicating its amorphous nature. The literature found that the spray-dried Lactose becomes amorphous and produces a single diffuse peak with no long-range order. The author also reported the effect of environmental (Temperature and Humidity) and the influence of other excipients on the transformation of amorphous Lactose into crystalline form (Wu *et al.*, 2014). Hence, the presence of new or definite sharp peaks in the diffractogram of the mixtures is of high interest in determining the compatibility.

The diffractogram of quench cooled amorphous clarithromycin and lactose mixture (c) shows halo peaks that represent the individual compounds, and no new or sharp crystalline peaks are seen. This indicates that both the compounds remain in amorphous form and can be considered as possible compatibility between Lactose and amorphous clarithromycin.

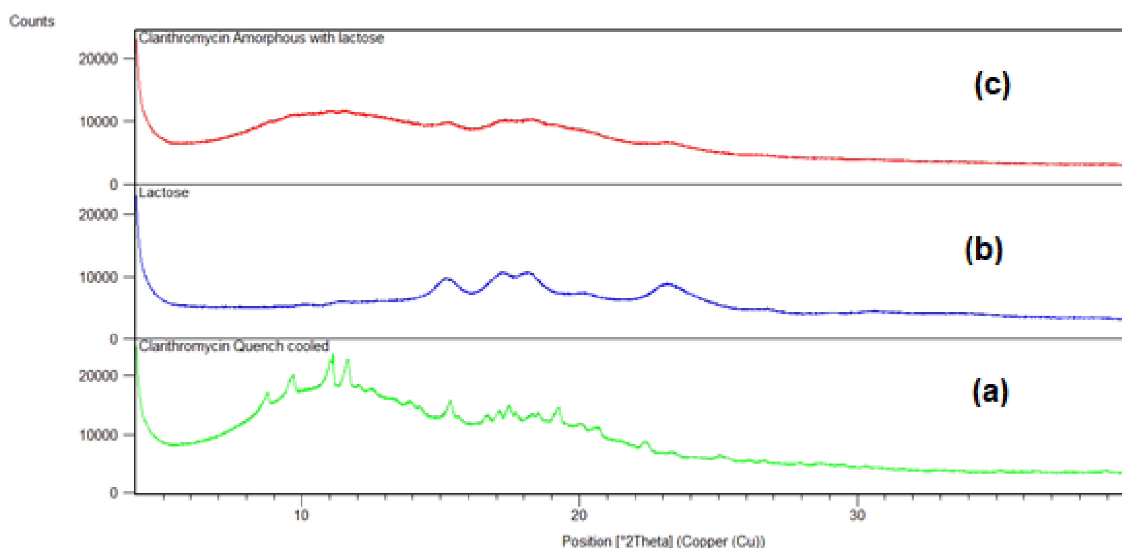


Figure 4.6: Overlay XRPD of amorphous clarithromycin with Lactose

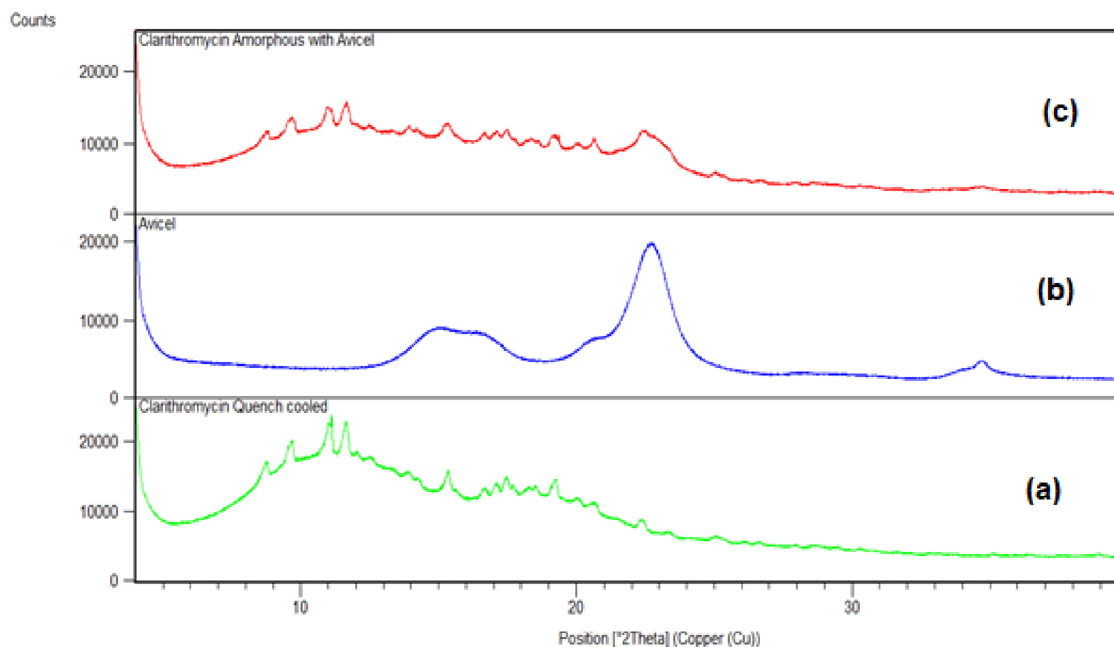


Figure: 4.7: Overlay XRPD of amorphous clarithromycin with Avicel® PH200

Microcrystalline cellulose (Avicel® PH200) is a partially depolymerized alpha-cellulose composed of crystalline and amorphous domains (Vehovec *et al*., 2012). As stated in the literature, the parallel arrangement of cellulose chains gives rise to some characteristic peaks to the Avicel® PH200. The characteristic diffraction peaks are noted at 14.8, 16.3, and 22.4° 2θ (Rojas *et al*., 2011). Figure 4.7 (b) above represents the diffractogram of the Microcrystalline cellulose (Avicel® PH200), which corresponds with the literature observations. The physical mixture of amorphous clarithromycin and Avicel® PH200 retain their characteristic peaks and the halo nature, respectively, indicating possible compatibility.

Figure 4.8 below depicts an overlay diffractogram of amorphous clarithromycin (a), Ac-Di-Sol (b), and the mixture (c). Windriyati *et al* (2019) reported the XRPD pattern of the Ac-Di-Sol and confirmed its amorphous nature through the lack of intense peaks in the diffractogram. From the Figure below, the same can be observed for the Ac-Di-Sol with a halo peak. The diffractogram (c) also shows broad halo peaks

confirming the compounds' amorphous nature when mixed. This is an indication of possible compatibility between the drug and the excipient.

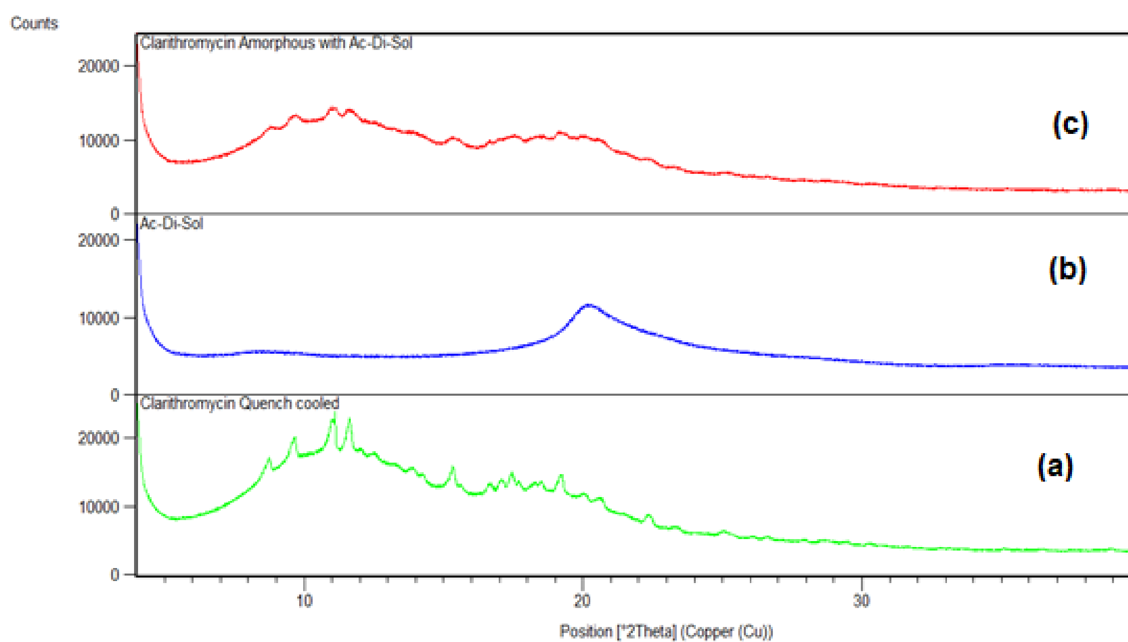


Figure 4.8: Overlay XRPD of amorphous clarithromycin with Ac-Di-Sol®

Commercially available magnesium stearates (anhydrate, monohydrate, dihydrate, and trihydrate) are available as a mixture of crystalline forms (Li & Wu, 2014). This can be confirmed from the diffractogram presented in the figure below (figure 4.10 (b)), where the presence of sharp peaks can be observed. However, combining the crystalline and amorphous compounds can cause stability issues as the crystalline compounds can act as a seed for amorphous forms to convert back into crystalline over time (Sun *et al*., 2012). Nevertheless, as depicted (figure 4.9 (c)) below, the diffractogram presents the characteristic peaks of both the amorphous clarithromycin and magnesium stearate, indicating the possible compatibility between the compounds.

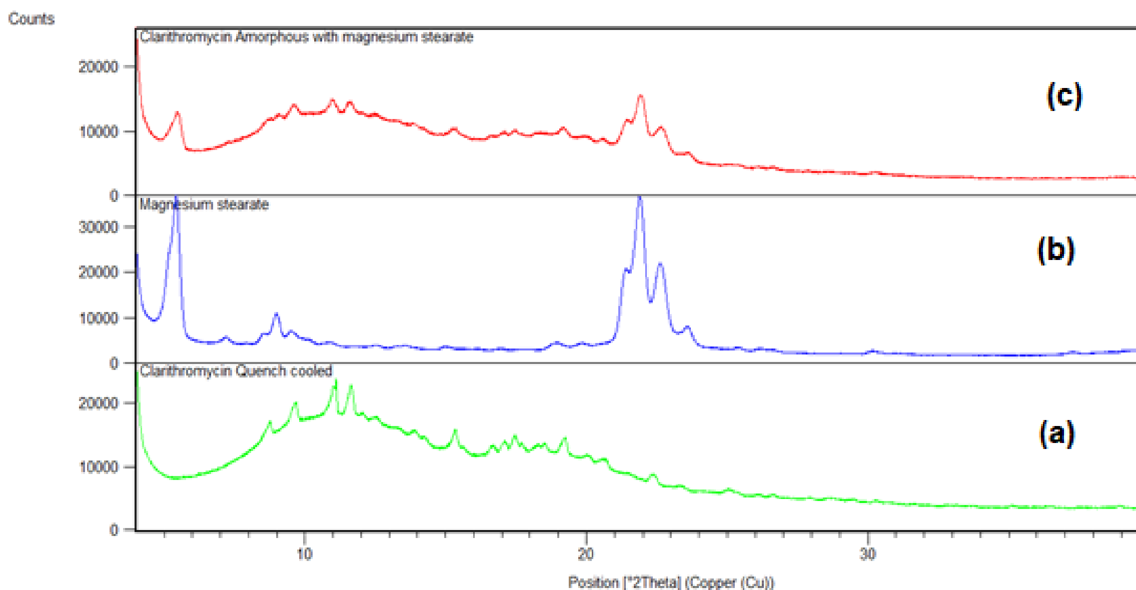


Figure 4.9: Overlay XRPD of amorphous clarithromycin with magnesium stearate

4.3.2 Infrared Spectroscopy

Amorphous clarithromycin and excipient interactions were further examined using IR as depicted in figure 4.10. The clarithromycin amorphous form in figure 4.10 shows the characteristic features as presented under 4.2.3 and figure 4.5 (b), which shows a sharp non-hydrogen bonded O-H stretch in the region 3500 cm^{-1} and band broadening of the -OH stretch was also observed due to hydrogen bond formation. There is also the -CH stretch in the 2900 cm^{-1} region with some broadened showing lower intensity. The band behaviour remains true for the ketone and lactone carbonyl peaks in the region of 1600 and 1740 cm^{-1}, respectively, which were broader and of lower intensity. The obtained results for the API, excipient combinations also retain the amorphous form's characteristic peaks, indicating the compatibility of amorphous clarithromycin with the chosen excipients.

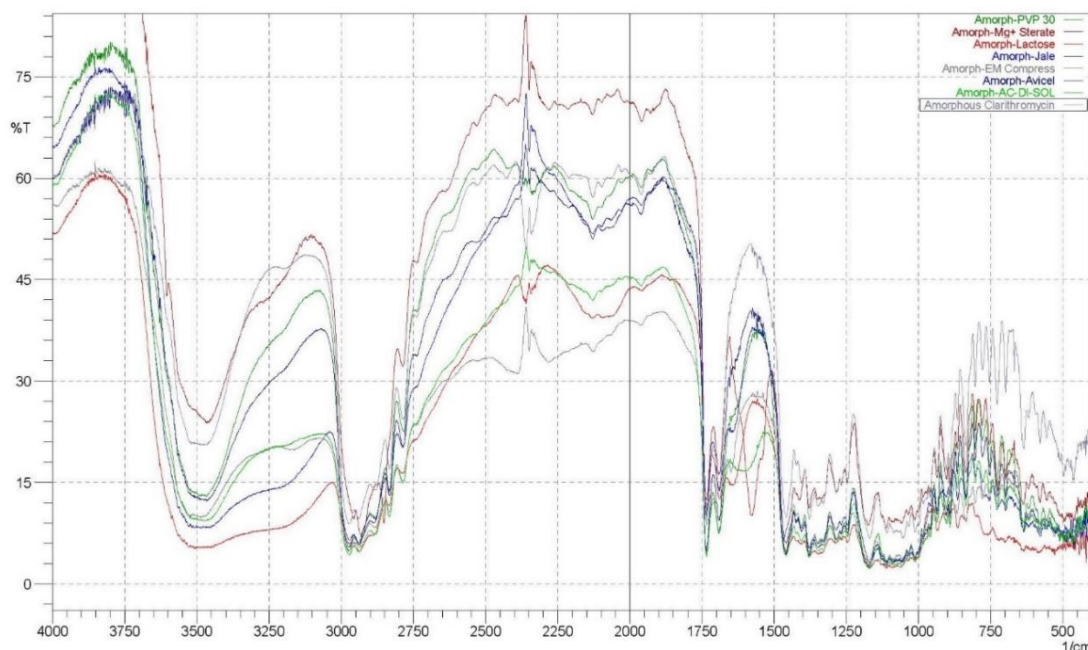


Figure 4.10: IR Overlay of Amorphous Clarithromycin with selected excipients

4.3.3 Hot-stage microscopy (HSM)

Visual thermal phase changes for a 1:1 ratio of crystalline clarithromycin raw material combined with the excipients were captured using HSM.

During the thermal microscopy analysis of clarithromycin/ Emcompress® mixture, which is depicted in figure 4.11, clarithromycin started to melt at 224 °C. This is in good correlation with the melting point (221 °C) observed for amorphous clarithromycin (figure 4.1). Therefore, the slight shift of melting point can be considered as insignificant. As the heating continued, all the clarithromycin melted by 268 °C, and the Emcompress® can be found intact, showing no signs of degradation. On the other hand, Emcompress® degrades from 370 °C (Rowe *et al.*, 2006) with no characteristic melting point. This indicates that there was interference of Emcompress® with the clarithromycin, indicating possible compatibility between the two compounds.

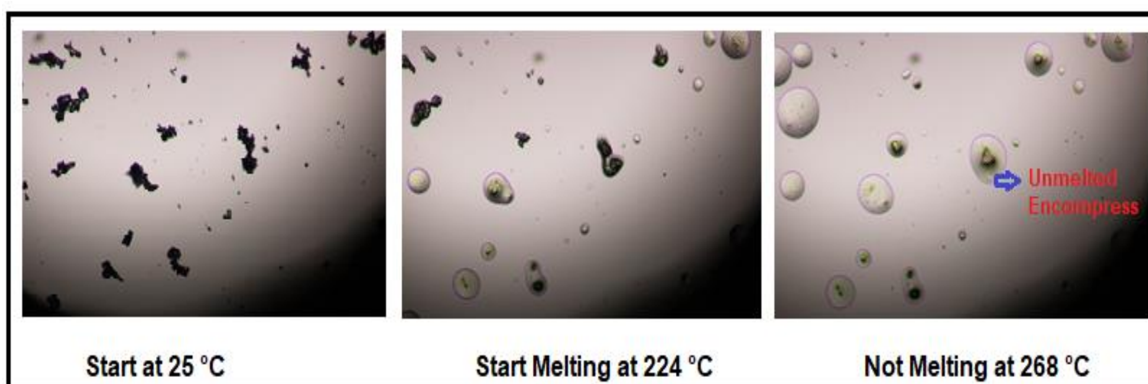


Figure 4.11: HSM images of clarithromycin and Emcompress® 1:1 ratio

For the combination of clarithromycin/lactose as depicted in Figure 4.12, it was observed that Lactose started to melt at a lower temperature of 185 °C than its melting point of 202.8 °C, followed by the melting of the rest of the clarithromycin at 213°C. The shift in the melting point of Lactose (Approximately 17 °C) and for clarithromycin (Approximately 9 °C). Despite the moderate shift in melting points, there are no signs of degradation observed, and both compounds after melting were found miscible. Harding *et al* (2008) reported similar observations in the case of acetylsalicylic acid and magnesium stearate, where both the compounds melt at a temperature far lower than their respective melting points. However, the miscibility of both the compounds in each other without signs of degradation was considered as signs of compatibility. Hence, the combination of clarithromycin and Lactose in the current study is considered as compatible.

Figure 4.12: HSM images of clarithromycin and Lactose 1:1 ratio

Figure 4.13 shows thermal microscopy of a mixture of amorphous clarithromycin and Avicel® PH200. It was observed that at 240°C, the melting of the mixture started, and it was completely melted at 262°C. Both the active ingredient and the polymer start to melt simultaneously, indicating good miscibility, hence possible compatibility.

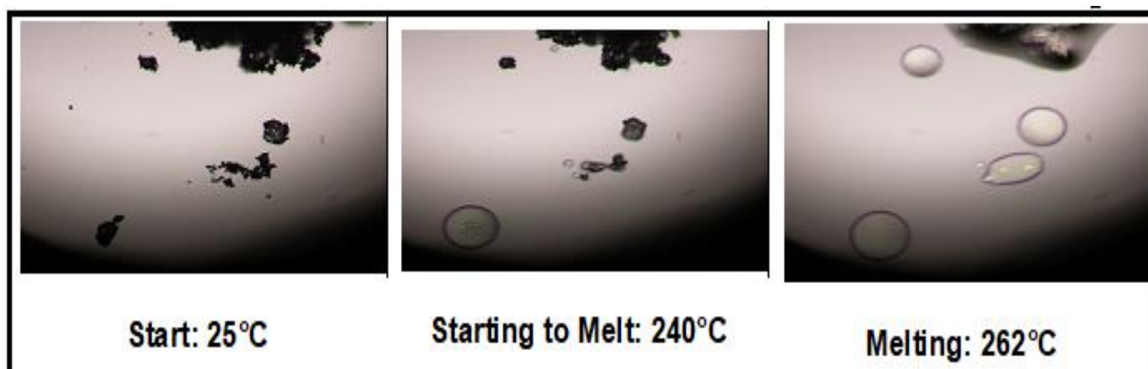


Figure 4.13: HSM images of clarithromycin and Avicel PH200 1:1 ratio

During the thermal microscopy analysis of clarithromycin/Ac-Di-Sol, depicted in figure 4.14, clarithromycin started to melt at 227°C while the Ac-Di-Sol formed a polymeric matrix in which the molten clarithromycin is encapsulated. This polymeric matrix does not melt and stays intact throughout heating. At approximately 280 °C, the matrix starts to turn yellowish-brown which indicates that degradation occurs. Therefore, it was concluded that no incompatibility exists between Ac-Di-Sol and clarithromycin.

Figure: 4.14: HSM images obtained during heating of clarithromycin and Ac-Di-Sol 1:1 ratio

The HSM of clarithromycin mixed with magnesium stearate (Figure 4.15) shows that the mixture started melting at 227 °C and both the materials melted completely by 230 °C, and the liquid colour changed to yellow. Although magnesium stearate's actual melting point was reported as 88 °C, the big shift in the melting point and possible degradation that occurred close to the melting point of clarithromycin could be a possible indication of incompatibility.

Figure 4.15: HSM images of clarithromycin and magnesium stearate 1:1 ratio

4.3.4 Summary of compatibility studies

The following table (Table 4.2) summarises the outcomes of the compatibility studies between XRPD, FTIR, and HSM.

Table 4.2: Summary of compatibility results of clarithromycin / Excipients

Excipient	XRPD	FTIR	HSM
Emcompress®	-	√	√
Ac-Di-Sol®	√	√	√
Lactose	√	√	√
Avicel PH200®	√	√	√
Magnesium stearate	√	√	X

From the summary data provided for compatibility studies, it can be concluded that the remaining excipients could be compatible except for magnesium stearate. The interactions observed between the drug and excipients may be due to chemical or physical incompatibility. In literature, it has been reported that an indication of interaction is not necessarily a chemical incompatibility but could be a result of physical incompatibility (Corvi *et al.*, 2006). Given that magnesium stearate will be used at a very low concentration, considering the temperature at which the degradation happened (230 °C), which is not commonly used during the storage of finished product and considering the positive results obtained for XRPD and FTIR, magnesium stearate was included in the formulation development.

4.4 TABLET FORMULATION

Upon consideration and complete assessment of all the compatibility testing results, a series of formulations were developed using the shortlisted as mentioned in Table 4.4. All the developed formulations were subjected to both pre and post-compression studies. From all of these results, the best formulation was then chosen to manufacture a bigger batch of tablets for stability studies.

Four different formulations (F1 – F4) of amorphous clarithromycin tablets were prepared using 500 mg of clarithromycin (CLA), 2% (10mg) of the Ac-Di-Sol as a disintegrant, 10% (50 mg) of Lactose as a binder, and 0.5% w/w of Magnesium stearate as a lubricant in each tablet. The excipients used in these formulations are the minimum concentrations proposed in the literature to execute the required functions. The lower concentrations were chosen to limit the final tablet weight and minimize possible incompatibilities. The variable between batches were the diluents Emcompress® and Avicel PH200®, limited to a maximum of 20% (100 mg). The composition of formulations F1 to F4 is given in Table 4.3 below.

Table 4.3: Composition of formulations F1 to F4

Batch No	Ingredients in mg / tablet						
	CLA	Avicel PH200®	Emcompress®	Ac Di-Sol	Lactose	Magnesium Sterate	Total weight
F1	500	100	-	10	50	2.5	662.5
F2	500	50	50	10	50	2.5	662.5
F3	500	25	75	10	50	2.5	662.5
F4	500	-	100	10	50	2.5	662.5

Clarithromycin tablets were prepared by using the manufacturing method described in chapter 3. The batch sizes of all the formulations were kept at 100 tablets per batch. The prepared powder mixtures were evaluated for pre-compression parameters and then compressed into tablets using a 12 mm punch on a Modul™D-tablet compression machine (Frankfurt, Germany). Finally, the compressed tablets were evaluated for post-compression parameters. Table 4.4 gives a summary of these parameters.

Table 4.4: Pre and post-compression parameters of formulations F1 to F4

Parameter	Mean± S.D			
	F1	F2	F3	F4
Carr's index (%)	28.20 ± 1.42	26.04 ± 2.13	19.03 ± 2.64	22.23 ± 1.22
Hausner's ratio	1.39 ± 0.03	1.35 ± 0.04	1.24 ± 0.04	1.28 ± 0.02
Angle of repose (θ)	42.50± 0.91	43.30± 1.1	35.31 ± 1.13	33.36 ± 1.01
Average Weight	656 ± 17.5	670 ± 15.9	664 ± 14.3	660 ± 12.1
Hardness	32N ± 7.61	41N ± 11.63	43N ± 11.33	44N ± 8.71
Friability	N/A	N/A	4.23%	4.71%
Disintegration	N/A	N/A	< 40 seconds	< 40 seconds

Pre-compression parameters (Table 4.4) of the granules from formulations F1 and F2 showed poor flow properties as confirmed by the angle of repose and indicated potential problems during compression as the Carr's index values were high. However, when the compression of the tablets commenced, the powder flow was acceptable, and die filling occurred, resulting in tablets passing for the weight variation test.

However, tablets compressed for these batches showed poor mechanical strength and broke into pieces during the friability test. Formulations F3 and F4 showed good flow properties and compressibility in compression with the F1 and F2. However, the tablets formed do not have enough hardness, and it reflected during the friability test (4.23% and 4.71%, respectively), failing for the USP limit of 1% or below. This could be attributed to a lack of sufficient binder.

Formulations that could form tablets (F3 and F4) passed for the disintegration, and it is very well within the limits of less than 15 minutes. Also, tablets formed for F3 and F4 were found rough on the sides, indicating resistance to ejection due to insufficient amounts of lubricant. From the first 4 formulations, F3 showed better performance for pre and post-compression parameters evaluated. Hence, F3 is further improved by changing the binder and lubricant concentration.

Four different formulations (F5 – F8) of amorphous clarithromycin tablets were prepared using 500 mg of clarithromycin (CLA), 2% (10mg) of the Ac-Di-Sol as a disintegrant, 5% (25 mg) of Avicel PH200®, 15% (75 mg) Emcompress® as diluents in each tablet. The variable between batches were the lactose (binder) at 20% (100 mg) and 30% (150 mg), magnesium stearate (Lubricant) at 1 (5 mg) and 1.5% (7.5 mg). The composition of formulations F5 to F8 is given in Table 4.5 below.

Table 4.5: Composition of formulations F5 to F8

Batch No	Ingredients in mg / tablet						
	CLA	Avicel PH200®	Emcompress®	Ac Di-Sol	Lactose	Magnesium Sterate	Total weight
F5	500	25	75	10	100	5	715
F6	500	25	75	10	150	5	765
F7	500	25	75	10	100	7.5	717.5
F8	500	25	75	10	150	7.5	767.5

Granules of all the formulations (F5 – F8), showed very good flow properties as confirmed by the angle of repose as all of them showed an angle of less than 25 degrees (Table 4.6), indicating excellent flow properties. This could be attributed to the increased concentrations of spray-dried Lactose and the magnesium stearate that reduced the resistance to the flow.

Table 4.6: Pre and post-compression parameters of formulations F5 to F8

Parameter	Mean± S.D			
	F5	F6	F7	F8
Carr's index (%)	16.66 ± 1.33	12.8 ± 1.13	14.01 ± 2.20	11.05 ± 1.01
Hausner's ratio	1.20 ± 0.04	1.15 ± 0.04	1.16 ± 0.04	1.13 ± 0.05
Angle of repose (θ)	22.30 ± 0.73	18.78 ± 0.63	20.22 ± 1.01	19.63 ± 0.98
Average Weight	706 ± 12.0	761 ± 10.6	723 ± 11.2	758 ± 10.1
Hardness	63N ± 4.53	81N ± 5.63	93N ± 6.72	108N ± 7.33
Friability	0.68%	0.26%	0.71%	0.59%
Disintegration	2 min 10 sec	2 min 33 sec	1 min 40 sec	3 min 26 sec

Tablets compressed for all these batches showed good mechanical strength as indicated under the friability results. However, despite the tablets passed for friability, formulation F7 and F8 needed to be compressed at high pressure as some tablets during the compression at low pressure were found to be chipping. Nevertheless, all the formulations passed for the dissolution and weight variation tests. Between the formulation, F5 and F6, the formulation of choice was F6 as all the pre and post-compression parameters for this batch were better than the F5. Hence, F6 is considered as the optimized batch for upscaling to a batch size of 500 tablets to conduct the stability tests. Table 4.7 below gives the composition of the final formulation used for preparing the stability study batch.

Table 4.7: Composition and tablet parameters of the final formulation of amorphous clarithromycin tablets

Composition			**Tablet parameters (post compression)**	
Ingredient	**Function**	**Concentration/ Tablet**	**Average tablet weight:**	765 mg
Clarithromycin	API	500 mg	**Hardness:**	80 – 110 N
Avicel® PH 200	Diluent	5% w/w (25 mg)	**Friability:**	<1%
Emcompress®	Diluent	15% w/w (75 mg)	**Disintegration:**	Within 15 minutes
Lactose	Binder/Diluent	30% w/w (150 mg)	**Diameter:**	16.00 mm
Ac-Di-Sol®	Super-diaintegrant	2%w/v (10 mg)	**Thickness:**	5.00 mm
Magnesium stearate	Lubricant	1%w/v (5 mg)		

4.5 STABILITY STUDIES

Stability tests are significant as tablet formulations containing the active pharmaceutical ingredient (API) in any high-energy amorphous form are expected to enhance solubility, dissolution rate, and consequently, oral bioavailability of poorly water-soluble drugs. However, the amorphous solid formulations are thermodynamically unstable and are therefore susceptible to recrystallization upon storage (Surampalli, 2014).

Long-term and accelerated stability studies were conducted on the tablets of scale-up batch according to the stability guidelines of the South African Health Products Regulatory Authority (SAHPRA, 2012). Long term and accelerated stability testing were carried out for 6 months at 25 °C ± 2°C / 60 ± 5% RH and 40 °C ± 2 °C / 75 ± 5% RH respectively.

The purpose of the present study was to determine the influence of environmental factors like temperature and humidity on the physico-chemical properties. *In addition, in-vitro* dissolution studies of both the crystalline and amorphous formulations were conducted to determine the solution-mediated phase transition of amorphous clarithromycin into the stable crystalline form.

4.5.1 Physico-chemical Parameters

Tablets subjected to stress conditions were tested for various physico-chemical parameters like colour, weight variation, dimensions, hardness, friability, and assay. All the tests were performed according to the procedures described in chapter 3. Results obtained for long-term and accelerated stabilities are given in Table 4.8 and 4.9, respectively.

Table 4.8: Stability results of F6 for Physico-chemical properties at 25 °C ± 2 °C / 60 ± 5% RH

Product name: Amorphous Clarithromycin immediate release tablets		**Packaging (material and pack size):** Securitainer with desiccant sachet (50's)						
Batch no:	T6	**Storage conditions:** 25 °C ± 2 °C / 60 ± 5% RH						
Batch size:	500 tablets	**Manufacturing date:** 14/09/2020						
Date of commencement of stability studies: 21/10/2020		**Time intervals (months)**						
Parameter	**Limits**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Colour	White	White	White	White	White	White	White	White
Average weight	765 mg ± 5% (727-803 mg)	761 ± 11.3	771 ± 8.6	769 ± 10.5	759 ± 8.5	767 ± 12.0	758 ± 11.6	763 ± 10.1
Thickness	5.00 mm ± 5% (4.75-5.25 mm)	4.90 ± 0.02	4.89 ± 0.03	4.83 ± 0.02	4.88 ± 0.01	4.91 ± 0.02	4.94 ± 0.02	4.92 ± 0.04
Diameter	16.00 mm ± 1% (15.84-16.16 mm)	15.98 ± 0.06	15.98 ± 0.04	15.99 ± 0.02	15.98 ± 0.04	16.01 ± 0.03	16.00 ± 0.02	16.01 ± 0.04
Hardness	100N (80-110 N)	94 ± 5.3	92 ± 4.6	89 ± 7.2	90 ± 7.8	85 ± 6.3	95 ± 4.2	93 ± 5.8
Friability	< 1%	0.11	0.23	0.21	0.31	0.28	0.18	0.24
Disintegration	Within 15 minutes	3 min 12 sec	3 min 45 sec	2 min 56 sec	3 min 28 sec	4 min 02 sec	3 min 10 sec	2 min 48 sec
Assay	95- 105%	98.3	98.9	98.6	97.2	98.4	98.1	97.6

Table 4.9: Stability results of F6 for Physico-chemical properties at 40 °C ± 2 °C / 75 ± 5% RH

<table>
<tr><td colspan="2">Product name: Amorphous Clarithromycin immediate release tablets</td><td colspan="7">Packaging (material and pack size): Securitainer with desiccant sachet (50’s)</td></tr>
<tr><td>Batch no:</td><td>T6</td><td colspan="7">Storage conditions: 25 °C ± 2 °C / 60 ± 5% RH</td></tr>
<tr><td>Batch size:</td><td>500 tablets</td><td colspan="7">Manufacturing date: 14/09/2020</td></tr>
<tr><td colspan="2">Date of commencement of stability studies: 21/10/2020</td><td colspan="7">Time intervals (months)</td></tr>
<tr><td>Parameter</td><td>Limits</td><td>0</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td></tr>
<tr><td>Colour</td><td>White</td><td>White</td><td>White</td><td>White</td><td>White</td><td>White</td><td>White</td><td>White</td></tr>
<tr><td>Average weight</td><td>765 mg ± 5%
(727-803 mg)</td><td>759 ± 8.2</td><td>761 ± 9.3</td><td>770 ± 9.5</td><td>756 ± 10.6</td><td>769 ± 11.0</td><td>768 ± 12.4</td><td>766 ± 9.3</td></tr>
<tr><td>Thickness</td><td>5.00 mm ± 5%
(4.75-5.25 mm)</td><td>4.87 ± 0.02</td><td>4.85 ± 0.02</td><td>4.89 ± 0.02</td><td>4.93 ± 0.03</td><td>4.91 ± 0.02</td><td>4.85 ± 0.03</td><td>4.91 ± 0.03</td></tr>
<tr><td>Diameter</td><td>16.00 mm ± 1%
(15.84-16.16 mm)</td><td>16.02 ± 0.03</td><td>15.99 ± 0.02</td><td>15.99 ± 0.02</td><td>16.03 ± 0.04</td><td>16.01 ± 0.02</td><td>16.02 ± 0.02</td><td>15.99 ± 0.04</td></tr>
<tr><td>Hardness</td><td>100N (80-110 N)</td><td>91 ± 4.8</td><td>98 ± 7.1</td><td>86 ± 8.1</td><td>93 ± 6.4</td><td>103 ± 5.1</td><td>107 ± 4.8</td><td>112 ± 7.1</td></tr>
<tr><td>Friability</td><td>< 1%</td><td>0.26</td><td>0.31</td><td>0.28</td><td>0.33</td><td>0.28</td><td>0.32</td><td>0.24</td></tr>
<tr><td>Disintegration</td><td>Within 15 minutes</td><td>3 min 05 sec</td><td>2 min 52 sec</td><td>2 min 45 sec</td><td>3 min 40 sec</td><td>4 min 20 sec</td><td>3 min 50 sec</td><td>4 min 30 sec</td></tr>
<tr><td>Assay</td><td>95- 105%</td><td>99.1</td><td>98.5</td><td>97.4</td><td>98.1</td><td>98.7</td><td>98.5</td><td>98.4</td></tr>
</table>

Tablets stored under real-time conditions, the organoleptic properties such as shape and colour were unchanged. Uniformity of the weight of the tablets was within the 5% deviation, and the average weight of 20 tablets was within the set limits. Tablets were found to be within limits for thickness, hardness, friability, and disintegration, as indicated in Table 4.8.

Tablets stored under accelerated conditions also did not exhibit any visible physical changes and passed for weight variation, thickness, and diameter at all the time intervals. However, a gradual increase in hardness was observed from the 4th month of the stability studies, and tablets failed for the hardness limit at 6 months (Table 4.9). Despite the increase in moisture content, no significant changes were observed for tablet weight, thickness diameter, and friability. Though the tablets did not fail for the disintegration test limit, there is also an increase in the disintegration time as compared to the first three months. These changes could be attributed to the loss of moisture due to exposure at high temperature rather than absorption of humidity.

Tablets stored under both real-time and accelerated stability conditions were assayed for clarithromycin to determine the chemical stability of the final formulation. Shelf-life is defined as the time required for a drug to decompose to 90% ((t90) of its initial concentration. However, a “significant change” under accelerated testing conditions is defined as a 5% potency loss from the initial assay value of a batch (SAHPRA, 2012). Data tabulated (Table 4.8 and 4.9), indicate that the tablets are stable chemically when stored at different temperature and humidity conditions.

4.5.2 *In Vitro* Dissolution Studies

Tablets manufactured from the amorphous clarithromycin and tablets commercial clarithromycin tablets (crystalline clarithromycin) were investigated, and their dissolution rate results were compared in this chapter. Katsidzira (2017) reported that water is the ideal medium to differentiate between the two forms of clarithromycin as it showed very poor solubility in water. Hence only water was

investigated as the dissolution medium. The dissolution profiles of the 6 month stability study and the respective overlays are shown in Figures 4.16 and 4.17.

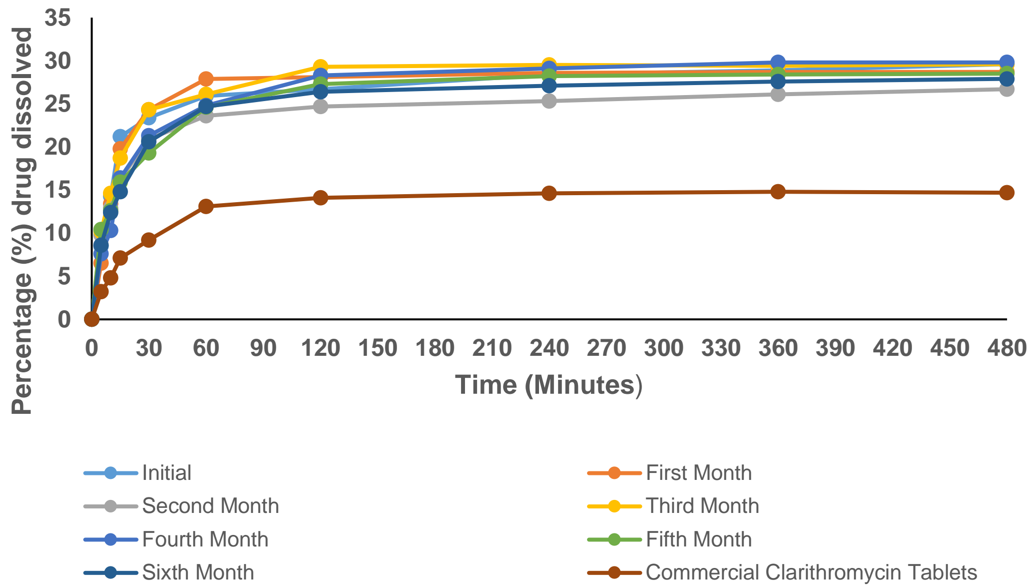


Figure 4.16: Comparative dissolution profile overlay of amorphous clarithromycin tablets from initial to 6 months with the commercial product at 25 °C ± 2 °C / 60 ± 5% RH (n=12)

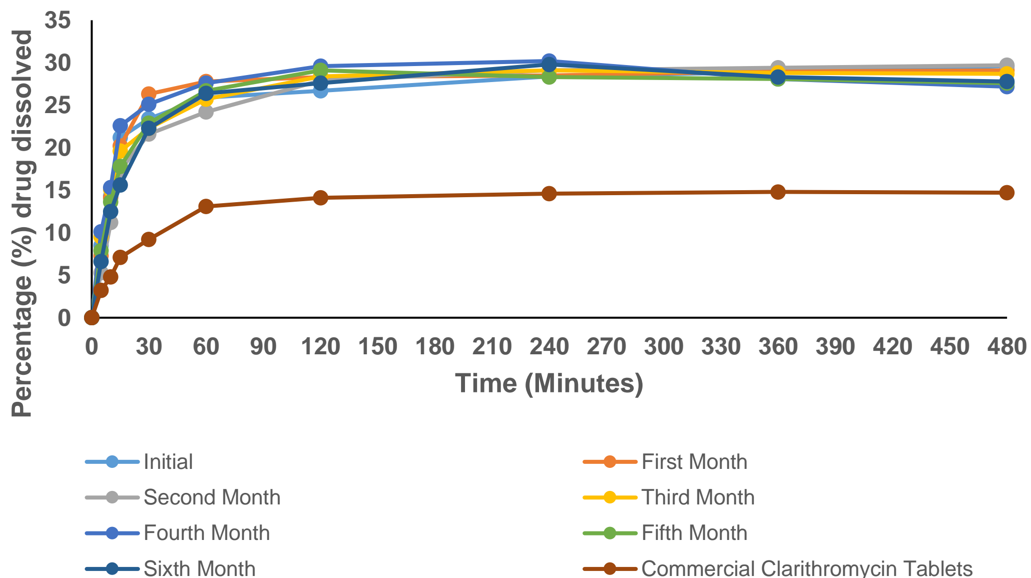


Figure 4.17: Comparative dissolution profile overlay of amorphous clarithromycin tablets from initial to 6 months with the commercial product at 40 °C ± 2 °C / 75 ± 5% RH (n=12)

Table 4.10: Stability results for *in-vitro* dissolution of F6 in comparison with commercial tablets from initial to 6 months with commercial product at 25 °C ± 2 °C / 60 ± 5% RH (n=12)

Sampling time (Minutes)	**Cumulative percentage drug release (mean ± S.D)**							
	Commercial Product	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5	3.2 ± 0.8	8.5 ± 0.7	6.5 ± 0.4	9.8 ± 1.0	10.2 ± 1.3	7.6 ± 0.8	10.4 ± 0.7	8.6 ± 1.2
10	4.8 ± 1.2	14.3 ± 1.4	13.4 ± 1.0	12.9 ± 1.5	14.6 ± 1.1	10.3 ± 1.6	12.6 ± 1.3	12.4 ± 1.6
15	7.1 ± 2.1	21.2 ± 2.1	19.8 ± 1.6	16.3 ± 1.8	18.7 ± 2.0	16.4 ± 2.1	15.9 ± 1.1	14.8 ± 2.2
30	9.2 ± 2.7	23.4 ± 2.5	24.3 ± 2.1	20.9 ± 2.1	24.3 ± 2.2	21.3 ± 2.1	19.3 ± 1.5	20.6 ± 2.1
60	13.1 ± 1.5	25.9 ± 2.9	27.9 ± 2.1	23.6 ± 1.9	26.1 ± 1.9	24.8 ± 2.5	24.6 ± 1.9	24.7 ± 2.4
120	14.1 ± 1.3	26.7 ± 1.8	28.1 ± 1.2	24.7 ± 1.2	29.3 ± 1.5	28.3 ± 1.4	27.3 ± 1.8	26.4 ± 2.1
240	14.6 ± 0.8	28.4 ± 1.4	28.6 ± 2.2	25.3 ± 1.6	29.5 ± 1.1	29.1 ± 1.6	28.2 ± 0.9	27.1 ± 1.2
360	14.8 ± 1.1	28.9 ± 2.1	28.7 ± 2.0	26.1 ± 2.5	29.4 ± 1.1	29.8 ± 2.0	28.4 ± 1.3	27.6 ± 1.1
480	15.1 ± 0.9	29.6 ± 1.9	28.7 ± 2.3	26.7 ± 1.7	29.6 ± 1.3	29.8 ± 1.7	28.5 ± 1.2	27.9 ± 1.7
f1	---	---	90	77	88	80	79	78
f2	---	---	05	11	05	08	08	08

Table 4.11: Stability results for *in-vitro* dissolution of T final in phosphate buffer (pH 6.8) in comparison with Niaspan® after 0, 3 and 6 months at 40 °C ± 2 °C / 75 ± 5% RH (n=12)

Sampling time (Minutes)	**Cumulative percentage drug release (mean ± S.D)**							
	Commercial Product	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5	3.2 ± 0.8	8.5 ± 0.7	7.3 ± 0.8	5.3 ± 1.3	9.5 ± 0.8	10.1 ± 1.6	7.9 ± 1.7	6.6 ± 1.0
10	4.8 ± 1.2	14.3 ± 1.4	14.1 ± 0.9	11.2 ± 1.0	15.1 ± 1.0	15.3 ± 1.2	13.6 ± 1.8	12.5 ± 1.8
15	7.1 ± 2.1	21.2 ± 2.1	20.2 ± 1.9	17.4 ± 1.2	19.6 ± 2.2	22.6 ± 2.1	17.8 ± 2.1	15.6 ± 1.8
30	9.2 ± 2.7	23.4 ± 2.5	26.3 ± 2.1	21.6 ± 1.8	22.3 ± 2.5	25.1 ± 1.5	22.9 ± 1.9	22.3 ± 3.1
60	13.1 ± 1.5	25.9 ± 2.9	27.8 ± 2.6	24.2 ± 1.7	25.7 ± 1.5	27.6 ± 2.6	26.7 ± 2.9	26.4 ± 2.6
120	14.1 ± 1.3	26.7 ± 1.8	28.3 ± 1.4	27.9 ± 2.1	28.4 ± 1.2	29.6 ± 1.1	29.1 ± 2.8	27.6 ± 2.5
240	14.6 ± 0.8	28.4 ± 1.4	28.5 ± 1.1	29.1 ± 1.5	29.1 ± 1.7	30.2 ± 2.6	28.3 ± 1.9	29.8 ± 2.2
360	14.8 ± 1.1	28.9 ± 2.1	29.0 ± 1.1	29.4 ± 1.5	28.8 ± 1.4	28.1 ± 1.8	28.1 ± 1.7	28.3 ± 2.1
480	15.1 ± 0.9	29.6 ± 1.9	29.1 ± 2.0	29.7 ± 1.9	28.7 ± 2.1	27.2 ± 2.2	27.6 ± 1.6	27.8 ± 2.3
f1	---	---	88	81	92	84	86	80
f2	---	---	05	08	04	07	05	08

As expected, the dissolution rate of amorphous clarithromycin was higher than that of crystalline clarithromycin because amorphous solid forms are more soluble than their crystalline counterpart. The amorphous clarithromycin dissolution rate was close to 30% as compared to the crystalline clarithromycin (Commercial product), which is 15%. As reported by the Katsidzira (2017), the equilibrium solubility of clarithromycin cannot be established due to its extremely poor solubility in water and in a basic environment. Good solubility is related to good dissolution characteristics. Dissolution data was therefore applied as an indication of apparent solubility for amorphous clarithromycin. Based on the dissolution data observed, a two-fold increase in the solubility of the amorphous clarithromycin can be reported from the study.

From the dissolution studies, no significant difference was observed for the drug release performance of tablets stored at room temperature and accelerated conditions for 6 months. Dissolution profiles are normally considered similar when the f1 values are between 0-15 and f2 values between 50-100 (SAHPRA, 2015). The f1 and f2 values calculated (Table 4.10 and Table 4.11) also suggest that the dissolution characteristics of the product are stable when stored at room temperature and accelerated conditions.

It was expected that the dissolution rate would drop as the amorphous form recrystallizes into a metastable form. This could be attributed to the mixture of amorphous clarithromycin with excipients that have been proven to stabilize the metastable amorphous clarithromycin. However, the recrystallization only occurred in month 3. 4, 5, and 6, whereby the dissolution rate at between 360 and 480 minutes dropped (Table 4.10 and Table 4.11).

4.6 SUMMARY

The physico-chemical characterization of the solid-state forms was well achieved through thorough pre-formulation studies conducted. Therefore, it can be concluded that the successful preparation of amorphous clarithromycin. Furthermore, the compatibility studies conducted between the amorphous clarithromycin and selected excipients showed only one incompatibility (Clarithromycin and Magnesium Stearate). As most of the excipients selected have direct compressible and good properties, product development became less strenuous.

The stability testing of the amorphous clarithromycin tablets showed that storage for six months at long-term temperature and humidity did not result in significant changes in the solid-state forms as no solution-mediated phase transition was observed. However, tablets stored under elevated temperature and humidity conditions showed this phenomenon from the 3rd month until the 6th month interval. Considering the time intervals at which this phenomenon occurred (360 and 480 minutes), which is not a regular practice for dissolution studies for immediate release tablets, it can be concluded that the quench cooled clarithromycin remained amorphous. Furthermore, it is also clear that various processing effects such as compression and handling also did not affect the amorphous nature. In terms of the stability of the amorphous form of clarithromycin, the outcome is regarded as positive.   This is not found in Bibliography

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 INTRODUCTION

There are numerous methods and procedures available to improve the solubility and enhance bioavailability of oral solid forms. However, the preparation of formulations in amorphous form have been realized as an extremely useful tool in improving the dissolution rate of poorly water-soluble drugs and ultimately improving bioavailability of the drug at the site of action. Recently, a great deal of knowledge has been accumulated about amorphous forms in terms of the feasible preparation techniques, characterization methods, and the effect of various factors on the phase transitional behaviour into a more stable crystalline form. In addition, several studies conducted so far to explore the physico-chemical behaviour of amorphous forms lead to understanding their potential advantages in the pharmaceutical industry.

Previous studies conducted by Katsidzira (2017), reported the improved dissolution rate of amorphous clarithromycin in the raw material form. However, the study did not explore the stability of the clarithromycin to a sufficient extent (Effect of elevated temperature and humidity for a longer period) to provide a basis for developing a stable dosage form.

Clarithromycin is a semisynthetic erythromycin-derivative that is highly potent against a wide variety of aerobic and anaerobic Gram-positive and Gram-negative organisms. Moreover, considering its potential use among various disease conditions and the limited oral bioavailability resulting in underperforming of the drug, it makes the perfect choice for improving the solubility. Therefore, this study focussed on the successful formulation of clarithromycin tablets by using the most straightforward formulation technique, namely direct compression.

5.2 PRE-FORMULATION STUDIES

Pre-formulation studies were conducted to characterize the amorphous clarithromycin formed through the quench cooling process and determine the compatibility between selected excipients and amorphous clarithromycin. Techniques such as FTIR, XRPD, and DSC were used to characterize. From all the characterization results, clarithromycin raw material was confirmed to be the crystalline Form II polymorph. It was successfully converted to an amorphous form (CLA) by quench cooling of the melt. Pre-formulation studies conducted to determine the excipient compatibility revealed possible interaction with the magnesium stearate, while others confirmed that the amorphous nature of the CLA is maintained.

5.3 FORMULATION DEVELOPMENT

Preliminary studies were designed to facilitate the manufacture of a prototype formulation that meets the physico-chemical requirements of the USP. A series of formulations were developed by varying tablet components, such as diluents, binder, and lubricant. The influence of these variables was studied regarding pre- and post-compression parameters. The ratio of the diluents used played an important role in improving the flow properties and compressibility with Emcompress® creating a more positive impact on these parameters. Formulation (F6) was identified as the suitable formulation as all the pre and post-compression parameters were found to be within the pharmacopeial limits, which was further upscaled to conduct stability studies.

5.4 REAL TIME AND ACCELERATED STABILITY TRIALS

The tablets manufactured on a larger scale (F6) were subjected to stability trials according to SAHPRA guidelines (SAHPRA, 2012). During the 6 month stability trial period, the tablets were analyzed at a monthly month interval. Tablets were tested for changes in qualitative and quantitative attributes such as organoleptic properties, weight variation, tablet dimensions, hardness, friability, assay, and *in*

vitro release rate. The product specifications for all parameters were set prior to initiating stability studies. Dissolution profiles were compared using an independent mathematical model, where the f1 and f2 values were used to determine the similarity and difference between the results obtained initially and at samples taken from various time intervals.

For this study, the method of dissolution was considered to be an indicative method of product stability. The reason being; the phenomena of solution-mediated phase transformation. Solution-mediated phase transformation is considered to be the only method that would indicate the recrystallization of the amorphous form to the stable crystalline form within the solution. During the 6 month stability study at long-term conditions, no solution-mediated phase transformation was observed, indicating no change in the solid-state form of amorphous CLA. However, at accelerated conditions, this phenomenon was observed from the 3rd month. The decrease in the concentration available from time points of 360 and 480 minutes indicates the conversion of amorphous CLA to stable crystalline. Therefore, amorphous CLA shows excellent stability in terms of physical and chemical properties at ambient conditions.

After the formulation of amorphous CLA tablets, it was determined that milling and mixing processes did not induce the recrystallization of the amorphous form back to the most thermodynamically stable dihydrate form. This was considered as a positive outcome. Therefore, amorphous clarithromycin can probably be included in a marketable product that provides improved treatment and patient compliance through proper formulation and storage strategies.

5.5 RECOMMENDATIONS

The study revealed that stable amorphous clarithromycin tablets can be developed. However, to produce a stable product under various environmental conditions for commercial purposes, the following interventions are recommended:

1. To develop solid dispersions of amorphous clarithromycin to further improve the stability of amorphous clarithromycin as polymers used in the process can significantly reduce the crystallization process by acting as a barrier.
2. To develop formulations using the solid dispersions of amorphous clarithromycin and assess the stability.
3. To improve the finished product stability, the tablets can also be coated with acrylate polymers such as Eudragit®, which prevent the uptake loss/uptake of the moisture.
4. Further *in-vitro / in-vivo* correlation studies in animals are recommended to establish the innovator's bio-equivalence of the developed product.

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ANNEXURE A: STANDARD CALIBRATION CURVE