

Stability of amorphous azithromycin in a tablet formulation

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ABSTRACT

It is a well-known fact that drugs can exist in different solid-state forms. These solid-state forms can be either crystalline or amorphous. Furthermore, significant differences are identified between the different solid-state forms of the same drug. Physico-chemical properties that are affected by the solid-state include: melting point, solubility, dissolution rate, stability, compressibility, processability, to name but a few. During the last two decades a significant amount of attention was directed towards the amorphous solid-state forms of drugs. The amorphous form is the direct opposite of the crystalline solid-state. While crystalline forms are constituted by unit cells arranged in a repetitive and structured nature, amorphous forms do not have a long-range order. This lack of order leads to an increase in the Gibbs free energy of such compounds which in turn leads to increased dissolution and solubility. The advantage of improved aqueous solubility and dissolution is a sought after characteristic within the pharmaceutical industry. Improved solubility ultimately could lead to improved bioavailability of a drug. In this study the amorphous nature and stability of amorphous azithromycin was studied. Although previous studies reported that amorphous azithromycin can be easily prepared, there is not a significant amount of data available on the stability of the amorphous form. Furthermore, the effect of milling, mixing, compression, handling and storage on the amorphous form was also investigated.

This study showed that amorphous azithromycin remains stable during milling, mixing and compression. A compatibility study on azithromycin when mixed with tableting excipients showed some incompatibilities and this was helpful information to assist with the choice of excipients to be included in the tablet formulation. During the formulation study it became evident that good formulation strategies can greatly improve the flow properties of a drug.

The stability of amorphous azithromycin was also studied. During this phase of the study an atypical stability indicating method was used in order to determine and demonstrate the stability of amorphous azithromycin. Dissolution studies were used to illustrate the stability of amorphous azithromycin due to the fact that dissolution is the only method that indicates the phenomena of solution-mediated phase transformation of an amorphous form to a stable crystalline form. During the stability study of six months at $40^{\circ}\text{C} \pm 75\% \text{ RH}$ no recrystallisation of the amorphous form to the crystalline form occurred. It was concluded that amorphous azithromycin will remain stable during processing steps, product formulation and manufacturing as well as during storage for a period of six months at elevated temperature and humidity.

Key words: azithromycin, amorphous, stability, solution-mediated phase transformation, physico-chemical properties

UITTREKSEL

Dit is 'n alombekende feit dat geneesmiddels in verskeie vastestof vorme kan bestaan. Hierdie vastestof vorme kan kristallyn of amorf wees. Verder kan daar beduidende verskille bestaan tussen die verskillende vastestof vorme van dieselfde geneesmiddel. Fisies-chemiese eienskappe wat geaffekteer word deur die spesifieke vorm waarin 'n geneesmiddel voorkom sluit in: smeltpunt, oplosbaarheid, dissolusietempo, stabiliteit, saampersbaarheid, om maar slegs 'n paar te noem. Gedurende die afgelope twee dekades is baie aandag gewy aan die amorge vorme van geneesmiddels. Die amorge vorme is direk teenoorgestel van die kristallyne vorme van 'n geneesmiddel. Kristallyne vorme word uitgekies deur die geordende en herhalende samestelling van molekulêre eenheidselle teenoor die amorge vorme wat geen molekulêre orde het nie. Hierdie gebrek aan molekulêre orde het 'n verhoogde Gibbs vrye-energie tot gevolg wat weer verhoogde dissolusie en oplosbaarheid tot gevolg het. Die voordeel van verbeterde wateroplosbaarheid en dissolusie is 'n gesogte eienskap van 'n geneesmiddel binne die farmaseutiese bedryf. Verbeterde oplosbaarheid het op die uiteinde verbeterde biobeskikbaarheid van 'n geneesmiddel tot gevolg. Tydens hierdie studie is die amorge aard en stabiliteit van amorge asitromisien ondersoek. Alhoewel vorige studies al gerapporteer het dat amorge asitromisien maklik berei kan word is daar tot op hede min data beskikbaar aangaande die stabiliteit van die amorge vorm. Die effek wat maling, vermenging, samepersing, hantering en berging op die amorge vorm van hierdie geneesmiddel het, is ook ondersoek.

Hierdie studie het bewys dat amorge asitromisien stabiel is gedurende maling, vermenging en samepersing. 'n Verenigbaarheidstudie waar asitromisien met verskillende tablettering hulpstowwe vermeng is, het 'n paar onverenigbaarhede uitgewys. Hierdie was waardevolle inligting wat gehelp met die keuse van hulpstowwe. Tydens die formuleringsfase van hierdie studie het dit duidelik na vore gekom dat die vloeie-eienskappe van 'n geneesmiddel drasties verbeter kan word deur middel van goeie formuleringsstrategieë.

Die stabiliteit van amorge asitromisien is ook ondersoek tydens hierdie studie. Gedurende hierdie fase van die studie is die stabiliteit van amorge asitromisien ondersoek deur die gebruik van 'n atipiese stabiliteitsaanduidende metode. Dissolusiestudies is gebruik om die stabiliteit van amorge asitromisien te illustreer. Die rede hiervoor was omdat slegs dissolusies die verskynsel van oplossing-gemedieerde fase oorgang akkuraat kan aandui. Tydens so 'n verskynsel kristalliseer die amorge vorm na die stabiele kristallyne vorm. Tydens die ses maande stabiliteitstudie is die tablette wat die amorge vorm bevat gestoor by $40^{\circ}\text{C} \pm 75\% \text{ RH}$. Die tablette wat die kristallyne vorm bevat is gebruik as kontrole. Gedurende die bergingstydperk was geen kristallasie van die amorge vorm na die

kristallyne vorm opgemerk nie. Die gevolgtrekking is gemaak dat amorge asitromisien stabiel sal bly tydens prosessering, produkformulering en vervaardiging sowel as tydens berging vir 'n periode van ses maande by verhoogde temperatuur en humiditeit.

Sleutelwoorde: asitromisien, amorf, stabiliteit, oplossing-gemedieërde fase oorgang, fisies-chemies eienskappe.

OBJECTIVES

During this study the following objectives was pursued:

- Preparation of amorphous azithromycin
- Solid-state characterisation of the amorphous solid-state form in comparison with the crystalline dihydrate form of azithromycin
- Selection of suitable excipients by conducting compatibility studies between excipients and azithromycin
- Manufacturing of the tablet dosage forms containing both amorphous and commercially available azithromycin dihydrate
- Testing of the physical tablet properties as well as drug content
- Stability studies at elevated temperatures and humidity to determine the effect of temperature and humidity on the amorphous form.
- Dissolution studies to evaluate the dissolution profiles and possible solution-mediated phase transformation of the amorphous azithromycin.

CHAPTER 1

SOLID-STATE PROPERTIES OF DRUGS

1.1 INTRODUCTION

It is a well-known fact that drugs may exist either in crystalline or amorphous solid-state forms. With the crystalline solid-state forms structural units are repeated in a regular pattern so that a well-defined crystalline lattice is formed. The main difference between the crystalline and amorphous solid-state forms of drugs is based on the presence or lack of unit cell orientation and positional long-range order. Therefore, meaning that the amorphous solid-state form does not exhibit a well-defined molecular packing but rather local molecular associations that shows only short-range unit cell order (Heinz *et al.*, 2007). Crystalline forms exhibit polymorphism, where polymorphism is the ability of a substance to exist in more than one distinct crystal form. Polymorphs are different crystalline forms of the same pure substance, in which the molecules have different arrangements and/or 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 *et al.*, 2001).

Different polymorphic forms exhibit different physico-chemical properties and therefore the biopharmaceutical factors may be different for each polymorphic form. Since polymorphism is related to the packing arrangements and/or conformations of the molecules, this characteristic is only exhibited by a substance when in its solid form (Vippagunta *et al.*, 2001). Polymorphs may display different chemical and physical properties. Properties that might be influenced by polymorphism 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 (Yu, 2001). Selection of the optimum solid-state form of an active pharmaceutical ingredient (API) is a critical aspect for the formulation and development of a dosage form (Zhang *et al.*, 2004).

Amorphous pharmaceuticals represent both opportunity and necessity in the pharmaceutical development. The opportunity arises from the potential to improve solubility and thus bioavailability *via* use of an amorphous form, rather than a crystalline form. On the other hand, it is sometimes the case that no crystalline form is available, in which case it is then necessary to deal with the amorphous form (Strachan *et al.*, 2005).

1.2 THE CRYSTALLINE SOLID-STATE

APIs can exist in the form of amorphous materials, crystalline forms, polymorphic forms, solvates or hydrates. Crystalline solids consist of unit cells and the unit cells are repeated

regularly in long range three dimensional order in space. Stahly (2007) reported that 80 % of organic substances can exist as polymorphic forms.

Phase transitions can occur during manufacturing process such as: crystallisation, milling, heating, storage of the dosage form, to name but a few. Phase transitions such as polymorph conversion, desolvation, and dehydration, crystalline to amorphous or amorphous to crystalline are important parameters since it can alter the stability, dissolution rate and bio-availability of the drug. Therefore, it is important to choose the best and suitable form at the initial stages of drug development. Studies of such transitions are important because a change in the physical form of a crystalline form can influence process development which could impact on the product performance at the end.

Polymorph screening is an important step in the pre-formulation process. The goal of polymorph screening is to find all possible polymorphic forms and to determine which one is the stable form. There are many ways to perform a polymorph screening test, but recrystallisation remains one of the most reliable and effective methods. Other methods include evaporation, slurrying, spray drying, and then recrystallisation from the melt or the amorphous form.

Solvates are crystalline solids which contain solvent molecules (usually recrystallisation solvents) within the crystal structure. The solvent molecules could be in stoichiometric or non-stoichiometric proportions within the crystal structure. Every solvate of a given API have unique and characteristic properties. As mentioned in previous section, when the incorporated solvent is water, it is termed a hydrate (Byrn *et al.*, 2010; Chieng *et al.*, 2011; Stahly, 2007; Vega *et al.*, 2007; Vippagunta *et al.*, 2001; Yu, 2001).

1.3 THE AMORPHOUS SOLID-STATE

As mentioned earlier amorphous materials have no long range order but consist only of short range order which is a characteristic of liquids. Amorphous materials exhibit greater chemical reactivity (Pikal *et al.*, 1978). This phenomenon leaves the pharmaceutical scientist with an interesting dilemma, the higher energy levels in comparison with the crystalline state, lead to enhanced solubility values but it can also convert back during storage or processing to the crystalline state (Huttenrauch, 1978; Yoshioka *et al.*, 1994).

1.3.1 Formulation of amorphous drugs – general considerations

The pharmaceutical significance and importance of the solid-state properties of amorphous materials could be discussed over three areas of interest: crystallisation, chemical degradation and mechanical responses to stress (Hancock & Zografi, 1997).

Crystallisation of amorphous materials can occur when the relative humidity reach a certain level, small amounts of absorbed water can plasticise amorphous forms leading to the recrystallisation of the amorphous form (Hancock & Zografi, 1997). Crystallisation during storage and handling is also a possibility since the amorphous form is thermodynamically metastable in comparison to the crystalline form (Hancock & Zografi, 1997). The storage temperature of an amorphous material can be reduced to prolong stability and shelf-life. An example being that of amorphous indomethacin having a T_g of about 50°C, and crystallising completely within a few weeks at a storage temperature of 20°C. After reducing the temperature below zero, the indomethacin amorphous form was stable for longer than a year (Hancock & Zografi, 1997).

Chemical degradation of substances in the solid-state at high temperatures and relative humidities is a common occurrence for drugs which degrade easily in solutions. With crystalline and amorphous drugs we would suspect that the amorphous drug would have a greater reaction rate than the crystalline forms due to the higher free energy within the amorphous systems. The general rule therefore is that crystalline material is more stable than amorphous materials. Pikal and Rigsbee (1997) reported that the stability observed in the crystalline state may not be the same for proteins. They found that freeze dried amorphous insulin is more stable than crystalline insulin.

With the processing of pharmaceuticals into a specific dosage form there is a number of factors which could influence the stability and performance of a given drug. The amount of water in the excipients should be kept at a specific level for a specific drug. Mechanical stress during tableting could alter the viscoelastic behaviour of crystalline and amorphous materials (Hancock & Zografi, 1997).

It is sometimes the case that no crystalline form, including salts, co-crystals or pharmaceutically acceptable solvates, is available which has a favourable pharmaceutical profile. The amorphous drug may then be prepared by a variety of methods including precipitation or desolvation of a solvate, and the ease of preparation will be affected by the glass transition temperature (T_g) and the extent to which the T_g is lowered by residual solvent (Hancock & Zografi, 1997). In some cases, amorphous salts have been found to have a higher T_g than the free acid or base (Towler *et al.*, 2008; Tong *et al.*, 2002), and amorphous dispersions may also be used to improve the physical properties.

Several methods can be employed to prepare amorphous solid-state forms (Figure 1.1). Amorphous forms can be prepared by solvent-based methods, melting or grinding. With solvent-based methods a drug and polymer are dissolved and after rapid removal of the solvent, an amorphous residue is formed. Normally roto-evaporation is used for rapid removing of the solvent. Spray-drying is another solvent based method. Hot melt extrusion is a process commonly used in the food industry during rubber and plastic manufacturing. Lately hot melt extrusion is frequently used in the preparation of amorphous materials. Hot melt extrusion is solvent free and anhydrous, highly suitable for drugs which are sensitive to water. The specific drug should however be thermally stable to the applied temperatures (Byrn *et al.*, 2010).

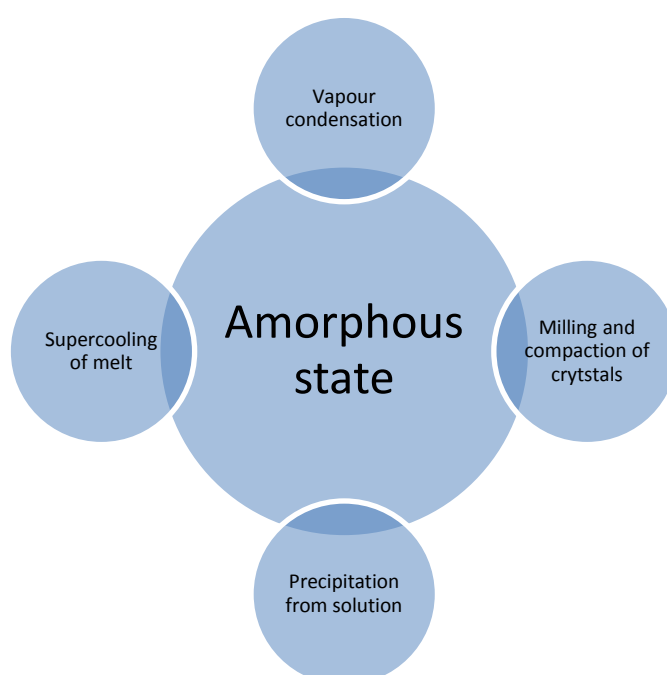


Figure 1.1: Ways in which the amorphous character is induced in pharmaceutical systems (Adapted from Hancock & Zografi, 1997).

Whenever amorphous material is the only option left, the following questions should be asked:

Based on the preliminary solubility data, will the conversion of the amorphous solid-state form to a crystalline form negatively impact on the bioavailability of the drug? and,

During formulation will the amorphous material be stable? What is the physical and chemical stability of the amorphous material? (Huang & Tong, 2004).

The advantage of amorphous materials is that it is significantly more soluble than the corresponding crystalline forms. The experimental solubility is however, normally less than the predicted solubility. This is because it is difficult to measure in amorphous systems the

true equilibrium solubility (Hancock & Parks, 2000). The amorphous systems are unstable and during solubility measurements the unstable amorphous material tends to recrystallise and change into a crystalline form of the drug. Aucamp *et al.* (2013) reported the solution-mediated phase transformation of roxithromycin in water. The metastable amorphous form of roxithromycin crystallises to the stable form during the solubility tests and therefore the solubility value obtained will only reflect that of the stable solid-state form. Solution-mediated transformation of an amorphous solid-state form to the crystalline form during dissolution testing involves three stages, i.e. pre-transformation -, transformation - and steady state stage.

(1) *Pre-transformation stage* – during this stage the metastable phase dissolves at a constant rate. Generally this rate is fast resulting in the attainment of relative high concentrations of dissolved drug.

(2) *Transformation stage* – this stage begins upon super-saturation of the dissolution solution with respect to the stable solid-state form. During this stage nucleation and crystal growth of the stable solid-state form will occur. This stage will also involve the simultaneous dissolution of both solid-state forms that are now available. The relative amount of the stable solid-state form will continue to increase and therefore leading to the overall decrease of the dissolution rate.

(3) *Steady-state stage* – This is the final stage during the process of solution-mediated transformation. During this stage only the dissolved concentration of the stable crystalline solid-state form will be detected (Aaltonen *et al.*, 2006).

Aucamp propose that for amorphous or unstable forms the solubility enhancement ratio should be calculated using the peak concentration during dissolution testing. Such an approach should allow for more accurate solubility values (Aucamp *et al.*, 2013).

1.4 PHARMACEUTICAL IMPORTANCE OF IMPROVED SOLUBILITY OF A DRUG

Drugs with low aqueous solubilities tend to dominate the new entities being developed recently, which could be problematic due to poor bioavailability (Van Eerdenburgh *et al.*, 2013). Hence the renewed interest in ways to enhance solubility and or formulate an entity with enhanced bioavailability.

For a drug to be effective and bioavailable, it should dissolve easily in the gastro-intestinal (GI) tract. Poor solubility can be overcome by formulation strategies, but sometimes even that is not enough to dissolve the drug. Also, the rate and extent of drug absorption is a complex process and many factors play a role in the absorption process, with the solubility factor being one of many (Dahan *et al.*, 2009).

The use of high throughput methods in drug discovery has led to compounds with more lipophilic properties and hence poor aqueous solubility (Keserü & Makara, 2009; Lipinski, 2000), resulting in drugs with dissolution-limited bioavailability. In the case of poorly soluble but well-permeable drugs (BCS class II), high free energy states such as the amorphous form can significantly improve 'apparent' solubility (Hancock & Parks, 2000; Hancock & Zografi, 1997). This often leads to large increases in dissolution rate in the GI tract, thus increasing the bioavailability. However, since the amorphous form is a highly metastable state, there is a thermodynamic drive towards crystallisation, in some cases even at temperatures below the glass transition temperature (T_g) (Wu & Yu, 2006). In cases where a crystalline drug has been rendered amorphous, it is common practice to prepare a dispersion of the drug in a pharmaceutically acceptable polymer in order to stabilise against crystallisation (Nagapudi & Jona, 2008). In some cases, the use of a solid dispersion may have a further role in acting as a crystallisation inhibitor *in vivo* (Marsac & Taylor, 2009).

1.5 TABLET FORMULATION – A GENERAL OVERVIEW

1.5.1 Tablets as a solid dosage form

The most common route of administering drugs is through the oral route due to the fact that it is a convenient and safe method of drug administration. There are two main solid dosage forms namely tablets and capsules. The compressed tablet is by far the most widely used dosage form. The formulation of a drug into an acceptable product still requires a substantial amount of expertise, knowledge and a basic understanding of the solid-state properties of powders. Different types of tablets collectively represent the largest dosage form type. Some tablets are swallowed whole, others are swallowed after being chewed, some types are dispersed in water and some are retained in the mouth where the drug is then dissolved and absorbed. Tablets are used mainly for systemic drug delivery and therefore the drug must be released from the tablet. The dissolution process usually occurs in the fluids of the mouth, stomach or intestine (Alderborn, 2013).

In order to successfully formulate a tablet it is imperative to firstly investigate the chemical and physical properties of the drug that needs to be incorporated into the solid dosage form. Not only is it important to understand the physico-chemical properties of a drug for formulation purposes, it is also important to be able to choose the most suitable excipients. Excipients are usually inert or inactive ingredients that do not react with the drug, but plays a specific role in the dosage form formulation. The formulation process for the production of acceptable tablets generally involves several developmental steps. The following flow diagram is a summary of all necessary steps that should be followed to produce pharmaceutically acceptable tablets.

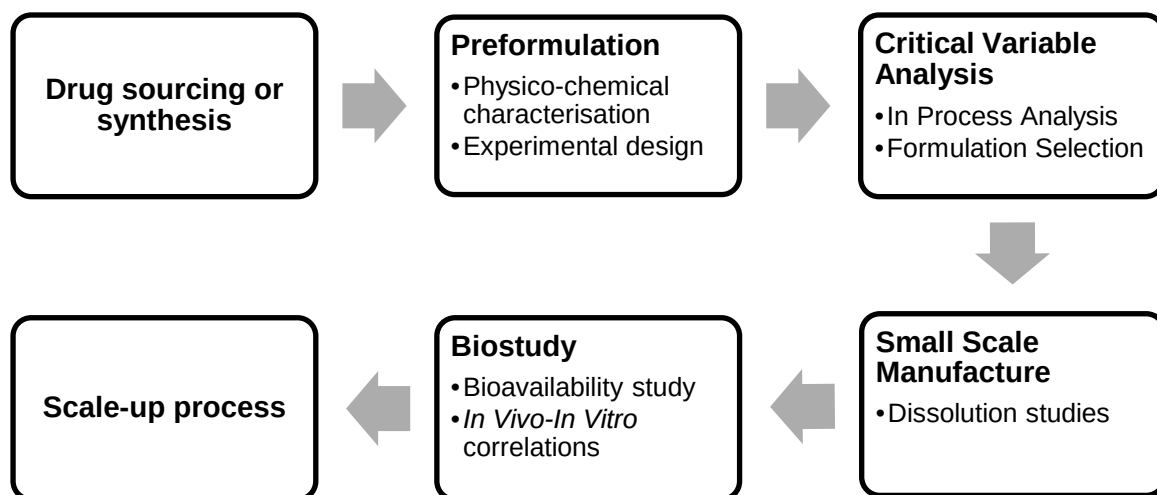


Figure 1.2: A formulation development model depicting the different steps in the development process of tablets (adapted from Augsburger & Zelhofer, 2007).

1.5.2 Tablet compression

All tablets are produced through the process of compression (Armstrong, 2007). The compression process entails the forcing of the powder particles into close proximity of each other to such an extent that the result is a porous solid sample with a defined shape. The powder particles are contained in a die and a compression force of several tons is applied to the powder by means of punches. The shape of the die determines the cross-sectional shape of the tablet and the distance between the punch tips determines the thickness of the tablet. Basically the tablet compression process can be divided into three main steps, namely; (1) die filling; (2) tablet formation and (3) tablet ejection.

1.5.3 Powder flow properties

The preparation of solid dosage forms greatly depends upon the characteristics of the ingredients forming the formulation. Powders are generally seen as assemblies of particle with interactions between gas and solid internal surfaces. Generally, powders are non-homogenous in nature but consist of discrete solid particles of different shapes and sizes inter dispersed with a gaseous phase (Howard, 2007). Powders are therefore composed of solid particles of either one compound or a mixture of compounds and of which the particles have a mean diameter of less than 1000 μm .

The flow properties of powders (drug and excipient) have great impact on the tableting process, since the powder mixtures need to flow from mixing or storage containers to filling

stations such as tablet dies. The weight uniformity of tablets is dependent on the uniform flow of the powder mixture. The flow properties of powders also influence the mixing and de-mixing of the drug and excipient mixture before tableting can commence. The following factors can impact the flow properties of powders: particle size distribution and specific surface area, particle shape distribution, cohesion, strength and adhesion, packing properties, rate and compressibility of packing, segregation and angle of internal friction (Howard, 2007).

(a) Adhesion and cohesion

Due to intermolecular forces that exist at the surfaces of particles can interact which would lead to the formation of bonds between the particles. These forces can include Van der Waals forces, electrostatic forces as well as hydrogen bonding. Adhesion and cohesion are basically aspects of the same phenomenon. Cohesion is described as non-specific Van der Waals forces that occur between like surfaces, therefore powder particles in a bulk solid. On the other hand adhesion occurs between two different particles or for example between a particle and a hopper wall (Aulton, 2013).

(b) Angle of repose

Although the angle of repose is a simple method for the indication of the powder flow characteristics of a drug or drug/excipient mixture it is still based on scientific principles. A powder particle will begin to move when the angle of inclination is large enough to overcome frictional forces. On the other hand a powder particle will stop sliding when the angle of inclination is below that required to overcome adhesion or cohesion. This cooperation of forces will cause powder poured from a container on to a horizontal surface to form a heap. This heap is initially formed as a stack of particles until the approach angle for joining particles is large enough to overcome frictional forces. This leads to the slip and roll of the particles until the gravitational forces balance the inter-particulate forces. The sides of the heap form a measurable angle with the horizontal surface. The angle of repose will be high for a cohesive powder and low for a non-cohesive one. Therefore, a high angle of repose will indicate a poor flowing powder while a low angle of repose will be indicative of a free flowing powder (Aulton, 2013).

(c) Particle size and size distribution

It is imperative to realise that all matter interacts. The dimensions of particles and any change in the nature of particles will also change the forces acting on them. Fine powder particles less than 100 μm in diameter possess over a significantly high surface-to-mass ratio which in turn will lead to more pronounced adhesion/cohesion. In the case of small particle sizes gravitational forces plays a significant role. With relative small particles, the

flow through an orifice may be restricted because the cohesive forces between the particles are of the same magnitude as the gravitational forces. Since gravitational forces are a function of the particle diameter raised to the third power, they will become more significant once the particle size increases and flow is facilitated.

Generally, the particle size of a powder is increased through the process of granulation. It is a general rule of thumb that particles larger than 250 μm are usually relatively free flowing. Care should however be taken not to increase the particle size to such an extent that flow from the hopper to the tableting die becomes problematic. Considering the above mentioned facts it is quite clear that a fine line exist between particle size and the attainment of optimum powder flow properties (Howard, 2007; Aulton, 2013).

(d) Particle shape and surface morphology

Particle size combined with the shape and morphology of the particles will also greatly influence the flow properties of a powder. It is generally accepted that the flowability of a powder will decrease as the shape of particles become more irregular (Howard, 2007). A good comparison is the flow properties of spheres versus that of flakes. Spherical particles has minimum inter-particulate forces therefore resulting in better flow characteristics, while irregular particle shapes possess over a high surface-to-volume ratio which will result in poorer flow properties. In the case of irregular particle shapes it must be taken into account that mechanical interlocking may also occur in addition to adhesion or cohesion (Aulton, 2013).

1.5.4 Tablet manufacturing

The most prevalent technique for producing tablets is through the method of powder compression. This process allows particles to cohere into a porous, solid form having a definitive geometry. Although it seems to be a simple process the compression of a powder or granulated mixture into a tablet is complex and irreversible (Leuenberger & Rohera, 1986). Mainly, the tableting process occurs in three stages namely, (1) die filling, (2) tablet formation and (3) tablet ejection.

(1) Die filling

The die is typically filled through the gravitational flow of the powder form the hopper via the die tablet into the die. The die is closed at the lower end by the lower punch.

(2) Tablet formation

The upper punch descends and enters the die cavity, it exerts pressure on the powder bed in the die while approaching the lower punch, and this allows the pressure to increase on the powder in the die (Wray, 1992). During the formation process the powder particles will

rearrange in such a way that a closer packing is obtained. As the upper and lower punch comes closer to one another the rearrangement process of the particles becomes more stunted and deformation of the particles commence, ultimately leading to the formation of points of contact between the particles (Rudnic & Kottke, 1996). After the maximum force is reached the upper punch will leave the die cavity and this is noted as the decompression phase of the whole tableting process (Aulton, 2013).

(3) Tablet ejection

The lower punch will rise until its tip reaches the same level as that of the die. The tablet is then pushed aside into a collection container (Aulton, 2013).

(a) Tablet production via the direct compression method

Direct compression of a mixture is considered to be a more cost-effective and less labour intensive tablet production method in comparison with granulation. This is understandable due to the fact that not only is the production steps minimised but less equipment or machinery are needed. Furthermore, direct compression is considered advantageous since the stability of most drugs is not affected detrimentally since heat and granulation fluid is not involved in the process. Also, tablets that were produced by means of direct compression holds the possible advantage that the dissolution rate might be faster due to the quicker disintegration of the tablet into individual drug particles (Alderborn, 2013).

A considerable amount of time and effort is put into the development of diluents and other excipients that are direct compressible (Carstensen, 2001). Diluents that are intended to be included into direct compression formulations have to possess certain qualities. Firstly, it should exhibit good flow properties since it should ensure uniform powder flow into the hopper and subsequently the tableting die. It should have a high bulk density so that it will truly act as tablet filler, meaning that it would result in an acceptable diameter and thickness of the tablet after compression. Another property that a direct compressible filler should have is a good pressure-strength profile that will result in the compression of acceptable tablets at relatively low compression pressures (Armstrong, 2000).

Despite the above mentioned advantages the process of direct compression also has some disadvantages. In order to have a powder mixture with acceptable flowability and bulk density, relative large particle sizes are necessary. This might lead to problems during mixing with a prevalence of powder segregation occurring. Furthermore, a powder mixture which consists mainly of a drug having poor compactibility will be difficult to compress directly and finally the uniform colouring of the tablets is difficult to obtain with a colourant in dry particulate form (Alderborn, 2013).

(b) Tablet production via the granulation method

Granulation of powder particles is a useful technique in the pre-tableting stage in order to improve the compaction characteristics of a specific powder. Generally, granulation is considered as a two stage particle enlargement process. The drug and diluent mixture are granulated before tableting to:

- Increase the bulk density of the powder mixture,
- Improve the flowability of the powder,
- Improve the mixing homogeneity and reduce the segregation of the powder,
- Improve the compactibility of the powder mixture by adding a solution binder that is distributed on the surface of the particles and in some instances,
- Ensuring a homogenous colouring of the tablet.

Different granulation methods may be use to obtain granules of the drug and filler mixture namely: convective mixers, fluidised-bed driers, spray driers and compaction machines (Alderborn, 2013).

1.5.5 Excipients used for tableting

In order to successfully produce acceptable tablets it is necessary to add a range of excipients to the drug(s). Pharmaceutical excipients may be defined as inert substances that are included in formulations to improve the manufacturing process, drug and product stability, bioavailability as well as patient compliance. Considering pharmaceutical excipients, the following criteria are essential: physiological inertness, physical and chemical stability, no interference with drug bioavailability, conformance to the requirements of regulatory bodies, absence of microbial organisms, it should be readily available and cost-effective (Armstrong, 2007). Depending on the main function of the excipient in a specific formulation, excipients are usually grouped into different categories. The function of the most common types of excipients is described in the following paragraphs.

(a) Filler or diluent

Fillers are bulking agents. Typically they are added to tablet formulations to produce tablets of an appropriate size. This type of excipient is used in large quantities and should be chemically inert, non-hygroscopic, biocompatible, possess good compactibility and dilution capacity, have an acceptable taste and since it is used in large quantities it must be cost-effective (Alderborn, 2013). Examples of typical diluents used in tablet manufacturing includes: sugars, lactose, mannitol, sucrose, inorganic salts, polysaccharides and microcrystalline cellulose (Armstrong, 2007).

(b) Binding agents

Binders are materials that act as adhesives to adhere the individual particles together. Most binding agents are polymeric characteristics and are derivatives of cellulose or starch (Khankari, 1993). The following binders are most commonly used in tablet formulations: sugars, glucose, polymers, natural gums, starch, gelatin, polyvinyl-pyrrolidone (PVP), polymethacrylate.

(c) Glidants

Glidants are incorporated into tablet formulations to improve the flowability of the powder mixtures. Most often glidants are added to formulations intended for direct compression but it can also be added to granules before the tableting process to ensure effective flowability during high production speeds. Some commonly used glidants and the typical concentration in tablet formulations are listed in Table 1.1.

Table 1.1: Typical tablet glidants used in tablet formulations (Armstrong, 2007)

Glidant	Concentration in tablet (%)
Calcium silicate	0.5-2
Powdered cellulose	1-2
Magnesium carbonate	1-3
Magnesium oxide	1-3
Magnesium silicate	0.5-2
Silicon dioxide	0.05-0.5
Starch	2-10
Talc	1-10

(d) Disintegrants

Disintegrants are added to tablet formulations to ensure that the tablet breaks up into smaller fragments once it comes into contact with a liquid. Ideally a tablet should disintegrate into smaller pieces in order to obtain the largest possible effective surface area during dissolution. Disintegrants are classified into two types: (a) disintegrants that facilitate water

uptake and (b) disintegrants that will rupture the tablet. Disintegrants that facilitate water uptake acts by facilitating the transport of liquid into the porous structure of the tablet with the consequence that the tablet will break into smaller fragments. On the other hand, the rupturing of tablets is facilitated by the swelling of the disintegrant particles during the sorption of water. The most commonly used disintegrants are starch, microcrystalline cellulose, clays, algin, gums and surfactants (Alderborn, 2013).

(e) Lubricants

Lubricants are added to tablet formulations to ensure that tablet formations and ejection occur without or with low friction between the tablet and the die wall. If high friction is encountered during the tableting process a series of problems may arise leading to substandard quality of the tablets. Capping or fragmentation of the tablets or vertical lines on the tablet edges may occur. High friction can even cause the production of tablets to stop. Typical lubricants are divided into water-insoluble and water-soluble lubricants and are listed as the following. *Water-insoluble:* Metal stearates, stearic acid, talc. *Water-soluble:* boric acid, sodium chloride, benzoate, sodium or magnesium lauryl sulphate (Alderborn, 2013).

(f) Anti-adherents

Anti-adherents prevent the adhesion of powder to the punch faces and therefore prevent powder particles sticking to the punches. Such a phenomenon is typically prone to occur if the punches have markings or symbols. The sticking of a thin layer of powder to the punches will lead to uneven, matt tablets with unclear markings or symbols. Typical anti-adherents that are being incorporated into tablet formulations include: talc, corn starch, metal stearates and sodium lauryl sulphate (Alderborn, 2013).

(g) Flavourants and colourants

Colourants are added to tablets mainly to assist in patient compliance. Furthermore, colouring plays a critical role in tablet identification. Typical colourants that are used included: natural pigments or synthetic dyes. Flavouring agents are added to give the tablet a more pleasant taste. General flavourants and sweeteners include: mannitol (natural) or aspartame (artificial) (Alderborn, 2013).

1.5.6 Drug-excipient compatibility studies

The compatibility of drugs and excipient when in combination is a critical factor during pre-formulation and product formulation stages that are most often overlooked. Usually drug-excipient incompatibilities are related to the moisture present in one or more of the components. Drug-excipient compatibility studies are typically performed at high

temperature and humidity conditions and on blends of pure drug and excipients in ratios similar to those that will be used in the final dosage form. The evaluation and testing of drug-excipient compatibility will be done through visual inspection of the blends for changes in colour or texture, qualitative results can be obtained if the blends are compared with unstressed sample blends and analysed by means of high performance liquid chromatography (HPLC) or thin layer chromatography (TLC) and lastly differential scanning calorimetry (DSC) can be applied and the appearance or disappearance of one or more thermal events might be an indication of incompatibility. Another powerful technique for the detection of incompatibilities between drug and excipient blends is isothermal microcalorimetry, which allows the detection of small and low energy interactions between the individual components (Augsberger & Zelhofer, 2007).

1.5.7 Evaluation of tablets

(a) Uniformity of content

Pharmaceutical dosage forms should provide a constant dose of drug between individual dosages. The test of the uniformity of content for tablets ultimately test whether each tablet in a batch will contain the labelled concentration of drug. In practice, it would be impossible to manufacture tablets that always contain the exact same drug concentration. Therefore small variations between individual tablets are acceptable and the limits for this variation are defined as standards in pharmacopoeias. In the case of tablets the aspect of dose uniformity or dose variation is tested in two separate tests, namely: uniformity of weight and uniformity of active ingredient (Alderborn, 2002).

For the test for uniformity of drug content a sample of tablets is randomly collected for each batch. Typically 10 tablets will be sampled, followed by a determination of the drug concentration in each tablet. The average drug content is calculated and the content of the individual tablets should fall within specified limits in terms of percentage deviation from the mean concentration value (Alderborn, 2002).

(b) Disintegration

For a substantial amount of time the disintegration test for tablets has been a tablet evaluation requirement. During the development of the science of tablet manufacturing the disintegration test for tablets was at some stage the only test used to evaluate the release of drugs from the tablet matrix. However, it was realised that using the disintegration test alone to determine drug release is not nearly sufficient. This resulted in the introduction and development of dissolution testing of tablets (Kootke & Rudnic, 2002).

A disintegration apparatus consists of six chambers. These chambers are tubes that are open at the upper end and closed by a mesh at the lower end. One tablet is placed in each tube and sometimes a plastic disc is placed upon it. The tubes are placed in a water bath, set to a temperature of 37°C. Subsequently the tubes are raised and lowered at a constant frequency in the water in such a way that at the highest position of the tubes, the screens remains below the surface of the water (Alderborn, 2002).

A disintegration test is considered complete when the particles remaining on the mesh (other than fragments of coating) are soft and without palpable core. A maximum time for disintegration to occur is specified for each tablet, and at the end of this time the aforementioned criteria must be met. The disintegration media used during disintegration testing is greatly dependent on the type of tablet to be tested. The disintegration apparatus should meet the pharmacopoeial specifications. Several modifications of the official method have been suggested in the literature, including a basket insert as an alternative to the disks (Kootke & Rudnic, 2002).

(c) Dissolution

“Dissolution is the process by which a chemical or drug becomes dissolved in a solvent” (Shargel & Yu, 2002). Dissolution testing of solid oral dosage forms is considered to be the most important test. The determination of the dissolution rate of not only tablets is considered imperative due to the fact that the dissolution step can be a rate limiting step for the absorption of drugs (Kramer *et al.*, 2005). As a rule the Noyes-Whitney equation is used to describe the rate of drug dissolution. This equation calculates drug dissolution in terms of the rate of drug diffusion from the surface to the bulk of the solution. In general, drug concentration at the surface is assumed to be the highest possible, i.e. the solubility of the drug in the dissolution medium. The drug concentration (C) is considered to be the homogeneous concentration in the bulk solution which is generally lower than that in the stagnant layer immediate to the surface of the solid. The decrease in concentration across the stagnant layer is called the diffusion gradient

$$dc/dt = DA(CS - C)h$$

Where;

dc/dt = rate of drug dissolution,

D = diffusion rate constant,

A = surface area of the particle,

CS = drug concentration in the stagnant layer,

C = drug concentration in the bulk solvent, and

h = thickness of the stagnant layer (Shargel & Yu, 2002).

Dissolution studies are imperative in order to investigate the effect of formulation variables on the possible bioavailability of the drug. Furthermore, dissolution studies can indicate whether any solid-state changes of the particular drug were induced during processing steps. The correct interpretation of dissolution results will also allow pharmaceutical scientists to correlate and predict the performance of the drug under *in vivo* conditions (Alderborn, 2002).

(d) Mechanical strength

The mechanical strength of a tablet is an indication of the resistance of the tablet towards fracturing and attrition. An acceptable tablet must remain intact during production steps, packaging and handling and even storage. Thus, an integrated part of the formulation and production of tablets is the determination of their mechanical strength.

The mechanical strength of the tablet is primarily due to two events that occur during compression: the formation of inter-particulate bonds and a reduction in porosity resulting in an increased density (Kootke & Rudnic, 2002).

A number of methods are available for measuring mechanical strength and they give different results. The most commonly used methods for strength testing can be subcategorised in two main groups: attrition-resistance methods (typically friability) and fracture-resistance methods (hardness testing) (Alderborn, 2002). It is imperative to test the ability of tablets to remain intact. Not only is it important for patient compliance, but also for proper patient treatment.

1.6 CONCLUSION

Solid-state characterisation of amorphous forms is imperative for the sake of patenting, therapeutic and commercial applications and is also a requirement for regulatory authorities. Furthermore, the design of quality products with enhanced performance greatly depends on the solid-state form used during manufacturing, the formulation design as well as the manufacturing process. To ensure consistent product quality it is imperative to anticipate, control or prevent phase transformation during product design and development. Currently, the pharmaceutical industry is highly interested in amorphous formulations because amorphisation techniques are very innovative and results in a substantial amount of advantages in terms of drug solubility and bioavailability. Stabilisation of amorphous drugs

has become an important aspect of developing formulations with amorphous forms of poorly soluble compounds, to produce a drug product, which will perform consistently over time. In the present study, the amorphous form of azithromycin was used to enhance the solubility and hence dissolution. Thus it is important to develop a formulation that can maintain the therapeutic and performance benefit of an amorphous form of azithromycin, while preventing phase transitions during storage.

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CHAPTER 2

AZITHROMYCIN

2.1 INTRODUCTION

The word macrolide is originated from the macrocyclic lactone ring which is the core of the erythromycin base. Erythromycin was the only macrolide antibiotic in use for several years until the arrival of the novel macrolides. Erythromycin was the primary member of the group, first purified in 1952 from Philippines soil samples that retained *Streptomyces erythreus* (Sood, 1999). The macrolides, particularly derivatives of erythromycin are amongst the most frequently prescribed antibiotics that have proved to be extremely well tolerated and safe (Miroshnyk *et al.*, 2008).

Azithromycin is an antibiotic of the macrolide group which differs structurally from erythromycin by introduction of the methyl substituted-nitrogen ring at a position 9a in the macrolide base (Girard *et al.*, 1987). The addition of the nitrogen methyl group in the lactone ring enhances the drug stability in acidic conditions. Azithromycin has an extended serum half-life, enhanced bioavailability and increased tissue penetration in comparison with erythromycin (Opitz & Harthan, 2012).

2.2 MACROLIDE GENERATIONS

Erythromycin has been developed at least four decades ago and was the first 14-membered macrolide to be used clinically. However, newer members of this group, comprising of josamycin, roxithromycin, spiramycin, rokitamycin, dirithromycin and clarithromycin, have also been derived (Amsden, 1996). The development of newer generations of macrolide antibiotics was mainly prompted by the instability of erythromycin in acidic environments. The second generation of macrolides, which comprises of the 14-membered structured macrolides, for instance, clarithromycin and roxithromycin exhibits improved acid stability in addition to an increased spectrum of antimicrobial action.

Azithromycin is a 15-membered lactone ring macrolide while antimicrobial agents like tylosin, carbomycin A and spiramycin are the 16-membered lactone ring macrolides. The development of the newer generation macrolides is considered to be the answer to the emerging occurrence of erythromycin resistant diseases. Regrettably, all of these molecules became prone to the selection of resistant strains. The new generation of macrolides, the ketolides, whose medical

use is in its starting phase showed enhanced activity on some of the resistant strains such as *Streptococcus pneumonia* (Gaynor & Alexander, 2003).

The new generations of macrolides, which include clarithromycin, dirithromycin, azithromycin and roxithromycin, could be considered as the 'advanced-generation' group of macrolides. Their pharmacokinetics are studied by a combination of low serum concentrations, high tissue concentrations and in the case of azithromycin, an extended tissue elimination half-life. Azithromycin is specifically known for relatively high and sustained concentrations at the area of infection (Amsden, 2001).

Advanced macrolide antibiotics have been synthesised by modifying the erythromycin basic structure resulting in derivatives with increased spectrum of activity, improved pharmacodynamics, once a day administration, and better tolerability. In 1991 and 1992, the US Food and Drug Administration (FDA) gave approval for two derivatives namely clarithromycin and azithromycin, for clinical use. These two advanced macrolides are mainly prescribed for the treatment of respiratory tract diseases, sexually transmitted infections, and infections due to *Helicobacter pylori* and *Mycobacterium avium* complex (Zuckerman, 2004).

2.3 MODE OF ACTION OF AZITHROMYCIN

Azithromycin exhibits activity against bacteria by attaching to the 50S subunit of the ribosome in the cells of bacteria. Azithromycin obstructs peptide translocation and the synthesis process of bacterial poly-peptide. It has been expected that the high ribosome binding affinity of azithromycin might account for its enhanced activity against Gram-negative micro-organisms (Hoepelman & Schneider, 1995). The mechanism of azithromycin is depicted in Figure 2.1.

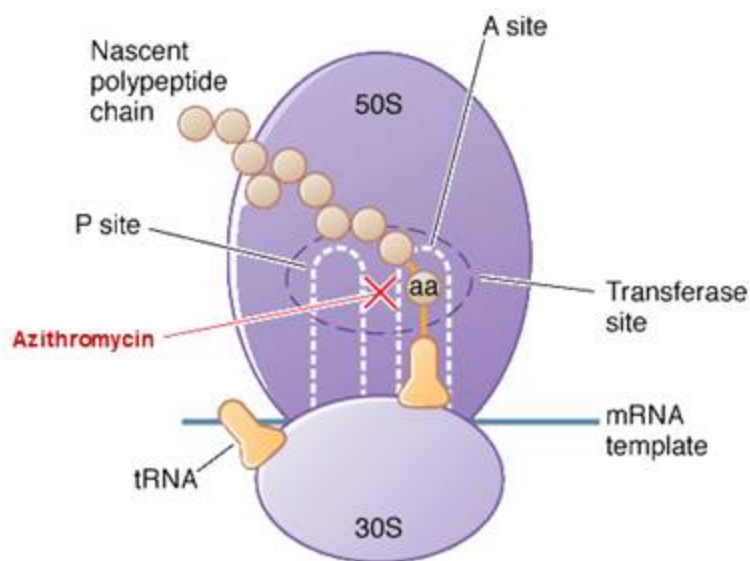


Figure 2.1: Mechanism of action of azithromycin (Adapted from NIPA, 2004).

2.4 STRUCTURAL ASPECTS OF AZITHROMYCIN

The molecular formula of azithromycin is $C_{38}H_{72}N_2O_{12}$ and it has a molecular weight of 749 g/mol (EP, 2014). Azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin) is produced by replacement of the methyl-substituted nitrogen with a carbonyl group in the glycone ring at the 9a position. This structural modification producing a dibasic 15-membered ring macrolide derivative, and is appropriately referred to as an “azalide”. This modification in structure makes the derivative more stable in acidic environments markedly enhances the serum half-life and tissue penetration, and hence therapeutic activity against gram-negative organisms is increased when compared with erythromycin (Zuckerman, 2004). The chemical structure of azithromycin is depicted in Figure 2.2.

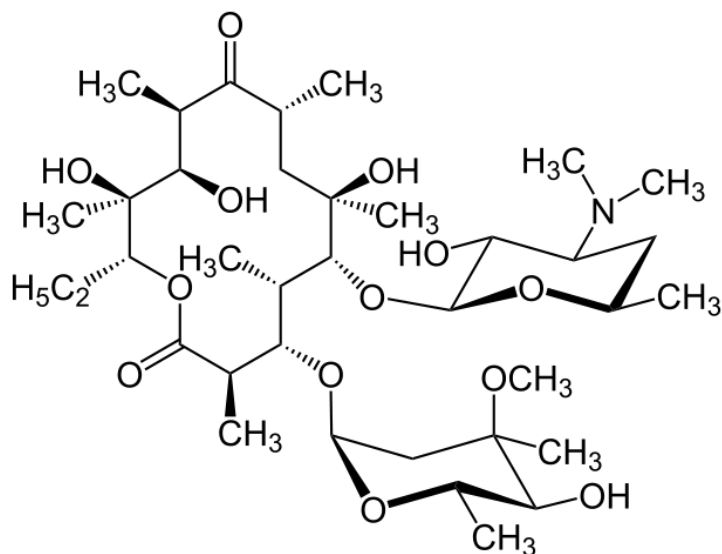


Figure 2.2: Chemical structure of azithromycin (Adapted from USP, 2014).

2.5 PHYSICO-CHEMICAL PROPERTIES

Azithromycin is commercially available in the form of a dihydrate, however a monohydrated form was also reported previously in literature (USP, 2014). The anhydrous solid state form of azithromycin appeared to be unstable since it transformed to the dihydrate during storage at room temperature. Furthermore, in the presence of moisture the monohydrate transforms to the stable dihydrate form (Gandhi *et al.*, 2002). According to the USP (2014), the labelled content of water for the anhydrate should be not more than 2.0 % when analysed by Karl Fischer titration. In addition, the water content for the monohydrate varies in the range of 1.8 - 4.0 % and a dihydrate will contain 4.0 - 5.0 % water (USP, 2014).

The melting points vary for the different hydrated solid-state forms of azithromycin. The anhydrous form of azithromycin has shown a melting point at 113 – 115°C and its dihydrated form at about 126°C (Odendaal, 2013).

Azithromycin is very poorly soluble in water, but is very soluble in some organic solvents, such as dehydrated alcohol and dichloromethane (USP, 2014). The aqueous solubility of azithromycin decreases as its hydration state changes. This means that azithromycin monohydrate is better soluble in water compared to the dihydrate and that the anhydrous form of azithromycin is the most water soluble solid-state form (Gandhi *et al.*, 2002; Hoepelman & Schneider, 1995; USP, 2014).

Timoumi *et al.* (2014) reported that azithromycin transformed from the dihydrate to the anhydrous form when heated up to a temperature of 80°C. The dehydration occurs with no physical or chemical modification of the crystalline lattice. The anhydrous solid-state form, transforms quickly to the dihydrate with the presence of moisture at ambient temperature. It is therefore imperative to manage the levels of moisture content throughout the drying procedure as well as during formulation processes. Furthermore, it is essential to make sure that excipients combined with azithromycin do not have an effect on the moisture content of azithromycin (Timoumi *et al.*, 2014). The physico-chemical properties of azithromycin are listed in table 2.1.

Table 2.1: Physico-chemical properties of azithromycin

Property	Description
Appearance	White or almost white crystalline powder (USP, 2014).
Solubility	Practically insoluble in water, freely soluble in ethanol and in methylene chloride (USP, 2014).
Purity (%)	94.5 to 103.0 % (anhydrous substance) (USP, 2014).
Melting point	The anhydrous form of azithromycin: 113-115 °C Dihydrate: 120-130 °C (Gandhi <i>et al.</i> , 2002).
Water content (%)	Anhydrate: Not more than 2.0 % (KF titration) Monohydrate: 1.8-4.0 % Dihydrate: 4.0-5.0 % (USP, 2014)
Stability in acidic conditions	Azithromycin is considered to be stable in an acidic environment (Sood, 1999).
Storage	Store in well-closed, light-resistant and air-tight containers. Keep in cool and dry place (BP, 2014).

2.6 PHARMACOKINETICS

The azithromycin efficacy can be best associated with the pharmacodynamic parameter of AUC/MIC. Elevated concentrations, attained by accelerated dosing or 'front-loading' (i.e. giving the entire dose of therapy as one dose), results in enhanced anti-bacterial efficacy (Lucchi *et al.*, 2008).

Azithromycin administered *via* the oral route is quickly absorbed through the gastrointestinal tract but the absorption is reduced by the intake of food. The absolute oral bioavailability of this drug is nearly 37 %. Peak blood plasma concentration levels are attained 2 to 3 hours after given a dose. Azithromycin is widely distributed into the tissue, and drug concentration levels in tissue remains higher than in the blood. Small concentrations of azithromycin are metabolised in the liver by demethylation, followed by the elimination in bile as unchanged drug and metabolites. Metabolites of azithromycin are thought to have insignificant antimicrobial action. Nearly 6 % of a dose administered orally is eliminated through the urine. The systemic half-life of azithromycin (terminal elimination) is approximately 68 hours (Harahap *et al.*, 2012; Amsden, 2001).

Azithromycin exhibits widespread tissue distribution, this leads to high concentrations that can be maintained for longer periods of time. Furthermore, animal model studies of human infections have revealed clinical efficiency when concentrations of azithromycin in extravascular tissue are above the minimum inhibitory concentration levels, whereas blood plasma concentrations stay below the minimum inhibitory concentration levels (Lode *et al.*, 1996).

The major concentration of administered azithromycin does not undergo any metabolic modifications whilst present in the human body. This macrolide antibiotic is eliminated in its non-metabolised form through the bile and faeces. About 50 % of azithromycin is identified in bile fluid (Hoepelman & Schneider, 1995; Kremer, 2002; Niederman, 2005; Reisner, 1996). Comparative pharmacokinetics of macrolide antibiotics are shown in Table 2.2.

Table 2.2 Comparative pharmacokinetics of macrolide antibiotics (Zuckerman *et al.*, 2009)

Parameter	Erythromycin Base	Azithromycin	Clarithromycin	Telithromycin
Bioavailability %	25	37	55	57
C _{max} (mg/L)	0.3-0.9	0.4	2.1-2.4	1.9-2.0
t _{max} (h)	3-4	2	2	1.0
t _{1/2} (h)	2-3	40-68	3-5	7.16-13
AUC (mg/L × h)	8	3.4	19	7.9-8.25

2.7 SPECTRUM ACTIVITY

Azithromycin is a broad spectrum antibiotic and is prescribed to treat the listed bacterial infections:

- Gram-positive micro-aerobes: *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Staphylococcus aureus*;
- Gram-negative micro-aerobes: *Hemophilus influenzae*, *Hemophilus ducreyi*, *Moraxella catarrhalis*, and *Neisseria gonorrhoeae*;
- Other pathogens: *Chlamydia trachomatis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* (Zuckerman, 2004; Kremer, 2002).

2.8 SAFETY OF AZITHROMYCIN

Macrolide antibiotics are regarded as safe, especially for treating children. Azithromycin and clarithromycin are used in the treatment of common paediatric infections (King, 1995; Abu-Gharbieh *et al.*, 2004.). Azithromycin can be administered to patients suffering from liver diseases since this drug has no influence on liver enzymes. Furthermore, azithromycin is safe to prescribe to patients with impaired kidney function as well as to pregnant and lactating patients (Amsden, 1996; Hoepelman & Schneider, 1995).

2.9 DOSAGE OF AZITHROMYCIN

In comparative studies with patients suffering from lower respiratory tract infections, azithromycin (500 mg daily for 3 days) was found to be equally effective as clarithromycin (250 mg twice a day for 10 days). A randomised controlled clinical study of azithromycin reported on comparable clinical results during the treatment of acute bronchitis in comparison with clarithromycin. A recent study revealed equivalent potency between a 5-day course of azithromycin and a 7-day course of levofloxacin in the treatment of acute bronchitis (Goldman & Longworth, 1993; Zuckerman, 2004).

However, due to the extended half-life of azithromycin, the 3 or 5 day dosing regimens are anticipated to cause drug concentration levels that are above the minimum inhibitory concentrations for target pathogens (Lode *et al.*, 1996).

2.10 DRUG INTERACTIONS

Azithromycin has fewer incidences of drug interactions in contrast with other macrolide antibiotics such as erythromycin. Azithromycin serum concentrations are negatively affected by antacids. Aluminium and magnesium containing antacids must not be administered with azithromycin. Theophylline and digoxin blood concentration levels can increase in the presence of azithromycin causing toxicity due to drug-drug interaction. The therapeutic activity of sodium warfarin has been enhanced by co-administration with azithromycin. The effect of food on the absorption of azithromycin can lead to a 50 % decrease in the absorbed concentration. Therefore, it is recommended that azithromycin must be administered at least 1 hour before a meal or otherwise 2 hours after a meal. The pharmacokinetic studies on human volunteers revealed that there is no confirmation of drug interactions between azithromycin, terfenadine and cimetidine. Azithromycin combined with zidovudine administered to a group of patients with HIV has indicated the disposition of zidovudine to be unaltered in comparison to the combination of zidovudine and clarithromycin. Furthermore, no significant drug-drug interactions were reported between azithromycin and carbamazepine (Hoepelman & Schneider, 1995; Kremer, 2002; Reisner, 1996; Rubinstein, 2001).

2.11 ADVERSE EFFECTS

The major side effects of azithromycin include diarrhoea (5 %), nausea (3 %), and abdominal pain (3 %). Some other side effects can occur in 1 % of patients and can include: palpitations, chest pain, dyspepsia, flatulence, vomiting, melena, cholestatic jaundice, monilia vaginitis, nephritis, dizziness, headache, vertigo, somnolence, fatigue, rash, photosensitivity, and

angioedema. Abnormalities in serum creatine phosphokinase, potassium, alanine aminotransferase, gamma glutamyl transpeptidase, and aspartate aminotransferase have also been reported in 1-5 % of cases (Kremer, 2002; Zuckerman, 2004).

2.12 CONTRAINDICATIONS

Azithromycin intake for respiratory tract infections should be avoided in patients with a known hypersensitivity to azithromycin or any of the macrolide antibiotics. Apart from this, azithromycin should also not be co-administered with ergot derivatives because a theoretical risk of ergotism exists (Hoepelman & Schneider, 1995).

2.13 TOXICITY

When a person is allergic to macrolide antibiotics, or exhibit symptoms or signs of drug sensitivity while treatment with a macrolide antibiotic is ongoing, the patient should be recommended not to take any antimicrobial substances in the macrolide category. While a person is diagnosed with septic shock or sepsis, the administration of azithromycin must be avoided, due to the patient's low blood plasma levels (Sood, 1999).

2.14 CONCLUSION

Azithromycin is one of the newer generations of macrolide antibiotics. Azithromycin was approved for clinical use in 1992 and has various benefits over conventional macrolides like erythromycin. Azithromycin is more stable in acidic environments and hence has better bioavailability than erythromycin when administered orally. It has a prolonged half-life and therefore could be given once a day. Azithromycin also attains greater tissue drug concentrations and increased anti-microbial therapeutic activity in contrast with erythromycin. However, the low oral bio-availability associated due to aqueous solubility is a disadvantage of the current commercially available azithromycin products. The present study is focused on developing a dosage form of azithromycin consisting of a higher soluble amorphous form that would ultimately lead to improved bioavailability and better patient treatment.

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CHAPTER 3

MATERIALS AND METHODS

3.1 PREPARATION OF AMORPHOUS AZITHROMYCIN

The preparation methods of amorphous forms of drugs (e.g., pure drug or polymer glass solution) in pharmaceutical formulations are classified according to the two most commonly used transformation mechanisms. Transformation of a crystalline substance into a thermodynamically less stable intermediate non-crystalline form (either a melt or a solution) is a commonly occurring process while formulating dosage forms. The other mechanism occurs mostly with milling when the crystalline form is directly transformed into an amorphous form (Einfalt *et al.*, 2013).

The amorphous form of azithromycin was prepared through the quench cooling melt technique. In this method, the crystalline raw material was melted by placing it in a petri dish and subjecting it to heat of approximately $140^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in an oven (Binder GmbH, Germany). The melt was removed from the oven and subsequently, the molten product was quenched on a cold surface. After the melt had reached room temperature, it was transferred into a mortar and pestle and ground into a homogenous powder. This product was used for the studies of this project. The amorphous habit was confirmed by X-ray powder diffraction and the purity by means of high performance liquid chromatography analysis (Aucamp *et al.*, 2014).

3.2 SOLID-STATE CHARACTERISATION

Looking into the aspect of solid-state characteristics of drugs, it is a well-known fact that drugs can exist in different solid-state forms which can either be crystalline or amorphous (Vippagunta *et al.*, 2001). Different solid-state forms of a given drug will lead to differences in terms of physical, chemical and mechanical properties. These differences have direct implications for processability, and formulation strategies, while the difference in solubility influences the absorption and bioavailability thereof (Brittain, 1995).

3.2.1 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) works on the principle of measuring the difference in heat flow between the sample and reference cell, observed over time and temperature (Raju *et al.*, 2009). When a thermal transition occurs in the sample, thermal energy is added to either the sample or the reference cells in order to maintain both the sample and reference at the same temperature. Because the energy transferred is exactly equivalent in magnitude to the

energy absorbed or evolved in the transition, the balancing of energy yields a calorimetric measurement of the transition energy (Dodd & Tonge, 1987).

In this study, a DSC-60 Shimadzu instrument (Shimadzu, Japan) was used to record the DSC thermograms. Samples weighing approximately 3-5 mg was placed in aluminium crimped cells and heated to the desired temperature, with a nitrogen gas flow of 35 ml/min and a heating rate of 10 °C/min. Experimental setup and conditions for DSC analysis are indicated in Table 3.1. The resulting thermograms were used to either determine the melting point or glass transition (T_g) of the different solid-state forms of azithromycin.

Table 3.1: Experimental setup and conditions for DSC analysis

Starting temperature	25°C
Maximum temperature	200°C
Heating rate	10°C/min
Nitrogen flow rate	35 ml/min

3.2.2 Thermogravimetric Analysis (TGA)

In thermogravimetric analysis a sample is placed into a TGA sample pan which is attached to a sensitive microbalance assembly. The sample holder portion of the TGA balance assembly is subsequently placed into a high temperature furnace. The balance assembly measures the initial sample weight at room temperature and then continuously monitors changes in sample weight as heat is applied to the sample (Wendlandt, 1986).

A Shimadzu TGA-60 instrument (Shimadzu, Japan) was used to determine the percentage weight loss (%) of the azithromycin solid-state forms. In this study the percentage weight loss, was considered to be an indication of hydration. As mentioned in the previous chapter, crystalline azithromycin can exist as an anhydrate, monohydrate or dihydrate and it is therefore important to know the hydration status of the azithromycin forms used in this study. Approximately 3-5 mg of the samples was weighed accurately in aluminium sample pans. The samples were heated from 25 to 250°C with a heating rate of 10°C/min and a nitrogen gas purge of 35 ml/min.

The percentage weight loss was calculated by taking into consideration the molecular weight of water and the molecular weight of azithromycin (Equation 3.1).

$$\% \text{ Weight loss} = \frac{\text{Molecular weight solvent}}{\text{Molecular weight solvent} + \text{Molecular weight azithromycin}} \times 100 \quad \text{Eq. 3.1}$$

3.2.3 Infrared Spectroscopy (IR)

IR spectroscopy is a technique based on the vibrations of the atoms of a molecule. An IR spectrum is commonly obtained by passing IR radiation through a sample and determining what fraction of the incidence radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule (Stuart, 2004). IR provides a “fingerprint” of the molecular solid (Yu, 2001) and is sensitive to short-range molecular order when studying molecular vibrations.

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

3.2.4 X-Ray Powder Diffraction (XRPD)

X-ray powder diffraction (XRPD) is a rapid analytical technique primarily used for the phase identification of crystalline substances and can provide information on unit cell dimensions (Brittain, 1995). During the XRPD analyses 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 PANalytical Empyrean diffractometer (PANalytical, Netherlands). The measurement conditions are represented in the Table 3.2.

Table 3.2: The measurement conditions of X-ray powder diffraction

The measurement conditions	
Target	Cu
Voltage	40 kV
Current	30 mA
Divergence slit	2 mm
Antiscatter slit	0.6 mm
Detector slit	0.2 mm monochromator
Scanning speed	2° /min(step size, 0.025°; step time, 1.0 sec).

3.3 PRE-FORMULATION STUDIES

Before the development of a pharmaceutical formulation, it is important that the solid-state characteristics, which include crystal form, stability etc., be determined. This in turn decides many of the successive trials and approaches in formulation development. This primary investigating phase is known as pre-formulation.

3.3.1 Compatibility studies

Compatibility studies of a drug with various excipients are an important aspect 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 *et al.*, 2006). For this study two methods were used to determine compatibility of azithromycin with different excipients, i.e. DSC and microcalorimetry.

3.3.1.1 Differential Scanning Calorimetry (DSC)

Compatibility studies were done by preparing physical mixtures of azithromycin 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 analysed by DSC analysis. The resulting DSC thermograms of the mixtures were compared to determine possible incompatibilities. The presence of a peak shift, appearance of new peaks, or

disappearance of peaks in the DSC thermograms of the mixtures is considered as a possible incompatibility.

3.3.1.2 Thermal microscopy

Thermal microscopy analyses of the individual compounds as well as the 1:1 ratios of excipients mixed with azithromycin will be performed with a Nikon Eclipse E4000 microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan Linkam THMS600 equipped with a T95 LinkPad temperature controller (Surrey, England). Thermal micrographs will be taken and the temperature at which the micrographs were taken will be recorded.

3.3.2 Powder properties

The powder mixtures obtained for all the formulations were used to determine the flow properties of the powder blends. The flow properties were evaluated by determining the angle of repose, Carr's index, Hausner ratio and powder densities.

3.3.2.1 Angle of repose

The angle of repose of all the formulations was determined by the fixed funnel method. A funnel is fixed at a height approximately of 10 cm above a flat surface. The loose powder is slowly passed through the funnel, until a cone of powder formed. The diameter and height of the powder cone is measured and the angle of repose was determined using equation 3.2.

$$\tan \theta = \frac{h}{r} \quad \text{Eq. 3.2}$$

Where;

θ = angle of repose,

h = height of the powder cone and

r = radius of the heap of the cone.

25 g of powder was weighed and the powder flow rate was measured as the mass per time using a glass funnel with an orifice of 10 mm in diameter ($n=3$). Angle of repose of the powder was measured by static angle of repose method (Figure 3.1). The height (h) and diameter (r) of the resulting cone were measured. Comparison between the angle of repose and flow properties of solids are indicated in the Table 3.3.

Table 3.3: Comparison between the angle of repose and flow properties of powders (USP, 2014)

Flow Property	Angle of Repose (degrees)
Excellent	25–30
Good	31–35
Fair - aid not needed	36–40
Passable - may hang up	41–45
Poor - must agitate, vibrate	46–55
Very poor	56–65
Very, very poor	>66



Figure 3.1: Powder clinical heap with reference lines to enable measurement of its height and base for the angle of repose calculation (Figure adapted from Manyama, 2012).

3.3.2.2 Powder densities

(a) Bulk Density

The definition of bulk density is described in the USP as, “the bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume” (USP, 2014). The density of powder particles and the spatial arrangement of the particles in the powder bed are two parameters which contribute to the bulk density.

When measurement of bulk density is done in a graduated cylinder, the bulk density is expressed in grams per millilitre (g/ml) although the international unit is kilogram per cubic meter ($1 \text{ g/ml} = 1000 \text{ kg/m}^3$) (USP, 2014).

For this study a definite quantity of powder was used, which was sufficient to complete the test. The powder was sieved through a sieve with apertures of 1.0 mm. Agglomerates were gently broken up that are formed during storage to avoid changing the nature of the material. The powder was gently introduced into a dry graduated cylinder of 250 ml (readable to 2 ml), without compacting. Approximately 100 g of the test sample was weighed with 0.1 % accuracy. Carefully level the powder without compacting, and noted the unsettled apparent volume to the nearest graduated unit (USP, 2014). A sample of each powder blend was placed in a graduated measuring cylinder. The weight (w) and bulk volume (V_0) was noted. Accordingly, the bulk density can thus be determined according to equation 3.3.

$$\rho_B = \frac{w}{V_0} \quad \text{Eq. 3.3}$$

(b) Tapped Density

The definition of tapped density according to the USP is, “an increased bulk density attained after mechanically tapping a container containing the powder sample” (USP, 2014).

All measurements for this study were conducted at controlled ambient conditions ($22 \pm 2^\circ\text{C}$, $40 \pm 5 \%$ RH) using a tapped density instrument fitted with 250 mL glass volumetric cylinders. All samples were tapped (14.25 mm height; 300 taps/min) until they reached a terminal density (typically after 2000 taps) (USP, 2014). The cylinder was placed on an Erweka[®] vibrating apparatus (Type SVM 223, Erweka, Germany) for 3 minutes. Subsequently the sample weight (w) and tapped volume (V_{tap}) was noted. The tapped density was calculated according to equation 3.4.

$$\rho_T = \frac{W}{V_{tap}} \quad \text{Eq. 3.4}$$

3.3.2.3 Hausner ratio

The ratio of the tapped density/bulk density can be related to interparticulate friction. A smaller ratio value, < 1.2 will be indicative of powders with low interparticulate friction whereas powders with a Hausner ratio of > 1.5 represents more cohesive, less free-flowing powders (Aulton, 2013). The ratio is calculated using equation 3.5.

$$\text{Hausner ratio} = \frac{\rho_{tap}}{\rho_{bulk}} \times 100 \quad \text{Eq. 3.5}$$

3.3.2.4 Carr's index (Compressibility index)

The Carr's index (%) is also considered an indirect method of measuring powder flow (Aulton, 2013). This index is calculated using equation 3.6.

$$\% \text{ Compressibility} = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}} \quad \text{Eq. 3.6}$$

3.4 FACTORIAL DESIGN FOR TABLET FORMULATION

A factorial design was used to assist in the development of a suitable formulation for azithromycin tablets. The factorial design was also used to perform compatibility testing and to eliminate unsuitable excipients. Table 3.4 indicates the different variables and levels of the formulation study.

Table 3.4: Factorial design indicating the variables which were investigated for diluent A in the formulation study

		Diluent A (Avicel® PH200)			
		Disintegrant			
		Ac-di-Sol® (0.5% w/w)	Ac-di-Sol® (1.0% w/w)	Ac-di-Sol® (0.5% w/w)	(Ac-di-Sol® 1.0% w/w)
Binding agent	Kollidon® VA64 (3% w/w)		X		X
		X		X	
	Kollidon® VA64 (5% w/w)	X		X	
			X		X

A concentration of 1-1.5 % w/w magnesium stearate was included in all test formulations to prevent sticking of the tablets to the tableting die during compression.

3.5 MANUFACTURING OF AZITHROMYCIN TABLETS

Tablets containing 500 mg of azithromycin, either in the amorphous or crystalline form were prepared using the direct compression method. Azithromycin and all the other excipients were weighed individually for a batch size of 100 tablets and transferred into a glass jar. The lid was sealed with Parafilm®. A Turbula® W2B (Bachofen, Switzerland) rotating mixer set at 47 rpm was used to thoroughly mix the contents for 10 minutes. The powder mixture was then transferred into the hopper of a single punch tablet press (Cadmach® SSF3, India). 12 mm diameter upper and lower punches were selected according to the size and weight of the desired tablets (1000 mg). Formulation summary of the ingredients used in the method are indicated in the Table 3.5.

Table 3.5: Ingredients used in the manufacturing of azithromycin tablets

Ingredient	Batch number	Manufacturer
Ac-di-Sol [®]	T017C	FMC Corporation, Philadelphia, Pennsylvania, USA.
Kollidon [®] VA64	62-8826	BASF Aktiengesellschaft, Ludwigshafen, Germany.
Avicel [®] PH200	M939C	FMC Corp., Cork, Ireland.
Magnesium stearate	472131	Kirsch Pharma, Isando, Johannesburg, RSA.

3.6 TABLET EVALUATION

The compressed tablets were evaluated in terms of the following parameters: 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) or British Pharmacopoeia (BP, 2014).

3.6.1 Appearance

Tablets were evaluated visually for properties such as colour and shape and the presence of lamination, cracking and chipping.

3.6.2 Weight variation

This test was carried out in accordance with the BP (2014). Twenty tablets of each batch were selected randomly and weighed individually on an analytical balance. The average tablet weight and standard deviation (SD) were calculated. The individual weights of the tablets were compared to the average weight. According to the BP, not more than two of the individual tablets weight should deviate from the average mass by more than the allowed percentage deviation whereas none should deviate by double the allowed percentage deviation (BP, 2014). Average tablet mass and corresponding percentage deviation allowed is indicated in Table 3.6.

Table 3.6: Average tablet mass and corresponding percentage deviation allowed (BP, 2014)

Tablet formulation	
Average mass	Percentage deviation allowed
80 mg or less	10 %
More than 80 mg and less than 250 mg	7.5 %
250 mg or more	5 %

3.6.3 Thickness and diameter

Ten tablets were randomly selected for measurement of thickness and diameter and it was measured individually using an Erweka® TBH 425 hardness and thickness tester (Erweka, Germany). The average and relative standard deviations (% RSD) was calculated.

3.6.4 Hardness

Hardness indicates the ability of a tablet to withstand mechanical shocks during handling, packing and shipping (Banker & Anderson, 1987). The hardness was determined on 10 randomly selected tablets. The instrument used for the determination of hardness was an Erweka® apparatus (Type TBH 425 TD, Erweka, Germany).

3.6.5 Friability

The friability test is a measure of the physical ability of the tablet to resist abrasion during such conditions. Tablets that abrade with ease result in an undesirable, progressive reduction in their weight and in a change in appearance (Manyama, 2012).

Twenty tablets from each formulation were weighed individually and then transferred to the friabilator. The friabilator is operated at 25 rpm for 4 minutes, using an Erweka® friabilator (Erweka®, Germany). The duration of 4 minutes was chosen in order to achieve 100 rotations of the tablets. The tablets were dusted and weighed on the analytical balance, prior to and after executing the test. According to the USP, the tablets should not lose more than 1 % of their total weight. The percentage weight loss was calculated using the following formula (equation 3.7):

$$\% \text{ Friability} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100 \quad \text{Eq. 3.7}$$

3.6.6 Disintegration

The disintegration times of the tablets were determined in distilled water at $37.0 \pm 2^{\circ}\text{C}$, using the Erweka[®] disintegration testing apparatus (Erweka, Germany). The disintegration test was done using 6 tablets from each batch and tablets introduced into each of the six cylindrical tubes, and the time taken for all of the six tablets to disintegrate and pass through the mesh wire was recorded. The BP (2014), recommends that conventional tablets should be disintegrated within 15 minutes of initialising the test.

3.6.7 Dissolution testing

An *in vitro* dissolution study of the manufactured tablets containing either amorphous or crystalline azithromycin was performed according to the "Dissolution procedure" (711) (USP, 2014). The study was performed on a Vankel instrument (Varian, USA). USP apparatus II (paddle). The dissolution testing was carried out at a temperature of $37 \pm 0.5^{\circ}\text{C}$, with the apparatus set at a rotating speed of 100 rpm. The dissolution medium used for the tablets was 900 ml of distilled water. Aliquots of dissolution media (5 ml) were withdrawn at 5, 10, 20, 30, 40, 55, 70, 180, 240, 360, 480 and 540 minutes and filtered using 0.45 μm PVDF filters. It is imperative to obtain a saturated solution of the drug in order to study solution-mediated phase transformation. Since these dissolution studies are designed to evaluate the solution-mediated phase transformation of amorphous azithromycin in tablet formulations, no dissolution media replacement was done. The samples were then analysed using the same HPLC method as for the assay. The data was analysed by calculating the amount of drug released and the cumulative percentage drug released at different time intervals.

3.6.8 Assay testing

Twenty tablets were selected randomly, weighed and crushed in a mortar and pestle. A quantity of powder equivalent to the weight of 1 tablet (1000 mg) was weighed and the powder was transferred into a 100 ml volumetric flask. The dilution was made with mobile phase. The resulting solution was filtered through a 0.45 μm PVDF filter into HPLC vials. The samples were analysed using a Shimadzu HPLC (Shimadzu, Japan) chromatographic system.

A Phenomenex[®] Luna C₁₈ (5 μm) 150 mm x 4.6 mm reverse phase column was used as stationary phase. A mobile phase consisting of 70 parts of 0.06 M phosphate buffer at pH 6.0 (pH adjusted with 1.0 M sodium hydroxide solution) and 30 parts of acetonitrile was used at a flow rate of 1.0 mL/min. All samples were analysed using an isocratic elution method at a

wavelength of 205 nm. The injection volume for each sample was 15 μ L (Odendaal *et al.*, 2012).

3.7 STABILITY STUDIES

Accelerated stability studies were conducted on the tablet formulations for 6 months at 40°C $\pm$ 2°C/ 75 $\pm$ 5 % RH. Samples were taken on monthly intervals (0, 1, 2, 3, 4, 5 and 6 months) and analysed for difference in solid-state characteristics, azithromycin content, rate of dissolution and physical tablet properties.

3.8 DATA ANALYSIS

Statistical analysis of the data was performed on the calculated data. One-way repeated analysis of variance (ANOVA) with Microsoft Excel[®] (Microsoft Corporation, USA) was used to calculate *p*-values of data sets. The differences between the values of the experimental groups would be considered statistically significant if $p \leq 0.05$.

3.9 CONCLUSION

This chapter describes the preparation of the amorphous solid-state form of azithromycin as well as the pre-formulation and formulation processes involved in order to obtain a successful product. During this study a strong focus was placed upon the stability of the amorphous form of azithromycin. The aim was to investigate whether the amorphous solid-state form of azithromycin will remain amorphous during pre-formulation and the formulation process. Furthermore, the influence of increased temperature and humidity on the amorphous form was investigated. Throughout this study the characteristics and behaviour of amorphous azithromycin was compared with that of crystalline dihydrate.

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CHAPTER 4

PRE-FORMULATION STUDIES OF AZITHROMYCIN

4.1 INTRODUCTION

The main objective of this study was to study the stability of amorphous azithromycin when it is incorporated into a tablet dosage form. As discussed in chapter 1 the use of amorphous solid-state forms of drugs in marketable dosage forms are a challenging task, due to the fact that these high free energy forms tend to be physically and chemically unstable. Pre-formulation studies form a critical part of any product development process. In order to successfully formulate a drug into a dosage form it is imperative to complete comprehensive pre-formulation studies. Generally, such studies involve the investigation of the powder properties of the drug that needs to be incorporated into a dosage form. Furthermore, an investigation into the compatibility of the drug in combinations with different excipients is also considered imperative.

Proper pre-formulation studies will reveal a substantial amount of information in terms of physical and chemical properties of the drug to be formulated. Since this study focusses on the formulation of amorphous azithromycin into tablets followed by the stability study of resulting formulations, a fair amount of attention was paid towards the stability of the amorphous solid-state form of azithromycin during processing and handling steps. This chapter will discuss all pre-formulation tests that were conducted with emphasis on the stability of amorphous azithromycin.

4.2 RESULTS

4.2.1 Physico-chemical characterisation of amorphous azithromycin

As discussed in chapter 3, section 3.1, amorphous azithromycin was prepared through the method of quench cooling of the melt. Considering the fact that this preparative method involves heating of azithromycin until it melts, followed by the rapid cooling of the molten product, it was important to confirm the purity of azithromycin after the preparation of the amorphous form. The purity of amorphous azithromycin was confirmed through HPLC analysis. The purity was calculated to be $99.5 \% \pm 2.0 \%$. This was a confirmation that the drug remained intact during the preparation method and that no degradation of azithromycin occurred. Furthermore, it was imperative to confirm the amorphous habit of the prepared solid-state form. This was done by means of DSC, IR and XRPD analyses (chapter 3, sections 3.2.1, 3.2.3 and 3.2.4).

Figure 4.1 depicts the DSC thermograms obtained with crystalline azithromycin dihydrate and amorphous azithromycin. For crystalline azithromycin a melting temperature of 123.6°C was observed (Figure 4.1a) while for amorphous azithromycin no melting endotherm was observed but rather a glass transition temperature (T_g) of 109.8°C (Figure 4.1b). The observed T_g is considered to be an indication of the stability of amorphous azithromycin upon exposure to relative high storing temperatures. According to literature a rule of thumb exists in terms of the storage temperatures of amorphous drugs. Generally, it is considered good practice to store an amorphous form of a drug at 50°C below the obtained T_g (Yu, 2001). Considering this it becomes evident that an approximate storage temperature of 60°C and below will not induce crystallisation of amorphous azithromycin. In terms of pharmaceutical stability this is considered an advantageous property of amorphous azithromycin.

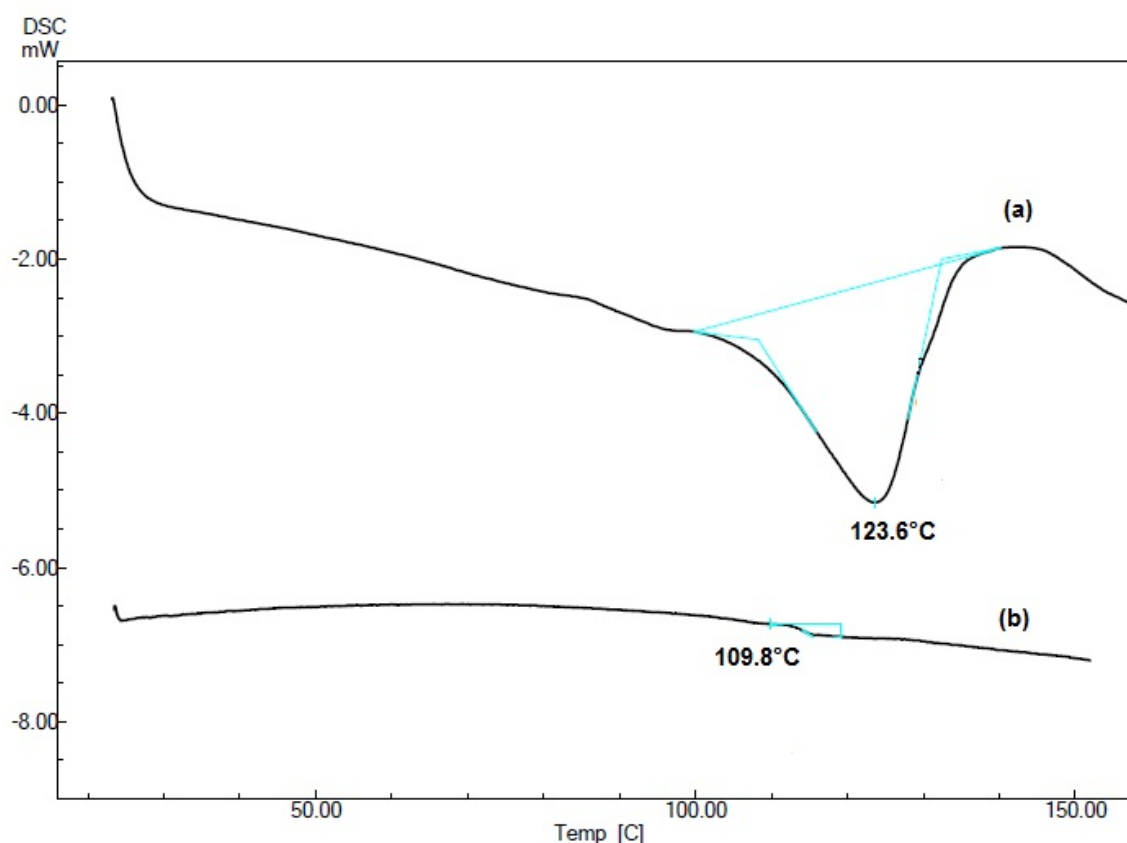


Figure 4.1: Overlay of the DSC thermograms obtained with (a) crystalline azithromycin dihydrate and (b) amorphous azithromycin.

Furthermore, it can be deduced from the DSC thermogram that an increase in temperature does not induce crystallisation of the amorphous form of azithromycin. Due to the thermodynamic instability generally associated with amorphous forms of drugs it is often observed that amorphous forms will recrystallise upon exposure to relative high temperatures (Yu, 2001).

Figure 4.2 depicts an overlay of the XRPD results obtained for crystalline azithromycin and amorphous azithromycin. The XRPD results confirm the amorphous habit of the amorphous solid-state form of azithromycin (Figure 4.2b). Therefore, it can be deduced that the preparative method rendered crystalline azithromycin into the amorphous solid state form.

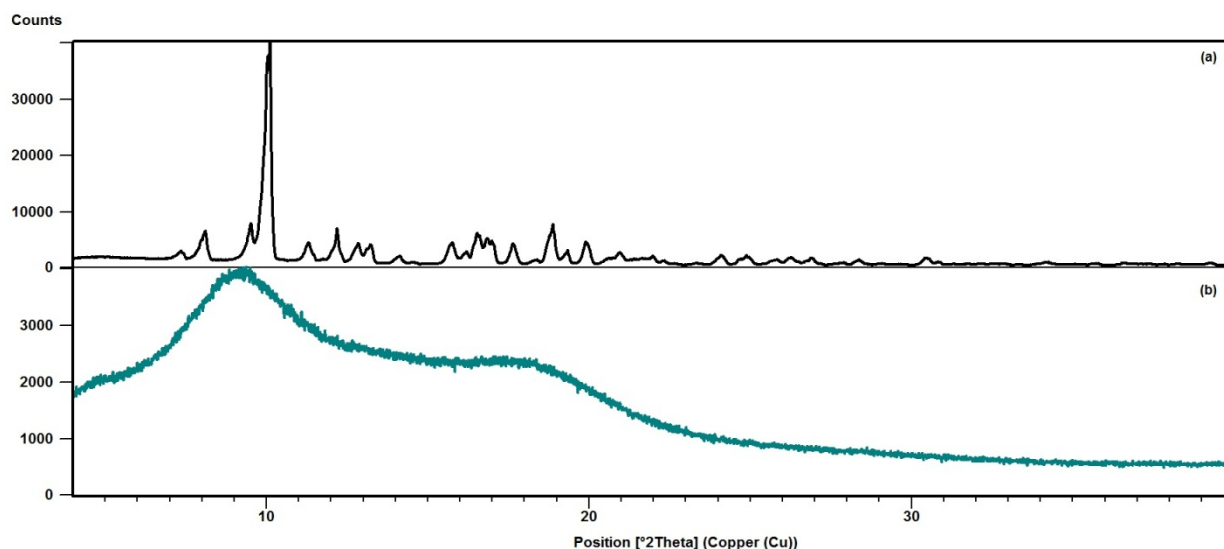


Figure 4.2: Overlay of powder x-ray diffractograms of (a) crystalline azithromycin dihydrate and (b) amorphous azithromycin.

The amorphous characteristics of the prepared azithromycin solid-state form were furthermore investigated by means of IR spectroscopy (chapter 3, section 3.2.3). Figure 4.3 shows an overlay of the IR spectra obtained for both crystalline and amorphous azithromycin. For crystalline azithromycin characteristic sharp absorbance peaks are displayed at 3567, 3496 and 3236 cm^{-1} . These absorbance peaks signifies free hydroxyl, hydrogen bonded hydroxyl and intramolecular hydrogen bonded hydroxyl functional groups, respectively (Brittain, 1999; West, 1999; Gandhi *et al.*, 2002). The IR spectrum obtained with amorphous azithromycin exhibits peak broadening in the region of 3500 – 3000 cm^{-1} . This peak broadening can be attributed to the random arrangement of azithromycin molecules due to the amorphous state. The absorbance peak broadening observed with amorphous azithromycin correlates well with what literature dictates (Yu, 2001; Chieng *et al.*, 2011).

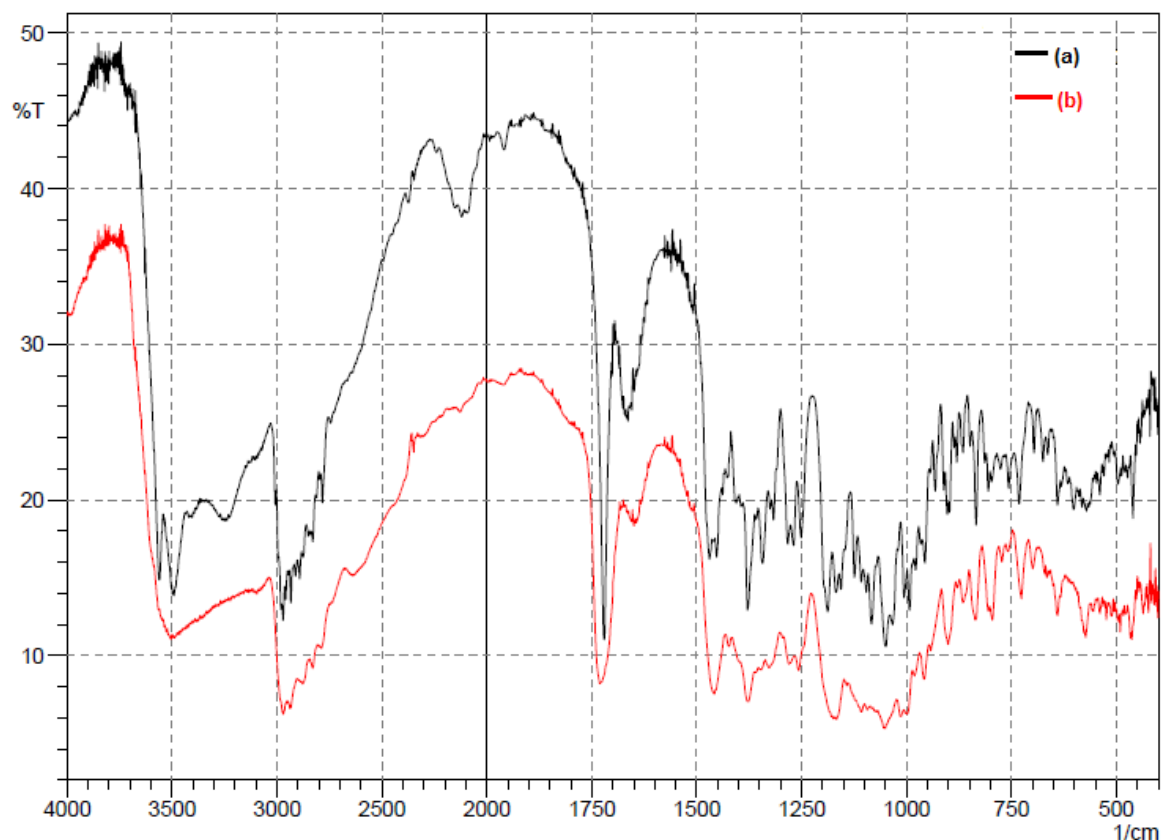


Figure 4.3: An overlay of the IR spectra obtained with (a) crystalline azithromycin and (b) amorphous azithromycin.

4.2.2 Characterisation of powder flow properties

The determination of the powder flow properties of a drug that is intended to be incorporated into a tablet is critical. Proper powder flow properties will influence the whole tablet manufacturing process. As outlined in previous chapters the aspect of powder flow properties influences the acceptability of the produced tablets. Poor powder flow will result in high tablet weight variations, high and unacceptable variations in drug concentrations which would ultimately lead to variable drug concentrations after patient administration. The powder flow characteristics were determined for both crystalline azithromycin dihydrate and amorphous azithromycin and included the angle of repose, the Carr's index, Hausner ratio and powder densities. The powder flow properties were determined as described in chapter 3 section 3.3.2.

Table 4.1 provides a summary of the determined powder flow properties for each of the solid-state forms of azithromycin.

Table 4.1: Summary of the powder flow properties determined for crystalline and amorphous azithromycin

Property	Crystalline azithromycin	Amorphous azithromycin
Angle of repose	-	-
Bulk density (g/ml)	0.58	0.53
Tapped density (mg/ml)	0.81	0.67
Hausner ratio	1.39	1.26
Carr's Index (%)	28	21

The angle of repose is the simplest and still the preferred method to test the flowability of a powder or a powder mixture. During the angle of repose measurements a substantial amount of difficulty was experienced and the accurate determination of the angle was not possible. The cohesiveness of both powders (crystalline and amorphous) impeded the flow of the powders through the funnel and consequently clogging of the funnel opening occurred, resulting in almost no powder flowing through the funnel. In terms of the angle of repose experiments it was not possible to classify the flow properties of the two solid-state forms of azithromycin.

The Hausner ratio and Carr's Index are the most effective methods for predicting the compressibility of powders. Both methods bring into account the volume of the powders. A Carr's Index greater than 25 is an indication of a poor-flowing powder and an index of less than 15 is indicative of a powder with good flow properties. The Carr's Index values obtained for both the crystalline and amorphous azithromycin powder samples is contradictory to some extent since one would assume that due to amorphous forms existing in a more viscous nature the flow properties would also be influenced detrimentally. However, according to the calculated Carr's indexes the crystalline solid-state form exhibited poorer flowability than the amorphous solid-state form. Although the flowability assessments of the powders of both solid-state forms differ to some extent it can still be concluded that both powders exhibit poor flow properties.

A Hausner ratio of less than 1.2 is indicative of a free-flowing powder with low interparticulate friction while a ratio of more than 1.5 represents powders with more cohesive forces acting on the powder particles. Considering the Hausner ratios obtained for both crystalline and amorphous azithromycin it is clear that both powders are not so free-flowing

but does exhibit workable flow properties to some extent. Considering the abovementioned data it is clear that although the powder flow properties of both solid-state forms are not ideal it is still reasonably acceptable and workable. It was concluded that the powder flow properties can be improved by means of good formulation strategies.

4.2.3 COMPATIBILITY TESTING

An important step in complete pre-formulation studies is that of compatibility testing. During this step mixtures of the drug and excipients are analysed in order to determine whether any interactions will occur between the different compounds. Although most excipients are usually inert of nature there is still the possibility of some kind of interaction between a drug and excipients. Compatibility testing is a prerequisite in order to manufacture an acceptable product without the possibility of the individual compounds influencing the performance of the product detrimentally.

During the compatibility testing a screening of different excipients in combination of azithromycin was conducted. Excipients were chosen randomly but also in such a way that fillers, disintegrants, binders, glidants and lubricants will be included in the screening stage. Therefore, the screening process included excipients that could possibly be used in the design of an appropriate formulation. Table 4.2 provides a list of all excipients screened for use in the tablet formulation. Only the compatibility screening results of those combinations that were questionable will be discussed in the following paragraphs.

For the 1:1 mixture of azithromycin and Emcompress[®] a shift of 36.9°C of the endothermic event was observed (Figure 4.4a,b). A shift in thermal events is considered an indication of incompatibilities. Further investigation for this mixture of azithromycin and excipient was therefore necessary in order to confirm or exclude the possibility of an incompatibility. Thermo-microscopy was performed on the mixture and the obtained micrographs are depicted in Figure 4.5a-c. During the thermal microscopy experiments it was observed that azithromycin starts to melt at approximately 128.0°C. This is in good correlation with the melting point observed for azithromycin raw material (Figure 4.1). However, after complete melting of azithromycin the degradation of Emcompress[®] was visible at approximately 187.4°C. During the DSC and thermal microscopy analyses of neat Emcompress[®] no degradation of the compound was observed. This could be a possible indication of an incompatibility between the two compounds but further and more precise investigation using HPLC and microcalorimetry will be needed in order to confirm this.

Table 4.2: List of excipients tested in combination with azithromycin for compatibility

Excipient	Compatibility confirmed
Microcrystalline cellulose (Avicel® PH101)	√
Microcrystalline cellulose (Avicel® PH200)	√
Tabletose® 70	√
Lactose	√
Anhydrous calcium hydrogen phosphate (Emcompress®)	X
Croscarmellose sodium (Ac-Di-Sol)	√
Sodium starch glycolate (Explotab®)	√
Polyvinyl acetate and povidone matrix (Kollidon® SR)	√
Polyvinylpyrrolidone (Kollidon® VA-64)	√
Magnesium stearate	√
Sodium stearyl fumarate (Pruv®)	X

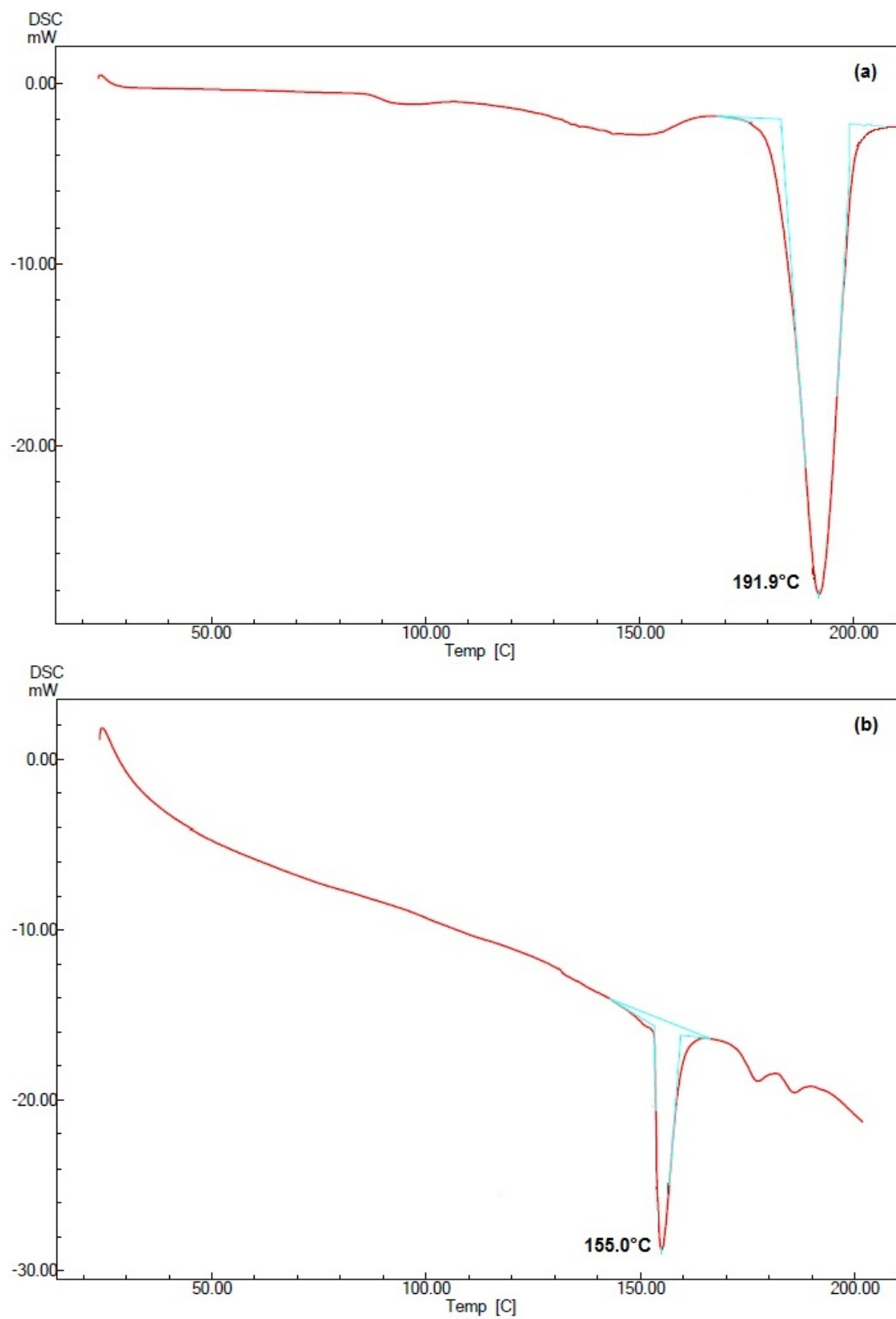


Figure 4.4: DSC thermograms obtained with (a) Emcompress[®] and (b) a 1:1 ratio mixture of Emcompress and azithromycin.

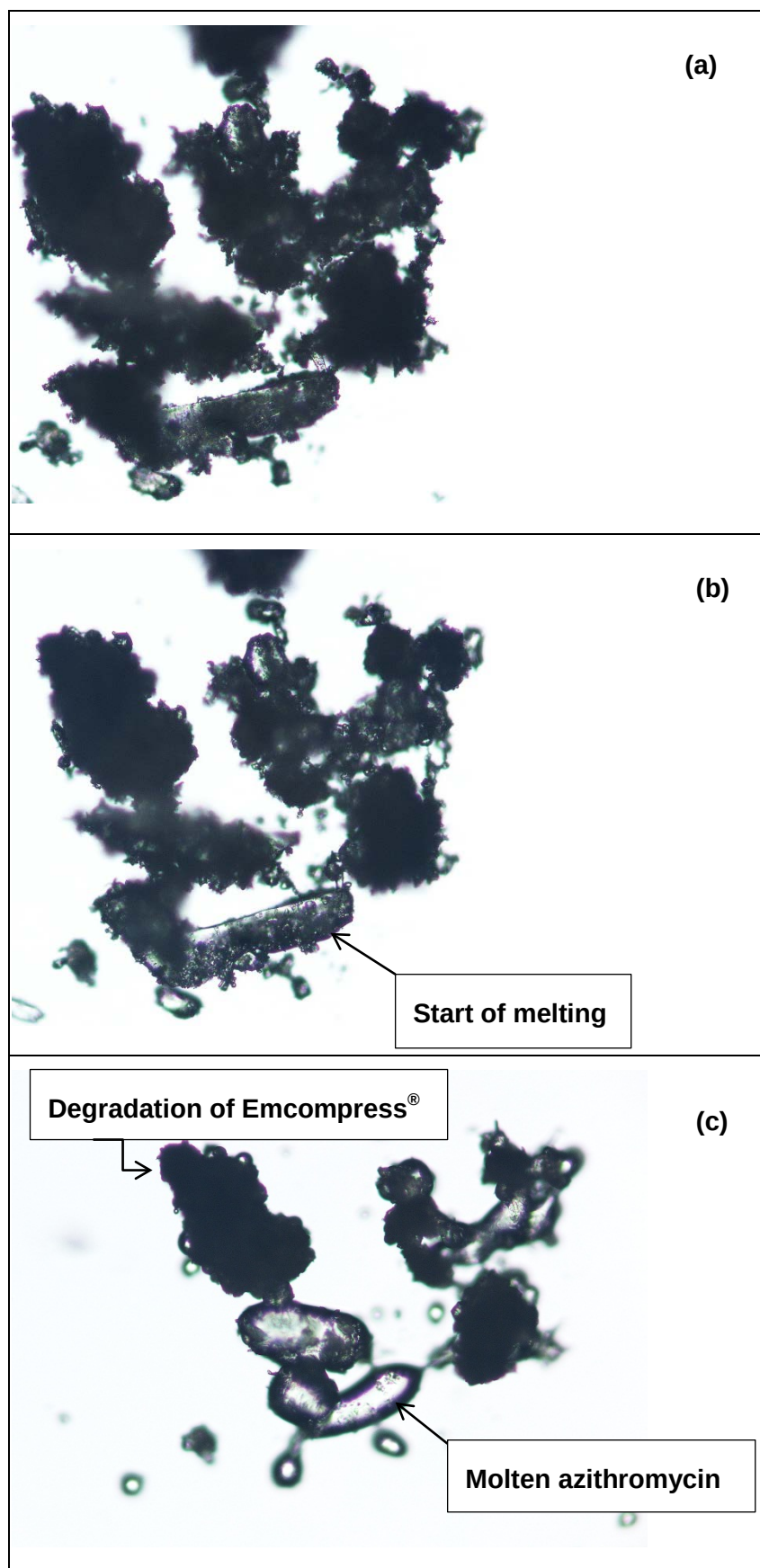


Figure 4.5: Thermo-micrographs depicting the melting of azithromycin raw material and the degradation of Emcompress®.

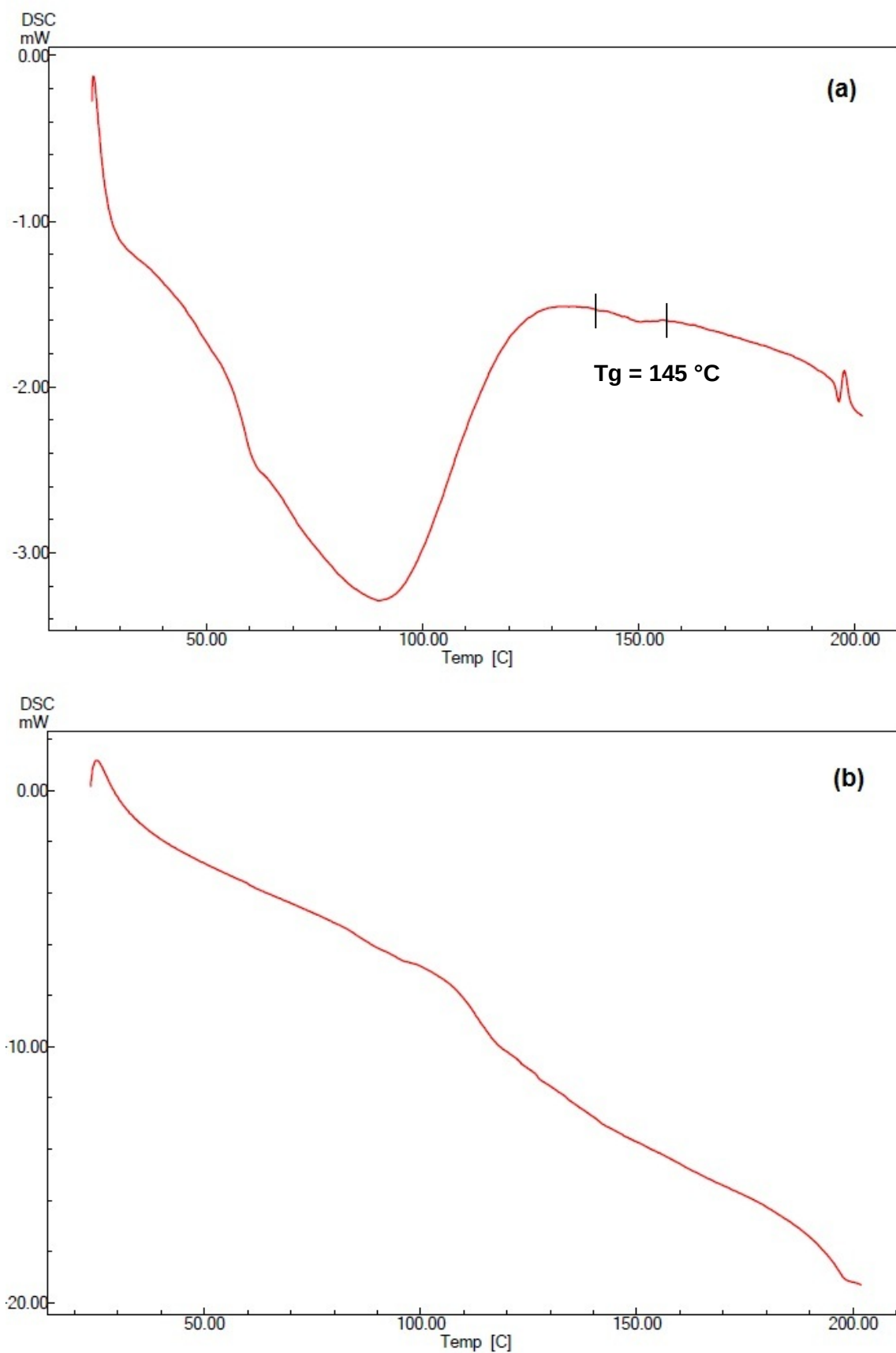


Figure 4.6: DSC thermograms obtained with (a) Kollidon[®] VA-64 and (b) a 1:1 ratio mixture of azithromycin and Kollidon[®] VA-64.

The DSC thermogram obtained with Kollidon® VA-64 did not indicate a melting endotherm but only a T_g at 145.3°C. This correlates well with the fact that Kollidon® VA-64 is a polymer with binding properties and therefore acts like a glassy/rubbery substance. However, it would be expected that a combination of the polymer and azithromycin will still exhibit the melting endotherm of azithromycin at approximately 123°C. This was not the case as can be clearly seen from Figure 4.6b, where no T_g or melting endotherm is visible. Due to this observation it was decided to perform thermal microscopy.

Figure 4.7a-c depicts the thermal micrographs obtained with the 1:1 mixture of azithromycin and Kollidon® VA-64. Figure 4.7a depicts the mixture at 25°C, figure 4.7b shows the start of liquefaction of the mixture at 132.8°C. The liquefaction is considered to be due to the melting of azithromycin as well as Kollidon VA-64 existing in a liquid state (the transition from solid to liquid at T_g). Upon further heating the mixture remains in a viscous liquid-like state without any further thermal events or degradation occurring (Figure 4.7c).

The fact that the mixture resulted in the formation of a viscous liquid upon heating without the clear melting of azithromycin raw material being visible is an indication that the drug : polymer mixture is miscible and not that the two compounds are incompatible with each other (Marsac *et al.*, 2009). From the presented data it can be deduced that although the DSC thermograms suggested degradation of azithromycin due to the absence of a melting endotherm, thermal microscopy data shows that no incompatibility exist but rather that the two compounds are merely miscible.

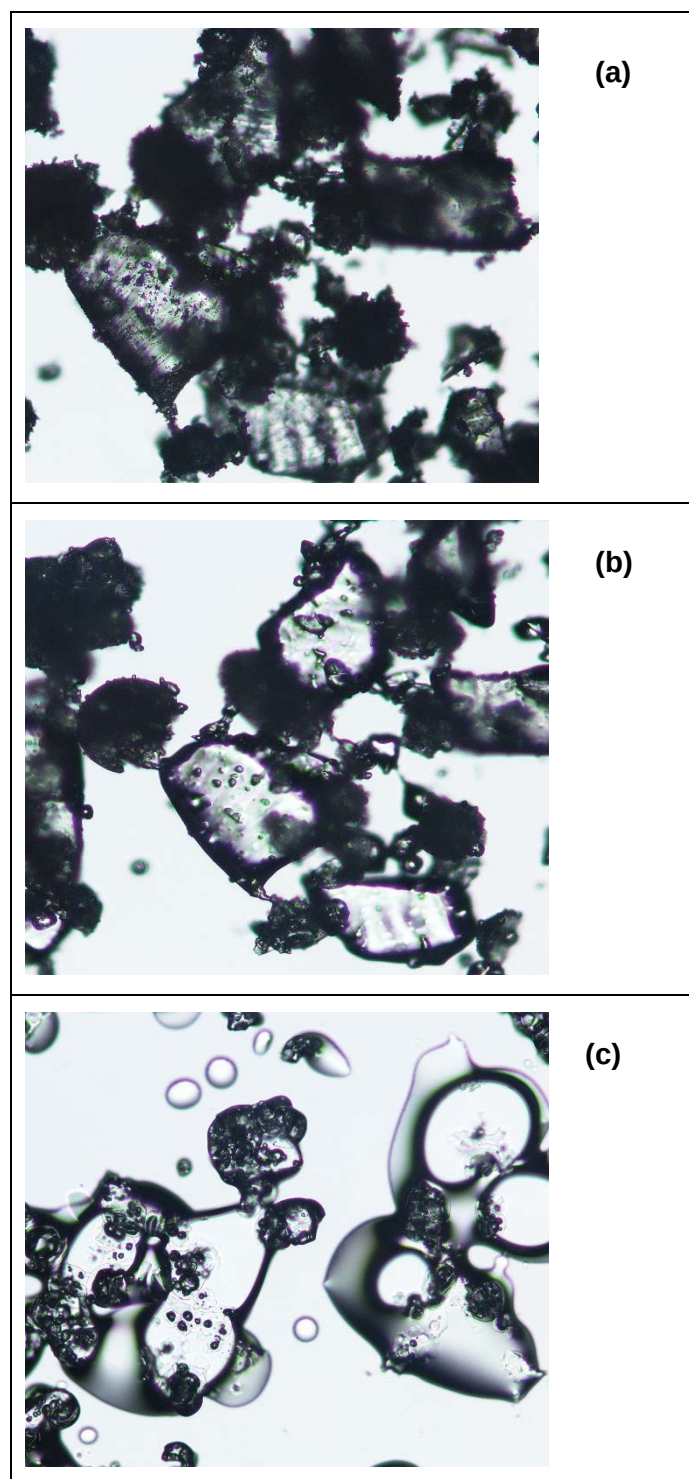


Figure 4.7: Thermal micrographs obtained with the 1:1 mixture of azithromycin and Kollidon® VA-64.

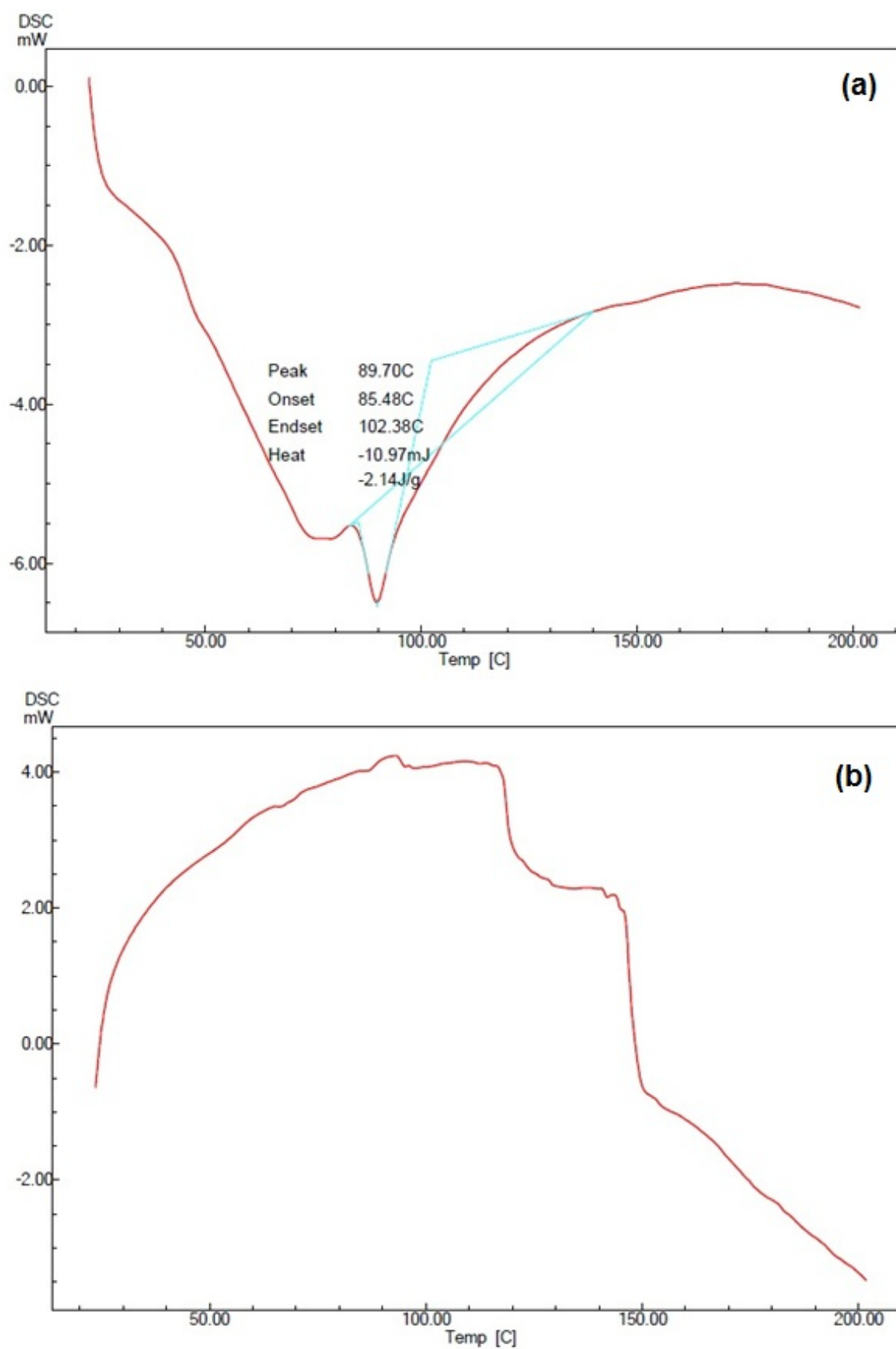


Figure 4.8: DSC thermograms of (a) Ac-Di-Sol and (b) a 1:1 mixture of Ac-Di-Sol and azithromycin.

From the DSC thermograms (Figure 4.8a,b) no conclusion could be made in terms of the compatibility of the two substances. The fact that no melting endotherms are visible might be due to the dissolution of one compound into the other or it might be due to degradation. Thermal microscopy was done in order to confirm the thermal events or lack thereof.

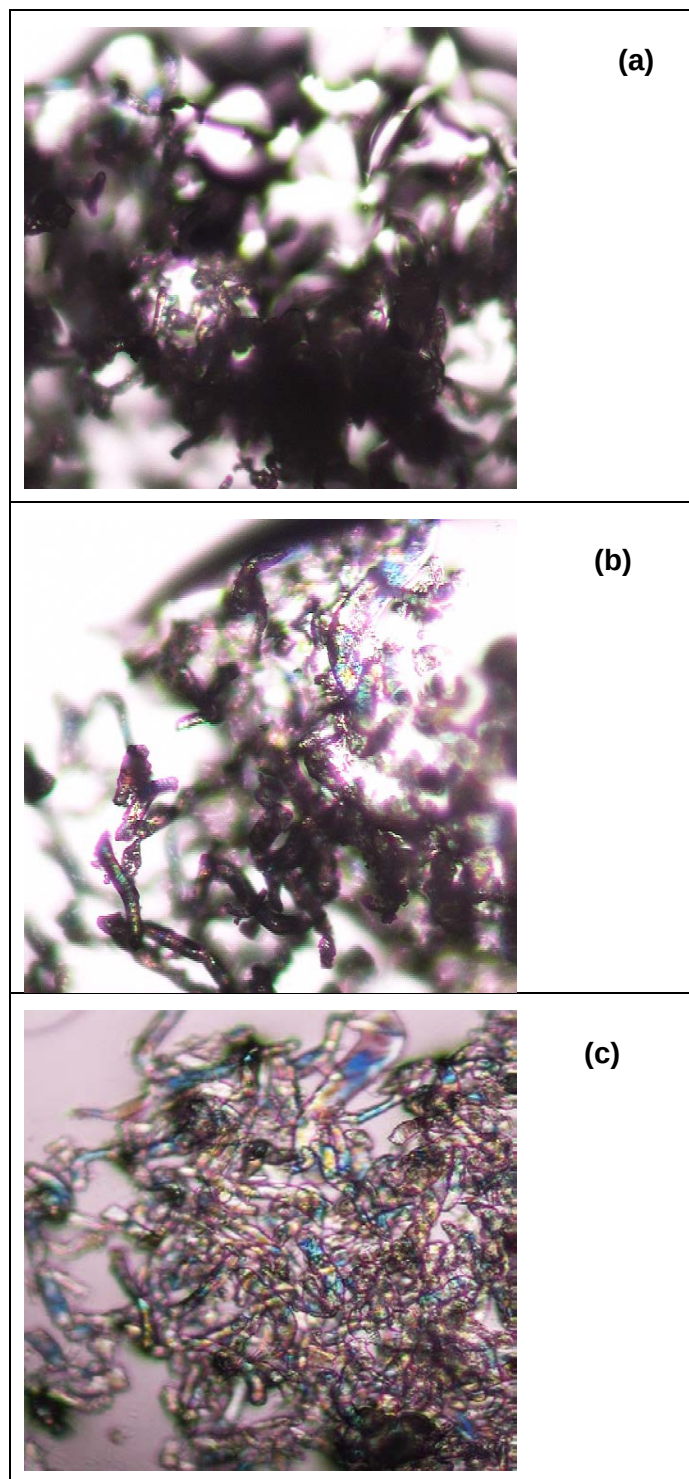


Figure 4.9: Micrographs obtained during the heating of a 1:1 mixture of azithromycin and Ac-Di-Sol.

During the thermal microscopy experiment it became clear that the Ac-Di-Sol does not melt but rather forms a polymeric matrix in which the azithromycin are encapsulated. This polymeric matrix does not melt and stays intact throughout heating. At approximately 245.7°C the matrix starts to turn yellowish-brown which indicates that degradation occurs. It was concluded that no incompatibility exists between Ac-Di-Sol and azithromycin.

4.2.4 TABLET FORMULATION

Upon consideration and complete assessment of all the compatibility testing results it was decided that four formulations will be mixed with the same excipients but with different concentrations of the excipients. The flow properties of the four formulations were tested by determining the angle of repose for all four powder mixtures. Furthermore, the mixtures were compressed and the resulting tablets were tested in terms of weight variation, hardness and disintegration. From all of these results the best formulation was then chosen for the subsequent manufacturing of a bigger batch of tablets.

Table 4.3 provides a summary of the first trial formulation (Formulation 1). After the mixing of the powder blend specified in Table 4.3, the angle of repose of the blend was tested in order to see if some improvement in the powder flow properties was obtained merely by mixing azithromycin with excipients. The angle of repose was determined to be 48.3°. Although the angle of repose was passable according to the specifications of the USP (2014) the compression of the tablets was challenging. The powder flow through the funnel of the tablet press hopper was erratic and poor. The compressed tablets chipped and cracked during compression.

Table 4.3: Composition and tablet parameters of formulation 1 of tablets containing azithromycin

Composition		Tablet parameters (post compression)	
Ingredient	Concentration	Average tablet weight:	950.7 mg
Azithromycin	500 mg	Weight variation of 20 tablets:	1.5 %
Ac-Di-Sol	2.0 %w/v	Hardness:	55.3 N ($\pm$ 9.4)
Kollidon® VA-64	3.0 % w/w	Friability:	All tablets were broken
Magnesium stearate	1.5 % w/w	Disintegration:	Within 10 seconds
Avicel PH 200	a.q		

Considering the poor and unacceptable results obtained with the first trial formulation it was decided to decrease the concentration of the magnesium stearate and to omit Kollidon® VA-64 from the formulation due to poor tableting properties. Prior to tableting of the powder blend the flow behaviour of the powder was investigated through the measurement of the angle of repose. The angle of repose was determined to be 42.3°. The angle of repose showed that this particular mixture was in the same range as formulation 1. Therefore, it was expected that the flow of the mixture through the tablet press hopper into the die will be a difficulty. However, when the compression of the tablets commenced the powder flow was acceptable and die filling occurred acceptably. Table 4.4 provides the formulation as well as the tablet parameters obtained after compression of the trial formulation.

Table 4.4: Composition and tablet parameters of formulation 2 of tablets containing azithromycin

Composition		Tablet parameters (post compression)	
Ingredient	Concentration	Average tablet weight:	1053 mg
Azithromycin	500 mg	Weight variation of 20 tablets:	0.02 %
Ac-Di-Sol	2.0 %w/v	Hardness:	110.7 N ($\pm$ 8.3)
Magnesium stearate	1.0 % w/w	Friability:	1.0 % ($\pm$ 0.6%)
Avicel PH 200	a.q	Disintegration:	Within 40 seconds

From the listed parameter it is clear that this formulation resulted in the tableting of acceptable tablets. Although the hardness was determined to be somewhat high it was still acceptable and the disintegration was not adversely affected by this.

Another and third formulation was mixed and compressed. With this formulation Kollidon[®] VA-64 was again added in the same concentration as with formulation 1. The only difference between this formulation and the first one is the concentration of the Ac-Di-Sol that was decreased with 50 %. The rationale for this formulation decision was to determine the influence that Ac-Di-Sol have on the disintegration properties of the tablets and to attempt to increase the disintegration time of the tablets. Table 4.5 lists the tablet formulation as well as the tested tablet parameters.

Table 4.5: Composition and tablet parameters of formulation 3 of tablets containing azithromycin

Composition		Tablet parameters (post compression)	
Ingredient	Concentration	Average tablet weight:	993.0 mg
Azithromycin	500 mg	Weight variation of 20 tablets:	0.01 %
Ac-Di-Sol	1.0 %w/v	Hardness:	95.6 N ($\pm$ 9.4)
Kollidon [®] VA-64	3.0 % w/w	Friability:	1.8 %
Magnesium stearate	1.5 % w/w	Disintegration:	Within 21 seconds
Avicel PH 200	a.q		

With the third formulation the angle of repose that was determined prior to tableting was determined to be 36.2°. According to the specifications provided by the USP (2014) this is an indication that the flow is fair. The flow properties of this formulation were indeed better than the previous two trial mixtures. The mixture also flowed freely through the hopper of the tablet press into the tablet die. Through assessment of the tablet parameters it is also clear that the weight variation and tablet hardness was acceptable. However, the friability was found to be out of specification and the disintegration was faster than with the formulation containing 2.0 % w/w Ac-Di-Sol. From this data it became clear that the effect of the disintegrating agent was not as pronounced. The high friability percentage is in fact affected by the presence of Kollidon[®] VA-64 and this could most possibly be ascribed by the moisture content of Kollidon[®] VA-64 (Bühler, 2008). Furthermore, the filler (Avicel PH200) is known for its ability to also act as a binding agent without the inclusion of moisture into the formulation.

Considering this, it was decided to prepare a fourth formulation in which Kollidon[®] VA-64 was again omitted and the concentration of the magnesium stearate was also decreased to 1.0 % w/w since sufficient lubrication of the die wall was achieved with formulation 2 which contained the same concentration of magnesium stearate. Table 4.6 lists the formulation with adjusted concentrations as well as the tablet parameters obtained after compression. The angle of repose was again determined using the powder mixture prior to compression

and 32.1° was measured. This showed that the powder mixture has good flow behaviour and no difficulty during compression was expected.

Table 4.6: Composition and tablet parameters of formulation 4 of tablets containing azithromycin

Composition		Tablet parameters (post compression)	
Ingredient	Concentration	Average tablet weight:	990 mg
Azithromycin	500 mg	Weight variation of 20 tablets:	0.01 %
Ac-Di-Sol	1.0 %w/v	Hardness:	101.2 N ($\pm$ 3.0)
Magnesium stearate	1.0 % w/w	Friability:	0.6 % ($\pm$ 0.6%)
Avicel PH 200	a.q	Disintegration:	Within 36 seconds

From the data provided in Table 4.6 it is clear that this formulation provided acceptable tablets that complied with all testing parameters. This formulation was therefore chosen as the best formulation for the manufacturing of azithromycin tablets. Subsequently, sufficient tablets were manufactured that would allow proper testing as well as stability testing for a period of six months.

4.3 CONCLUSION

The pre-formulation studies emphasised the importance of proper physico-chemical characterisation of the solid-state form that needs to be included into the dosage form. Furthermore, the compatibility studies showed only two incompatibilities between mixtures of excipients and azithromycin. It became also clear that the flow properties of a drug can significantly be improved with painstaking investigation of proper formulation strategies. The next chapter will discuss the stability of the tablets manufactured from the amorphous form, during a six month stability testing period.

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CHAPTER 5

TABLET EVALUATION AND STABILITY STUDY OF AZITHROMYCIN TABLETS

5.1 INTRODUCTION

Testing of the tablets was conducted over a 6 month period at 75 % relative humidity and 40°C. The physical integrity and strength of tablets can be tested by a variety of methods, like disintegration, thickness and diameter, friability and tablet hardness. The analytical tests conducted for this study includes an assay to determine tablet content and dissolution testing to indicate the availability of azithromycin from the manufactured tablets.

5.2 PHYSICAL PROPERTIES OF TABLETS

The tablets were evaluated in terms of the following physical parameters: appearance, weight variation, thickness, diameter, hardness, friability and disintegration using the specifications as stated in the United States Pharmacopoeia (USP, 2014) or British Pharmacopoeia (BP, 2014).

5.2.1 Results

Weight variation

According to the BP, not more than two of the individual tablets weight should deviate from the average mass by more than the allowed percentage deviation whereas none should deviate by double the allowed percentage deviation (BP, 2014). The tablets were manufactured as 1000 mg tablets. And according to the BP for a tablet of mass above 250 mg, a 5 % deviation is allowed. Results for azithromycin amorphous tablets and azithromycin raw material materials are given in Figures 5.1 and 5.2 respectively.

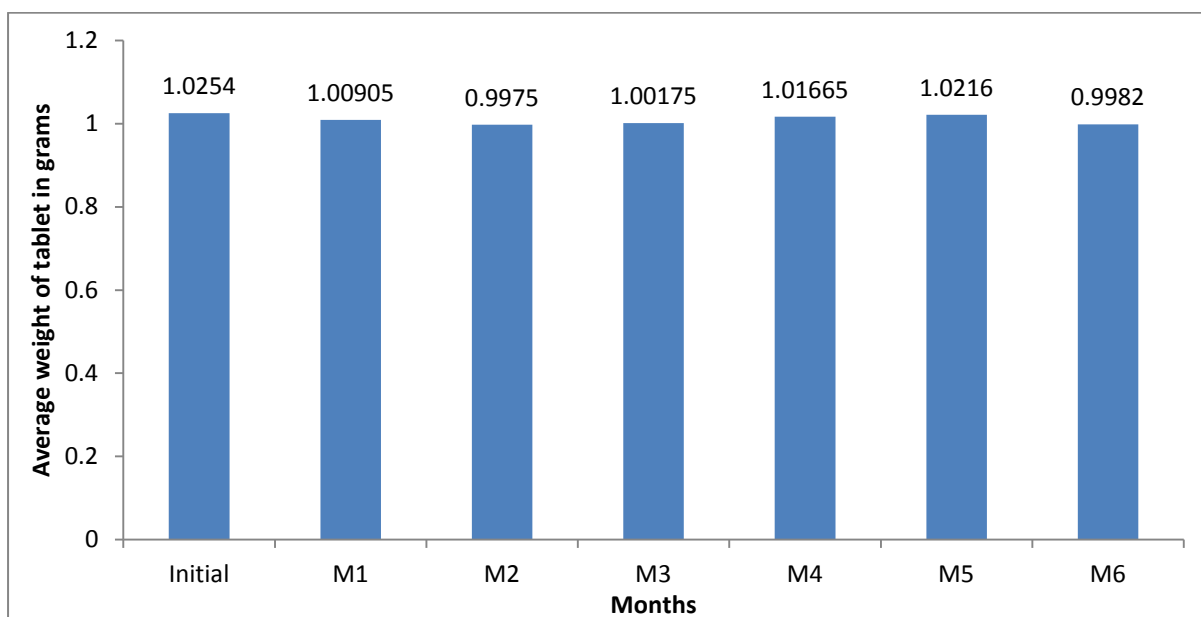


Figure 5.1: Average weight of azithromycin amorphous tablets in stability testing.

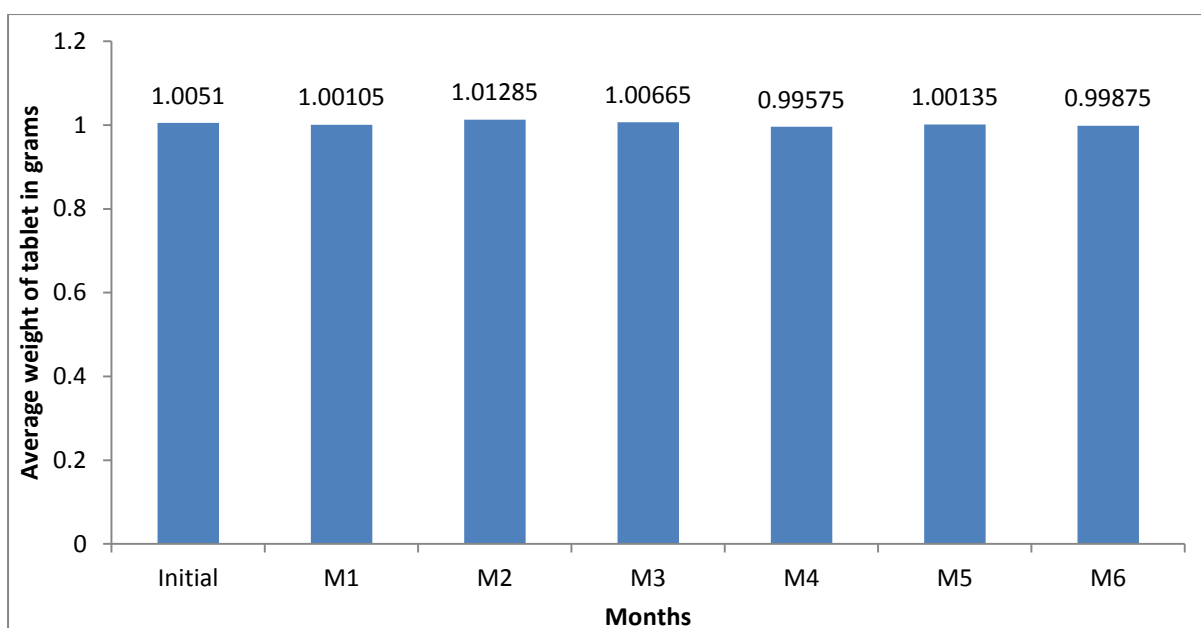


Figure 5.2: Average weight of azithromycin raw material tablets in stability testing.

The average weight of both the tablet batches manufactured from the amorphous and crystalline material remains stable over the 6 month stability trial period. Both batches comply with the BP specification (BP, 2014).

Friability

According to the USP, the tablets should not loose more than 1 % of their total weight. The percentage friability was calculated using equation 3.7 (USP, 2014).

Table 5.1: Percentage friability results of the azithromycin tablets

	Raw material tablets		Amorphous tablets	
	% Friability			
Initial	1.6	*dnc	1.6	*dnc
Month 1	1.0	*c	0.9	*c
Month 2	0.3	*c	0.3	*c
Month 3	0.5	*c	1.7	*dnc
Month 4	0.3	*c	1.3	*dnc
Month 5	0.5	*c	1.2	*dnc
Month 6	1.2	*dnc	0.9	*c

**dnc = does not comply*

**c = comply*

Summary and discussion of physical testing

Tables 5.2 and 5.3 listed a summary of all the physical test results conducted on the two batches of azithromycin tablets. For both batches there is an increase in the % RSD for the tablet hardness. Subsequently, the friability stabilised up to month 5 with a sudden increase in month 6. The great variation in the hardness can be attributed to moisture loss rather than moisture adsorption. This is due to the fact that the tablets were stored in closed containers in the stability chamber. Therefore, the elevated temperature of 40 °C had a greater effect on the sample than the higher humidity. However, moisture loss will result in harder tablets but only to a certain extent at which point the tablets will become brittle due to too much moisture loss. This is clear from the sudden increase in the friability at 6 months for the crystalline azithromycin tablet batch.

Table 5.2: Summary of the physical properties of azithromycin tablets manufactured from the raw material

Month	Hardness N (% RSD)	Diameter mm (% RSD)	Thickness mm (% RSD)	Friability %	Disintegration sec (RSD)
0	116.9 (7.60)	15.97 (0.12)	4.68 (2.98)	1.6	35.33 (1.33)
1	128.0 (5.34)	15.95 (0.16)	4.73 (3.10)	1.0	39.16 (1.75)
2	111.5 (10.23)	15.94 (0.04)	4.69 (3.11)	0.3	39.83 (1.72)
3	107.2 (6.50)	15.95 (0.03)	4.76 (2.76)	0.5	29.83 (2.30)
4	121.6 (17.37)	15.97 (0.21)	4.82 (2.16)	0.3	30.50 (1.63)
5	115.9 (15.85)	15.94 (0.04)	4.77 (3.08)	0.5	41.50 (1.20)
6	115.5 (32.31)	16.00 (0.06)	4.84 (5.63)	1.2	43.50 (2.20)

Table 5.3: Summary of the physical properties of azithromycin tablets manufactured from the amorphous material

Month	Hardness N (% RSD)	Diameter mm (% RSD)	Thickness mm (% RSD)	Friability %	Disintegration sec (% RSD)
0	114.3 (5.34)	15.97 (0.12)	4.99 (2.36)	1.6	37.50 (2.55)
1	120.5 (8.62)	15.95 (0.13)	4.88 (6.02)	0.9	40.16 (0.92)
2	90.8 (13.16)	15.96 (0.08)	4.96 (2.76)	0.3	41.83 (1.64)
3	102.6 (13.95)	15.94 (0.12)	5.03 (2.39)	1.7	33.00 (3.49)
4	121.6 (17.36)	15.95 (0.10)	5.10 (4.18)	1.3	30.50 (1.63)
5	105.6 (14.10)	16.01 (0.14)	5.17 (2.38)	1.2	44.16 (2.41)
6	113.9 (9.261)	16.01 (0.13)	4.98 (3.55)	0.9	45.83 (3.42)

With the batch containing amorphous azithromycin the same behaviour was not observed. Although the hardness of the tablets increased with a significant increase in the % RSD the same variation was not observed with the friability. For the batch containing amorphous azithromycin the friability was significantly higher when compared to the crystalline azithromycin batch. This can be explained by the fact that the amorphous azithromycin tablets contain even less moisture due to the fact that the amorphous solid-state form contains approximately 0.1 % moisture while the crystalline solid-state form contains 4.6 %.

5.3 DISSOLUTION STUDY

Tablets manufactured from the amorphous azithromycin and the raw material (azithromycin dihydrate) will be investigated and the results reported in this chapter. The dissolution medium for the testing was water. This medium will be the best to distinguish between the two forms of azithromycin, since azithromycin is sparingly soluble in water. Odendaal (2013) performed also tablet testing on tablets manufactured from amorphous and crystalline azithromycin. The dissolution media were pH 4.5 acetate buffer, pH 6.8 phosphate buffer and water. The dissolution results in water showed significant differences between the amorphous and dihydrate tablets. Then Odendaal (2013) performed accelerated stability studies (3 months) on the two batches of tablets. Instead of using water as a discriminative dissolution medium he choose pH 6.8 phosphate buffer in which both forms of azithromycin is very soluble. In both dissolution studies the tablets manufactured from amorphous and crystalline raw material were completely dissolved within 10 minutes. Thus no difference in the dissolution results were observed between the tablets manufactured from amorphous and crystalline forms. No conclusions could then be drawn about the stability or release of the amorphous tablets in comparison with the tablets manufactured from crystalline material. Therefore, for this study the dissolution medium will be water to differentiate between the amorphous and crystalline forms.

5.3.1 Results

The dissolution testing (see section 3.6.7) was carried out at a temperature of $37 \pm 0.5^{\circ}\text{C}$, with the paddles set at a rotating speed of 100 rpm. The dissolution medium used for the tablets was 900 ml of distilled water. Aliquots of dissolution media (5 ml) were withdrawn at 5, 10, 20, 30, 40, 55, 70, 180, 240, 360, 480 and 540 minutes, filtered using $0.45\ \mu\text{m}$ PVDF filters. Since these dissolution studies are designed to evaluate the solution-mediated phase transformation of amorphous azithromycin in tablet formulations, no dissolution media replacement was done. The samples were then analysed using the same HPLC method as for the assay. The data was analysed by calculating the amount of drug released and the

cumulative percentage drug released at different time intervals. The batches tested were tablets manufactured from amorphous material and crystalline material. The dissolution profiles of the 6 month stability study and the respective overlays are shown in Figures 5.3 – 5.9. Results for the respective tablets are graphically represented in figures 5.10 – 5.11.

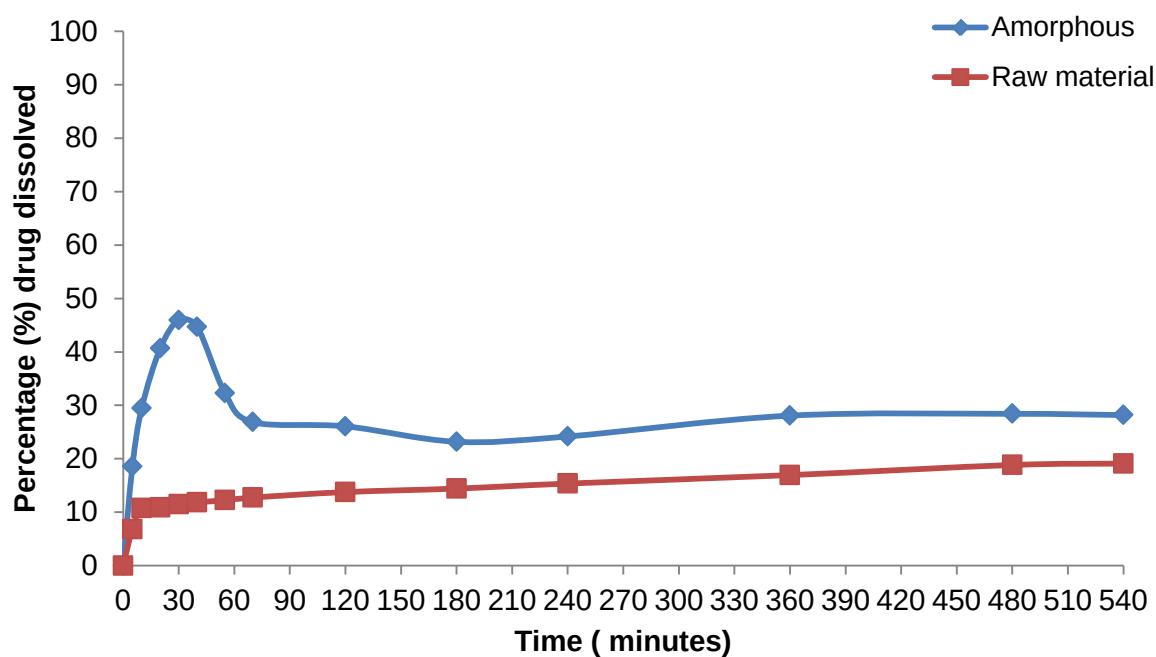


Figure 5.3: Dissolution profile overlay of azithromycin amorphous and crystalline tablets in water - initial testing.

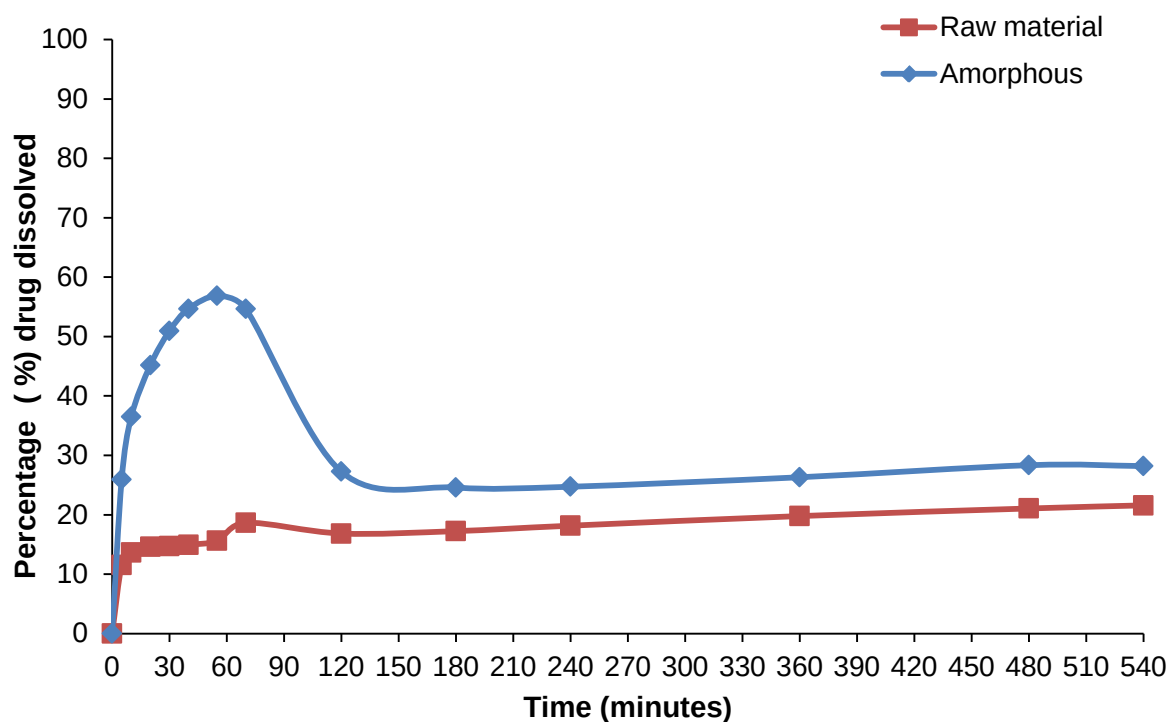


Figure 5.4: Dissolution profile overlay of azithromycin amorphous and crystalline tablets in water – month 1.

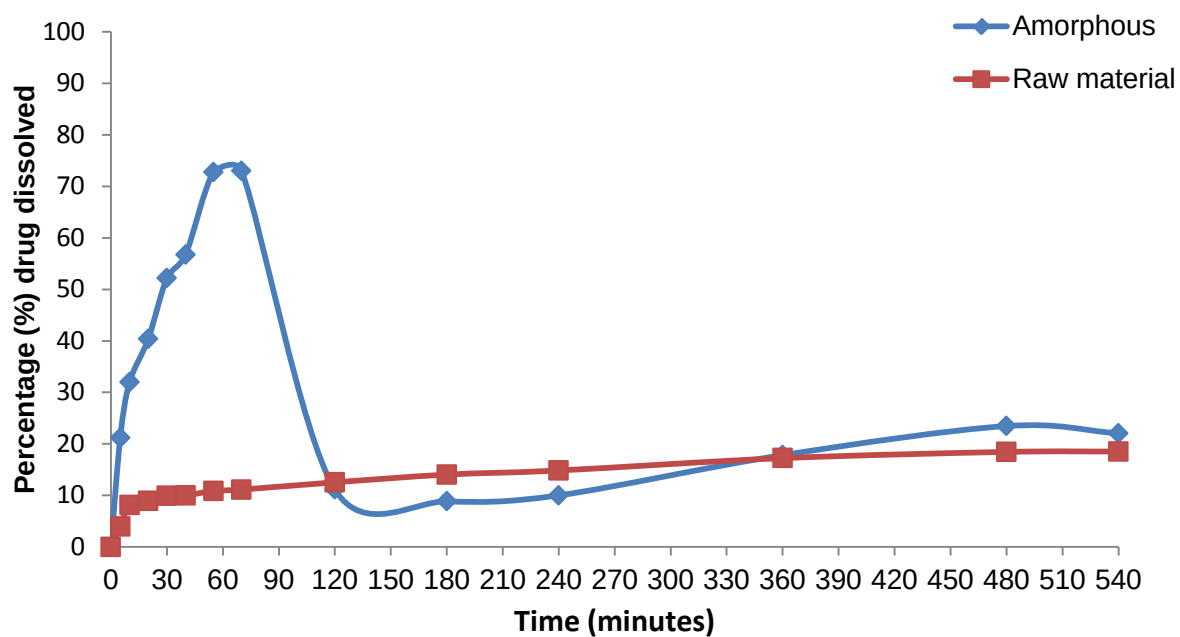


Figure 5.5: Dissolution profile overlay of azithromycin amorphous and crystalline tablets in water – month 2.

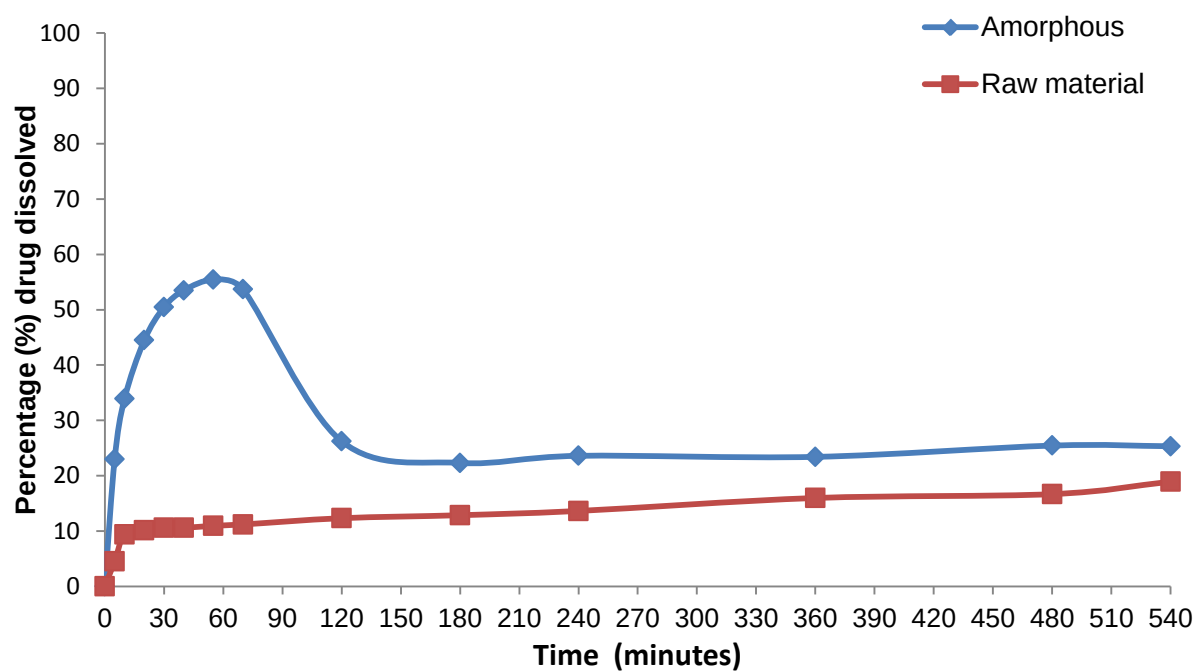


Figure 5.6: Dissolution profile overlay of azithromycin amorphous and crystalline tablets in water – month 3.

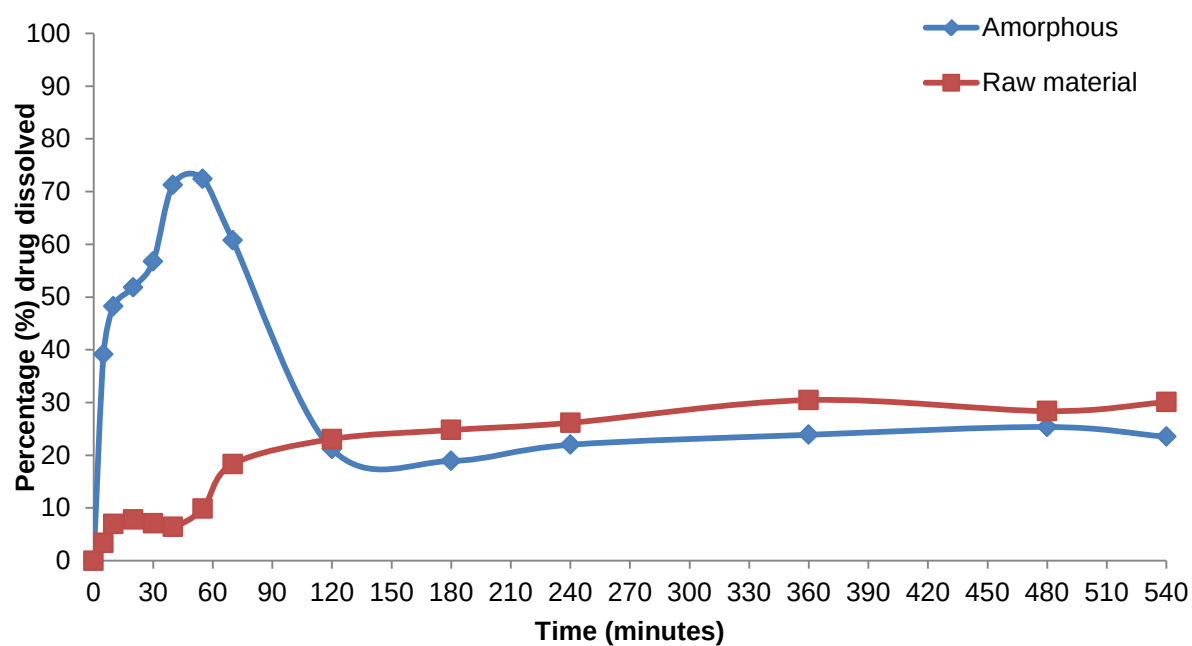


Figure 5.7: Dissolution profile overlay of azithromycin amorphous and crystalline tablets in water – month 4.

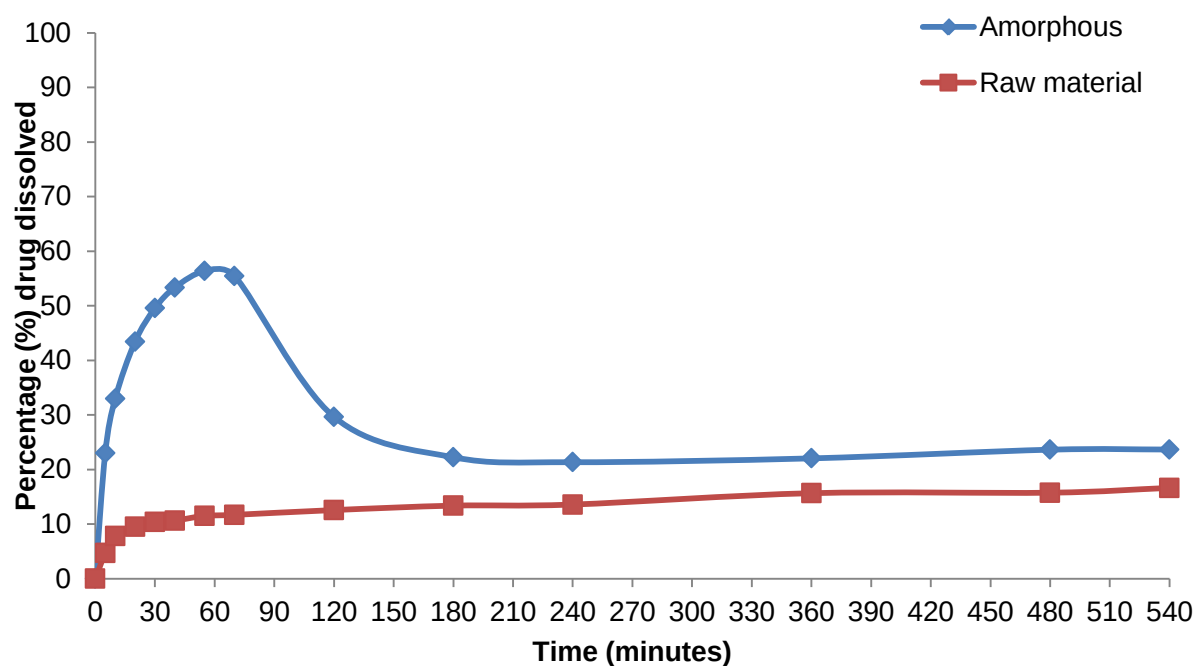


Figure 5.8: Dissolution profile overlay of azithromycin amorphous and crystalline tablets in water – month 5.

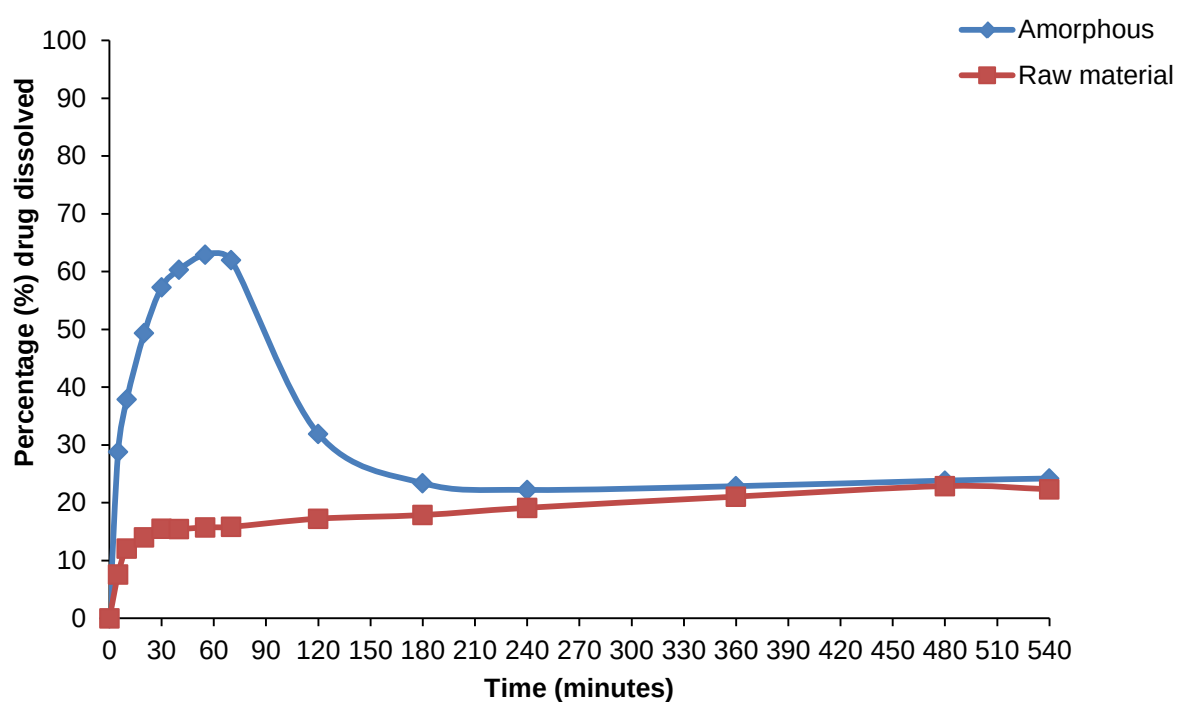


Figure 5.9: Dissolution profile overlay of azithromycin amorphous and crystalline tablets in water – month 6.

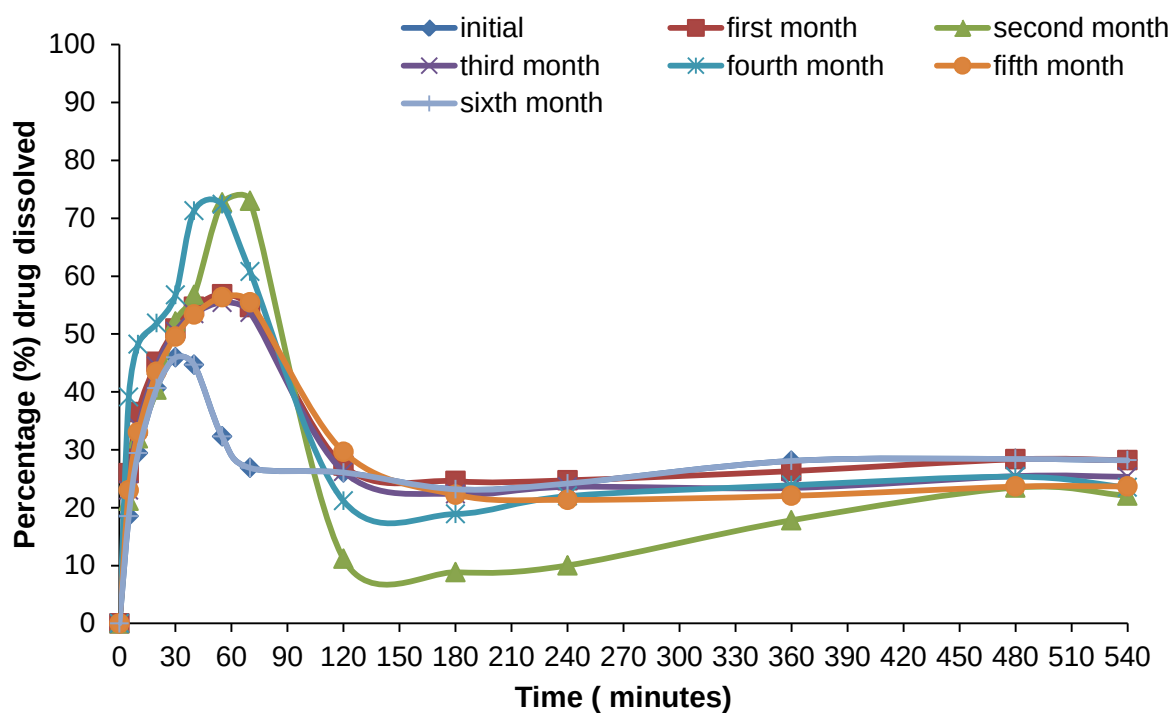


Figure 5.10: Dissolution profile overlay of azithromycin amorphous tablets in water – initial unto month 6.

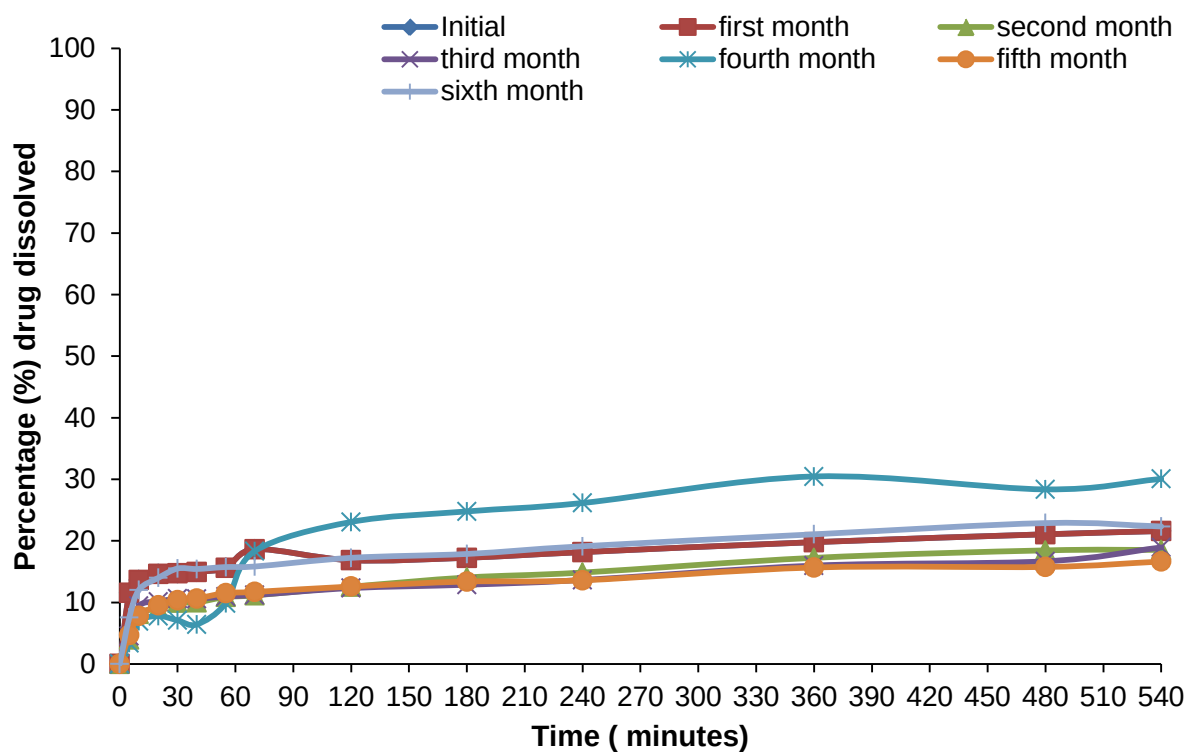


Figure 5.11: Dissolution profile overlay of azithromycin crystalline tablets in water – initial unto month 6.

5.3.2 DISCUSSION

The dissolution profiles of the tablets manufactured from the raw material, i.e. crystalline material were as expected. Since azithromycin is very poorly soluble in water, the average % dissolved never reached values above 30 %.

The profiles of the amorphous tablets show typical solution-mediated phase transformations. The identical profile was reported by Aucamp *et al.* (2013) for another macrolide, roxithromycin. In that particular study the transformation of the roxithromycin amorphous glass to the less soluble form of roxithromycin was described. The initial high dissolution values for the azithromycin amorphous tablets were typical of a metastable form, then it recrystallise into the less soluble form and the dissolution values decline until it reach the identical concentration of the raw material. At that point the transformation from the more soluble meta-stable form to the less soluble form is theoretically 100 % complete.

The 6 month stability period did not change the integrity of the tablets and its contents. Both the tablets manufactured from the amorphous material and the tablets manufactured from the crystalline raw material, dissolution values remained almost identical from initial to month 6.

5.4 ASSAY

The assay testing was performed according to the method described in par 3.6.8. Twenty tablets were selected randomly, weighed and crushed in a mortar and pestle. A quantity of powder equivalent to the weight of 1 tablet (1000 mg) was weighed in duplicate and the powder was transferred into a 100 ml volumetric flask. The dilution was made with mobile phase. The resulting solution was filtered through a 0.45 µm PVDF filter into HPLC vials. The samples were analysed using a Shimadzu HPLC (Shimadzu, Japan) chromatographic system. A summary of the assay results were shown in Table 5.4.

5.4.1 Results and discussion

Table 5.4: Assay summary of azithromycin tablets during stability testing

Azithromycin raw material tablets	Azithromycin amorphous tablets
Initial (mg/tab)	
1. 499.32	1. 504.21
2. 502.23	2. 505.14
Average: 500.77	Average: 504.67
SD: 1.455	SD: 0.465
% RSD: 0.290	% RSD: 0.092
Percentage: 100.15	Percentage: 100.93
Month 1 (mg/tab)	
1. 498.65	1. 502.74
2. 499.98	2. 499.21
Average: 499.31	Average: 500.97
SD: 0.665	SD: 1.765
% RSD: 0.133	% RSD: 0.352
Percentage: 99.86	Percentage: 100.19
Month 2 (mg/tab)	
1. 498.10	1. 512.23
2. 499.46	2. 501.69
Average: 498.78	Average: 506.96
SD: 0.68	SD: 5.27
% RSD: 0.136	% RSD: 1.039
Percentage: 99.756	Percentage: 101.39
Month 3 (mg/tab)	
1. 500.11	1. 512.57
2. 500.87	2. 510.57
Average: 500.49	Average: 511.57
SD: 0.38	SD: 1.00
% RSD: 0.0759	% RSD: 0.195
Percentage: 100.09	Percentage: 102.31

Month 4 (mg/tab)	
1. 501.48	1. 513.27
2. 503.27	2. 516.48
Average: 502.38	Average: 514.89
SD: 0.895	SD: 1.605
% RSD: 0.178	RSD: 0.311
Percentage: 100.47	Percentage: 102.97
Month 5 (mg/tab)	
1. 498.10	1. 512.23
2. 499.46	2. 501.69
Average: 498.78	Average: 506.96
SD: 0.68	SD: 5.27
% RSD: 0.136	% RSD: 1.0395
Percentage: 99.75	Percentage: 101.39
Month 6 (mg/tab)	
1. 496.50	1. 504.74
2. 484.46	2. 505.88
Average: 490.48	Average: 505.31
SD: 6.02	SD: 0.57
% RSD: 1.227	% RSD: 0.112
Percentage: 98.09	Percentage: 101.06

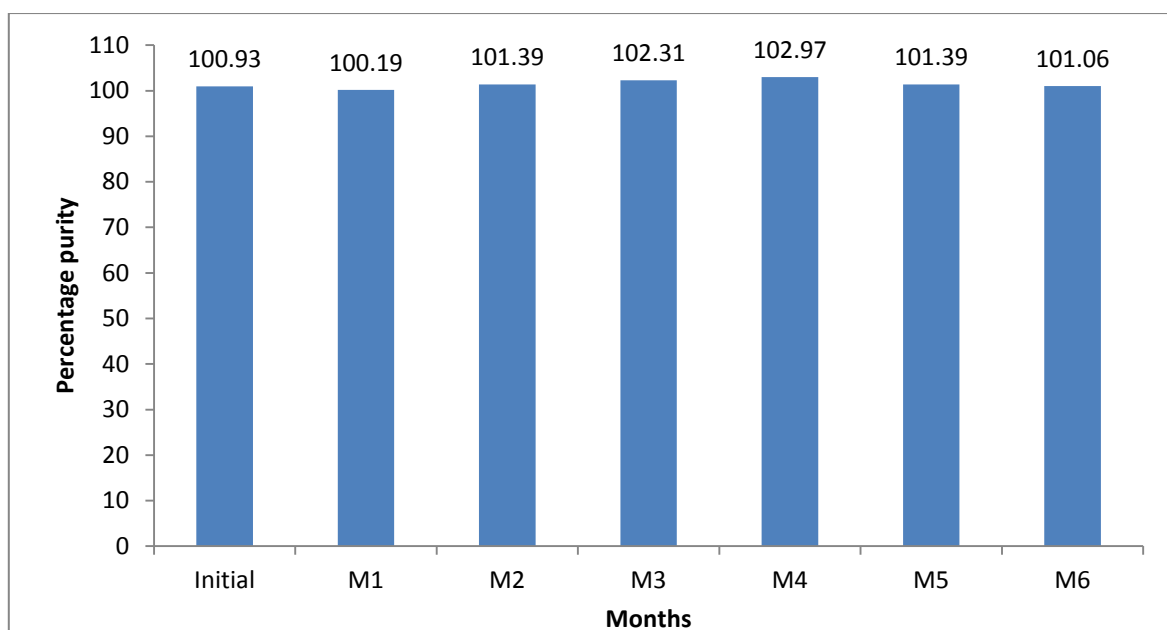


Figure 5.12: Assay results of the azithromycin tablets manufactured from amorphous material.

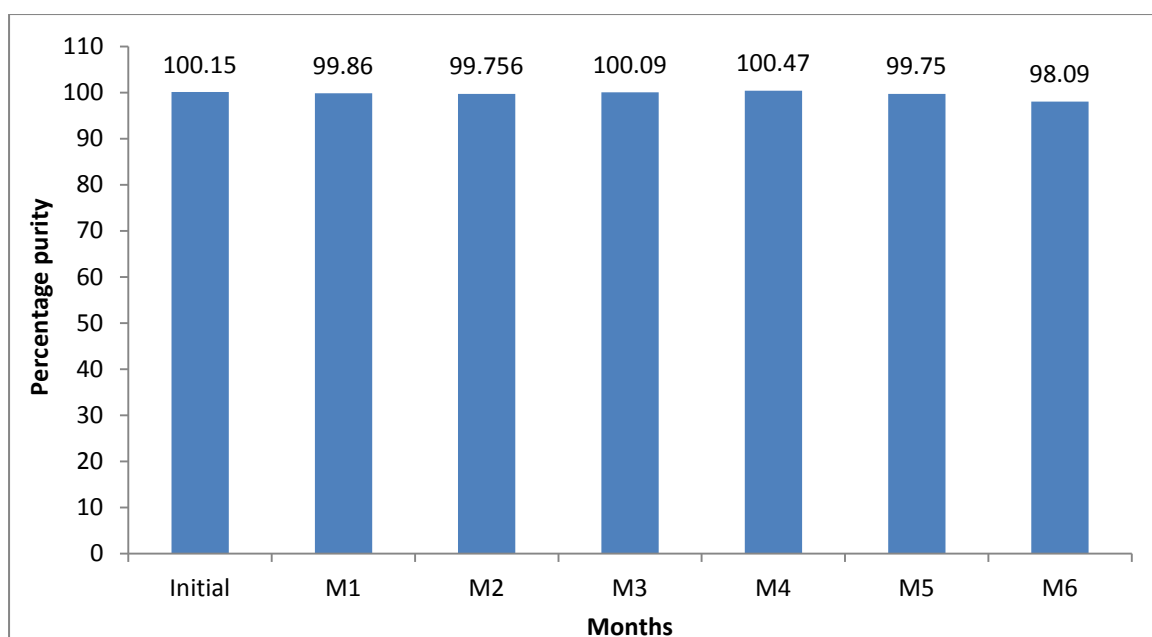


Figure 5.13: Assay results of the azithromycin tablets manufactured from raw material (crystalline material).

The content of the tablets over the 6 month period remained stable and comply with the USP criteria which state that not less than 90 % and not more than 110 % of the labelled amount of azithromycin should be present (USP, 2014).

5.5 CONCLUSION

During the stability testing of the two batches of azithromycin containing different solid-state forms of the same drug, showed that storage for a period of six months at elevated temperature and humidity did not result in significant changes of the solid-state forms. This conclusion is made from the dissolution data. From the initial data right through to the last stability time interval the phenomena of solution-mediated phase transformation were observed. This is a distinct indication that the solid-state form remained amorphous. Furthermore, it is also clear that even during compression, handling and storing the amorphous form of azithromycin remains amorphous. In terms of formulation strategies it can be concluded that whenever amorphous solid-state forms are formulated into tablets it must be kept in mind that moisture loss due to storage might detrimentally influence the friability of such tablets. Therefore, there exist a fine line between too much moisture (that would induce recrystallisation of the amorphous form) and too little moisture. In terms of the stability of the amorphous form of azithromycin the outcome is regarded positive. In terms of product formulation further developmental work would be necessary to formulate a stable dosage form.

5.6 REFERENCES

AUCAMP, M., STIEGER, N., BARNARD, N & LIEBENBERG, W. 2013. Solution-mediated phase transformation of different roxithromycin solid-state forms: Implications on dissolution and solubility. *International journal of pharmaceutics*, 449: 18-27.

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ODENDAAL, R.W. 2013. Amorphism and polymorphism of azithromycin. Ph.D Thesis, North-West University, Potchefstroom. 65-77p.

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CHAPTER 6

CONCLUDING REMARKS

From the literature search done during the course of this study it became clear that drugs have the ability to exist in more than one solid-state form. These different solid-state forms show significant differences between one another with regard to physico-chemical properties. One of the most important physico-chemical properties that is influenced by different solid-state forms is the aqueous solubility. Even after decades of research and development the ultimate factor that influences the performance of a drug remains the issue of improved solubility. During the last two decades a mentionable shift was observed in the application of amorphous solid-state forms of drugs. At first the strategy of including amorphous forms of drugs into dosage forms was almost unthinkable and even to some extent prohibited by regulatory bodies. However, in-depth investigation and exploring of the physico-chemical behaviour of amorphous forms lead to the realisation that these forms can actually be an advantage to the pharmaceutical industry.

This study illustrated the advantages of amorphous solid-state forms in terms of aqueous solubility but also emphasised the disadvantages of amorphous forms when considering the higher free energy and therefore the higher physical and/or chemical instability. Previous studies reported on the amorphous form of azithromycin, a 15-membered macrolide antibiotic. These studies showed that the amorphous form of azithromycin did result in improved aqueous solubility of the given antibiotic, however none of these studies mentioned whether amorphous azithromycin is sufficiently stable so that it can be formulated in a dosage form and that it would remain stable during a relative acceptable storage time.

Azithromycin is currently the drug of choice for the treatment of the co-infection *Mycobacterium avium* complex (MAC) infection. This infection is a common systemic infection in patients suffering from HIV. Usually patients suffering from HIV also suffer from life-threatening co-infections and most often these co-infections are the cause of high morbidity and mortality rates. Considering this it makes sense that improved treatments for these co-infections should be investigated.

Therefore, this study focussed on the successful formulation of azithromycin by using the simplest formulation technique; namely direct compression. After the formulation of amorphous azithromycin it was determined that milling and mixing processes did not induce the recrystallisation of the amorphous form back to the most thermodynamically stable dihydrate form. This was considered as a positive outcome.

Subsequently, the stability of the tablets containing the amorphous form was investigated. 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 recrystallisation of the amorphous form to the stable crystalline form within solution. It is indicative of the time that it takes for the recrystallisation process to complete as well as the time that an elevated concentration of the drug will be available for absorption after patient administration. During the six month stability study no change in any solid-state form was observed (i.e. amorphous to crystalline or crystalline to another solid-state form). Therefore it can be concluded that amorphous azithromycin shows excellent stability in terms of physical and chemical properties. Through proper formulation and storage strategies amorphous azithromycin can most probably be included into a marketable product that would provide improved treatment and patient compliance.