

This dissertation is dedicated in loving memory of my dearest Grandparents,
Carlos and Adelina Farinha

Wherever a beautiful soul has been there is a trail of beautiful memories

The development of sulfadoxine and nevirapine pharmaceutical amorphous solid dispersions

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ABSTRACT

Solid oral dosage forms are the most convenient and prevalent dosage form in the pharmaceutical industry although an estimated 90% of drugs currently in development can be classified as poorly soluble. These drugs will subsequently present with poor dissolution which can lead to inadequate bioavailability. Sulfadoxine (SULF) and nevirapine (NEV) both present with poor dissolution and solubility properties. Sulfadoxine is a long-acting sulfonamide with antibacterial and antimalarial properties. Regardless of the dissolution problem, artemisin-based combination therapies, such as artesunate in combination with sulfadoxine-pyrimethamine are still the recommended treatment for uncomplicated *Plasmodium falciparum* malaria. Nevirapine, an NNRTI is used to reduce morbidity and mortality caused by HIV-1 and AIDS. Nevirapine is especially used as prophylaxis against mother-to-child HIV transmission.

Limited information regarding improvement in dissolution for sulfadoxine has been reported. Nevirapine has undergone various studies, but with no outstanding success rate regarding the improvement of its limited solubility. The aim of this study was to improve the aqueous solubility, and dissolution rate, of the chosen drugs (sulfadoxine and nevirapine) by creating stable pharmaceutical amorphous solid dispersions (PhASDs) using a modified screening of polymers for amorphous drug stabilisation (SPADS) process. The use of dispersions, especially with polymers, enhances drug solubility and inhibits recrystallisation; this leads to an increase in stability and an extended shelf-life.

The amorphous form presents with a higher thermodynamic activity, is more reactive and has a greater dissolution and solubility rate than its crystalline equivalent. Various polymers were initially screened in order to find the combination and ratio, API:polymer, which was miscible and deemed to be successful. After thorough screening the optimal combination API:polymer was found (both APIs delivered most successful results with PVP 25 as polymer) and further experiments were concluded. Manufacturing methods such as hot-melt and solvent-evaporation were experimented with in order to find the most successful method for the development of the PhASD. Hot-melt was an unsuccessful method for both the APIs, though solvent evaporation (by means of spray drying and rotary evaporator) delivered promising results. The SULF:PVP 25 1:2 mixture prepared through rotary evaporation was the only SULF mixture which proved to be mainly amorphous. The NEV spray dried product (NEV:PVP 25 1:4) was a pharmaceutical amorphous solid dispersion (PhASD) and completely amorphous. The product (NEV:PVP 25 1:4) obtained through rotary evaporation was a nanocrystalline solid dispersion (NCSD).

Though amorphous forms are susceptible to reconvert to the crystalline form over time, accelerated stability studies were performed on the resulting PhASDs and NCSD under extreme conditions of 45°C / 75% RH in order to determine whether recrystallisation occurred or degradation took place. The PhASD SULF:PVP 25 1:2 indicated that the exposure to humidity had a plasticising effect as minimal crystal growth occurred. The NEV:PVP 25 1:4 NCSD did not deliver pronounced crystal growth as was found with the sulfadoxine PhASD (SULF:PVP 1:2). HPLC analyses revealed little to no chemical degradation of either sulfadoxine or nevirapine occurred after three month stability testing.

The PhASD, SULF:PVP 25 1:2 delivered a dissolution value of 214.39 µg/ml which is an improvement of 66.95 µg/ml within the first five minutes of dissolution testing when comparing to the SULF raw material (147.44 µg/ml).

The nevirapine NCSD prepared through rotary evaporation yielded a five-fold improvement and the PhASD prepared by spray drying, yielded a six-fold improvement in dissolution within the first five minutes of the study. The PhASDs and nanocrystalline solid dispersion maintained the solubility advantage throughout the remainder of the three hour dissolution study.

The solubility of both APIs remains challenging, as a drastic improvement in dissolution was not achieved for SULFA especially. The improvement of the solubility of NEV in the NEV dispersions, both NCSD and PhASD proved to be more significant.

Key words: sulfadoxine, nevirapine, pharmaceutical amorphous solid dispersion (PhASD), nanocrystalline solid dispersion, polymers, PVP25, solvent evaporation, spray-dry, dissolution, solubility, accelerated stability studies

AIM AND OBJECTIVES

Aim

The aim of this study is to improve the aqueous solubility, and dissolution rate, of sulfadoxine and nevirapine by creating stable pharmaceutical amorphous solid dispersions (PhASDs) using a modified screening of polymers for amorphous drug stabilisation (SPADS) process.

Objectives

Eliminate polymers and drug:polymer ratios that will potentially result in PhASDs having theoretical combined T_g values too low for stability;

Determine the miscibility of the remaining drugs and polymers at the chosen ratios to eliminate any ratios or polymers that will not result in PhASDs dispersed at molecular level;

Produce PhASDs for testing using the hot-melt method, rapid solvent evaporation, rapid precipitation or grinding (with or without solvents);

Perform *in vitro* solubility and dissolution tests on the most promising PhASDs, eliminating all but those with the best dissolution profiles;

Just a few of the very best performing PhASDs will go through to the next round of testing which will involve accelerated stability testing; and

From all the above it should be possible to select a PhASD with improved solubility and good stability for product development (not part of this study).

CHAPTER 1

PHYSICO-CHEMICAL PROPERTIES OF PHARMACEUTICAL ACTIVES

1.1 Introduction

Solid oral dosage forms are the most convenient, chemically and physically stable, as well as the most prevalent dosage forms in the pharmaceutical industry (Zhang *et al.*, 2004; Hilfiker *et al.*, 2006). Many drugs are capable of crystallising in multiple forms, each having different free energy states and physico-chemical properties such as solubility, melting point, density, hardness, refraction index etc. (Hörter & Dressman, 2001). The pharmaceutical behaviour of drug substances can be influenced by different physical and chemical properties. Therefore, the control over solid-state reactions of pharmaceutical solids, crystallisation as well as the solubility and phase stability is an important matter to understand (Lin, 2015). Crystalline solids can also exist in different sub phases namely polymorphs, solvates, desolvates, hydrates, co-crystals and amorphous solids (Vippagunta *et al.*, 2001; Vishweshwar *et al.*, 2006).

Generally, the stability of a molecule in the solid form is much greater than in a solution, consequently many drugs are often stored in the solid-state and dissolved just before administration (Hilfiker *et al.*, 2006).

1.2 Solubility and dissolution

Loftsson & Brewer (2010) reported that approximately 40% of pharmaceutical products which are currently on the market are poorly soluble while an estimated 90% of drugs in development can be classified as poorly soluble. Poorly water-soluble drugs do not present with satisfactory dissolution within the gastro-intestinal tract. This leads to inadequate bioavailability and challenges medicinal chemists to ensure that drugs are not only pharmacologically active but also have adequate solubility (Ashford, 2013a).

Solubility and dissolution rate are closely related to solid-state properties and need to be sufficiently high for every drug product (Hilfiker *et al.*, 2006). In general, poor solubility is associated with poor dissolution and consequently poor oral bioavailability (Hecq *et al.*, 2005). Dissolution is known as the transfer of molecules or ions from a solid-state into a solution. The relative affinity between the molecules of the solid substance and those of the solvent is in control of the dissolution process. On the other hand, solubility refers to the extent to which the dissolution proceeds under a particular set of experimental conditions. The amount of a substance that passes into solution when equilibrium is established between the solute in solution and the excess substance is known as the solubility of the substance (Aulton, 2013a).

Dissolution as well as solubility can control or limit the release of a drug substance after oral administration of a solid dosage form (Stahl & Sutter, 2006). Solubility, dissolution and permeability are fundamental characteristics in defining the rate and extent of absorption of the active pharmaceutical ingredient and thus the oral bioavailability thereof (Van de Waterbeemd, 1998).

Ionisation constants of both acidic and basic drugs are usually expressed in terms of pK_a . The symbol pK_a represents the negative logarithm of the acid dissociation constant K_a in an analogous way. The pK_a of the molecule and the pH of its surrounding environment control the degree of ionisation of the compound (Aulton, 2013b). When the pH is at least 2 pH units below the pK_a , a weakly acidic drug will be completely unionised although, when the pH is at least 2 pH units above the pK_a , complete ionisation will occur for a weak acidic drug (the opposite is of relevance for a basic drug) (Gaisford, 2013). Most drugs intended for oral administration are weak bases, therefore these drugs will be fully ionised in an acidic (low pH) environment such as the stomach, but once the drug reaches the alkaline (high pH) small intestine it will be non-ionised and easily absorbed. The pK_a of a drug is thus extremely important in peroral drug delivery (Aulton, 2013b).

Another important aspect regarding solubility, is the partition coefficient of the drug which is given by its ability to partition between water and a lipid-like solvent. This coefficient indicates the lipophilicity of the drug and whether the drug is likely to be transported across membranes. A drug which is more lipid soluble exhibits greater affinity for the gastrointestinal tract and is thus better absorbed than a drug whose ionised forms prevail (Ashford, 2013a).

Bioavailability of drugs can be influenced by the presence or absence of food in the gastrointestinal tract as well. It is important that certain drugs are not taken with certain food groups as insoluble complexes can be formed and drugs become unavailable for absorption. Although, the presence of food can be favourable as well, food increases the pH of the stomach by acting as a buffer which will result in an increase in dissolution and absorption of a weakly acidic drug but decrease that of a weakly basic drug. Certain foods, especially those with a high proportion of fat tend to delay gastric emptying which results in a delay in absorption of the drug taken therewith. There are many other factors associated with the simultaneous use of food and medication such as stimulation of gastrointestinal secretions, increased viscosity of gastrointestinal contents, food-induced changes in presystemic metabolism and blood flow etc. (Ashford, 2013b).

1.3 Biopharmaceutical classification system

A scientific framework identified as the Biopharmaceutical Classification System (BCS), classifies a drug substance based on its aqueous solubility and intestinal permeability (Yu *et al.*, 2002). Drug substances may be grouped into one of four categories as shown below (Fig. 1.1).

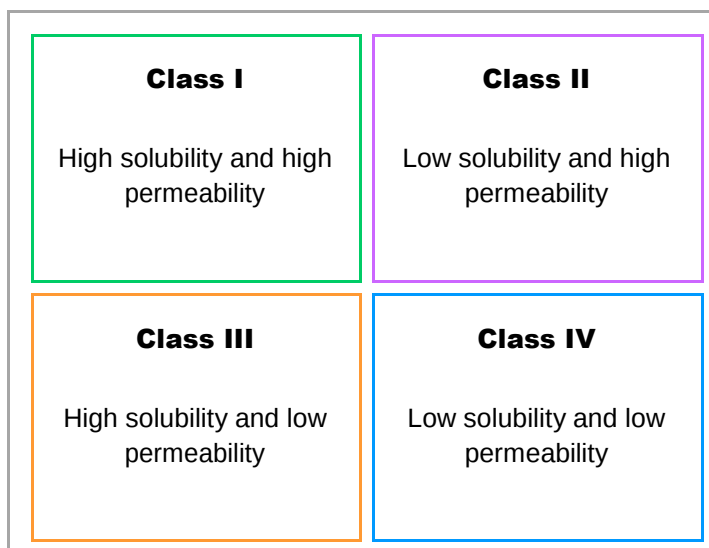


Figure 1.1: Biopharmaceutical classification system.

The solubility classification of a drug is based on the highest dose strength of the substance; if this dose cannot be dissolved in 250 ml in a pH range varying between one and seven, the compound is poorly soluble (Van den Mooter, 2012). The extent of intestinal absorption of a drug substance in humans is used for the direct permeability classification of drugs. Indirectly, the measurements of the rate of mass transfer across the human intestinal membrane can be used as well. A drug compound with a 90% or higher extent of intestinal absorption is considered to be highly permeable (Yu *et al.*, 2002).

The FDA reported that polymorphism is less likely to have an impact on bioavailability for BCS classes 1 and 3 but for class 2 and 4, polymorphism is a critical aspect. Compounds that belong to the last mentioned classes are also of primary interest from a formulation perspective. Class 2 drugs are dissolution rate limited and are therefore our first choice for the preparation of amorphous solid dispersions. Uncertainty occurs with class 4 drugs as it is not clear which property is weaker, the solubility or permeability.

1.4 Polymorphism

According to Byrn *et al.* (1999), “Polymorphism refers to the crystallization of the same compound in different crystal forms, in different crystal packing arrangements.” Different physical and chemical properties occur in pharmaceutical polymorphic solids due to the difference in their internal solid-state structure (Yu *et al.*, 2003). A substance can crystallise into more than one lattice and therefore possess different lattice energies, depending on the crystalline form, which leads to a variance in properties. For example, all polymorphs are crystalline forms although there will always be one polymorph which is most stable under certain conditions, this form will have the lowest free energy and the highest melting point (Aulton, 2013a). Any other metastable form whether it be amorphous or crystalline, presenting with higher potential energy is physically unstable and is susceptible to convert to the thermodynamically most stable form with the lowest energy over time (Cui, 2007).

1.4.1 Phase transformations

Phase transformations or a solvent interactive process can lead to interconversion of solid-state forms (Byrn, 1982; Aucamp *et al.*, 2015). Phase transformations can be induced by thermal-, pressure- or mechanical stresses (Hilfiker *et al.*, 2006). Favourable conditions for a change in solid-state form include heating, milling and exposure to a solvent. A change in crystal form may influence the physical, chemical and mechanical properties of the solid. Interconversion among polymorphic forms is known as polymorphic transition (Zhang *et al.*, 2004). Polymorphs are classified as either enantiotropes or monotropes based on the differences in thermodynamic properties. This classification depends on whether one form can transform reversibly to another or not. A reversible transition between polymorphs is possible at a definite transition temperature below the melting point in an enantiotropic system. In a monotropic system, no reversible transition is perceived between polymorphs below the melting point (Vippagunta *et al.*, 2001).

1.5 Inclusion compounds

1.5.1 Hydrates, solvates, clathrates and co-crystals

Hydrates, solvates and co-crystals are sometimes referred to as “pseudopolymorphs”, because unlike polymorphs, they consist of more than one type of molecule (Cui, 2007). As there will never be any doubt regarding the chemical identity of solvates, this term has been suggested to be abandoned (Seddon, 2004; Bernstein, 2005). Bernstein (2005) regards this term as a misnomer, “solvates and hydrates are just that – they are not pseudo anything, and they should be called what they are”.

Crystalline solid adducts containing solvent molecules within the crystal structure are known as solvates (Vippagunta *et al.*, 2001). Solvates can be classified into one of 2 main classes: stoichiometric or non-stoichiometric. Stoichiometric solvates are molecular compounds with a fixed ratio of solvent to compound. Non-stoichiometric solvates are a type of inclusion compound with the most important feature being that the structure of this class of solvates may often be retained for some time after desolvation. The host and guest molecules usually share weak bonds and the guest can often escape without causing a change in the crystal structure or only a slight change in molecular arrangement (Bērziņš *et al.*, 2017). The solvent acts as a space filler of the voids within the crystal lattice in which it is usually located. The solvent content can take on values between zero and a manifold of the molar compound ratio. Stieger *et al.* (2010) reported rare stoichiometric isostructural solvates for nevirapine where the guest-host ratio varied between 0.5 and 0.32.

When a solid adduct consists of the parent compound/API and water, it is known as a hydrate. Hydrogen bond(s) and/or coordinate covalent bond(s) are formed between water and the anhydrate drug molecules due to water occupying definite positions in the crystal lattice (Khankari & Grant, 1995).

“Desolvated solvates” often retain the crystal structure of the original solvate form, and show minor changes in lattice parameters. However, the solvent may also play an important role in stabilising the lattice whereby the process of desolvation may give rise to an amorphous form, a new crystal form or at least a change in the lattice parameters (Cains, 2009). Desolvation or dehydration describes the transformation from a solvate/hydrate to an unsolvated form or to an anhydrous crystalline form (Aucamp *et al.*, 2015). In practice this is usually achieved by simply removing the recrystallisation solvent and exposing the crystals to air at ambient temperature, although heat and/or vacuum may also be applied (Stieger & Liebenberg, 2012).

In cases where the solvent molecules are entrapped within voids of the structural network of the host molecules, and no significant interaction occurs, the resultant crystal form is known as a clathrate (Griesser, 2006). Solvates and pharmaceutical co-crystals are considered to being closely related from a supramolecular perspective, as components within the crystal interact by hydrogen bonding or other non-covalent interactions. The physical state of the isolated pure components is the main difference between solvates and pharmaceutical co-crystals. If one compound is a liquid at room temperature the compound is a solvate but if both compounds exist as solids under ambient conditions it is referred to as a co-crystal (Vishweshwar *et al.*, 2006; Zaworotko, 2007).

1.6 Amorphous forms

Amorphous solids have no crystal shape and are therefore different to crystalline solids (Byrn, 1999). The molecules of amorphous solids are not ordered in a specific arrangement and these solids do not possess a unique crystal lattice (Yu *et al.*, 2003; Newman & Byrn, 2003). Amorphous solids exhibit short-range order over domains which are too small to show crystalline properties; the consequence of the lack in long-range order is an increase in average molecular separation and weaker attractive forces between the molecules (Bellantone, 2014). The amorphous form has a higher thermodynamic activity and is usually more reactive than its crystalline equivalent. Amorphous solids have higher solubility, higher dissolution rate and are usually physically and chemically less stable than corresponding crystals (Yu, 2001; Zhang *et al.*, 2004). Thus, these forms are susceptible to reconvert to the crystalline form over time which could lead to a limited shelf-life (Gaisford, 2013).

When exposed to a humid environment, amorphous forms are considerably more hygroscopic and absorbed moisture acts as a plasticiser. This causes a dramatic increase in molecular mobility (Zhang *et al.*, 2004) with an obvious decrease in the energy barrier for recrystallisation (Fig. 1.2). An increase in molecular mobility leads to a reduced amount of energy required for recrystallisation to occur. Amorphous solids can be prepared by various methods such as quenching of a melt, rapid evaporation from solution, rapid precipitation by cooling or anti-solvent addition, desolvation/dehydration, physical mixture with amorphous excipients, physical vapour deposition, freeze- and spray-drying, milling and wet granulation (Sun *et al.*, 2012; Petit & Coquerel, 2006; Yu, 2001).

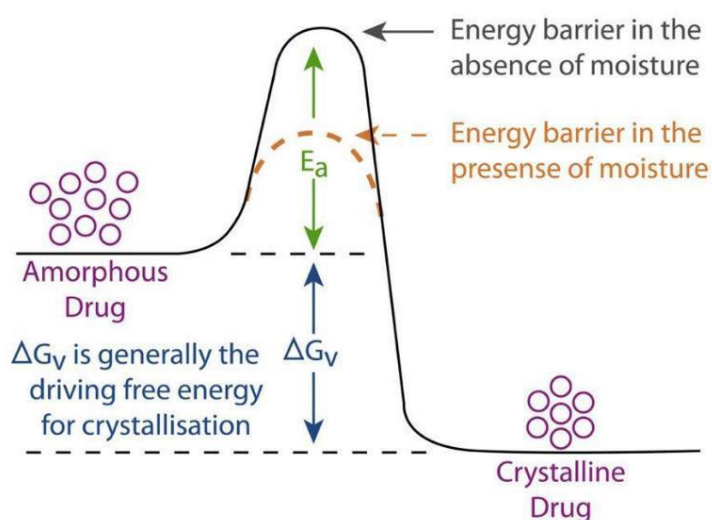


Figure 1.2: Schematic representation of the effect of moisture on the energy barrier for recrystallisation (adapted from Zografi & Newman, 2017).

According to Bellantone (2014) two types of amorphous solids are relevant to pharmaceutical sciences; neat active pharmaceutical ingredient (neat API) which is a pure single component amorphous material and multi-component amorphous solid dispersions (ASDs) which are solids dissolved/dispersed at a molecular level. Both of the amorphous solids mentioned are capable of increasing the solubility and the dissolution rate.

1.6.1 Amorphous solid dispersions

As defined by Chiou and Riegelman (1971), the term solid dispersions is a dispersion of an API in an inert carrier in the solid-state prepared by solvent, melting or solvent-melting methods. It is debateable whether amorphous systems were considered in this definition, therefor, a more recent study by Newman *et al.* (2011) stated that an amorphous solid dispersion is a system where the API is combined with a water-soluble polymer to produce a single-phase amorphous entity. Amorphous solid dispersions increase the solubility as well as the dissolution rate and thus enhance bioavailability (Yu, 2001; Vaka *et al.*, 2014; Van den Mooter, 2012).

Amorphous solid dispersions have many desirable advantages over liquid or semisolid formulations due to their amenability to be developed into solid dosage forms. These advantages include lower manufacturing cost, improved physical and chemical stability, etc. (Qian *et al.*, 2010). Although ternary systems have been reported (Leuner & Dressman, 2000; Al-Obaidi *et al.*, 2011), most amorphous solid dispersions are binary systems where the API is combined with a water-soluble polymer (Newman *et al.*, 2011; Al-Obaidi *et al.*, 2011).

Many of the polymers that can be used for the preparation of solid dispersions are already extensively used in the pharmaceutical industry which is a big advantage of solid dispersions (Leuner & Dressman, 2000). The choice of polymer, hygroscopicity, dissolution, wettability as well as biological aspects are merely a few factors which should be considered when developing these materials into drug products (Newman *et al.*, 2011).

Figure 1.3 is indicative of the difference in molecular arrangement of amorphous solids, crystalline solids and pharmaceutical amorphous solid dispersions.

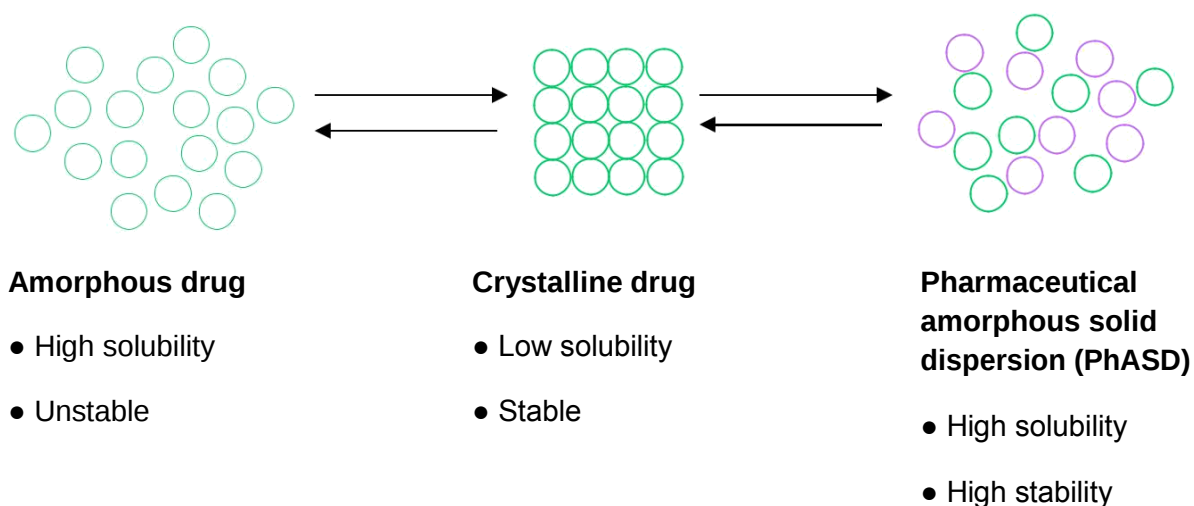


Figure 1.3: Representation of the molecular arrangements of (a) amorphous drugs, (b) crystalline drugs and (c) PhASD as well as principle characteristics of each.

1.6.2 Preparation methods of pharmaceutical amorphous solid dispersions (PhASDs)

Rapid solvent evaporation and fusion/melting methods are the two processes which are most commonly used for the preparation of PhASDs (Jermain *et al.*, 2018; Fujii *et al.*, 2005; Chiou & Riegelman, 1971). The use of dispersions, especially with polymers, enhances drug solubility and inhibits recrystallisation. This leads to an increase in stability and extended shelf-life (Singhal & Curatolo, 2004).

1.6.2.1 Solvent methods

The API and carrier are dissolved in an organic solvent chosen for its suitability to solubilise both API and carrier. Various techniques may be used to rapidly remove the solvent, such as vacuum evaporation and spray-drying (Fujii *et al.*, 2005; Leuner & Dressman, 2000). Due to the toxicity of most organic solvents and negative influence of residual solvent on the drugs' chemical stability a secondary drying step is often required (Jermain *et al.*, 2018; Leuner & Dressman, 2000).

1.6.2.1.1 Solvent evaporation by spray-drying

A common organic solvent or mixture of solvents is used to prepare a solution of API and carrier where after the solution is atomised through a spray drying nozzle. Due to heating and the internal flow of an inert gas, the solvent rapidly evaporates which contributes to the amorphous state of the solid dispersion. A fine dry powder, which is the solid dispersion, can

then be collected. In order to have acceptable residual solvent levels, this powder usually undergoes further drying (Van den Mooter, 2012).

1.6.2.1.2 Solvent evaporation by conventional means

Solid dispersions are attained by evaporating a mutual solvent from an API and carrier solution (Jermain *et al.*, 2018). Temperatures in the range of 23-65°C are typically used for solvent evaporation under vacuum (Leuner & Dressman, 2000). These temperatures are generally lower than the temperatures used in the fusion/melting method which makes this a suitable technique for thermolabile APIs.

1.6.2.2 Fusion (melting) methods

A composition of API and carrier are heated above their melting point or an API could dissolve in the polymer matrix to form a homogeneous glass solution of the API and polymer (Mahieu *et al.*, 2013). This composition is then cooled in such a way as to keep the API in its amorphous state (Jermain *et al.*, 2018). When hot melt extrusion is used as up-scaled preparation for solid dispersions, thermal stability and melt viscosity need to be considered as part of the production design (Van den Mooter, 2012). Decomposition or evaporation may occur if the temperature required is too high (Leuner & Dressman, 2000). This method is appropriate for thermostable drugs and carriers (Fujii *et al.*, 2005; Leuner & Dressman, 2000).

1.6.3 Glass transition temperature

Amorphous materials are typically characterised by their glass transition temperature (T_g), this temperature is representative of a dramatic change in the molecular mobility (Lubach & Munson, 2006). When a sample is at temperatures below its T_g the sample will be brittle and is in the glassy state. Once the sample is at temperatures above its T_g it is in the rubbery state and has increased molecular mobility which dramatically increases the probability of conversion to the crystalline phase (Buckton, 2013; Zhang *et al.*, 2004). The T_g of a sample is thus known as the point where the sample moves from a glass into a rubbery state (Lubach & Munson, 2006).

During the process of glass formation, molecular motions become increasingly slower with cooling which also causes relaxation processes to slow down. When the so-called glass transition temperature T_g is reached, the system can no longer reach internal equilibrium. Therefore, due to the high viscosity, glasses become kinetically frozen and have the appearance of a solid (Van den Mooter, 2012; Sun *et al.*, 2012). When the sample is in the glassy state, there is a lack of mobility which allows the amorphous form to exist for a longer time period (Buckton, 2013). Therefore, under long term conditions amorphous solids are

proposed to have adequate kinetic stability at $T < T_g - 50^{\circ}\text{C}$ as the molecular mobility can be neglected (Qian *et al.*, 2010; Jermain *et al.*, 2018).

1.7 Conclusion

The percentage of developmental drugs with poor solubility is on the increase. As a consequence it is very important to obtain an APIs optimal solid-state form with physico-chemical properties that will achieve the best balance between solubility and stability. One of the most promising strategies to improve these characteristics is the production of amorphous solid dispersions (Vasconcelos *et al.*, 2007).

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CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction

For this study, two of the older generation drugs with poor water solubility, and a history of studies with limited success in addressing these problems (Ahire *et al.*, 2010; O'Neil, 2006) were chosen. The aim of this study was to improve the aqueous solubility and dissolution rate of the chosen drugs, sulfadoxine and nevirapine, by creating stable pharmaceutical amorphous solid dispersions (PhASDs) using a modified screening of polymers for amorphous drug stabilisation (SPADS) process.

2.2 Materials

2.2.1 Active pharmaceutical ingredients

2.2.1.1 Sulfadoxine

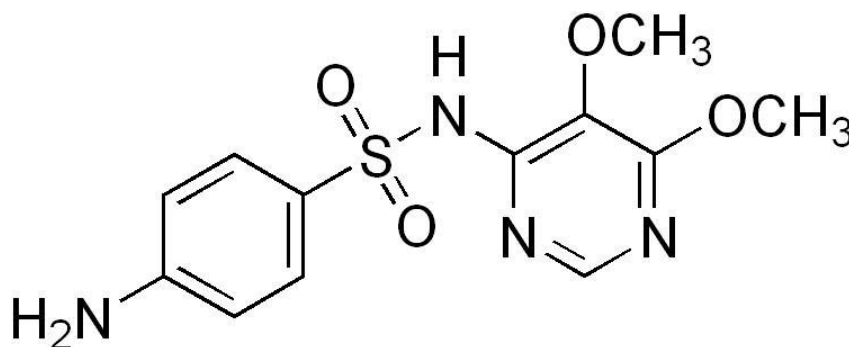


Figure 2.1: Chemical structure of sulfadoxine (O'Neil, 2006).

Sulfadoxine (Fig. 2.1) is a long-acting sulfonamide with antibacterial and antimalarial properties and presents with slow dissolution and poor solubility in water (O'Neil, 2006). According to the World Health Organization, artemisinin-based combination therapies such as artesunate, in combination with sulfadoxine-pyrimethamine are still the recommended treatment for uncomplicated *Plasmodium falciparum* malaria (WHO, 2017). In many African countries chloroquine has been replaced by the sulfadoxine-pyrimethamine combination which is also commonly used for the prophylaxis and suppression of chloroquine-resistant *Plasmodium falciparum* malaria (Odeniyi *et al.*, 2003; Minzi *et al.*, 2003).

2.2.1.1.1 Chemical and physical properties

Each API has its own characteristic chemical and physical properties. Other chemical names for sulfadoxine include the IUPAC name 4-amino-N-(5,6-dimethoxypyrimidin-4-yl)benzenesulfsulphonamide and the molecular formula $C_{12}H_{14}N_4O_4S$ (PubChem, 2018). Sulfadoxine has a molecular weight of 310.328 g/mol and has a physical appearance described as a white or creamy white crystalline powder (Kapoor, 1988). Other physical properties of sulfadoxine include a melting point of 190-194°C (O'Neil, 2006) and a glass transition temperature (T_g) of 38°C which was determined at a heating rate of 10°C per minute.

2.2.1.1.2 Solubility

Sulfadoxine has proven poor aqueous solubility which was reported by Badenhorst (2017) to be 269.12 µg/ml at a temperature of 37°C ($\pm 2^\circ\text{C}$). Sulfadoxine is slightly soluble in ethanol and methanol and practically insoluble in ether (Kapoor, 1988). According to Deck & Winston (2012) sulfonamides are inclined to be more soluble at alkaline pH than at acidic pH as sulfadoxine has an acidic nature.

2.2.1.1.3 Pharmacology

Sulfonamides are structural analogues and competitive inhibitors of para-aminobenzoic acid (PABA) and also inhibit dihydropteroate synthase which is an essential enzyme in the conversion of PABA to dihydrofolic acid (Deck & Winston, 2012). Folic acid is a vital nutrient for synthesis, repair and cell growth in *Plasmodium falciparum* (Ferone, 1977).

2.2.1.1.4 Pharmacokinetics of sulfadoxine

Sulfonamides are absorbed from the stomach and small intestine and are distributed extensively to tissues, body fluid, the placenta and foetus. The percentage protein-binding varies from 20% to above 90%. Blood levels of sulphonamides usually peak 2-6 hours after oral administration and the therapeutic concentration is 40-100 µg/ml of blood. The average half-life of sulfadoxine has been reported to be approximately 170 hours with an intermediate oral absorption.

Sulfonamides are mainly metabolised in the liver where a portion of the absorbed drug is acetylated or glucuronidated. Glomerular filtration is mainly responsible for the excretion of inactive metabolites into the urine (Deck & Winston, 2012).

2.2.1.1.5 Adverse effects

The most common adverse effects experienced with sulphonamides are fever, skin rashes, exfoliative dermatitis, photosensitivity, urticaria, nausea, vomiting and diarrhoea. Other undesirable effects include conjunctivitis, arthritis, stomatitis, hematopoietic disturbances, hepatitis, polyarteritis and psychosis, although the last two mentioned are uncommon (Deck & Winston, 2012).

2.2.1.1.6 Sulfadoxine: In conclusion

Sulfadoxine is administered in a fixed combination with pyrimethamine (sulfadoxine 500 mg and pyrimethamine 25 mg - Fansidar®). It is known that this drug is generally used for the prophylaxis and treatment of chloroquine-resistant *Plasmodium falciparum* malaria (Rosenthal, 2012; Odeniyi *et al.*, 2003). Amin & Kokwaro (2007) and Odeniyi *et al.* (2003) stated that sulfadoxine-pyrimethamine tablets have extremely poor *in vitro* dissolution profiles which is problematic as this drug will most likely fail an *in vivo* (bioavailability) test resulting in low plasma levels and possible therapeutic failure (Amin & Kokwaro, 2007).

2.2.1.2 Nevirapine

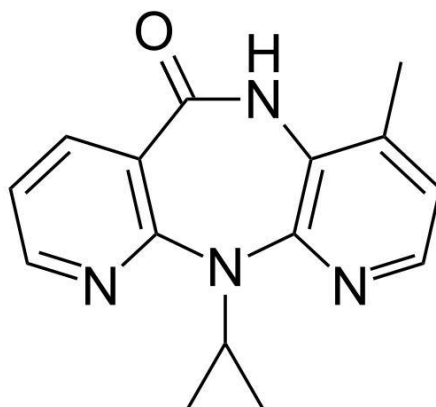


Figure 2.2: Chemical structure of nevirapine (O'Neil, 2006).

Nevirapine (Fig. 2.2) is a synthetic non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used in combination with other antiretroviral drugs to reduce the morbidity and mortality related to infection with human immunodeficiency virus (HIV-1) and subsequent acquired immune deficiency syndrome (AIDS) (Bardsley-Elliot & Perry, 2000). Nevirapine has been widely applied as single drug prophylaxis against mother-to-child HIV transmission in developing countries (Lallemant *et al.*, 2004). However, nevirapine is a BCS class II compound and this classification indicates that nevirapine has poor aqueous solubility despite of high permeation rates. These characteristics pose a challenge in reaching optimal dissolution kinetics from dosage forms (Ahire *et al.*, 2010; Chadha *et al.*, 2010).

2.2.1.2.1 Chemical and physical properties

Nevirapine is available in two commercial forms, nevirapine Form I which is the anhydrous, more stable form, and nevirapine Form II, the hemihydrate. Nevirapine possesses its own unique chemical and physical properties and has a few other familiar chemical names including its IUPAC name 11-cyclopropyl-4-methyl-5H-dipyrido[2,3-e:2',3'-f][1,4]diazepin-6-one and the molecular formula $C_{15}H_{14}N_4O$ (Form I); $C_{30}H_{30}N_8O_3$ (Form II) (PubChem, 2018). Nevirapine is a white or almost white powder (BP, 2017) and has a molecular weight of 266.304 g/mol (Form I) and 550.623 g/mol (Form II). Nevirapine has a melting point of 247-249°C (O'Neil, 2006) and an experimentally determined glass transition temperature (T_g) (heating rate 10°C per minute) of 87°C.

2.2.1.2.2 Solubility

The solubility of the two commercial forms of nevirapine (anhydrous and hemi-hydrate) was determined by Stieger *et al.* (2009) in three different solvents. The results reported for the anhydrous form of nevirapine in water, 0.1 N HCl and methanol were 9.76 mg/100 ml, 394.38 mg/100 ml and 938.81 mg/100 ml respectively. And for the hemihydrate the results were as follow, 5.82 mg/100 ml in water, 233.38 mg/100 ml in 0.1 N HCl and 939.40 mg/100 ml in methanol. All of the above mentioned solubility values were determined at a temperature of 37°C (Stieger *et al.*, 2009). Macha *et al.* (2009) reported that the solubility profile of nevirapine indicated a gradual decline in solubility with an increase in pH from 1.9 mg/ml at pH 1.5 to 0.1 mg/ml at pH 4. At a higher pH, the solubility remained steady at 0.1 mg/ml at pH 8, this can be attributed to nevirapine being a weak base.

2.2.1.2.3 Pharmacology

Nevirapine is a NNRTI which binds directly to HIV-1 reverse transcriptase. This binding leads to the allosteric inhibition of RNA- and DNA-dependent DNA polymerase activity. Resistance towards NNRTIs appear rapidly when used as monotherapy. Therefore, this drug is primarily used in combination therapy (Stieger *et al.*, 2009; Safrin, 2012).

2.2.1.2.4 Pharmacokinetics of nevirapine

Nevirapine, given in adult doses of 200 mg twice a day, is highly lipophilic with a serum half-life of 25-30 hours. It is mainly excreted in the urine after being extensively metabolised by the CYP3A isoform to hydroxylated metabolites. Nevirapine is a moderate inducer of CYP3A metabolism and should be taken into account when administered simultaneously with drugs such as amprenavir, lopinavir, indinavir, zidovudine, efavirenz and methadone as this will cause a decreased level of these drugs (Safrin, 2012).

2.2.1.2.5 Adverse effects

Within the first 4-6 weeks of therapy up to 20% of patients present with a mild and self-limiting maculopapular rash. Steven-Johnson syndrome and toxic epidermal necrolysis which are severe, life-threatening skin rashes have rarely been reported. Other known unwanted effects include: hepatitis (seldom fulminant), fever, headache, nausea and somnolence (Safrin, 2012).

2.2.1.2.6 Nevirapine: In conclusion

Nevirapine is an effective antiretroviral drug and is one of the most prescribed drugs for the treatment of HIV-1 and AIDS (Kuo & Chung, 2011). Although this drug is effective for abovementioned treatment it has been stated by Chadha *et al.* (2010) and Ahire *et al.* (2010) that the rate limiting step for absorption is attributed to the slow dissolution of the drug.

2.2.2 Polymers

All polymers which were used during this study are generally regarded as safe (GRAS) polymers which are commonly used for other purposes as well as in the final production of both food and medicine. Acceptable and safe concentrations of each polymer were adhered to in combination with each API and the quantity which is acceptable for intake was not exceeded.

2.2.2.1 Polyvinylpyrrolidone (PVP)

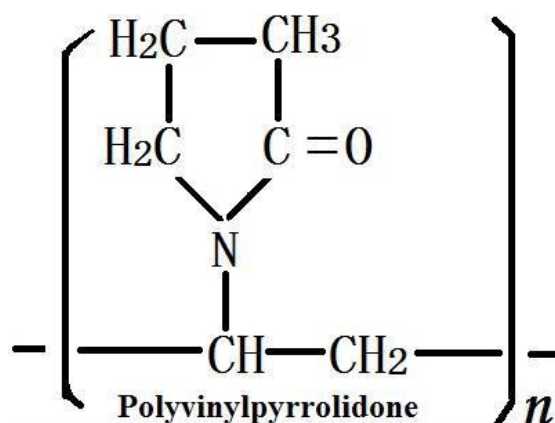


Figure 2.3: Chemical structure of polyvinylpyrrolidone (PVP) (Loraine, 2008).

Polyvinylpyrrolidone is a synthetic, water-soluble polymer which is synthesised by polymerisation of vinylpyrrolidone in water or isopropanol (Guo *et al.*, 1998; Kadajji & Betageri, 2011). This polymerisation leads to PVP with molecular weights ranging from 2500 to

3 000 000 (Leuner & Dressmann, 2000). Different grades of PVP are available which are based on the molecular weight (MW) thereof. PVP has various properties which are valuable in the pharmaceutical industry. PVP is essentially used during tablet formulations where it serves as a binder, although PVP is also known to increase the dissolution of the active ingredient (Kadajji & Betageri, 2011). The high MW of PVPs prevents absorption from the GI tract therefore, when given orally, they are regarded as non-toxic (Leuner & Dressmann, 2000).

The glass transition temperature of PVPs is generally high, this makes them of limited use in the preparation of solid dispersions by means of the hot-melt method. Preparation of solid dispersions by the solvent method is more appropriate due to their good solubility in an extensive selection of organic solvents. PVPs can also improve the wettability of the dispersed compound because of their good water solubility (Leuner & Dressmann, 2000). Chain length has a major influence on the dissolution rate of the dispersed drug as the aqueous solubility of PVP becomes poorer with increasing chain length.

Polyvinylpyrrolidone-vinylacetate (PVP-VA) is a copolymer belonging to the polyvinyl group and has been used to improve the solubility of many drugs with poor water solubility, particularly by means of hot-melt extrusion (HME) (Kadajji & Betageri, 2011).

2.2.2.2 Hydroxypropyl methylcellulose (HPMC)

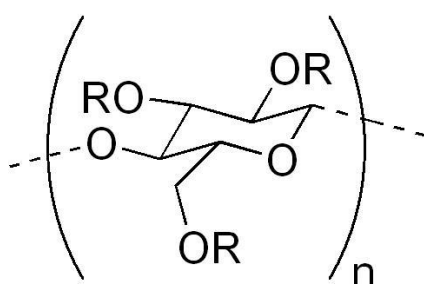


Figure 2.4: Chemical structure of hydroxypropyl methylcellulose (Anon, 2018a).

HPMC is a semisynthetic derivative of cellulose which, by appropriate alkylation, is derivatised to form hydroxypropyl methylcellulose (HPMC) (Lee *et al.*, 1999; Leuner & Dressmann, 2000). HPMCs can range in molecular weight from about 10000 to 1 500 000 and are soluble in water as well as in mixtures of ethanol with dichloromethane and methanol with dichloromethane (Leuner & Dressmann, 2000). The viscosity and extent of substitution of HPMCs differ which, together with the biocompatibility, contribute to the extensive use thereof in the

pharmaceutical, cosmetic and food industry. Pharmaceutical formulations where HPMCs are used include: oral products, film-coating, tablet-binder, emulsifying- and stabilising agent, tablet disintegrants and as a controlled release matrix, which is the main use. The release of water-soluble drugs can be delayed through the use of a high-viscosity grade HPMC (Guo *et al.*, 1998).

2.2.2.3 Hydroxypropyl methylcellulose acetate succinate (HPMCAS)

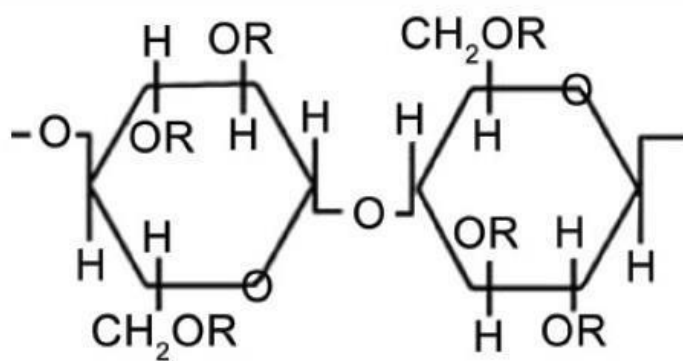


Figure 2.5: Chemical structure of hydroxypropyl methylcellulose acetate succinate (Sarode *et al.*, 2014).

HPMC is functionalised with a combination of mono succinic acid and acetic acid esters to form hydroxypropyl methylcellulose acetate succinate (HPMCAS). The ratio of succinyl and acetyl substituents on the HPMC backbone is used to differentiate between the three commercial granular grades which are currently available. HPMCAS, also known as hypromellose acetate succinate, is soluble in a wide variety of organic solvents which makes it an excellent candidate for the production of PhASDs. PhASDs with HPMCAS are frequently prepared through spray-drying, resulting in supersaturated levels of the drug compound in solution (Grasman, 2012; Anon, 2018). The flexibility in the substitution levels of acetate and succinate are advantages for solubility enhancement with a successive increase in bioavailability and material processing. HPMCAS is capable of maintaining stable solid dispersions and inhibits API crystallisation. All the above mentioned properties make HPMCAS worthy of consideration for PhASD production (Anon, 2018).

HPMCAS may be used with many different APIs due to its solubility in a wide range of organic solvents such as, methanol, acetone, ethanol/water (4:1) and more. It is incompatible with strong acids, bases and strong oxidising agents. A broad processing window for the invention of APIs is available due to the stability of HPMCAS under high temperatures. The degradation onset temperature of HPMCAS was reported as 200°C. Several toxicological studies were performed with HPMCAS in animals and no adverse effects were observed (Anon, 2018).

2.2.2.4 Polyethylene glycol (PEG)

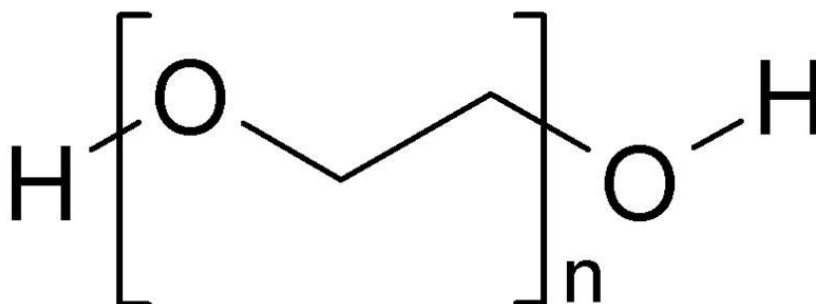


Figure 2.6: Chemical structure of polyethylene glycol (PEG) (Anon., 2018b).

PEGs are polymers which are synthesised by the interaction of ethylene oxide with water, ethylene glycol, or ethylene glycol oligomers (Kadajji & Betageri, 2011). PEGs have a ranging molecular weight (MW) of 200-300 000. The viscosity of PEGs increase as their MW increases. PEGs with molecular weights of 1500-20000 can be used for the manufacture of solid dispersions and solutions. Although, PEGs with a MW of 4000-6000 are more frequently used for the manufacture of solid dispersions, the reason for this is that the solubility in this range is still very high and hygroscopicity is not a concern (Leuner & Dressman, 2000). A great advantage of PEGs, especially for the development of solid dispersions, is their high solubility in organic solvents (Leuner & Dressman, 2000; Kadajji & Betageri, 2011). Another benefit of PEGs for the production of PhASDs by the melting method, is the relatively low melting point which lies under 65°C in every case (Leuner & Dressman, 2000). Due to acceptable water solubility and low intrinsic toxicity, PEG is suitable for biological applications as well. PEG has many more advantageous properties, such as enhancing compound wettability as well as solubility of hydrophobic drugs or carriers (due to the high hydrophilic nature thereof) (Leuner & Dressman, 2000; Kadajji & Betageri, 2011). The dissolution rate of a fairly soluble drug can also be improved by formulating it as a solid dispersion in PEG 6000, this was proven by Asker & Whitworth (1975) with acetylsalicylic acid. Improvement of the physical and chemical stability of drugs as well as prevention of aggregation of the drugs *in vivo* and during storage are also benefits of PEG. Although PEG has many positive characteristics there are a few negatives as well. PEGs with low molecular weight are prone to show slightly greater toxicity than those of higher MW. PEGs are approved for many purposes as excipients due to the limited concerns associated with toxicity. Stability problems during manufacture by the hot-melt method have also been observed with PEG (Leuner & Dressman, 2000).

2.2.3 Solvents

Solvents used during this study were ethanol (99.9%) and water. Ethanol was chosen as solvent for its solubilising properties as all of the APIs and polymers used during this study were soluble in ethanol, except HPMCAS. When this polymer was used, a mixture of ethanol and distilled water in a ratio of 4:1 was used. Ethanol presents with low toxicity and viscosity, the latter mentioned made it an excellent choice of solvent for spray-drying purposes as it can be fully spray-dried under mild process conditions (inlet temperature $T_{in} = 130^{\circ}\text{C}$) (Saß & Lee, 2014).

2.3 Methods

Figure 2.7 is a flow diagram indicating the optimal and economic process which streamlines polymer selection and PhASD production to minimise time and resources spent in a well-equipped academic laboratory setting such as the Pharmatech labs.

The general method is known as SPADS (screening of polymers for amorphous drug stabilisation) (Narayan *et al.*, 2015) and enables a researcher to find the correct combination and ratios of polymer and API. This method is designed to eliminate polymer candidates which are unsuitable for stabilising a specific API in PhASD form. The process was modified for greatest efficiency in the Pharmatech laboratories. In the end, only the best polymer and ratio (for each drug in this study) which remained after all the assessments, was deemed suitable for PhASD product formulation (Stieger & Liebenberg, 2017).

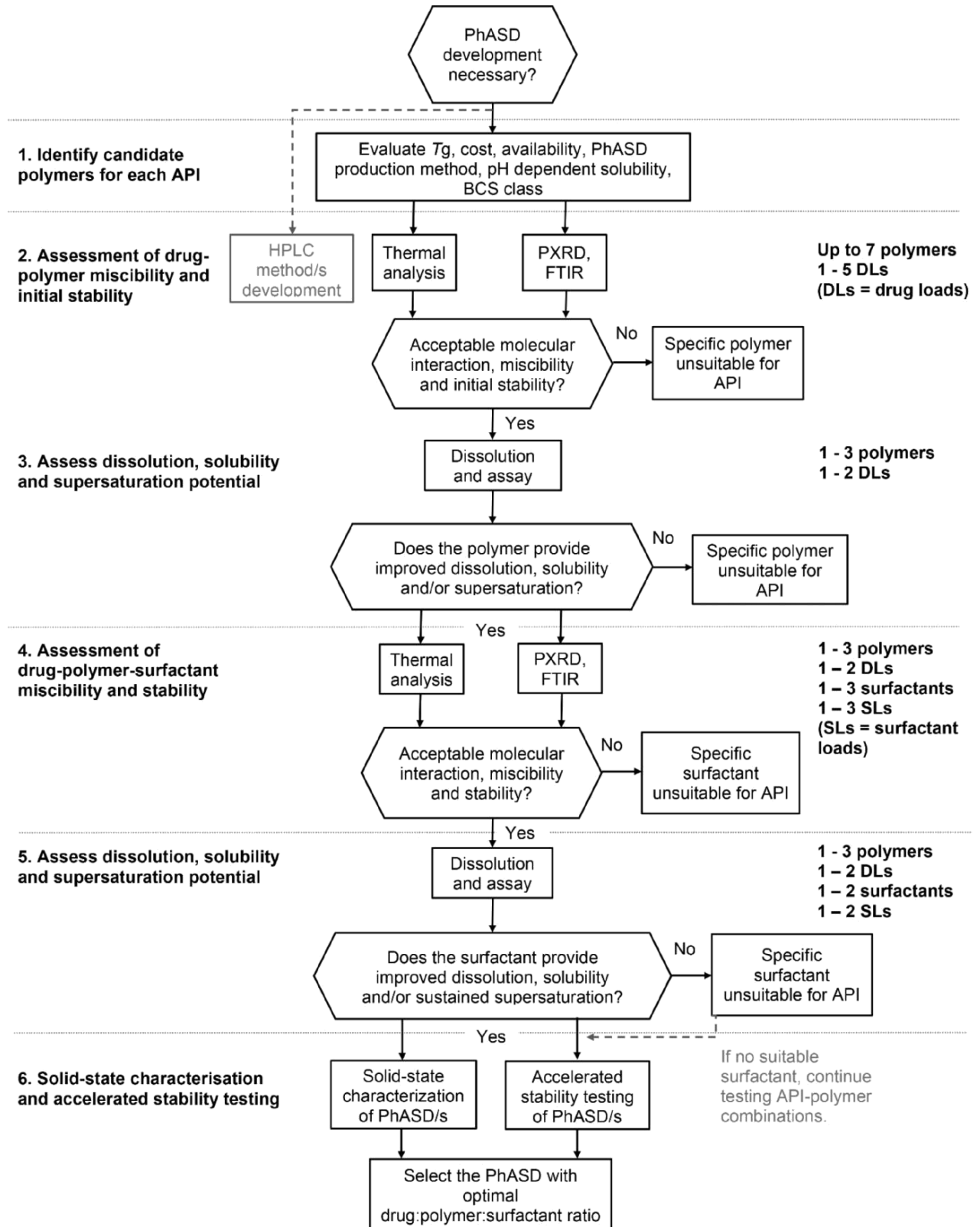


Figure 2.7: Modified SPADS process (Stieger & Liebenberg, 2017).

2.3.1 Simultaneous thermal analysis (STA:TGA/DSC)

A STA simultaneously measures the mass loss (TGA) and heat flow (DSC) of a sample during the heating process. With this combined technique, information about the mass loss, melting point, glass transition, solid-state transformation(s), loss of solvents and degradation of a sample can be obtained (Brown, 2001).

A Mettler DTG 3⁺ (Mettler Toledo, Greifensee, Switzerland) was used to record the DSC and TGA thermograms during this study. Powder samples, weighing approximately 3 - 5 mg was placed in aluminium crimp cells, open or sealed (100 µl) and heated to an end temperature dependent on the melting point of the API and/or polymer, at a heating rate of 10°C/min, with a nitrogen gas flow of 35 ml/min. The samples were then cooled and subjected to the same thermal program once more. This indicated whether a glass was formed, if a T_g could be observed and whether crystallisation occurred upon heating.

2.3.2 Differential scanning calorimetry (DSC)

A Mettler DSC 3 Star System (Mettler Toledo, Greifensee, Switzerland) was used for the DSC analyses. Approximately 3 - 5 mg of each sample was placed in an aluminium pan and hermetically sealed with an aluminium lid. Samples were scanned at a heating rate of 10°C/min from 30°C to the specific melting point of the API. The purge gas was nitrogen, and it had a flow rate of 35 ml/min for the DSC analyses.

2.3.3 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) scans a sample with a focused beam of electrons to produce an image of that sample. It is used to examine the morphology, crystal habit and surface topography of a sample (Bernstein, 2002).

An FEI Quanta 200 FEG SEM with an X-Max 20 EDS system (FEI, USA) was used to obtain micrographs of the various crystal and amorphous forms. In preparation, samples were adhered to a small piece of carbon tape, mounted onto a metal stub and coated with a gold-palladium film (Eiko Engineering ion Coater IB-2, Japan).

2.3.4 X-ray powder diffraction (XRPD)

X-ray powder diffraction (XRPD) is a useful technique to obtain a unique diffraction pattern of a specific crystal structure of an API which acts as a “fingerprint”. A diffractogram can be used to differentiate and identify different solid-state forms of the same API (Bhattacharya *et al.*, 2009).

X-ray powder diffraction patterns were obtained using a PANalytical Empyrean diffractometer (Malvern Panalytical, Almelo, Netherlands). The measurement conditions were: target, Cu;

voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; antiscatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/min (step size, 0.025°; step time, 1.0 sec).

2.3.5 Nano Spray Dryer B-90

This method involved spray drying of nevirapine from a 2% (m/v) solution in ethanol. The spray-dryer used was a Büchi (Flawil, Switzerland) B-90 Nano Spray-Dryer equipped with a B-295 Inert Loop and supplied with an inert atmosphere in the form of nitrogen gas. Spray-drying parameters were as follows: 3 µm spray nozzle; inlet temperature = 92°C; pump speed = 3; and gas flow = 100 l/min. The resulting amorphous nevirapine was collected and stored in a vacuum desiccator at ambient temperature until further testing.

2.3.6 High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) is a technique that is used to quantify and identify individual components present in a sample (Hassan, 2012). In HPLC, an elevated pressure produced by pump causes the mobile phase to move through a column. The column, packed with very small porous particles, is the stationary phase (Moldoveanu & David, 2013).

For the HPLC analysis of this study a Shimadzu (Kyoto, Japan) UFLC chromatographic system was used. The system consists of a SIL-20AC auto-sampler fitted with a sample temperature controller, a UV/VIS photodiode array detector (SPD-M20A) and an LC-20AD solvent delivery module. The mobile phase was degassed and filtered prior to use. The mobile phase and column used was dependent of the API. Official monograph methods were followed.

For the HPLC analysis of sulfadoxine an official monograph found in The International Pharmacopoeia (Ph.Int., 2017) was used. A Venusil XBP C₁₈₍₂₎ 250 x 4.6 mm 5 µg stainless steel column was used as the stationary phase, and the mobile phase was prepared in accordance with the above mentioned assay and used at a flow rate of 2 ml/min. An ultraviolet spectrophotometer set at a wavelength of 227 nm and an isocratic elution method was used to analyse all the samples. The injection volume of each sample was 20 µl. A linear regression of $r^2=0.9961$ was obtained with this validated HPLC method.

An official monograph stipulated in the USP (2017) was used for the HPLC analysis of nevirapine. A LC Luna C₁₈₍₂₎ 150 x 4.6 mm 5 µg stainless steel column was used as the stationary phase. A mobile phase consisting of 23 parts dehydrated alcohol (96% EtOH) and 77 parts of ultra-pure water was used at a flow rate of 1 ml/min. All of the samples were analysed using an isocratic elution method at a wavelength of 214 nm. The injection volume for each sample was 20 µl. This validated HPLC method had a linear regression of $r^2=0.9991$.

2.3.7 Stability testing

2.3.7.1 Temperature and humidity

The effect of increased temperature and humidity was determined by storing approximately 500 mg of each selected PhASD in a Petri dish. The samples were distributed evenly on the surface of Petri dishes and 3 samples of each PhASD were prepared. The samples were stored in a climatic chamber (Binder, Germany) at 40°C / 75 % RH for a period of 3 months. A sufficient amount of sample was removed at 0, 1, 2 and 3 month intervals. The samples were assayed to determine purity and possible degradation, by means of HPLC analysis. HPLC, DSC, TGA, and XRPD analyses were performed to determine if any solid-state change occurred during storage of the samples.

2.3.8 Solubility studies

The solubility of a drug determines not only its availability for absorption in the gastrointestinal tract, but also the formulation possibilities that exist for the manufacturing of dosage forms.

An excess of powder was weighed and placed into amber test tubes with screw cap closures. R.O. water (Merck Millipore, Wadeville, South Africa) was added to each of the test tubes and placed in a water bath at 37°C ± 2°C, fixed to a submerged rotating axis (54 rpm) for a sufficient period of time (to be determined experimentally). The subsequent solutions were then filtered through a 0.45 µm PVDF filter and diluted, after which HPLC analyses were performed on the filtrates according to the specifications of the HPLC.

2.3.9 Powder dissolution studies

The dissolution rate of a drug has an important influence on bioavailability and bioequivalence (Byrn *et al.*, 1999). Amorphous drugs or multi-component drug systems exhibit an initial apparent solubility that is much greater than that of the crystalline drug. However, concentration over time will decrease as the amorphous form crystallises to the stable and less soluble form. Dissolution studies yield information pertaining to the apparent solubility acquired in a particular medium at a certain temperature, but also shows how long the solubility advantage can be maintained (Stieger *et al.*, 2017).

A VanKel700 (Varian, Palo Alto, USA) dissolution bath was used for dissolution testing. USP apparatus 2 (paddle) was set up at 37°C ± 2°C with a rotational speed of 75 rpm, 900 mL dissolution media (R.O. water) was added to each dissolution vessel. A powder mass which was determined experimentally (SULF = 300 mg and NEV = 200 mg) was weighed into 10 mL test tubes, to which the half of this experimentally determined mass glass beads, ≤ 106 µm (Sigma-Aldrich, South Africa) were added. 5 mL of dissolution medium maintained at 37°C ±

2°C was added to each test tube. The mixtures were agitated for a period of 60 seconds, using a vortex mixer. The resulting mixtures were then transferred to each dissolution vessel. 2 mL of solution was withdrawn from each dissolution vessel at predetermined time intervals.

The dissolution medium was not replaced after each withdrawal since a supersaturated solution is required to observe solution-mediated transformations. After withdrawal, the samples were filtered through a 0.45 µm PVDF filter into an HPLC vial or UV cuvette. The filtered solutions were analysed by HPLC.

2.4 Conclusion

The modified SPADS method (Fig. 2.7) was followed to eventually identify the most promising PhASD candidate, with the listed APIs and polymers, presenting with enhanced solubility and stability. All of the methods mentioned were used in order to achieve this. Results will be discussed in the following two chapters.

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CHAPTER 3

THE DEVELOPMENT OF SULFADOXINE PHARMACEUTICAL AMORPHOUS SOLID DISPERSIONS

3.1 Introduction

The solid-state characteristics and physico-chemical properties of drugs are essential benchmarks for drug development in the pharmaceutical industry (Lin, 2015). A huge concern is that approximately 90% of drugs currently under development are poorly soluble (Jermain *et al.*, 2018). As previously mentioned in Chapter 2, Section 2.2.1.1.2, sulfadoxine (SULF) is an example of a drug with poor aqueous solubility. In this chapter, the outcomes of following a modified SPADS process, in order to find the best SULF PhASD, are presented.

3.2 Basic screening - Identify candidate polymers for sulfadoxine

3.2.1 Glass transition temperature (T_g)

A general proposal, with the storage temperature (usually ambient) being less than $T_g - 50^\circ\text{C}$, has been provided as a method to predict whether a PhASD will be stable under long-term conditions. This general rule of thumb assumes that if the combined T_g is greater than 50°C above storage temperatures, the molecular mobility in the glassy state will be suppressed sufficiently to provide long-term stability (Narayan *et al.*, 2015). Table 3.1 was drawn up after determining the theoretical resulting glass transition temperature for each API and polymer combination (Formula 3.1) in mass ratios ranging from 1:1 – 1:4, assuming each were to produce a PhASD. The mass units x and y in Formula 3.1 may have any practical unit of mass as long as it is the same for both API and polymer. The mass ratio of 1:4 API:polymer was not exceeded, as doing so would result in impractically large or numerous tablets or capsules.

Formula 3.1

$$\text{Resulting theoretical } T_g = \left(\frac{x}{x+y} \cdot T_g(\text{API}) \right) + \left(\frac{y}{x+y} \cdot T_g(\text{polymer}) \right)$$

Where:

x = Ratio/mass of API in the mixture

y = Ratio/mass of polymer in the mixture

$T_g(\text{API})$ = Glass transition temperature of the API

$T_g(\text{polymer})$ = Glass transition temperature of the polymer

Only the results conforming to the T_g -50°C “rule of thumb” are included in Table 3.1. This served as an initial screening process in order to determine which polymers and mixtures could, theoretically speaking, be potential candidates to undergo further investigation.

Table 3.1: Resulting theoretical T_g values when combining sulfadoxine ($T_g = 38^\circ\text{C}$) with various polymers in different ratios

API and Polymer	Ratio	Theoretical T_g ($^\circ\text{C}$)
SULF:HPMC	1:1	106.5
SULF:HPMC	1:2	129.3
SULF:HPMC	1:3	140.8
SULF:HPMC	1:4	147.6
SULF:HPMC	2:1	83.7
SULF:HPMCAS	1:2	88.0
SULF:HPMCAS	1:3	94.3
SULF:HPMCAS	1:4	98.0
SULF:PVP 25	1:1	99.0
SULF:PVP 25	1:2	119.3
SULF:PVP 25	1:3	129.5
SULF:PVP 25	1:4	135.6
SULF:PVP 30	1:1	106.5
SULF:PVP 30	1:2	129.3
SULF:PVP 30	1:3	140.8
SULF:PVP 30	1:4	147.6
SULF:PVP 30	2:1	83.7
SULF:PVP 90	1:1	114.0
SULF:PVP 90	1:2	139.3
SULF:PVP 90	1:3	152.0
SULF:PVP 90	1:4	159.6
SULF:PVP 90	2:1	88.7
SULF:PVP/VA 64	1:3	89.0
SULF:PVP/VA 64	1:4	92.4

3.3 Solid-state characterisation of PhASD

3.3.1 Thermal analysis

3.3.1.1 Miscibility screening utilising simultaneous thermal analysis (STA)

After theoretically determining the T_g , experimental STA (DSC) runs were performed in order to establish whether a glass was formed, if a T_g could be observed (and at what temperature) and whether crystallisation occurred upon reheating. This was done in accordance with the method described in Section 2.3.1.

The three combinations and ratios which are highlighted in Table 3.1 proved to be the most successful after initial thermal screening as the creation of pharmaceutical amorphous solid dispersions appeared to have been accomplished. Only these mixtures underwent further testing.

Binary systems are usually regarded as miscible when just one glass transition temperature is observed. However, according to Marsac *et al.* (2009) the presence of a single glass transition temperature is not always a certain or reliable indicator of miscibility.

The figures below are the thermogram components (DSC) of STA analysis of the mixtures which proved to be most successful during the first experimental screening process.

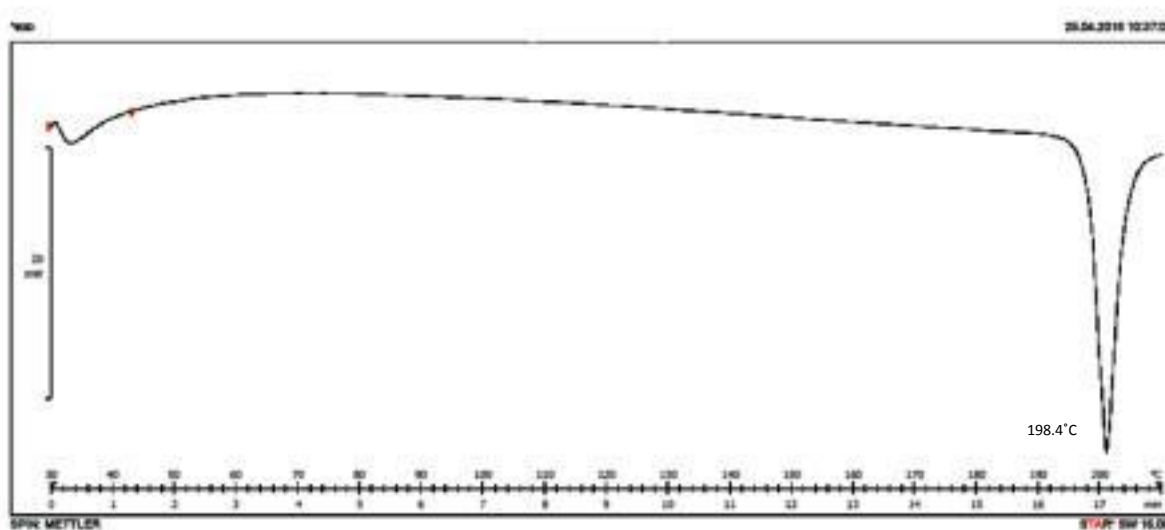


Figure 3.1: STA thermogram of SULF raw material with a peak melting temperature of 198.4°C.

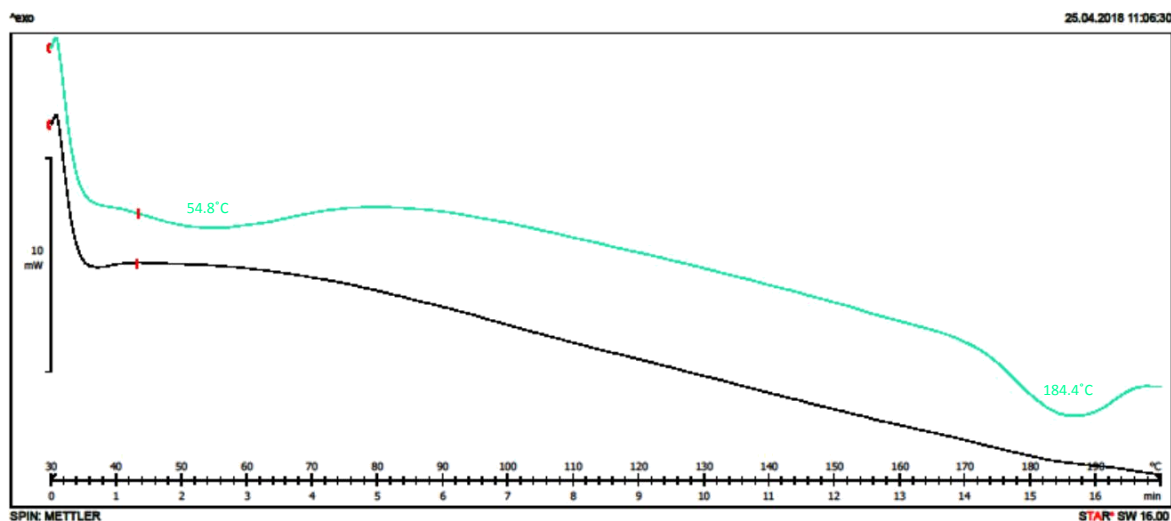


Figure 3.2: STA thermogram of SULF:PVP 25 1:1 physical mixture, displaying two endothermic events of the binary mixture (green). Then a second run with the same sample showed no melting points suggesting a miscible system was formed (black).

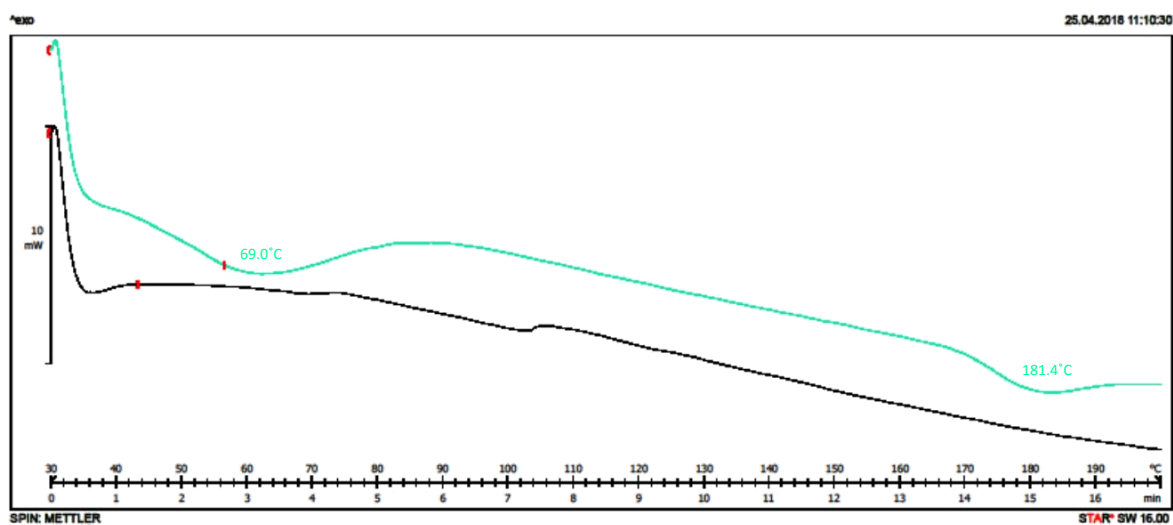


Figure 3.3: STA thermogram of SULF:PVP 25 1:2 physical mixture, displaying two endothermic events of the binary mixture (green). Then a second run with the same sample showed no melting points suggesting a miscible system was formed. A glass transition temperature was observed at 105°C.

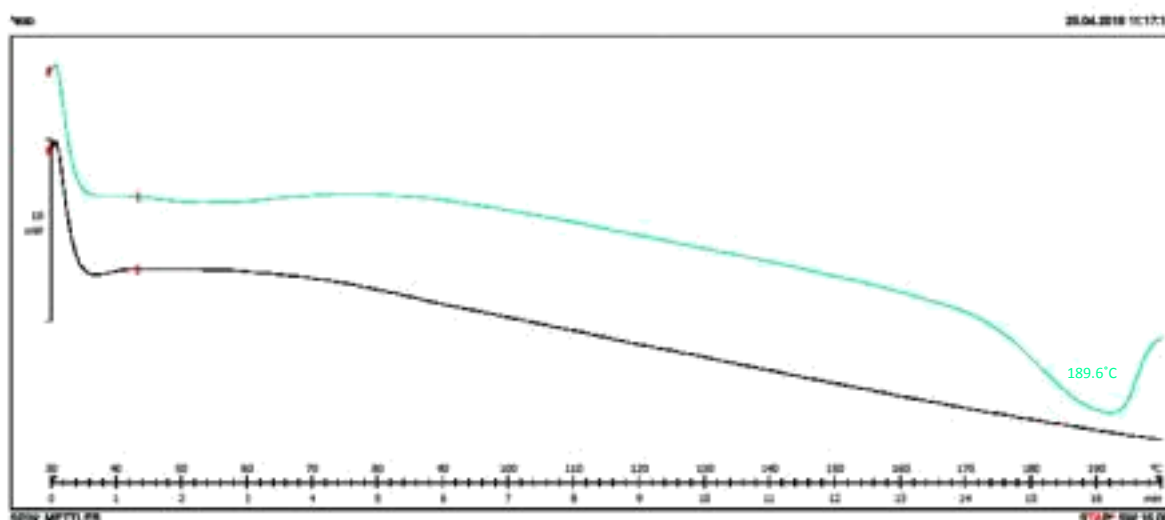


Figure 3.4: STA thermogram of SULF:PVP 30 2:1 binary physical mixture, displaying only one endothermic event (green). Then a second run with the same sample showed no melting points suggesting a miscible system was formed (black).

Figures 3.2 – 3.3 show that two separate endotherms were present during the first STA run of the mixtures containing PVP 25, *i.e.* the melting point of both the polymer and SULF could be distinguished (The STA of the mixture containing PVP 30 displayed only one observable endothermic event). The second run of each suggests that the system was miscible, since the two endothermic events of the physical mixture disappeared. Normally for a system to be miscible, a single glass transition temperature will be observed. For this study, miscibility screening was done on an STA system (simultaneous DSC and TGA), which is not always sensitive enough to detect a small glass transition. At the time of pre-screening, only an STA was available, but a sensitive dedicated DSC was acquired prior to characterisation of the PhASDs produced at laboratory scale (Section 3.5.2, to follow).

3.4 PhASD production methods

Various methods were investigated for the production of a SULF PhASD to find the one most suitable and acceptable. A hot-melt method was performed on laboratory scale by physically mixing the API and polymer with a mortar and pestle and placing it on a hot plate at a temperature of approximately 190°C (as the melting point of SULF is 190°C-194°C). This method was very challenging and ultimately unsuccessful, due to the uneven melting and discolouration of the powder as seen in Figure 3.5b-c. Hot melt extrusion techniques are rapidly growing in the pharmaceutical industry as it has several advantages over traditional processing techniques including less processing steps, absence of solvents, continuous operations as well as the possibility of forming a solid dispersion with improved solubility. This is another possible technique which could be investigated for SULF. During hot-melt extrusion

raw materials are pumped with a rotating screw under elevated temperatures through a die into a product of uniform shape. An API is often combined with a plasticiser, usually a polymer with a low melting viscosity, during this process. Good efficiency, stability, permanence and good compatibility are important properties for plasticisers used in the preparation of pharmaceutical dosage forms. Stability problems using plasticisers during hot-melt extrusion have been reported, especially when highly volatile plasticisers were used therefore the success of this method for SULF will remain unknown and will require a complete and thorough study (Crowley *et al.*, 2008).

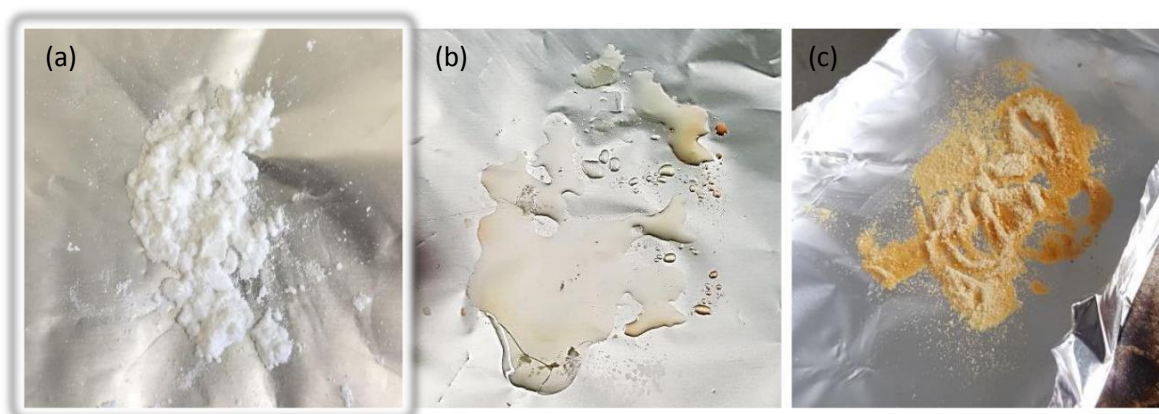


Figure 3.5: (a) Pure crystalline SULF raw material (b) Pure amorphous SULF prepared on a hot plate at approximately 190°C (c) SULF:PVP 25 1:4 physical mixture after being heated on a hot plate to approximately 190°C.

Another method which was investigated is “rapid solvent evaporation” which was performed using a rotary evaporator. The API and polymer were accurately weighed to the exact mass needed for each mixture. This mixture of API and polymer was then dissolved in 99.9% ethanol and evaporated using a rotary evaporator after which it was left to dry in an oven at 60°C. This method was successful as the product in each case showed no degradation. The clear amorphous products were ground using mortar and pestle prior to further analysis.

3.5 Characterisation of the PhASDs

3.5.1 Morphology

The SEM images as seen in Figures 3.6 and 3.7 were generated as described in Chapter 2, Section 2.3.3.

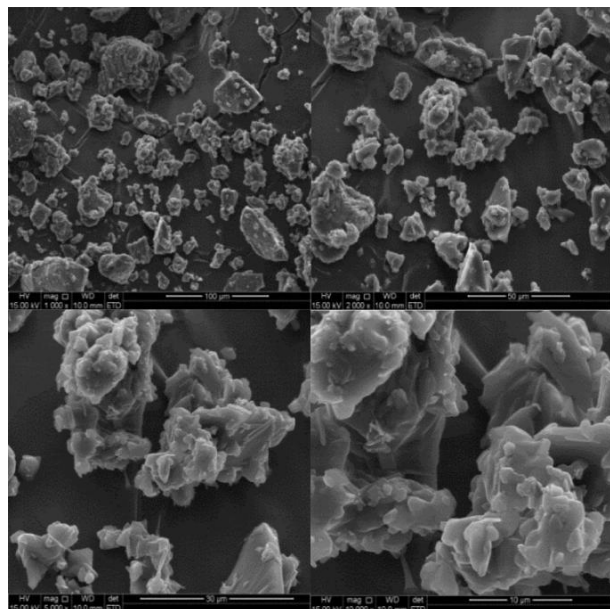


Figure 3.6: SEM images of crystalline SULF raw material magnified x1000, x2000, x5000 and x10000.

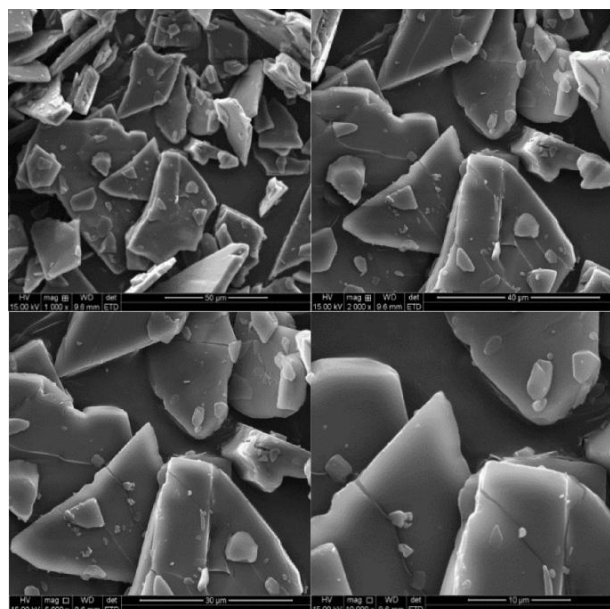


Figure 3.7: SEM images of SULF:PVP 25 1:2 PhASD, prepared by means of rapid solvent evaporation, magnified x1000, x2000, x5000 and x10000.

3.5.2 Differential scanning calorimetry (DSC)

DSC experimental runs, being more sensitive than STA, were performed in order to determine whether pharmaceutical amorphous solid dispersions did indeed form.

The melting endotherm of SULF raw material (black) is shown in Figure 3.8, as is the second run of SULF:PVP 1:1 physical mixture (blue). The remaining two thermograms of SULF:PVP 25, in ratios 1:1 (green) and 1:2 (purple), show that solvent removal *via* rotary evaporator appears to be a successful method for producing PhASDs of these combinations. No melting endotherms were measured, despite DSC being much more sensitive than STA. The amorphous nature of these dispersions will be clarified with XRPD.

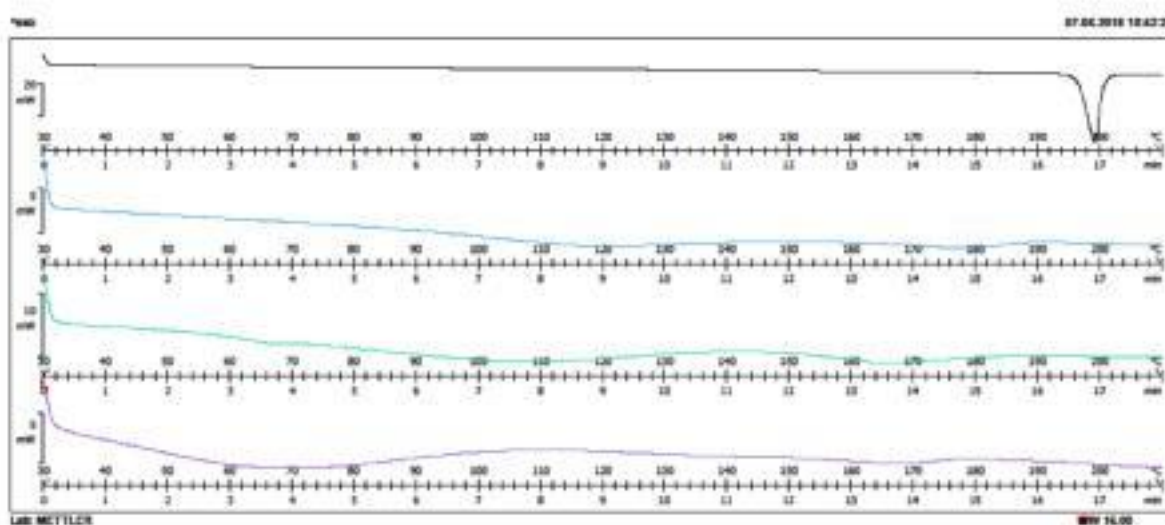


Figure 3.8: DSC overlay of SULF raw material (black), STA melt of SULF:PVP 25 1:1 physical mixture (blue), SULF:PVP 25 1:1 solvent evaporation (green) and SULF:PVP 25 1:2 solvent evaporation (purple).

The DSC thermogram for SULF:PVP 30 2:1 (Fig. 3.9) indicates two melting endotherms for the product obtained from rotary evaporation. Although miscibility screening, performed on a very small sample with an STA showed a miscible system, this DSC thermogram of product obtained from rapid solvent evaporation is clearly that of a binary system. This could be due to the system not being truly miscible, the method of production not being suitable or the DSC being more sensitive than the STA. The first option is probably the most likely explanation.

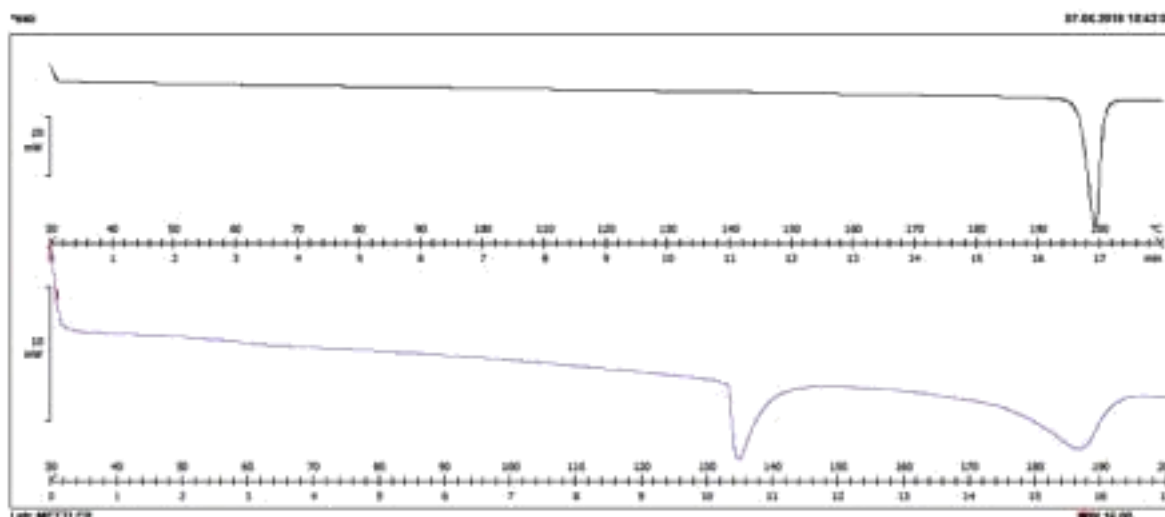


Figure 3.9: DSC overlay of SULF raw material (black) and SULF:PVP 30 2:1 solvent evaporation (purple).

3.5.3 X-ray powder diffraction (XRPD)

The XRPD diffractograms were obtained in accordance with the method, as described in Section 2.3.4. From these patterns one can clearly see that SULF raw material, with the greatest peak intensities, is the most crystalline. The diffractograms (Fig. 3.10b-c) obtained from the SULF:PVP 25 1:1 mixtures (physical mixture co-grinding and solvent evaporation) both proved to be mainly crystalline. On the other hand, SULF:PVP 25 1:2 prepared by the solvent evaporation method confirmed to be completely amorphous (Fig 3.10d).

The diffractogram (Fig. 3.11b) of the SULF:PVP 30 2:1 mixture prepared through solvent evaporation is crystalline confirming the DSC results (Fig. 3.9).

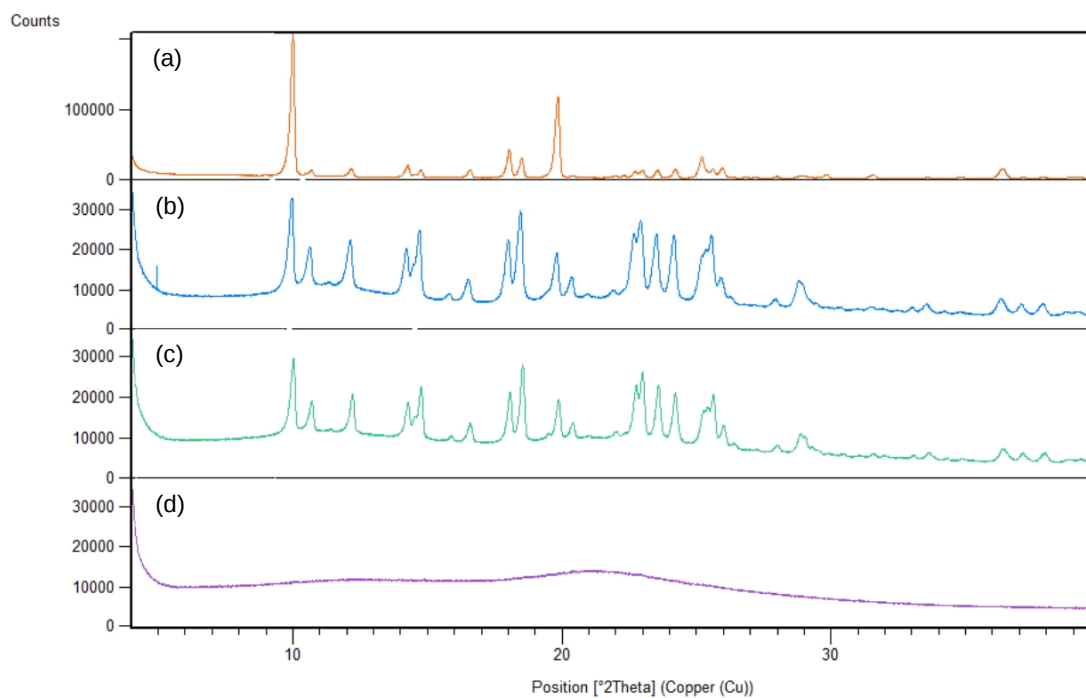


Figure 3.10: XRPD of (a) SULF raw material, (b) SULF:PVP 25 1:1 physical mixture, (c) SULF:PVP 25 1:1 solvent evaporation and (d) SULF:PVP 25 1:2 solvent evaporation.

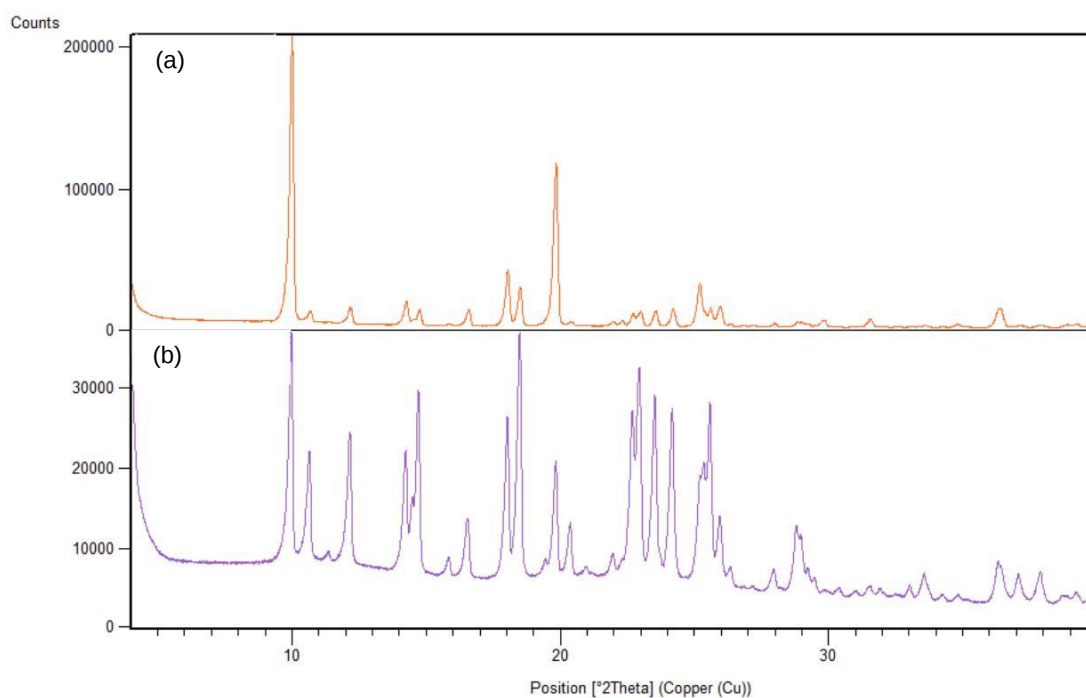


Figure 3.11: XRPD of (a) SULF raw material and (b) SULF:PVP 30 2:1 solvent evaporation.

According to the XRPD results, only the SULF:PVP 25 1:2 solid dispersion seemed to be amorphous.

3.6 Assess dissolution and solubility potential

3.6.1 Validation of the HPLC analytical method

The purpose of the validation process is to ensure that the analytical method used (as mentioned in Section 2.3.6) was both reliable and sensitive in the determination of the amount API found in the samples (Fig.3.12).

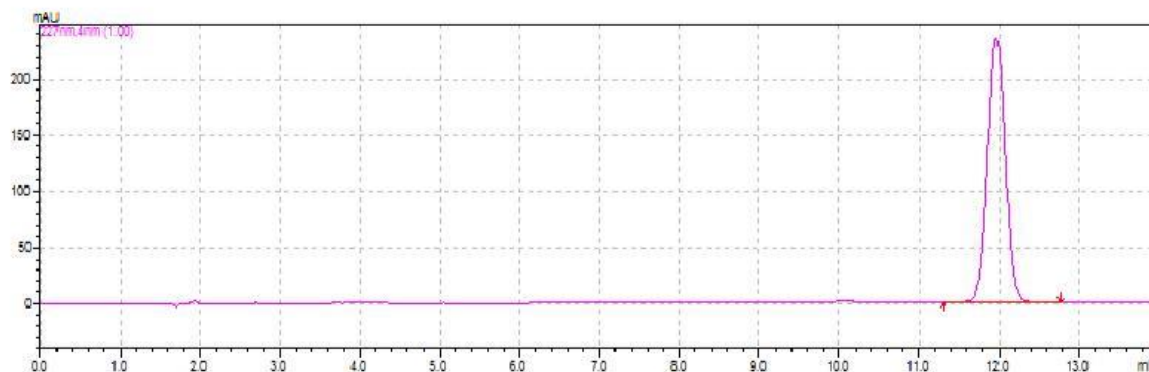


Figure 3.12: HPLC chromatogram of SULF.

3.6.1.1 Linearity

The ability of an analytical method to obtain results that are directly proportional to the concentration of analyte in the sample is known as linearity. It is essential that the linearity determined be across the entire range of the analytical procedure (Dong, 2006). A regression coefficient (r^2) of 0.99, obtained from the data, proves linearity.

Linear regression analysis was performed by injecting four different concentrations: 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$, 500 $\mu\text{g/ml}$ and 1000 $\mu\text{g/ml}$ of API in duplicate into the HPLC. The linearity of SULF was determined by performing linear regression analysis on the plot of the peak area ratios, versus concentration ($\mu\text{g/ml}$). A regression coefficient of 0.9961 was obtained, thus linearity was verified (Fig. 3.13).

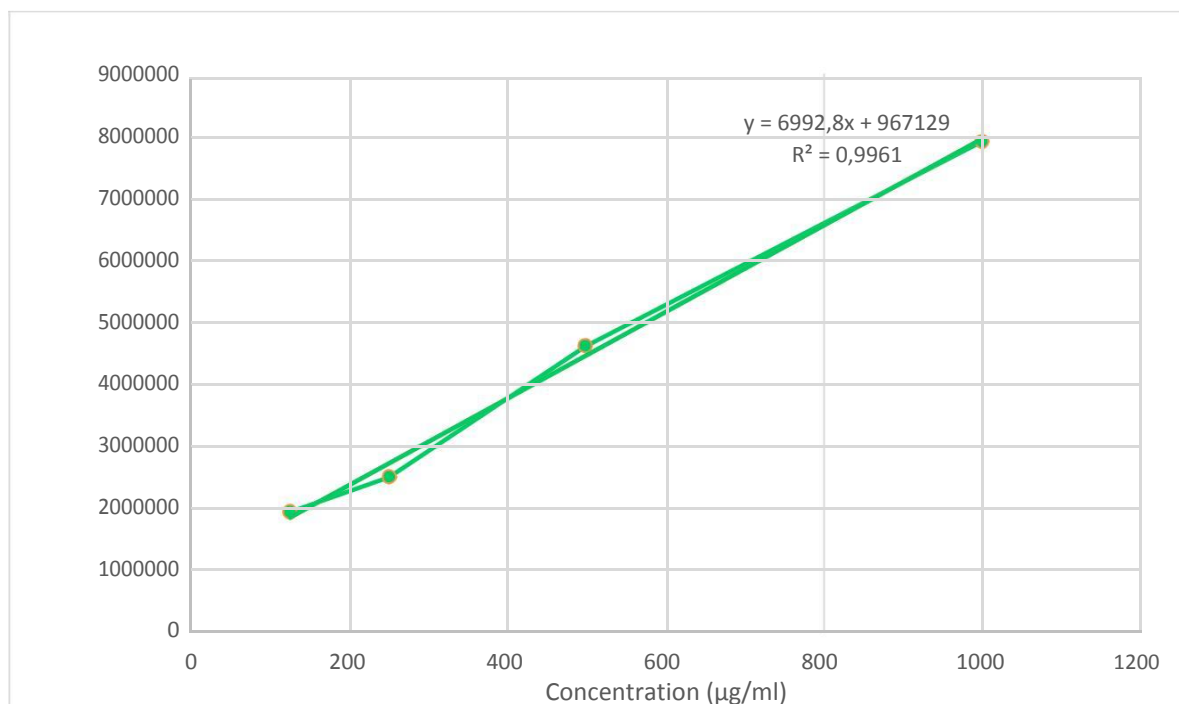


Figure 3.13: Linear regression curve of SULF.

3.6.2 Dissolution and assay

Dissolution studies were performed on the raw material, as well as the mixtures which proved to be stable and miscible after the above mentioned screening experiments. The dissolution profiles below clearly state the poor dissolution and solubility of SULF. After five minutes the dissolution value of this raw material was 147.44 µg/ml (44.23%) and after a period of three hours the maximum dissolution was a mere 213.52 µg/ml (64.06%).

Dissolution results with PVP 25 dispersions

The figures below (Fig. 3.14 - 3.15) prove that the mixtures with the selected polymers definitely improved the dissolution of the SULF raw material. According to the DSC and XRPD results, the only amorphous solid dispersion was the SULF:PVP 25 1:2 combination prepared by the solvent evaporation method. This was the most successful combination when comparing the dissolution results as well.

This combination of SULF:PVP 25 1:2 prepared by solvent evaporation had a dissolution value of 214.39 µg/ml (64.32%) after five minutes and after three hours a percentage of 76.1 (255.28 µg/ml) (Fig. 3.14).

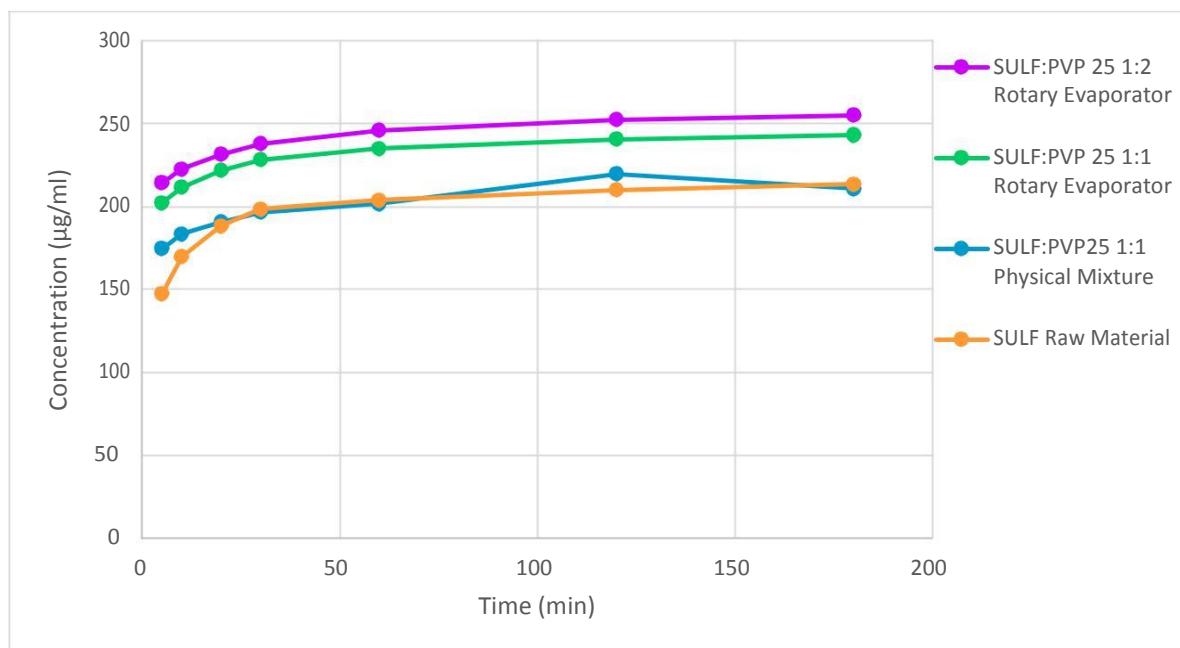


Figure 3.14: Dissolution of SULF raw material and various combinations of SULF:PVP 25.

Dissolution results with PVP 30 dispersion

Even though the SULF:PVP 30 2:1 mixture prepared by means of solvent evaporation, showed to be crystalline, a slight improvement in dissolution values were observed. The increase in dissolution value was however not significant (Fig. 3.15).

The dissolution results of both PhASDs were evaluated with Moore and Flanner's equation (Moore & Flanner, 1996) as discussed below (Formula 3.2).

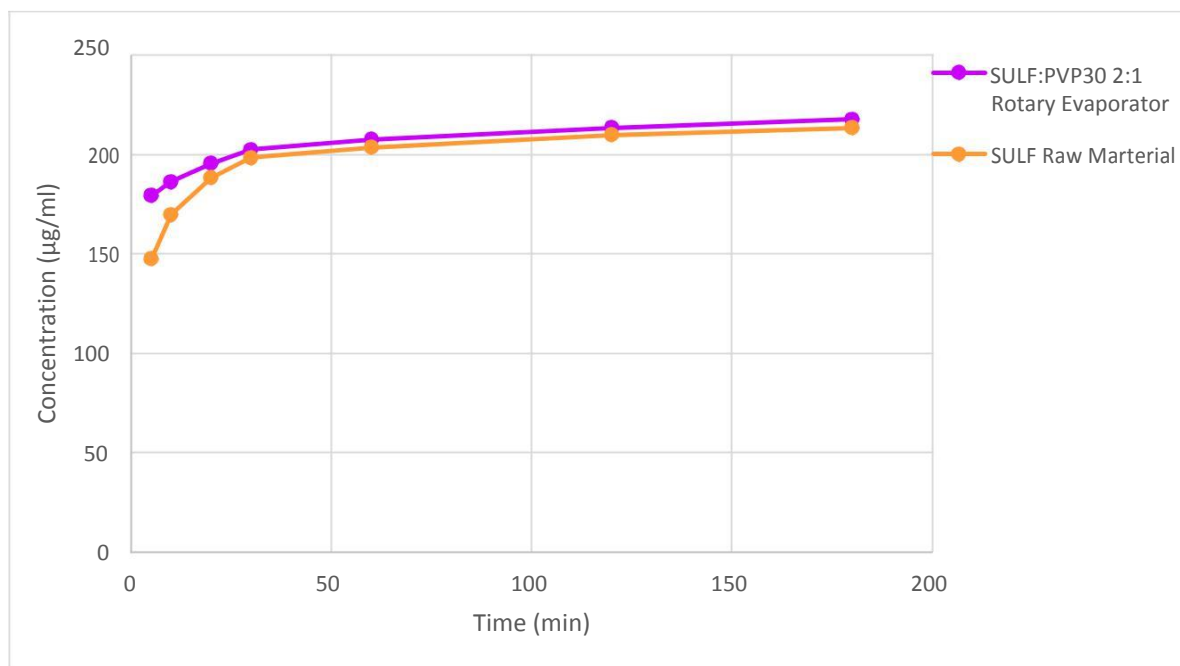


Figure 3.15: Dissolution of SULF raw material comparing with SULF:PVP 30 2:1 prepared by solvent evaporation.

Formula 3.2: Moore and Flanner's equation (Moore & Flanner, 1996).

$$f_2 = 50 \log \left[1 + \frac{1}{n} \sum_{t=1}^n \left(\frac{wt}{Rt} - \frac{Tt}{Rt} \right)^2 \right]^{0.5}$$

Where:

- n = Number of dissolution time points.
- Rt = Reference dissolution value, at time t.
- Tt = Test dissolution value, at time t.
- wt = Optional weighing factor.

The value of f2 will be 100 when the test and reference mean profiles are identical. A clear acceptance criterion for profile similarity has been established as $f_2 \geq 50$ (Diaz *et al.*, 2016). Therefore, any value below 50 is indicative of non-similarity, and in this study was the anticipated outcome to ensure improvement in dissolution.

Table 3.2: Similarity values as determined with Moore and Flanner's similarity equation

SULF and PVP Mixtures	Similarity factor (f2)
SULF:PVP 25 1:1 Physical mixture	71.01
SULF:PVP 25 1:1 Rotary evaporator	47.59
SULF:PVP 25 1:2 Rotary evaporator (PhASD)	42.07
SULF:PVP 30 2:1 Rotary evaporator	67.96

The similarity factor was determined using Formula 3.2 and as indicated in Table 3.2 the similarity factor for the pharmaceutical amorphous solid dispersion (SULF:PVP 25 1:2 rotary evaporator and SULF:PVP 25 1:1 rotary evaporator) was below 50, indicative of a non-similarity between the dissolution values. Which in this case is a positive finding, meaning that the dissolution profiles of the PVP 25 dispersions prepared with rotary evaporator were indeed better than that of the SULF raw material.

The profile similarity value of the SULF:PVP 25 1:1 physical mixture was above 50, indicative of similarity.

The similarity factor for the SULF:PVP 30 2:1 mixture was 67.96 which is according to the equation similar to the raw material, which corresponds with Figure 3.15.

After the dissolution studies, XRPD analysis was performed on the SULF:PVP 25 1:2 dissolution residues in order to determine whether recrystallisation occurred. When comparing Figure 3.16 to Figure 3.10d it is clear that crystallisation did occur during the dissolution process. This can be attributed to the presence of moisture, as illustrated in Chapter 1 (Fig. 1.2), i.e. moisture causes a decrease in the energy barrier for recrystallisation to occur.

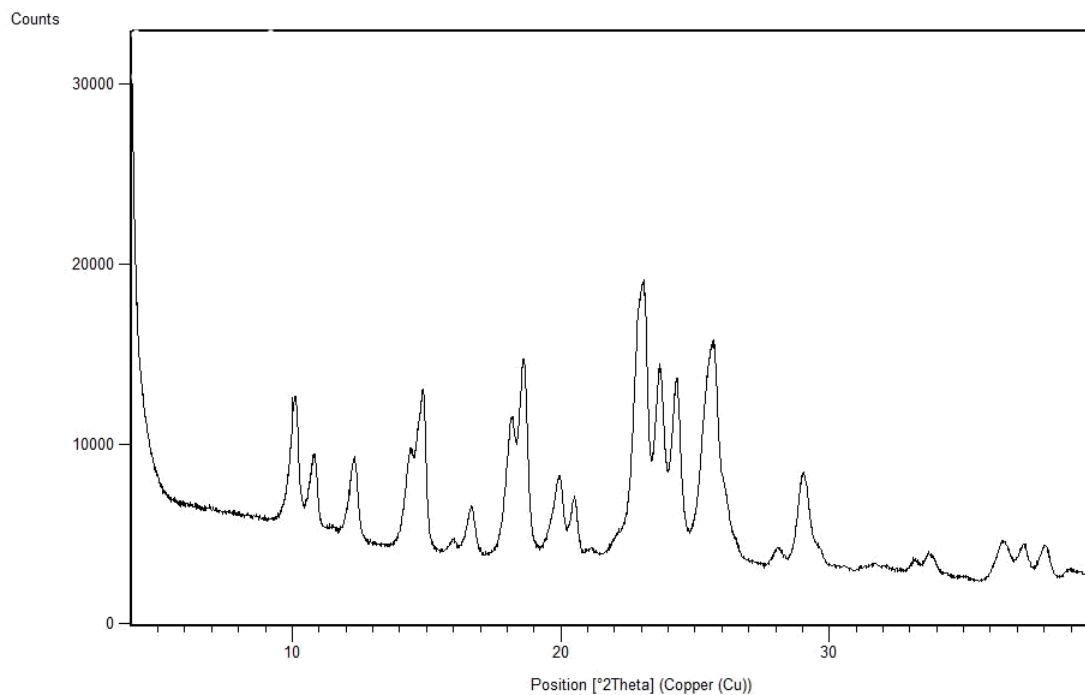


Figure 3.16: XRPD of the SULF:PVP 25 1:2 dissolution residues.

3.6.3 Solubility

The solubility of SULF was tested over a period of 24 hours in as stated in 2.3.8. The solubility of each of the six samples is given in the table below after being calculated by use of the equation as shown in Figure 3.13 in 3.6.1.1: $y = 6992.8x + 967129$.

Table 3.3: Solubility data for SULF in R.O water

Sample	Solubility (µg/ml)
1	202.0142646
2	156.8981934
3	177.7365719
4	170.5025075
5	153.7030583
6	130.8642704
Average	165.29

Sulfadoxine is extremely hydrophobic. Therefore, the variable results obtained with testing of sulfadoxine solubility in water (table 3.3), according to described methods, can most likely be attributed to aggregation of the powder particles. Another possibility or contributing factor could be that the 24 hour test period was insufficient for equilibrium to be attained. The average solubility of SULF was determined and a value of 165.29 µg/ml was obtained. This value does not correspond with the value (269.12 µg/ml) published by Badenhorst (2017), as previously mentioned in Chapter 2. According to O'Neil (2006) SULF is very slightly soluble in water. This term was defined by Anon. (2018) that 1000 to 10000 ml of solvent is needed to dissolve 1 g of solute. The value determined correlates with the information stated in the Merck index, SULF is very slightly soluble in water (O'Neil, 2006). When using the abovementioned value as a guideline, approximately 6050 ml of water will be needed to dissolve 1 g of SULF which falls in the range of 1000 - 10000 ml.

3.7 Accelerated stability testing of PhASD

Photos of each sample were taken monthly for a period of three months of which the samples were exposed to 40°C and 75% RH in order to determine whether there was any difference in the physical appearance of the samples. As one can clearly see, no significant colour change occurred, which could be indicative of no pronounced degradation. After month 3, only a slight colour change was observed for all the test samples.

Table 3.4: Images of samples exposed to three month stability test

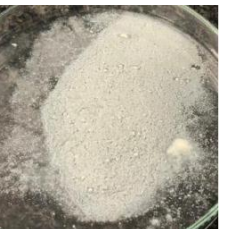
Sample	Initial	Month 1	Month 2	Month 3
SULF raw material				
SULF:PVP 25 1:2 Prepared by solvent evaporation method				

Table 3.5 was drawn to indicate the concentration of the API which was still present in each sample after exposed to temperature and humidity. The concentration of SULF raw material stability results remained stable, varying mainly between 97.35% and 100.59%.

There is more variation in the assay values of the PhASD (SULF:PVP 25 1:2), which could be expected. The dispersion is a binary mixture and the concentration can therefore vary throughout the dispersion and with each sample. This could have an impact on the assay value of the initial assay.

Table 3.5 HPLC assay results of SULF over the stability test period

	Initial	Month 1	Month 2	Month 3
SULF raw material	98.54	97.35	*	100.59
SULF:PVP 25 1:2	89.00	102.50	*	102.68

*Instrument failure

Together with the other experiments, STA analysis were also performed on the samples subjected to the stability test in order to determine whether the exposure to both heat and humidity would affect the stability of the API and whether recrystallisation of the amorphous SULF:PVP 25 mixture occurred. The STA was used because it can measure melting events as well as weight loss simultaneously. From Figure 3.17 it is clear that the API remained stable when exposed to such drastic temperatures and humidity conditions. The mixture SULF:PVP 25 also proved to be stable (Fig. 3.18), as no melting temperature of the API is visible on the DSC graph which proves that the mixture remained amorphous.

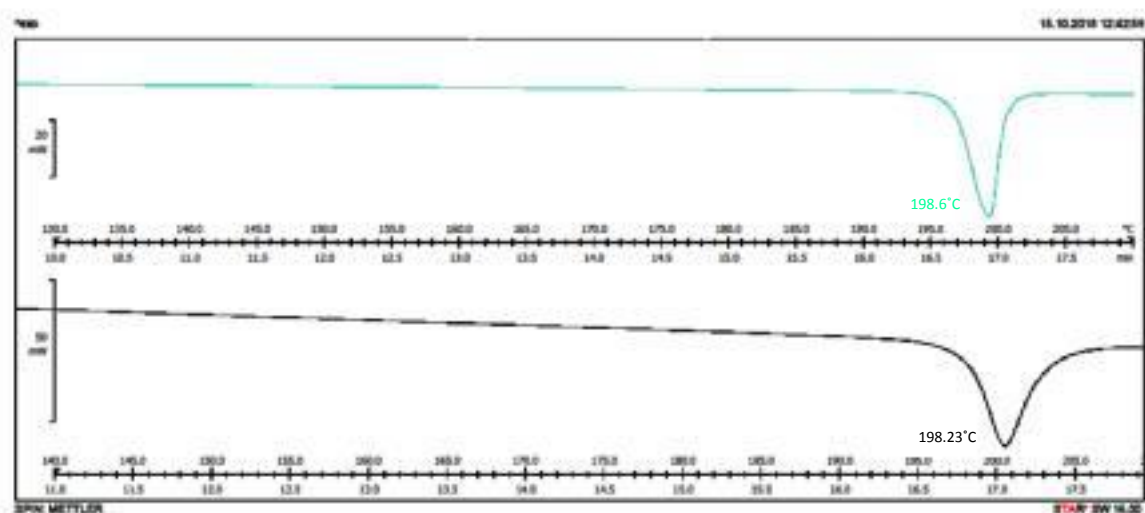


Figure 3.17: STA overlay of SULF raw material at month 0 (green) and 3 (black) after stability testing.

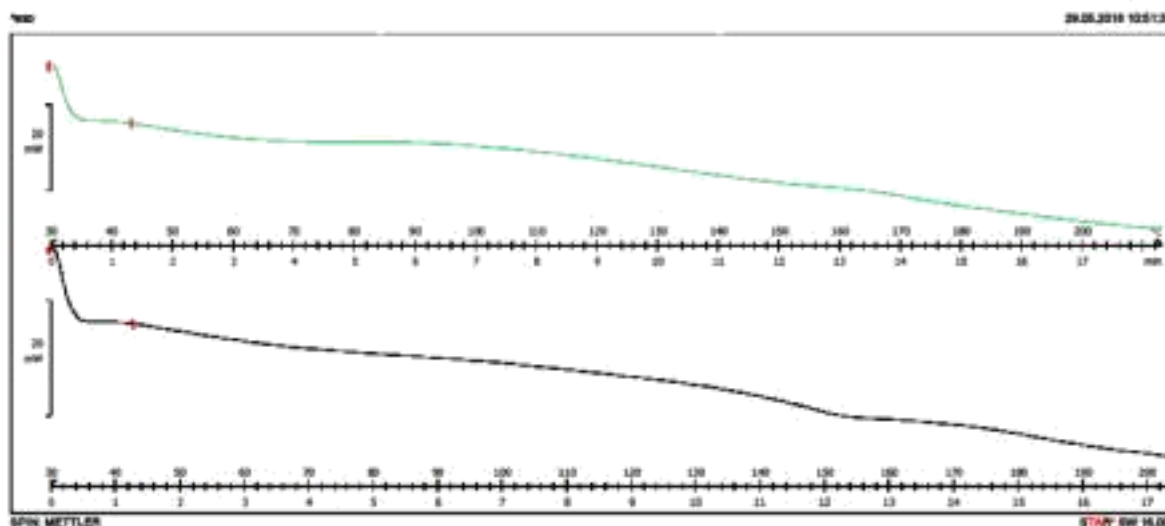


Figure 3.18: STA overlay of SULF:PVP 25 1:2 PhASD at month 0 (green) and 3 (black) after stability testing.

DTG measurements were conducted to determine whether the samples exposed to the three month stability test, SULF raw material and SULF:PVP 25 1:2, had adsorbed or lost any moisture. The moisture content determined on the samples of the SULF raw material was not indicative of a significant amount of adsorbed moisture, month two, however showed a higher percentage moisture adsorbed. The moisture did not induce any solid-state transformations in the SULF raw material as shown in the X-ray powder diffractograms (Fig. 3.19).

The PhASD SULF:PVP 25 1:2 indicated much more variety in the percentage. The moisture content of the initial sample was measured as 6.98% probably due to moisture in the PVP 25. Month 1 and 2 displayed a slight increase in moisture measurements, then at month 3, it dropped below 6%.

Fitzpatrick *et al.* (2002) stated that PVP changes from a glass to a rubbery state when exposed to elevated temperature and humidity and that moisture uptake depresses the glass transition temperature. The change from the glass to rubbery state could be responsible for the weight loss experienced at month 3 of the stability studies. Fitzpatrick *et al.* (2002) also stated that on exposure to 80% RH, PVP will absorb approximately 40% moisture. In this solid dispersion with sulfadoxine and PVP 25 the PhASD lost some moisture after month 3 in comparison to the initial value. When comparing the moisture content of the mixture to the initial value, month 1 and 2 showed an increase where after month 3 indicated a rather significant decrease.

Table 3.6: Moisture content measured by means of TGA

Sample	Initial	Month 1	Month 2	Month 3
SULF raw material	0.3275%	0.2459%	1.5769%	0.8070%
SULF:PVP 25 1:2 PhASD	6.9783%	8.3927%	9.9261%	5.7182%

Once again, XRPD analysis were performed on the samples exposed to the stability test to confirm, whether the samples remained in their original state or if the heat and humidity lead to a change in the physico-chemical properties of the mixture. As expected, the SULF raw material remained stable and crystalline.

Despite thermal analysis and initial XRPD characterisation of SULF:PVP 25 1:2 having indicated that the product was a PhASD (Fig. 3.10), XRPD analysis of the batch produced for stability testing revealed a largely amorphous product with slight crystallinity evident as tiny peaks on the diffractogram (Fig. 3.20). These results illustrate the difficulties encountered regarding batch reproducibility and up-scaling using this production method. At month 0 (Fig. 3.20) this batch was largely amorphous, but displayed tiny peaks on the XRPD pattern indicative of a small crystalline component. After one month of exposure to heat and humidity clear crystalline peaks appeared. The solid dispersion became more crystalline after a month (Fig. 3.20), which could be attributed to the moisture absorbed serving as plasticiser and allowing the small crystallites initially present to grow. From month 1 to 3, the intensity of the XRPD peak signals did not increase. The intensity counts of the diffractograms of month 1, 2 and 3 appeared identical. The individual samples were not prepared any differently at the consecutive intervals, nor were they ground further after initial placement into the stability chamber. XRPD is primarily a qualitative method of analysis, but an increase in overall peak intensities may be interpreted as an increase in crystallinity (barring other sample manipulation). The observed XRPD peak intensities of the patterns of samples taken at 1, 2 and 3 months were unchanged. Also, there was pronounced peak broadening indicative of very small individual crystal size. We conclude that the polymer present in the SD prevented further crystal growth.

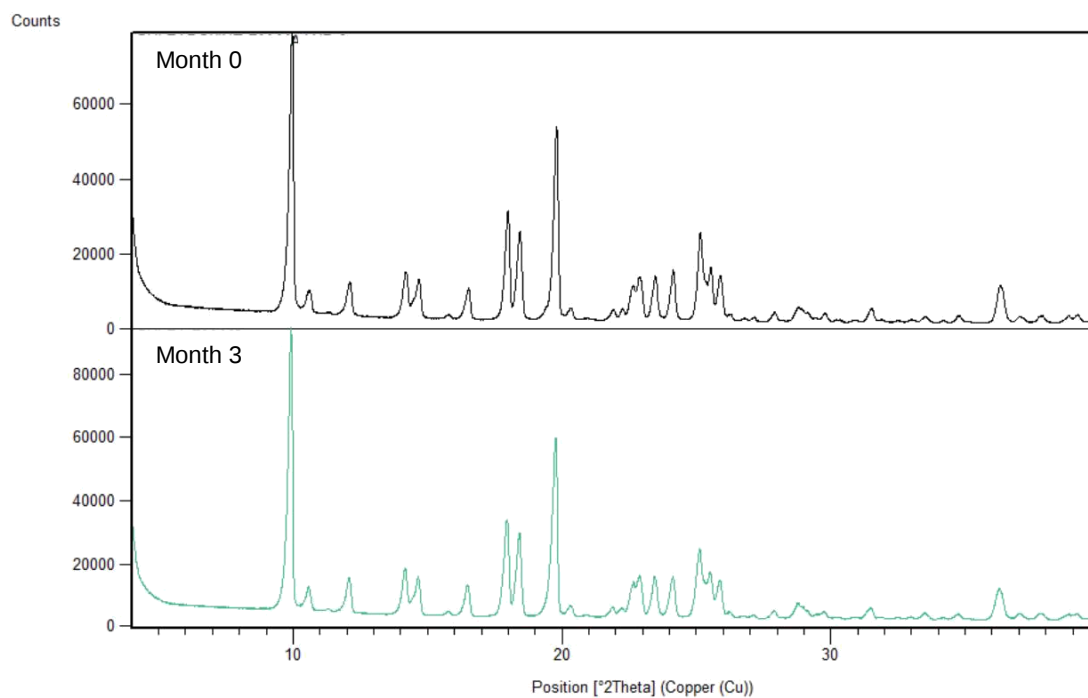


Figure 3.19: XRPD overlay of SULF raw material after three months stability testing, month 0 (black) month 3 (green).

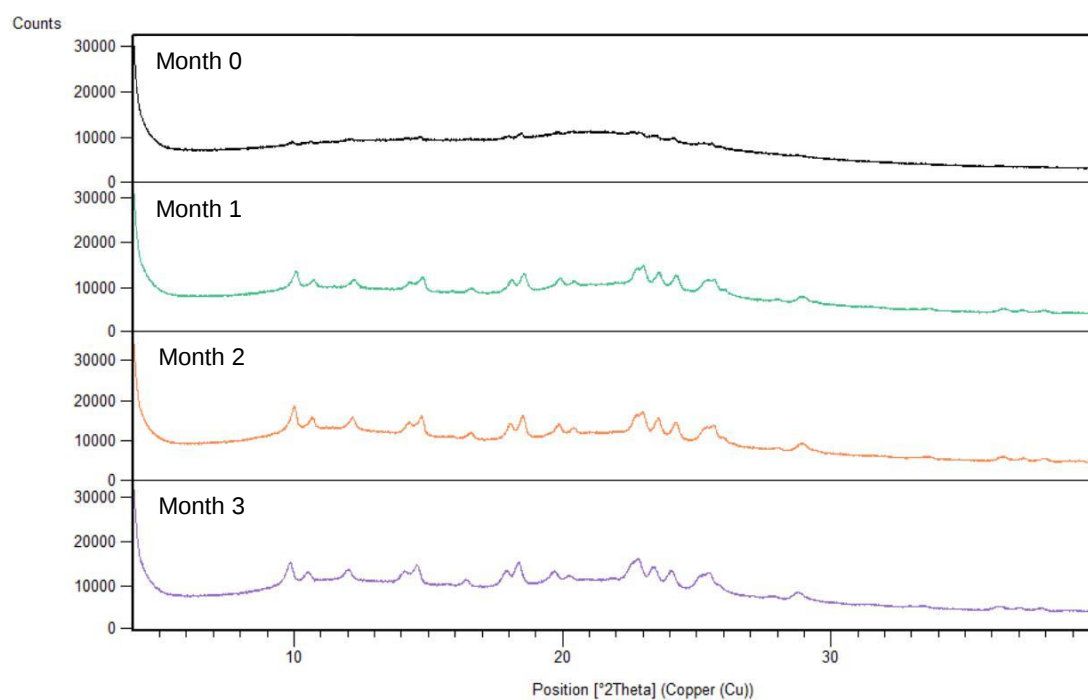


Figure 3.20: XRPD overlay of PhASD SULF:PVP 25 1:2 after three months stability testing, month 0 (black), month 1 (green), month 2 (orange) and month 3 (purple).

3.8 Conclusion

After following the modified SPADS process in order to find the most stable PhASD with acceptable physico-chemical properties, it was found that SULF:PVP 25 in a ratio of 1:2 was the most successful candidate having been prepared by small scale rapid solvent evaporation. Up-scaling of production yielded a solid dispersion (SD) with a small crystalline component prone to limited crystal growth. The SD, SULF:PVP 25 1:2 delivered a dissolution value of 214.39 µg/ml which is an improvement of 66.95 µg/ml within the first five minutes of dissolution testing when comparing to the SULF raw material (147.44 µg/ml). Improvement in dissolution is likely a result of smaller crystalline particles, as well as the solubilising effect of the polymer. Extreme stability testing resulted in limited growth of the crystalline component of the SD during the first month. In this case, SPADS failed to produce an ASD that may be up-scaled using the best method as identified for lab-scale production. Further research into alternative production methods (like spray drying), broader polymer screening, multi-polymer systems and the possible addition of a surfactant might yield an improved product. That said, the SD as is, is more soluble than sulfadoxine alone and appears to have limited crystal growth under extreme test conditions.

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CHAPTER 4

THE DEVELOPMENT OF NEVIRAPINE PHARMACEUTICAL AMORPHOUS SOLID DISPERSIONS

4.1 Introduction

Nevirapine Form I is poorly soluble (0.976 mg/ml) in water at neutral pH, as previously mentioned in chapter 2, but absorbs well from the gastrointestinal tract. It is therefore a BCS Class II drug. Consequently, the absorption of this model drug is dissolution rate limited. As nevirapine is unable to form salts, various methods have been investigated for the improvement of solubility.

As with sulfadoxine (chapter 3), the modified SPADS process was adhered to in order to find the best composition of a PhASD with nevirapine as active ingredient. A solid dispersion refers to a mixture with at least two components, a polymer and typically a hydrophobic drug. Normally a pharmaceutical amorphous solid dispersion (PhASD) will form which was the main goal in this study, but it is possible that the formation of nanocrystalline solid dispersions (NCSDs), as well as eutectic mixtures to name a few, can occur (Dhirenda *et al.*, 2009).

4.2 Basic screening – Identify candidate polymers for nevirapine

4.2.1 Glass transition temperature (T_g)

Formula 3.1 was used to determine the theoretical glass transition temperature for each combination of nevirapine:polymer in ratios varying from 1:1 – 1:4. Once again, only the combinations adhering to the T_g -50°C “rule of thumb” as mentioned in chapter 3 – Section 3.2.1 were included in table 4.1.

An abbreviation of NEV will be used for nevirapine.

Table 4.1: Resulting T_g when combining NEV ($T_g = 87^\circ\text{C}$) with various polymers in different ratios

API-Polymer	Ratio	Resulting T_g ($^\circ\text{C}$)
NEV:HPMC	1:1	131.0
NEV:HPMC	1:2	145.7
NEV:HPMC	1:3	153.0
NEV:HPMC	1:4	157.4
NEV:HPMC	4:1	104.6
NEV:HPMC	3:1	109.0
NEV:HPMC	2:1	116.3
NEV:HPMCAS	1:1	100.0
NEV:HPMCAS	1:2	104.3
NEV:HPMCAS	1:3	106.5
NEV:HPMCAS	1:4	107.8
NEV:HPMCAS	4:1	92.2
NEV:HPMCAS	3:1	93.5
NEV:HPMCAS	2:1	95.7
NEV:PVP 25	1:1	123.5
NEV:PVP 25	1:2	135.7
NEV:PVP 25	1:3	141.8
NEV:PVP 25	1:4	145.4
NEV:PVP 25	4:1	101.6
NEV:PVP 25	3:1	105.3
NEV:PVP 25	2:1	111.3
NEV:PVP 30	1:1	131.0
NEV:PVP 30	1:2	145.7
NEV:PVP 30	1:3	153.0
NEV:PVP 30	1:4	157.4

Table 4.1(continued): Resulting T_g when combining NEV ($T_g = 87^\circ\text{C}$) with various polymers in different ratios

NEV:PVP 30	4:1	104.6
NEV:PVP 30	3:1	109.0
NEV:PVP 30	2:1	116.3
NEV:PVP 90	1:1	138.5
NEV:PVP 90	1:2	155.7
NEV:PVP 90	1:3	164.3
NEV:PVP 90	1:4	169.4
NEV:PVP 90	4:1	107.6
NEV:PVP 90	3:1	112.8
NEV:PVP 90	2:1	121.3
NEV:PVP VA64	1:1	96.5
NEV:PVP VA64	1:2	99.7
NEV:PVP VA64	1:3	101.3
NEV:PVP VA64	1:4	102.2
NEV:PVP VA64	4:1	90.8
NEV:PVP VA64	3:1	91.8
NEV:PVP VA64	2:1	93.3
NEV:PEG 6000	4:1	81.4
NEV:PEG 6000	3:1	80.0

4.3 Solid-state characterisation of PhASD

4.3.1 Thermal analyses

4.3.1.1 Miscibility screening utilising simultaneous thermal analysis (STA)

After initial screening, only the combinations and ratios highlighted in the table above delivered successful amorphous solid dispersions. These are the mixtures that underwent further experimentation.

An experimental STA (DSC) run was performed, after theoretically determining the T_g , to establish whether heating the sample lead to glass formation, a visible glass transition

temperature or if crystallisation occurred. This was done in accordance with the method described in chapter 2 Section 2.3.1. Thermograms of the mixtures proving to be successful during the first screening process are illustrated in the figures below.

STA runs were performed on the abovementioned mixtures in order to establish which of these combinations were successful during the first screening process. As one can see in Figure 4.1, NEV melted at a temperature of 246.2°C therefore the remainder of the runs for the mixtures were performed up to a temperature of 250°C.

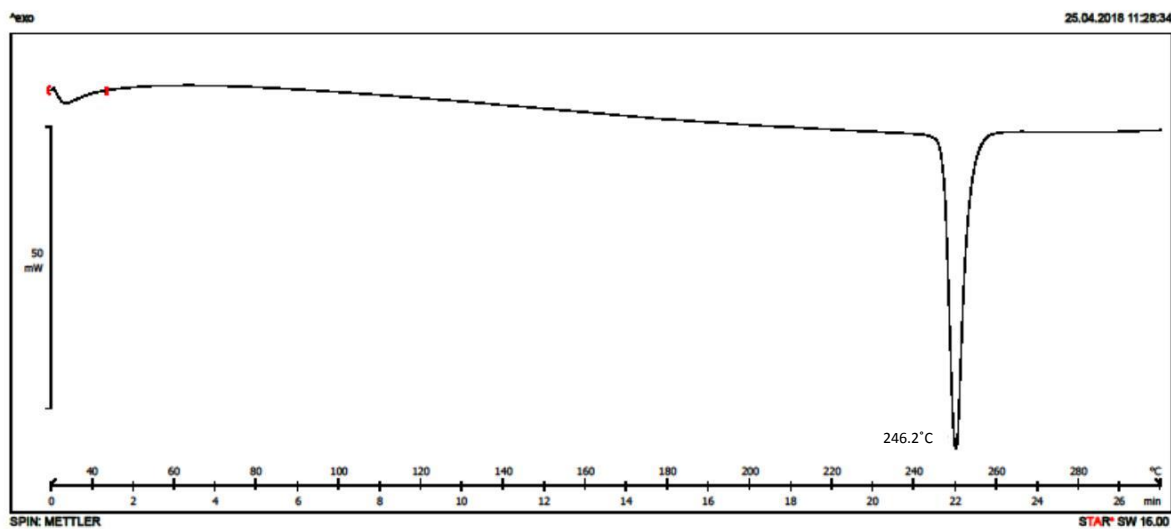


Figure 4.1: STA thermogram of NEV raw material with a melting temperature of 246.2°C.

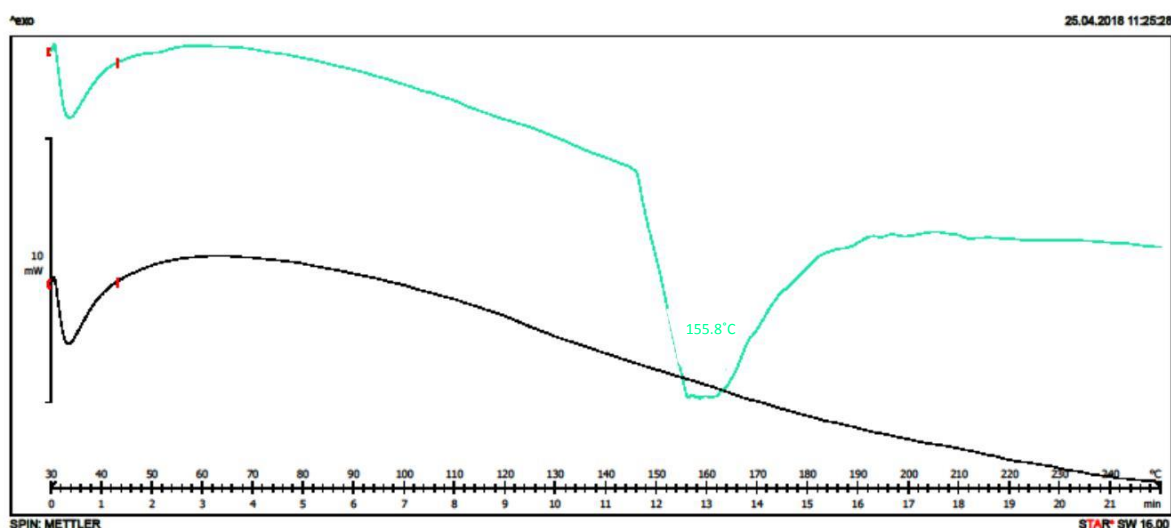


Figure 4.2: STA thermograms of NEV:PVP 25 1:4 physical mixture of powder; run 1 (green) and 2 (black). The first run only displays one endothermic event at 155°C.

This temperature (Fig 4.2 green) is neither the melting point of PVP 25 or NEV. With drug-polymer mixtures this is a normal phenomenon, whereby dissolution of the API into the amorphous polymer occurred which then depressed the melting point of the API (Knopp *et al.*, 2015). The second run (black) showing no melting points, suggests that a miscible system was formed.

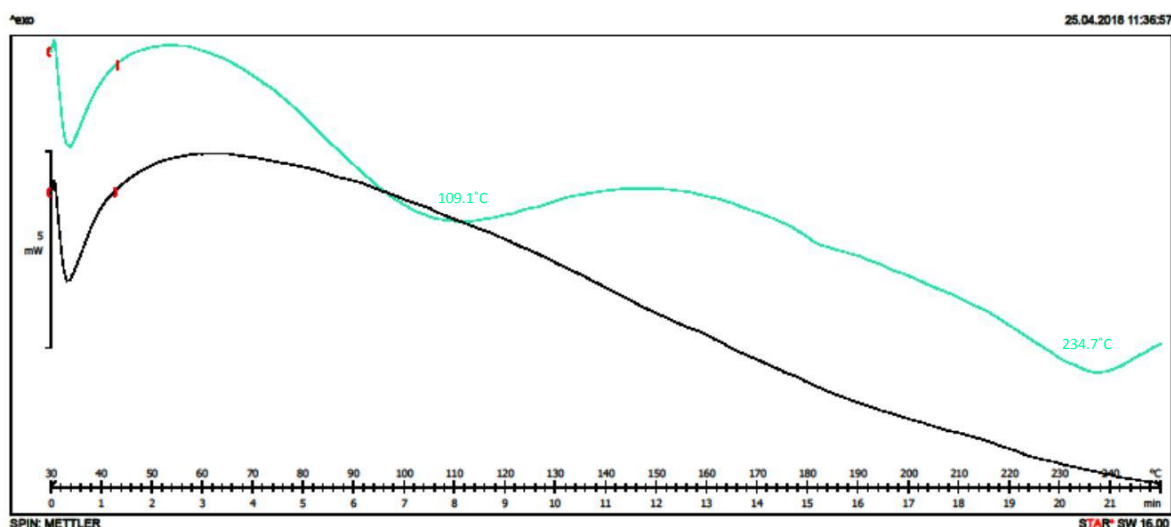


Figure 4.3: STA thermograms of NEV:PVP 90 1:4 physical mixture of dry-powder; run 1 (green) and 2 (black). Two endothermic events were visible (green), melting of the polymer and NEV. The second run (black) showed no endothermic events, suggesting that the system formed a miscible dispersion after the melt.

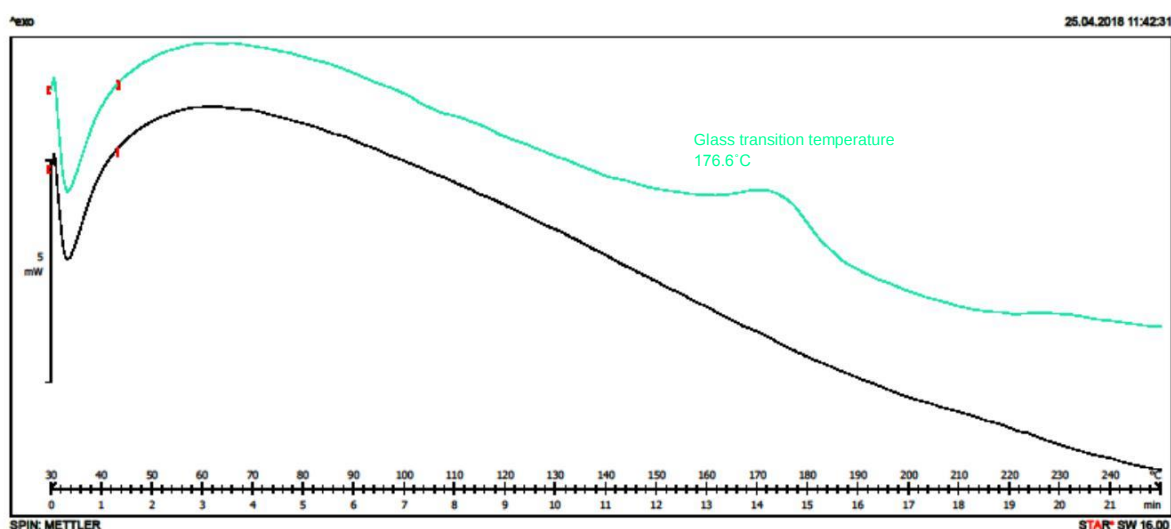


Figure 4.4: STA thermograms of NEV:HPMCAS 1:4 physical mixture of dry-powder; run 1 (green) and 2 (black). With the first run, a glass transition temperature was visible at 176°C. The glass transition temperature of HPMCAS is approximately 120°C.

After this initial screening only the following three mixtures and ratios were chosen for further experimentation:

NEV:PVP 25 1:4

NEV:PVP 90 1:4

NEV:HPMCAS 1:4

4.4 PhASD production methods

Multiple production methods were investigated in order to find the most successful method for producing a stable PhASD with NEV as API. Hot-melt by heating on a hot plate at 250°C as well as in a vacuum oven (200°C) was unsuccessful with NEV as it was impossible to obtain uniform melting of the powder mixture. Discoloration occurred at certain areas of the powder as the heat was not distributed evenly, this occurred on both the hot plate as well as in the oven.

After unsuccessful results with abovementioned methods, solvent evaporation (rotary evaporator and spray drying) was performed. The exact mass of API and polymer was separately weighed for each mixture and dissolved in 99.9% ethanol. This solution was then placed in a rotary evaporator followed by oven drying at approximately 60°C to remove any solvent residue. A 2% w/v solution (NEV and polymer in 99.9% ethanol) was prepared for the spray drying.

4.5 Characterisation of the PhASDs

4.5.1 Morphology

SEM images of NEV raw material can be seen in Figure 4.5, the particles look as if it has been milled to reduce particle size. Figure 4.6 represents an image of the solid dispersion, NEV:PVP 25 1:4 prepared by rotary evaporator. The particles are mostly plate-like, amorphous particles. The spray-dried particles of the NEV:PVP 25 1:4 dispersion, (Fig. 4.7) are much smaller and spherical in size. The smaller particles lead to a bigger surface area for wettability and should theoretically improve the solubility. The solid dispersion prepared with NEV:PVP 90 by solvent evaporation (Fig. 4.8) presented with a thin, but hard film which was very difficult to remove from the glass container. The product obtained after solvent evaporation through rotary evaporation from NEV:HPMCAS 1:4 (Fig. 4.9) can be described as amorphous agglomerates.

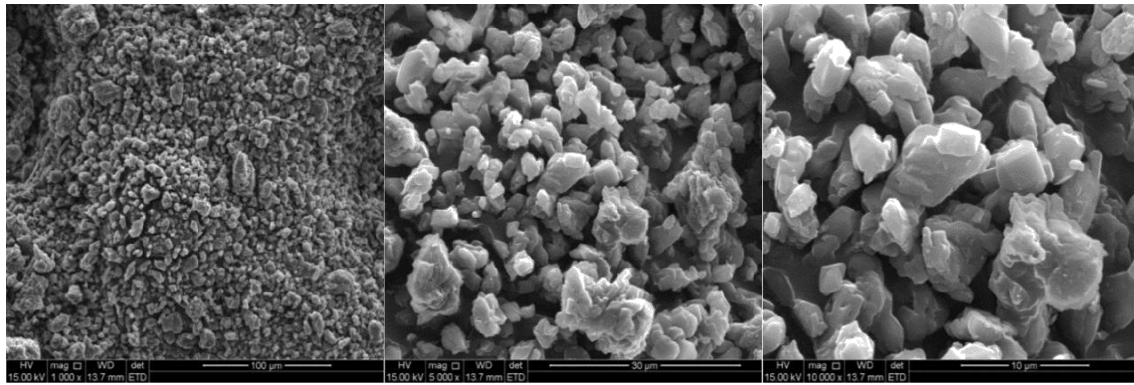


Figure 4.5: SEM images of NEV Form 1, raw material.

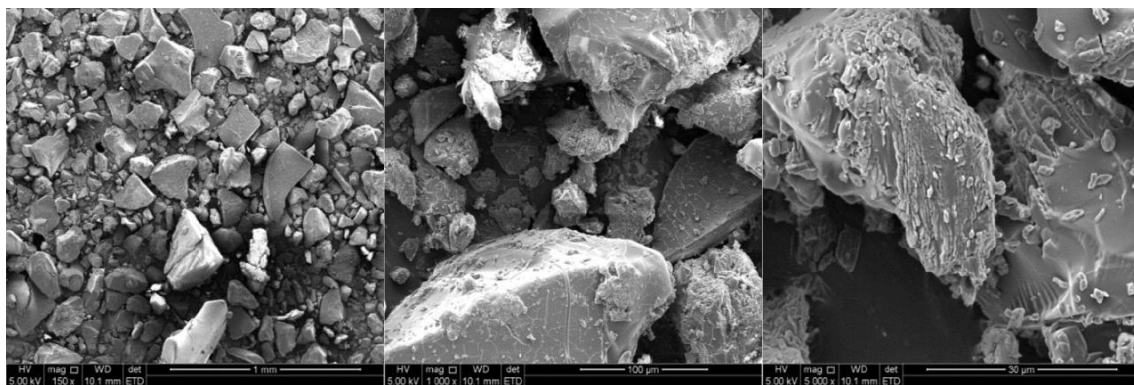


Figure 4.6: SEM images of NEV:PVP 25 1:4 prepared by rotary evaporator.

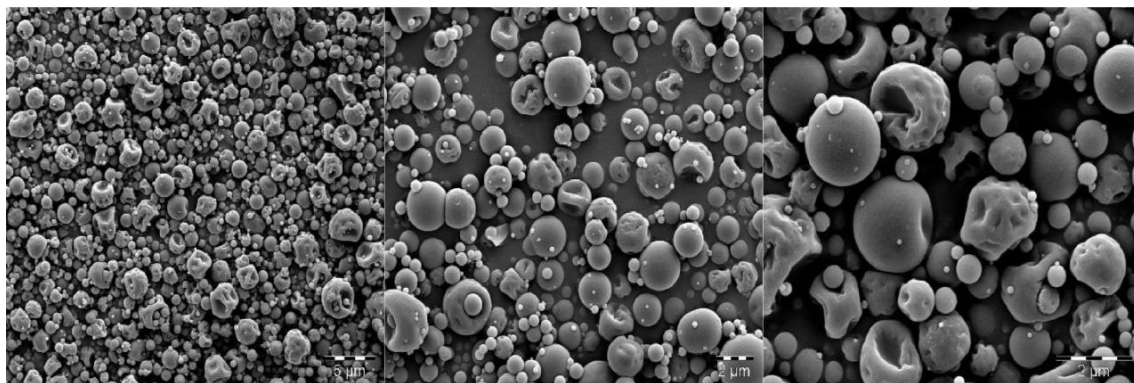


Figure 4.7: SEM images of NEV:PVP 25 1:4 prepared by spray-dryer.

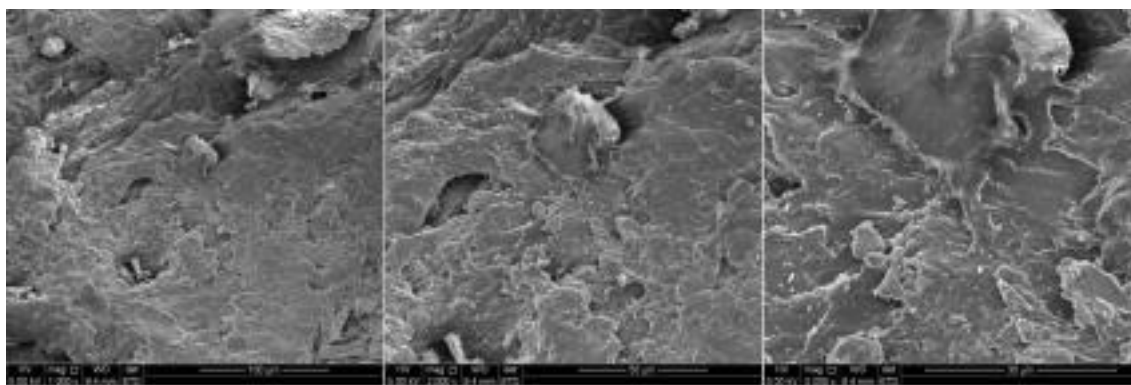


Figure 4.8: SEM images of NEV:PVP 90 1:4 prepared by rotary evaporator.

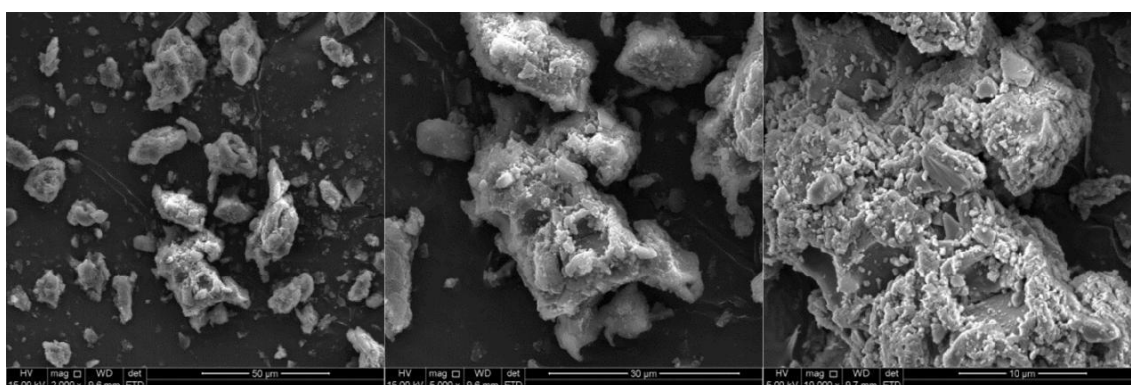


Figure 4.9: SEM images of NEV:HPMCAS 1:4 prepared by rotary evaporator.

4.5.2 Differential scanning calorimetry (DSC)

Occurrence of recrystallisation is a key component in the stability of any PhASD, as this will lead to the transformation of the most stable form, resulting in poorer dissolution profiles than that obtained with the amorphous form. Therefore, DSC runs were performed on these mixtures prepared by means of solvent evaporation in order to determine whether they remained amorphous or returned to the crystalline state.

Figure 4.10 showed a DSC thermogram of NEV raw material (black), and a melting endotherm is clearly visible at approximately 250°C. In Figure 4.10 the thermogram of NEV:PVP 25 1:4 (purple), prepared by solvent evaporation, illustrates a broad melting endotherm at approximately 130°C. As previously mentioned, the depression of the melting point of the API is a normal phenomenon with solid dispersions. No glass transition point was visible, it is possible that this mixture was only a solid dispersion and not an amorphous solid dispersion. The XRPD results which follow will probably clarify this.

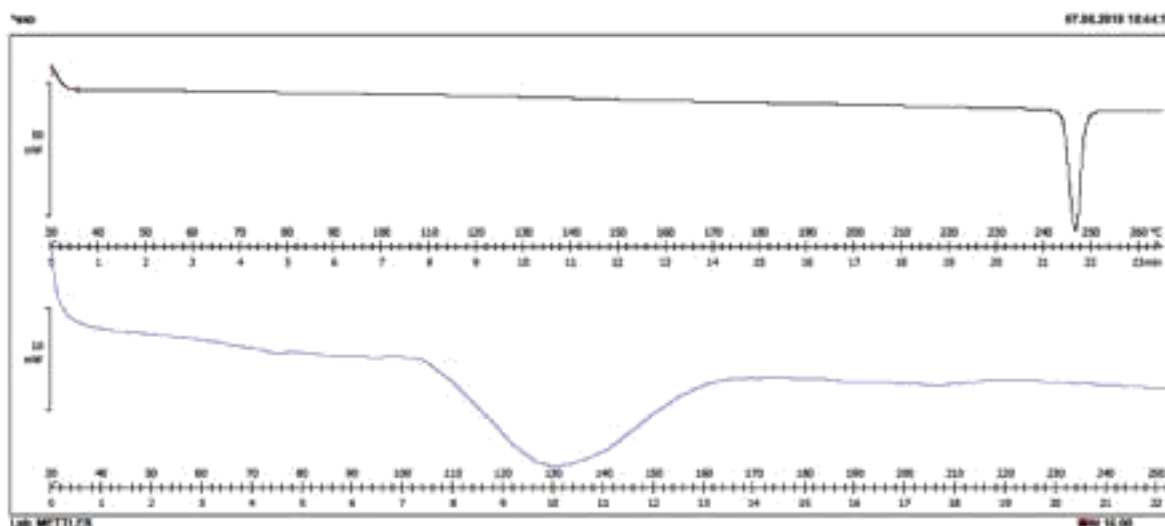


Figure 4.10: DSC overlay of NEV raw material (black) and NEV:PVP 25 1:4 solid dispersion prepared by rotary evaporator (purple).

In Figure 4.11 a DSC thermogram of the NEV:PVP 25 1:4 spray-dried solid dispersion is displayed (purple). A glass transition at 146.76°C was visible, and then one single endothermic event at 160.99°C which could be indicative of an amorphous solid dispersion. The glass transition temperature recorded with the DSC was identical to that of the calculated theoretical melting point (Table 4.1).

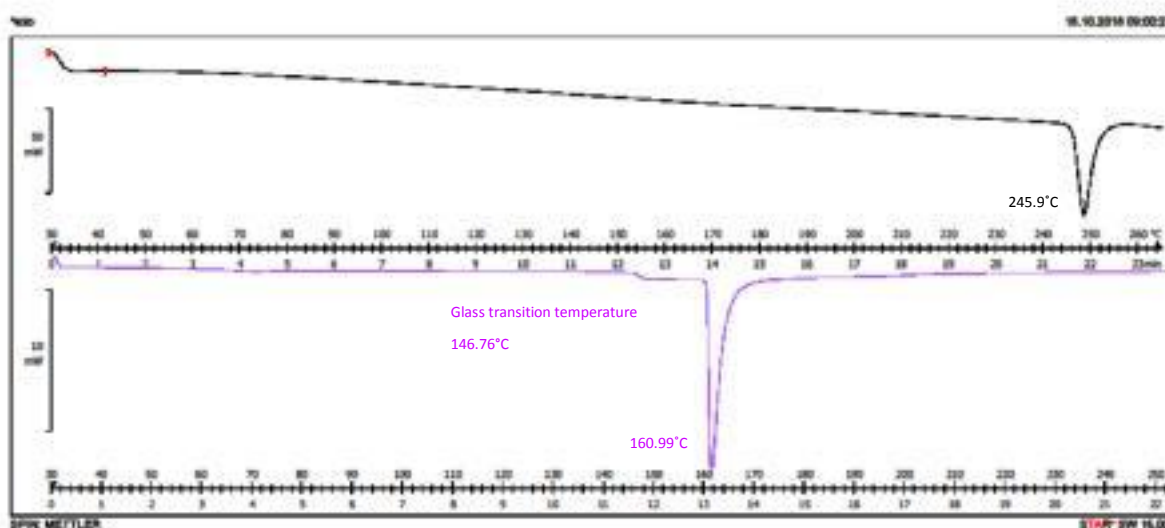


Figure 4.11: DSC overlay of NEV raw material (black) and NEV:PVP 25 1:4 solid dispersion prepared by nano spray drying (purple).

Figure 4.12 is indicative of the difference in the thermal behaviour of NEV raw material (black) and the NEV:PVP 25 1:4 mixtures prepared by different solvent evaporation methods; rotary evaporation (green) and nano spray drying (purple).

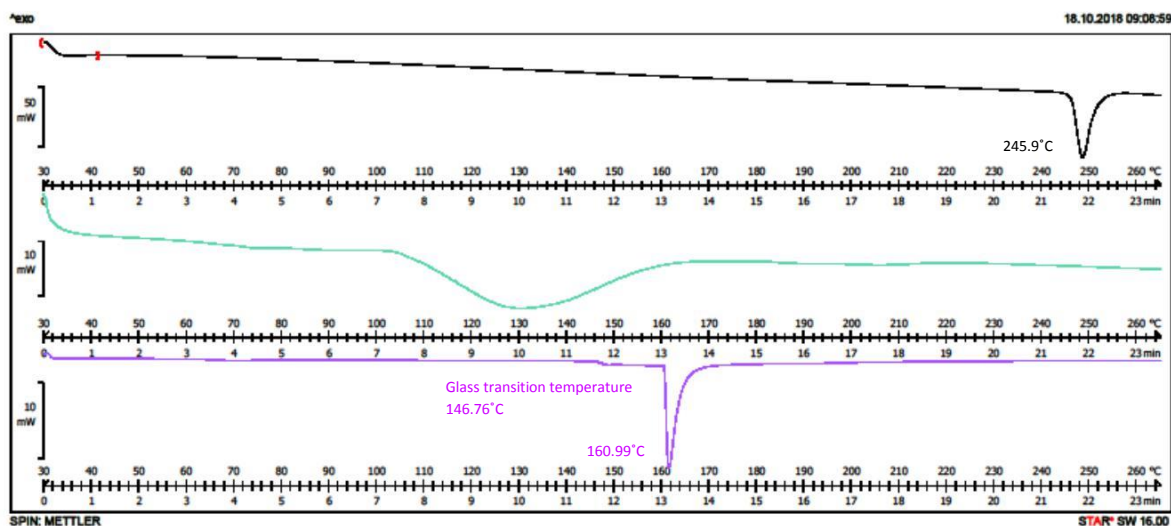


Figure 4.12: DSC overlay of NEV raw material (black), NEV:PVP 25 1:4 solid dispersion prepared by rotary evaporator (green) and NEV:PVP 25 1:4 solid dispersion prepared by nano spray drying (purple).

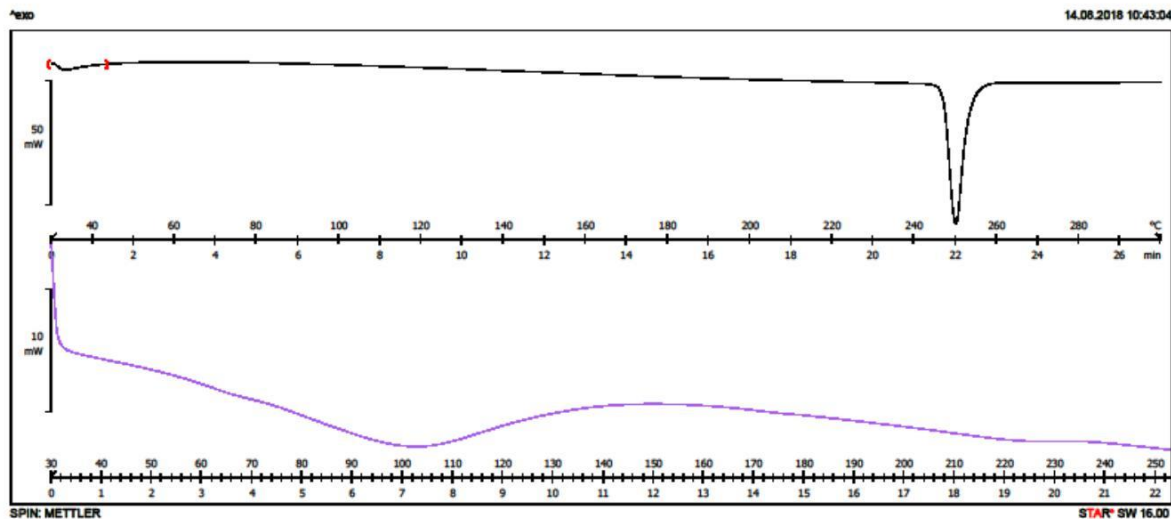


Figure 4.13: DSC overlay of NEV raw material (black) and NEV:PVP 90 1:4 prepared by rotary evaporator (purple).

Figure 4.13 is an overlay of NEV raw material (black) and that of NEV:PVP 90 1:4 prepared by rotary evaporation (purple). Only a broad endothermic event was visible at about 100°C, once again this can be attributed to the depression of the melting point occurrence in solid dispersions. Both melting points of NEV and the polymer were absent, indicating a miscible system. No glass transition temperature again, was visible.

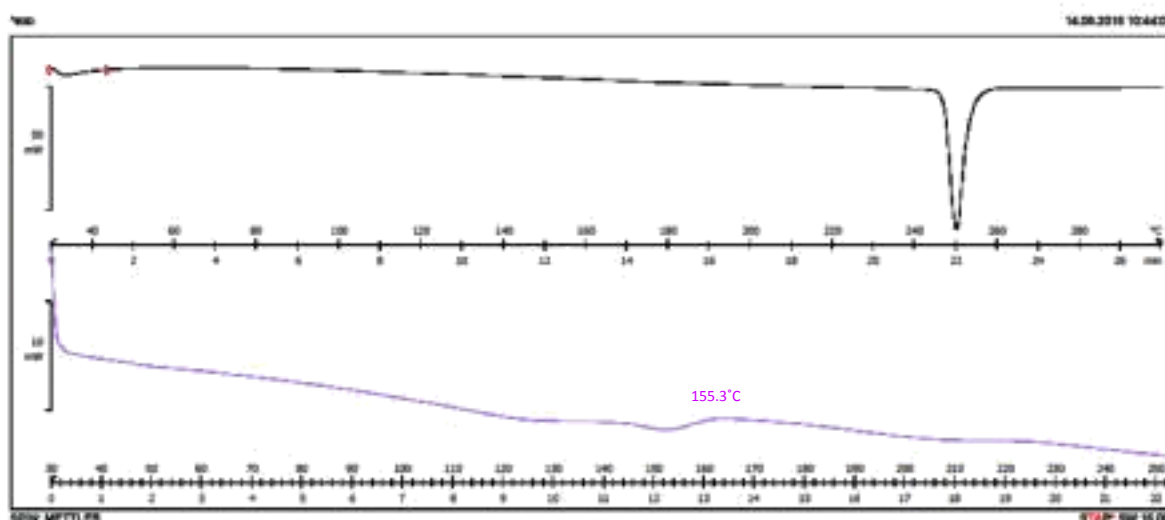


Figure 4.14: DSC overlay of NEV raw material (black) and NEV:HPMCAS 1:4 prepared by rotary evaporation (purple).

In Figure 4.14 an overlay of NEV raw material (black) and NEV:HPMCAS 1:4 mixture (purple). No prominent thermal events were visible with this dispersion. A small thermal event was visible starting at 150°C. No obvious explanation for this event.

4.5.3 X-ray powder diffractometry (XRPD)

The XRPD diffractograms were obtained as described in Section 2.3.4. These diffractograms of NEV (Fig. 4.15a-4.17a) showed that NEV raw material is completely crystalline. The physical mixture (co-grinding) of Figure 4.15b, NEV:PVP 25 proved to be mainly crystalline with a slight amorphous component.

The diffractogram of the NEV:PVP 25 1:4 product obtained through rotary evaporation (Fig. 4.15c) exhibits broad peaks in comparison to those of the crystalline NEV (Fig. 4.15a). The broadening of the peaks are consistent with nano-ordered domains (Jenkins & Snyder, 1996) and can thus be described as a nanocrystalline solid dispersion (NCSD).

The diffractogram of the NEV:PVP 25 1:4 product obtained through spray-drying (Fig 4.15d) showed no distinct peaks at all, indicating an amorphous nature.

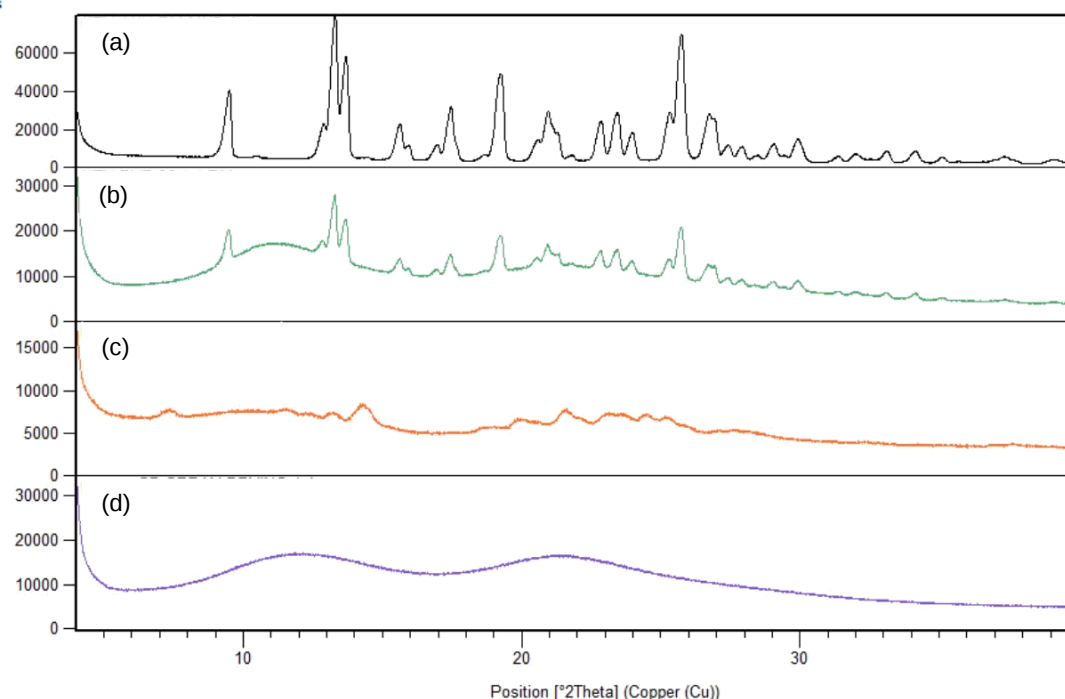


Figure 4.15: XRPD of (a) NEV raw material (b) NEV:PVP 25 1:4 physical mixture (c) NEV:PVP 25 1:4 NCSD prepared with a rotary evaporator and (d) NEV:PVP 25 1:4 PhASD prepared by spray-drying.

Figure 4.16(b) illustrated the mixture of NEV:PVP 90, solvent evaporation prepared by means of rotary evaporator. As with the previous polymer (PVP 25), it seems that the rotary evaporation preparation method produced a NCSD, due to the characteristic broadening of the peaks and low peak intensity.

As the polymer (PVP 90) is extremely hard and impossible to grind to a fine powder, this mixture was not prepared by physical mixture since uniform distribution was not possible to achieve.

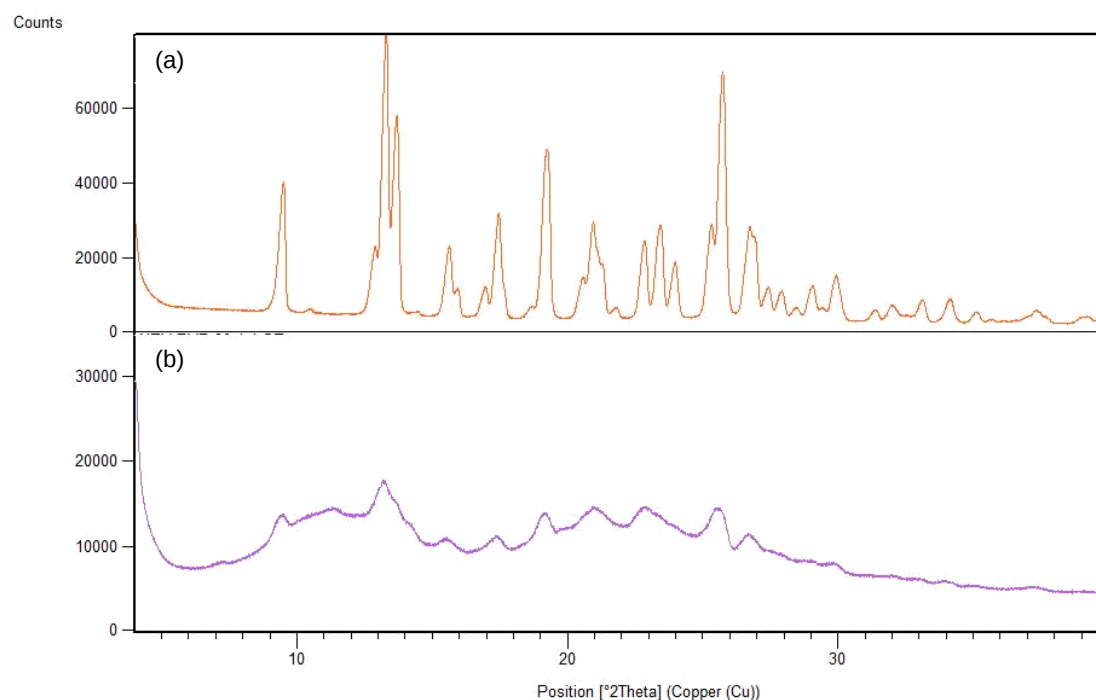


Figure 4.16: XRPD of a) NEV raw material and b) NEV:PVP 90 1:4 prepared with a rotary evaporator (NCSD).

Figure 4.17(b) indicates the diffractogram of NEV:HPMCAS 1:4 physical mixture, showing a bit of amorphous character due to the grinding. Almost all the peaks of the NEV raw material are visible on the diffractogram, only broader. The diffractogram of the product produced by rotary evaporation (Fig 4.17c) looks like a NCSD. The diffraction pattern differed significantly from that of the NEV raw material and that of the physical mixture. The peaks had different $^{\circ}2\theta$ values.

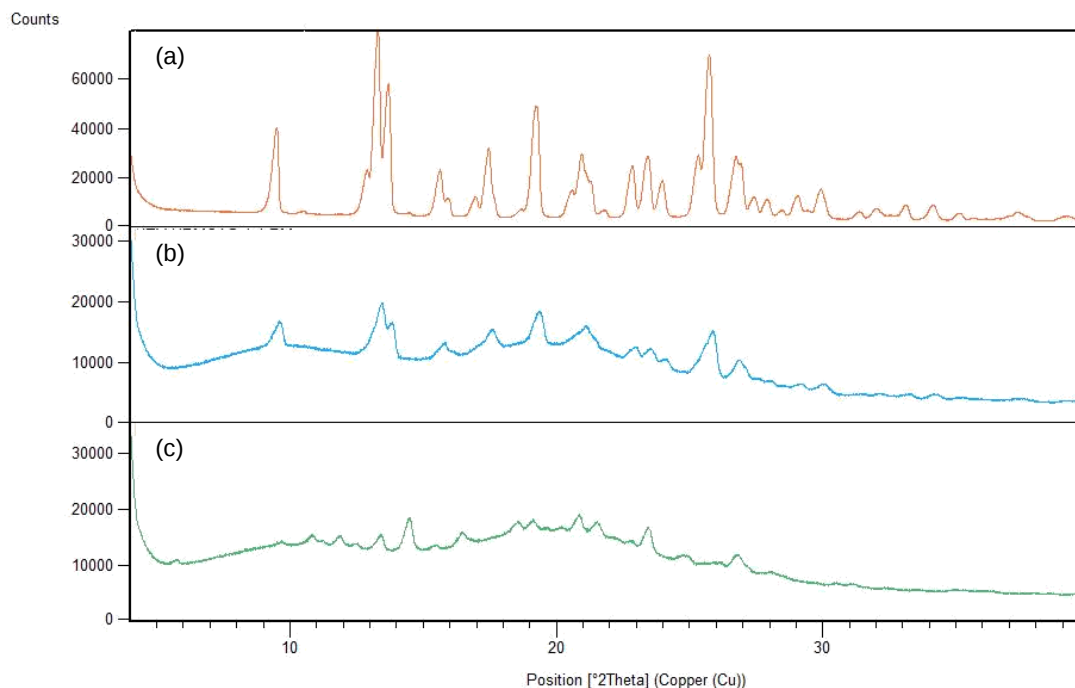


Figure 4.17: XRPD of a) NEV raw material), b) NEV:HPMCAS 1:4 physical mixture and c) NEV:HPMCAS 1:4 prepared with a rotary evaporator (NCSD).

To conclude: It seems that only the spray dried technique produced an amorphous solid dispersion (PhASD), the rotary evaporator produced nanocrystalline solid dispersions (NCSDs) and the physical mixture (prepared by co-grinding) only resulted in a mixture of amorphous and crystalline components.

4.6 Assess dissolution and solubility potential

4.6.1 Validation of the HPLC analytical method

The validation of the HPLC analytical method is important to guarantee consistency and sensitivity in the determination of the amount API found in the samples (Fig. 4.18).

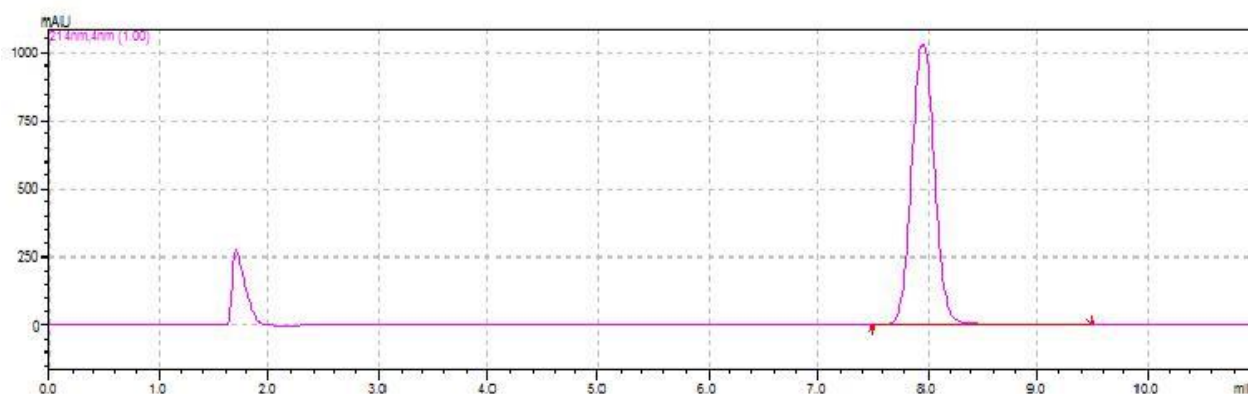


Figure 4.18: HPLC chromatogram of NEV.

4.6.1.1 Linearity

As mentioned in Section 3.6.1.1, linearity is an important aspect to be determined and results should be directly proportional to the concentration of analyte in the sample. A regression coefficient (r^2) of 0.9991 was obtained after injecting six concentrations (150 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 15 µg/ml and 5 µg/ml) of API in duplicate into the HPLC. The linearity of NEV was determined as it was in Section 3.6.1.1 and thus linearity was verified (Fig. 4.19).

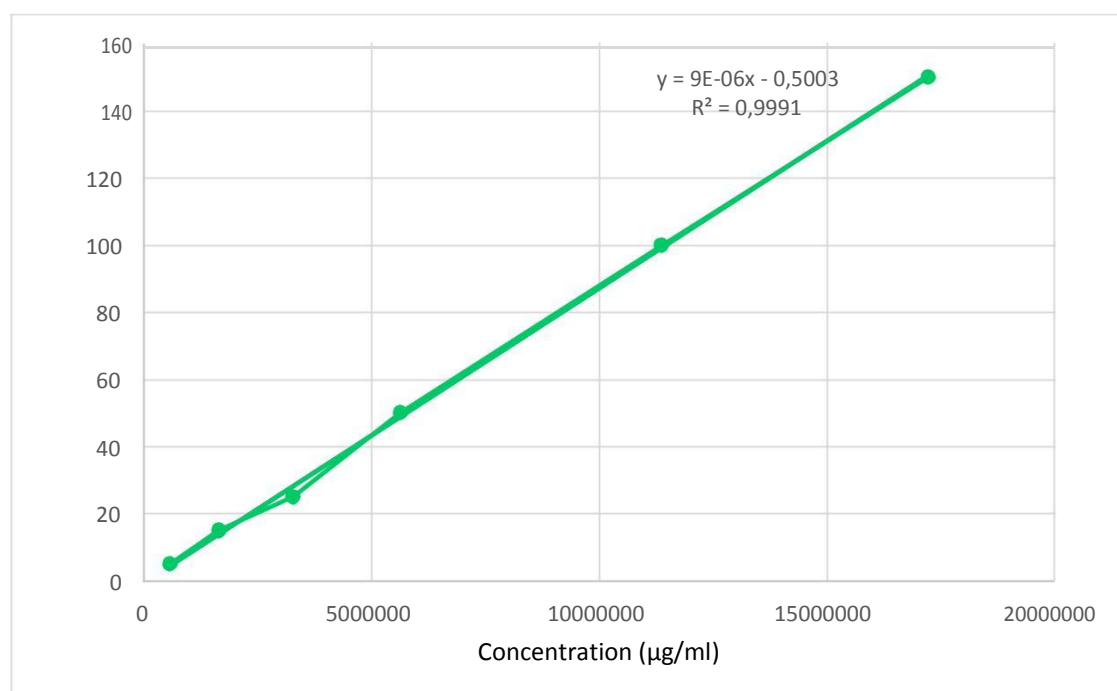


Figure 4.19: Linear regression curve of NEV.

4.6.2 Dissolution and assay

Dissolution studies were once again performed in accordance with the method as explained in Section 2.3.9. As the PhASD of NEV:PVP 25 1:4 prepared by nano spray drying appeared to be the most successful combination after initial screening, dissolution studies were performed on this mixture. Figure 4.20 indicates the improvement of dissolution results of NEV Form 1 when compared to the NCSD (prepared by means of rotary evaporation) and the PhASD (spray drying product). An approximate five-fold improvement in apparent solubility over NEV Form I (0.02 µg/ml) was observed after 5 minutes of powder dissolution testing for the NCSD (0.11 µg/ml). A solubility advantage was maintained for at least 180 minutes. The spray dried PhASD, with smaller, spherical and more uniform particle size (as seen in Fig. 4.7 above) performed even better during the same test period and the improvement in apparent solubility at 5 minutes was six-fold (0.13 µg/ml) compared to that of Form I. Not only did both the PhASD and the NCSD dissolve much faster, but the presence of the polymer (once dissolved) inhibited crystallisation and precipitation to the stable polymorphic form, as would

typically occur with a pure amorphous form. The fact that the dissolution values of the PhASD was superior, indicates the true amorphous nature as demonstrated with the XRPD analysis. The NCSD did however also show a significant improvement in the dissolution values when compared to that of the raw material.

Formula 3.2 was used to evaluate the dissolution results of the NCSD as well as the PhASD. Discussion follows below.

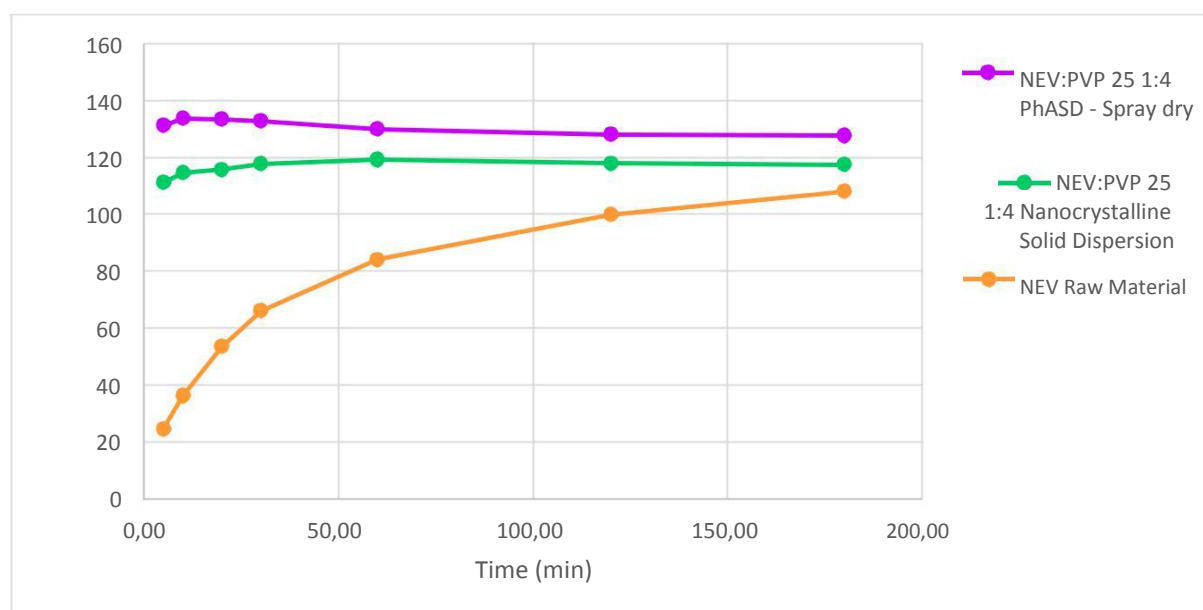


Figure 4.20: Dissolution profile of NEV raw material and NEV:PVP 25 1:4 NCSD prepared by means of rotary evaporator and PhASD prepared by spray-dry.

The dissolution results of both the NCSD and PhASD were evaluated with Moore and Flanner's mathematical comparison of dissolution profiles (Formula 3.2). As seen in Table 4.2, both f2-values were below 50 which was expected. No similarity was obtained. The dissolution profiles of the NCSD and PhASD were better than that of the NEV raw material.

Table 4.2: Similarity values as determined with Moore and Flanner's similarity equation

Mixture	Similarity factor (f2)
NEV:PVP 25 1:4 (NCSD)	29.95
NEV:PVP 25 (PhASD)	24.83

Dissolution studies were performed on the NEV:HPMCAS 1:4 NCSD as well, but these results were not included as this experiment delivered dissolution results lower than that of the raw

material. HPMCAS has a pH dependant solubility in aqueous media (Anon, 2018), with the optimum pH being 6.5 (phosphate buffer). In this case, the polymer did not help improve the solubility of the nevirapine in die solid dispersion since the dissolution medium was water. This SD might be further investigated for delayed- or prolonged release products.

The NEV:PVP 90 1:4 NCSD proved to be miscible as well, as seen above during the screening process, although it was impossible to create this NCSD in sufficient amounts needed for dissolution studies. This polymer was extremely hard and practically impossible to ground into a fine powder after the ethanol was completely evaporated. Thus, it was not possible to perform dissolution studies on this mixture.

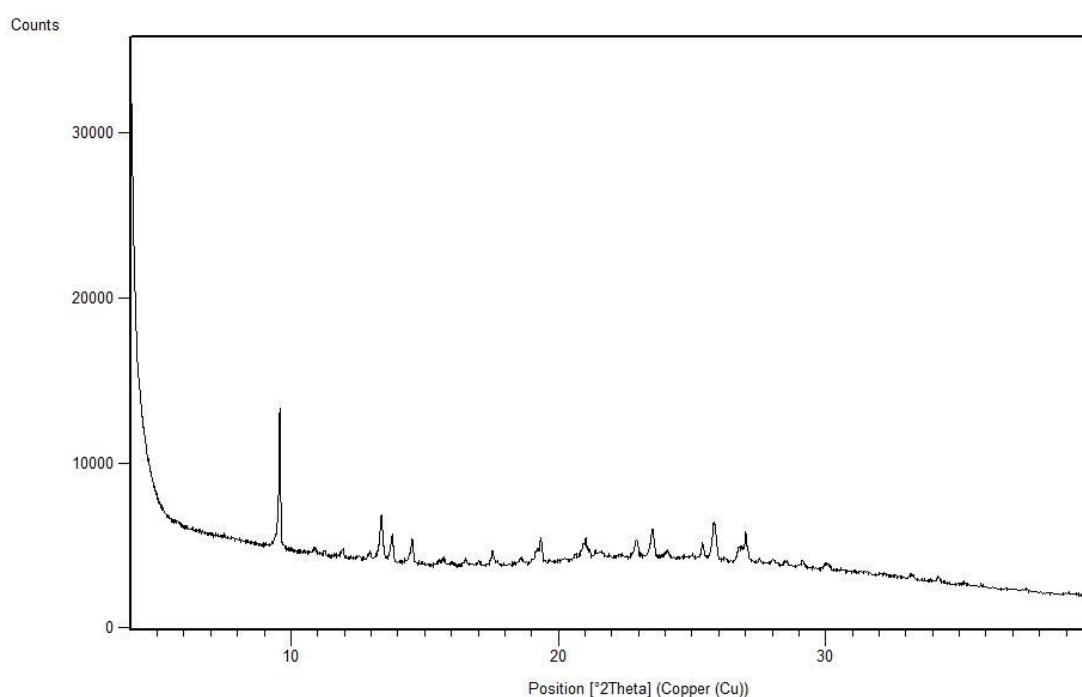


Figure 4.21: XRPD of the NEV:PVP 25 1:4 NCSD dissolution residues.

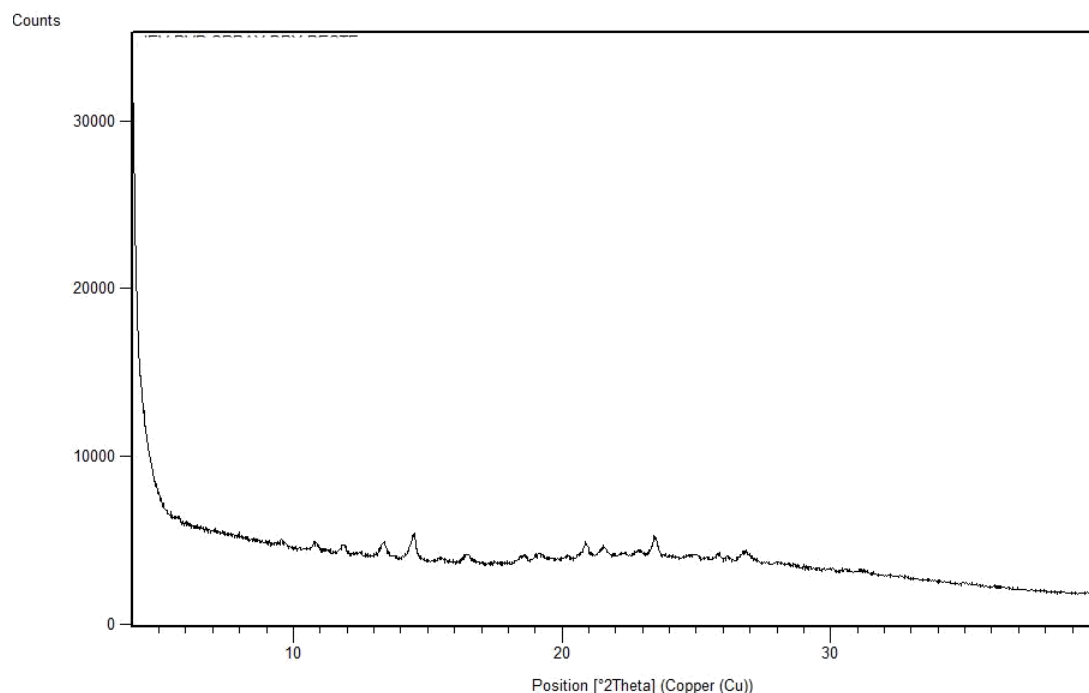


Figure 4.22: XRPD of the NEV:PVP 25 1:4 PhASD dissolution residues.

After the dissolution studies, XRPD analysis was performed on the dissolution residues in order to determine whether recrystallisation occurred or not. When comparing Figures 4.21 and 4.22 to Figure 4.15(c – d) it is clear that crystallisation did occur during the dissolution process, although it is more prominent in the NCSD, as expected due to the partial crystalline component which was originally present. The PhASD product produced by means of spray drying showed some recrystallisation as well, but not as much as the NCSD.

4.6.3 Solubility

A 24 hour solubility study was performed for NEV Form 1 as described in Section 2.3.8. The solubility of six samples were determined separately and delivered the following results.

Table 4.3: Solubility data for NEV in R.O water

Sample	Relative solubility (µg/ml)
1	86.51
2	103.38
3	103.38
4	109.50
5	112.18
6	107.86
Average	103.80

The average solubility of NEV was determined as 103.80 µg/ml. This value is relatively close to the value published by Stieger (2009) as mentioned in Section 2.2.1.2.2 and can therefore be accepted to be credible.

4.7 Accelerated stability testing of PhASD

Samples were exposed to stability testing of extreme conditions of 40°C and 75% RH for a period of three months. Photos were taken at each month in order to see whether there was any change in the physical appearance of the samples visible to the naked eye. For the raw material, no change in colour occurred which confirms that this powder remained stable during the test. For the NEV:PVP 25 dispersion, once again no visible colour change occurred, although the powder looks like it was hydrated and then dried out again. The powder was extremely hard to pulverize. As this did not occur with the raw material, we accept that this is due to the presence of PVP 25. Although dissolution studies were not possible to perform with the PVP 90 mixture, stability testing was still done as this could be a potential successful candidate for the formation of a solid dispersion with NEV. As with the two former mentioned, no colour change occurred as well as no visible moisture absorption although it appeared that the powder particles formed bigger, hard clots. This can, again, be attributed to the presence of the polymer (PVP 90).

No stability data was collected on the spray dried product due to the limited amount produced with the Buchi nano spray drier.

Table 4.4: Images of samples exposed to three month stability test

Sample	Initial	Month 1	Month 2	Month 3
NEV raw material				
NEV:PVP 25 1:4 NCSD				
NEV:PVP 90 1:4 NCSD				

The figure below (Fig. 4.23) is indicative of the amount of API which remained present in each sample (month 0 - 3) after being exposed to the stability testing conditions. All three of the month 0 samples presented with results below that of the remainder of the months, this can be attributed to possible experimental error. The concentration of NEV raw material (month 1 - 3) proved to be similar, ranging from 97.20 - 102.07% (green). The NCSD NEV:PVP 25 (purple) remained consistent in the amount of NEV present varying with a slight 3.675% (month 1 - 3). For the NCSD NEV:PVP 90 mixture (orange), variation was more present with values ranging from 91.87% - 96.20% (excluding month 0). This can be due to uneven distribution of the NEV and polymer particles.

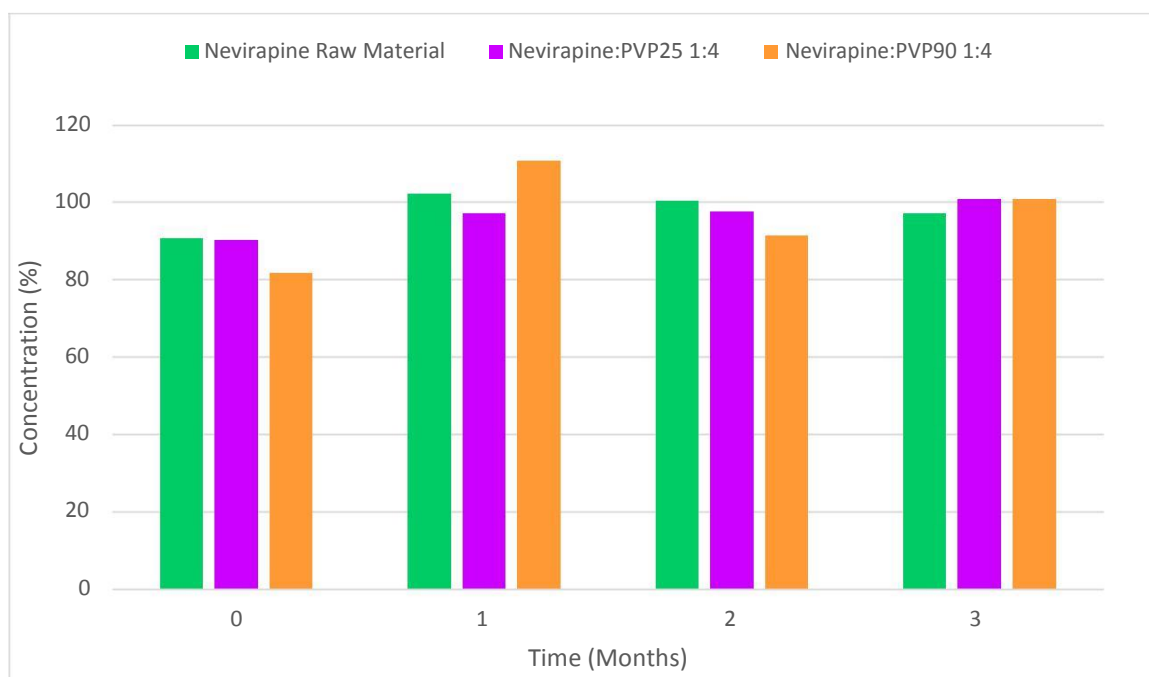


Figure 4.23: NEV concentrations in samples subjected to temperature and humidity conditions of 40°C / 75% RH over the stability test period of three months (NEV = green; NCSD NEV:PVP 25 1:4 = purple; NCSD NEV:PVP 90 1:4= orange).

It is of great importance to determine whether the exposure to temperature and humidity had any impact on the API and whether it caused the NCSD samples to recrystallise. Therefore STA analyses were performed on all of the samples subjected to the stability conditions. It is evident that these extreme conditions had no impact on NEV as the STA thermograms remained identical after the three month test period (Fig. 4.24). The NCSD NEV:PVP 25 1:4 and NCSD NEV:PVP 90 1:4 remained amorphous as no melting point is visible on the thermograms (Fig. 4.25 and 4.26).

**For the stability testing, the thermal analysis was done on the STA instrument which simultaneously measures weight loss and melting points. The STA instrument is not that sensitive for glass transition points, and therefore the NCSD thermograms will differ from those obtained with the DSC instrument.*

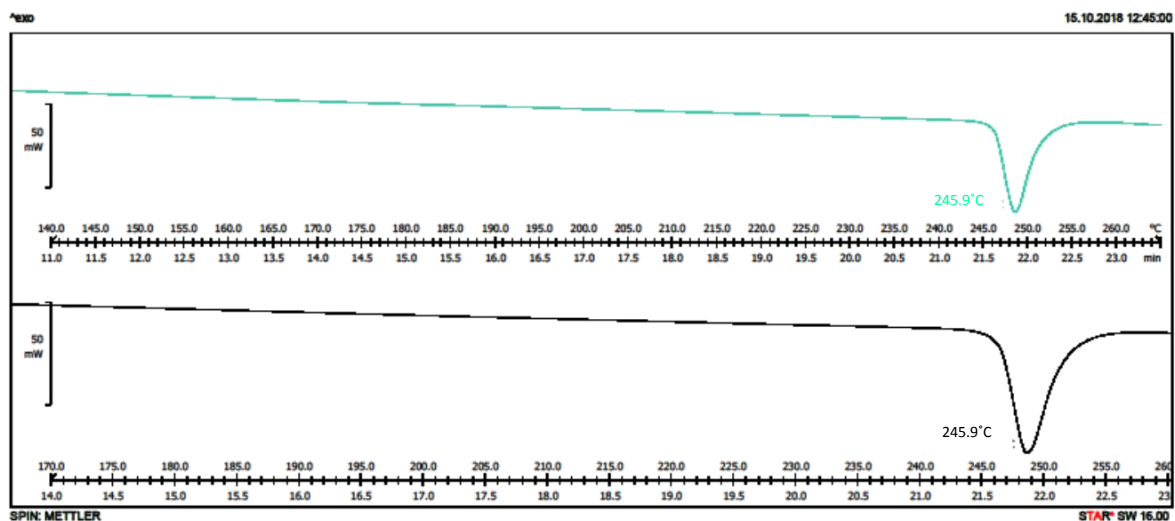


Figure 4.24: STA thermograms of NEV raw material of month 0 (green) and 3 (black) after stability testing.

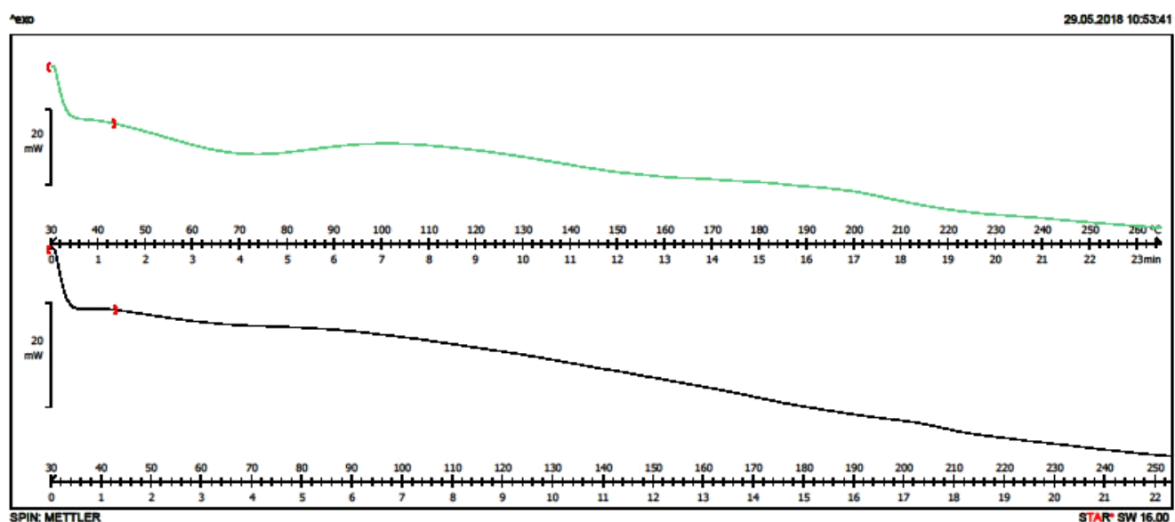


Figure 4.25: STA thermograms of NEV:PVP 25 1:4 (NCSD) month 0 (green) and 3 (black) after stability tests.

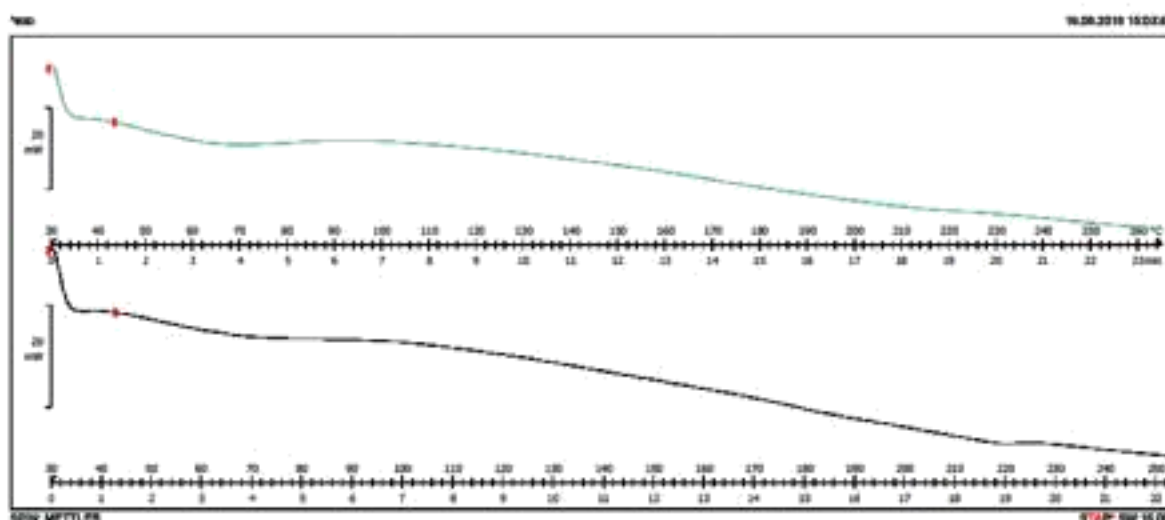


Figure 4.26: DSC thermograms of NEV:PVP 90 1:4 (NCSD) month 0 (green) and 3 (black) after stability tests.

In order to determine whether any of the samples exposed to the stability conditions absorbed any moisture during this period, TGA measurements were conducted. NEV raw material had moisture values ranging from 0.355% - 1.556%, which according to the manufacturer's COA is within the limits for this powder. The two NCSD mixtures showed much higher percentages of moisture than that of the raw material and in fact, the initial samples already had a moisture content of more than 10%. This can be attributed to the presence of the PVP which, as mentioned in the description of Table 4.4, is the responsible factor for absorption of moisture. The values for both solid dispersions seemed to differ minimally up until month 2. For both solid dispersions the weight loss at month three declined which correlates with the results of SULF as mentioned in the discussion of Table 3.5. This can, once again, be attributed to the change of PVP from a glass to a rubbery state. The NEV mixtures never gained a significant amount of moisture (less than 1%) when compared to that of the initial value, which is an excellent property from a stability point of view.

Table 4.5: Weight changes in NEV and the NCSDs over a three month accelerated stability study (40°C / 75% RH), as measured with TGA

Sample	Initial	Month 1	Month 2	Month 3
NEV raw material	1.5564%	0.3550%	0.6399%	0.4780%
NEV:PVP 25 1:4	10.7039%	10.4640%	11.6261%	6.1569%
NEV:PVP 90 1:4	11.0482%	11.0549%	11.8126%	6.4091%

To confirm the abovementioned results, XRPD scans were done as well. It is evident from Figure 4.27 that the API remained stable when exposed to extreme conditions as after the three months there was no change in the crystallinity.

The XRPD of the NEV raw material (Fig. 4.27) indicates that the API did not form a hydrate or hemi-hydrate during the stability testing.

Both nanocrystalline solid dispersions (Fig. 4.28 and 4.29), NEV:PVP 25 1:4 and NEV:PVP 90 1:4, showed a slight change after the first month of stability testing as a few of the peaks became more noticeable, indicating a bigger component being crystalline than originally present at month 0, this can be due to the moisture absorbed. *Again no stability testing was performed on the spray dried product due to yield restrictions during manufacturing with the nano spray drier.

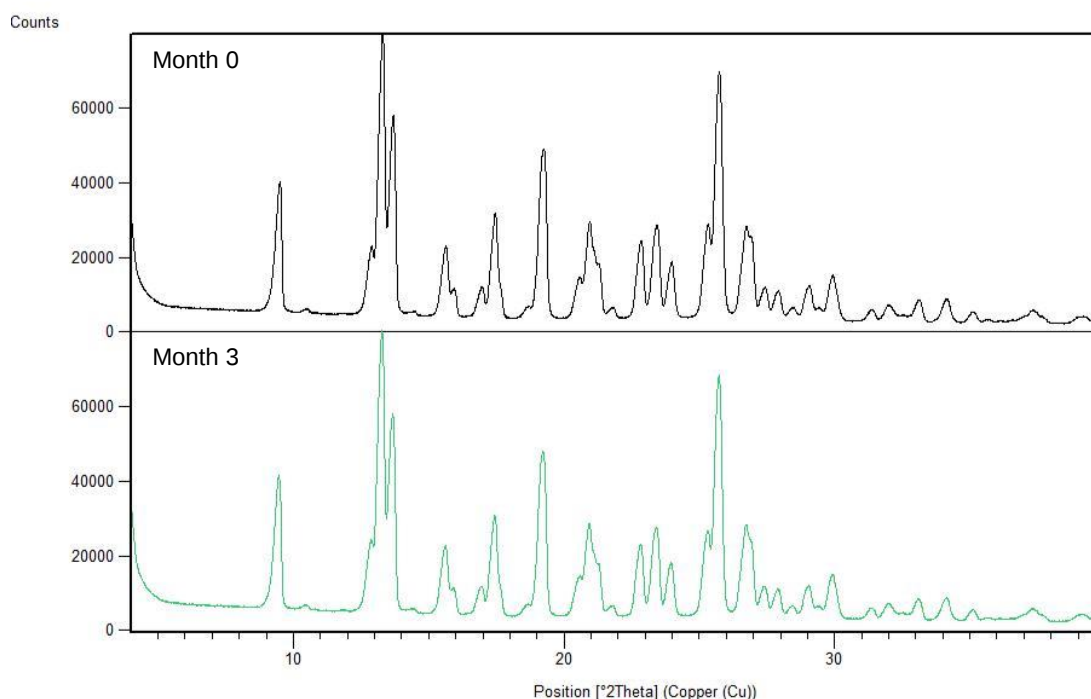


Figure 4.27: XRPD overlay of NEV raw material after exposed to stability tests, month 0 (black), month 3 (green).

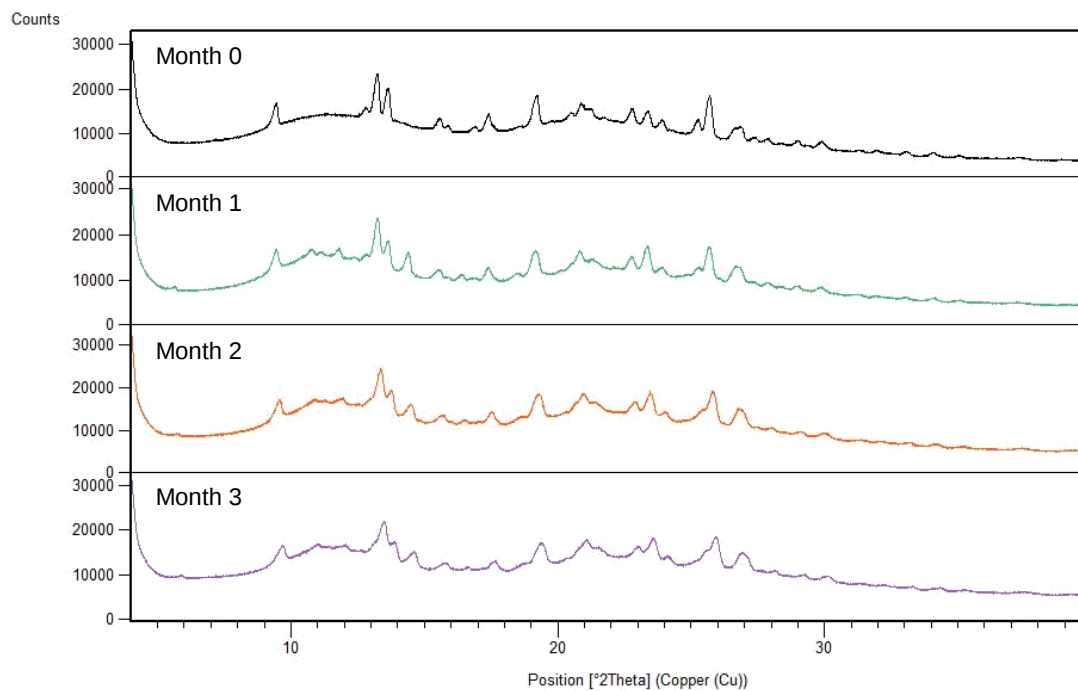


Figure 4.28: XRPD overlay of the NCSD NEV:PVP 25 1:4 after exposed to stability tests, month 0 (black), month 1 (green), month 2 (orange) and month 3 (purple).

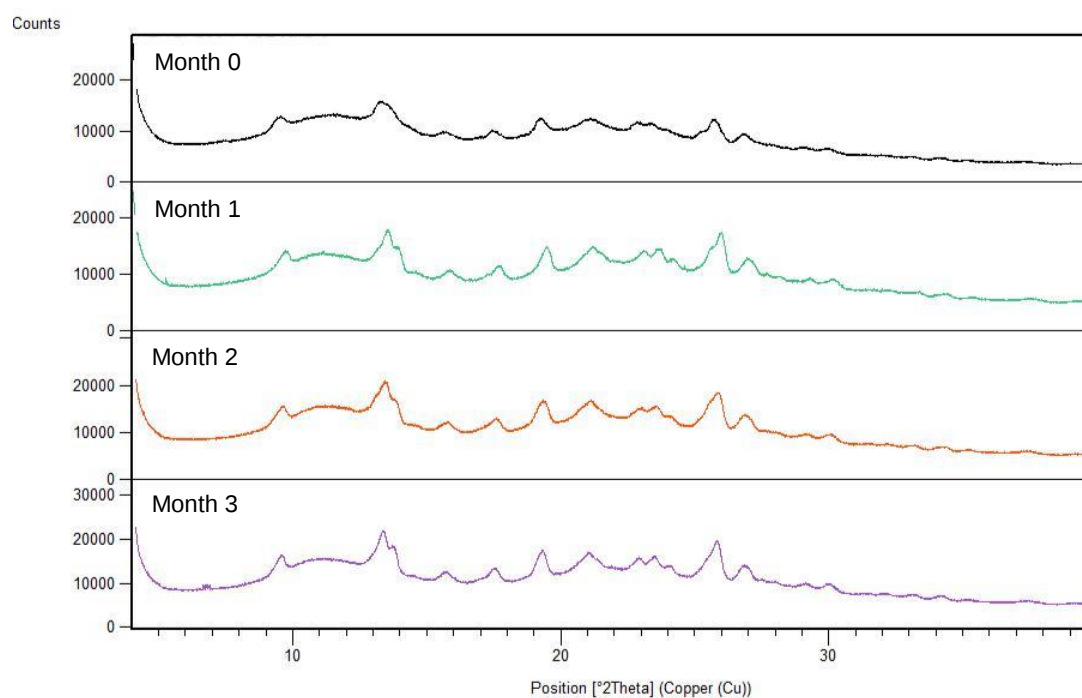


Figure 4.29: XRPD overlay of the NCSD NEV:PVP 90 1:4 after exposed to stability tests, month 0 (black), month 1 (green), month 2 (orange) and month 3 (purple).

4.8 Conclusion

Three methods of preparation were used in the search for a NEV amorphous solid dispersion. The physical mixture did not deliver an amorphous solid dispersion. Rotary evaporation delivered a NCSD with a five-fold improvement within the first five minutes of the dissolution test.

When preparing the same mixture with a spray dryer, a PhASD with a six-fold improvement within the first five minutes of powder dissolution studies was achieved. This improvement in apparent solubility remained throughout the three hour dissolution test. The NCSD (prepared by means of rotary evaporation) was exposed to stability testing under extreme conditions and remained stable for the three month period.

Due to the fact that the NCSD was very similar to the PhASD in dissolution performance, upscaling and production of this dispersion will probably be easier and more economic than the spray dried PhASD.

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CHAPTER 5

CONCLUSION

The physico-chemical properties of pharmaceutical actives, especially those of poorly-water soluble drugs were discussed in chapter 1. The importance of adequate solubility and the effect thereof on the bioavailability of drugs were thoroughly emphasised as poorly-water soluble drugs do not present with satisfactory dissolution within the gastro-intestinal tract.

Both of the APIs, sulfadoxine and nevirapine, presented with poor aqueous solubility, therefore they were excellent candidates in fulfilling the aim of this study which was to formulate stable PhASDs using a modified SPADS process. Sulfadoxine is an antimalarial, most commonly used in combination with pyrimethamine for the treatment of uncomplicated *Plasmodium falciparum* malaria as well as for the prophylaxis and suppression of chloroquine-resistant *Plasmodium falciparum* malaria (WHO, 2017; Odeniyi *et al.*, 2003; Minzi *et al.*, 2003). Limited studies have been performed on sulfadoxine for the improvement of dissolution.

Nevirapine, used for the treatment of HIV-1 and AIDS is dissolution rate limited and is classified as a BCS Class II drug. Extensive research has been done on nevirapine with the aim of improving solubility, though no pronounced success has been reported.

In Chapter 2, the screening (SPADS) method and techniques which were going to be used were discussed and the data collected from each technique was used to decide which candidate polymers should be eliminated or should remain for further screening. The initial screening begun with a total of 64 API:polymer ratios and combinations for each of the APIs.

Chapter 3 elaborated on the most successful PhASD which was prepared through rotary evaporation with sulfadoxine as API. Discussions regarding the elimination of certain polymer candidates and the reason thereof were explained. The most successful candidate and ratio, SULF:PVP 25 1:2 was completely amorphous and delivered an improvement in the dissolution profile with a solubility advantage over the crystalline raw material for 180 minutes. Although, after the dissolution studies, XRPD analysis indicated that crystallisation occurred as the dissolution residues proved to be more crystalline than the original PhASD. The PhASD, SULF:PVP 25 1:2 was exposed to extreme accelerated stability tests over a period of three months and it was evident that the increased temperature, or more likely the extreme humidity conditions led to crystal growth when comparing the samples of month 0 to month 3 (Figure 3.20). As moisture can act as a plasticiser it is important for this PhASD to be prepared in special conditions preventing exposure to moisture as this proved to cause recrystallisation. Further experiments with addition of a surfactant could lead to even greater dissolution profiles, but should be investigated as stability could be compromised.

Noteworthy discoveries regarding the preparation of solid dispersions for nevirapine were discussed in Chapter 4. Once again, solvent evaporation was the most successful method of preparation, which included spray drying and rotary evaporation. The NEV:PVP 25 1:4 mixture was prepared by rotary evaporator as well as spray-drying. The mixture prepared with the rotary evaporator was mainly amorphous with a slight crystalline component. It was proved that this product was a nanocrystalline solid dispersion, due to the low peak intensity and peak broadening on the X-ray powder diffractogram. The PhASD prepared through spray drying was completely amorphous. As with sulfadoxine, a solubility advantage was maintained for 180 minutes. XRPD analysis on the dissolution residues showed that crystal growth did occur, although the three month accelerated stability test did not indicate extreme crystal growth within the nanocrystalline solid dispersion.

The solubility of both APIs remain challenging as a drastic improvement was not attained. After completion of the powder dissolution studies, a mere 29.81 µg/ml improvement over the sulfadoxine raw material was obtained. Nevirapine yielded a significant improvement in dissolution within the first five minutes, five-fold for the NCSD and six-fold for the PhASD compared to the nevirapine raw material. Due to the fact that the NCSD was very similar to the PhASD in dissolution performance, upscaling and production of this dispersion will probably be easier and more economic than the spray dried PhASD. Other methods, such as hot-melt extrusion and the addition of a surfactant should definitely be further investigated for the development of a PhASD with NEV and SULF as APIs.

A manuscript is in preparation regarding the solid-state properties of the nevirapine nanocrystalline and amorphous solid dispersions, which will be submitted as soon as the patent registration is finalised.

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WHO see World Health Organization

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ANNEXURE A

POWER POINT PODIUM PRESENTATION:

**DEVELOPING A NEVIRAPINE PHARMACEUTICAL AMORPHOUS
SOLID DISPERSION, PRESENTED AT THE FIRST CONFERENCE OF
BIOMEDICAL AND NATURAL SCIENCES AND THERAPEUTICS**

At Stellenbosch, South Africa

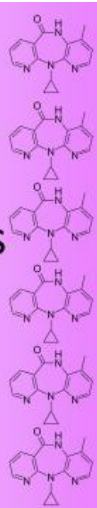
CoBNeST 2018



Developing a nevirapine pharmaceutical amorphous solid dispersion

Patent pending (ZA 2018/05052 – Nevirapine Amorphous Solid Dispersion).

Nadine de Melim



Good afternoon ladies and gentleman, my name is Nadine de Melim and I will be discussing my masters study – Developing a nevirapine pharmaceutical amorphous solid dispersion.

Background

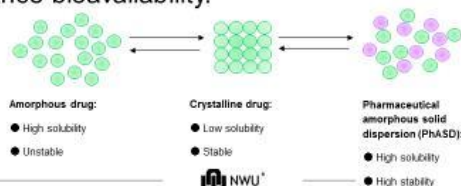
- Solid oral dosage forms are the most convenient dosage forms.
- Many drugs are capable of crystallising in multiple forms.
- All polymorphs are crystalline forms although there will always be one polymorph which is most stable under certain conditions
- Approximately 40% of pharmaceutical products which are currently on the market are poorly soluble.
- Estimated 90% of drugs in development can be classified as poorly soluble¹.

Solid oral dosage forms are the most convenient dosage forms ensuring patient compliance and present with the best stability. Many drugs are capable of crystallising in multiple forms, each having different free energy states and physico-chemical properties, this phenomenon is known as polymorphism. Approximately 40% of pharmaceutical products which are currently on the market are poorly soluble while an estimated 90% of drugs in development can be classified as poorly soluble. Poorly water-soluble drugs do not present with satisfactory dissolution within the gastro-intestinal tract. This leads to inadequate bioavailability.



Amorphous Solid Dispersions

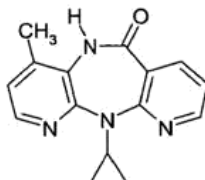
- An amorphous solid dispersion (ASD) is a system where the active pharmaceutical ingredient (API) is combined with a water-soluble polymer to produce a single-phase amorphous entity.
- Amorphous solid dispersions increase the solubility as well as the dissolution rate and thus enhance bioavailability.



An amorphous solid dispersion is a system where the active pharmaceutical ingredient is combined with a water-soluble polymer to produce a single-phase amorphous entity. ASDs increase the solubility as well as the dissolution rate and thus enhance bioavailability. This image is indicative of the difference in molecular arrangement of an amorphous drug which has no crystal shape or long range order. A crystalline drug, ordered in a specific arrangement and possessing a unique crystal lattice. And a pharmaceutical ASD where the polymer is homogeneously distributed through the API.

Nevirapine

- Nevirapine (NEV) is a synthetic non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used for the treatment of HIV-1 and AIDS.
- Nevirapine Form I is poorly soluble (0.976 mg/ml in water at neutral pH and 37°C) but absorbs well from the gastrointestinal tract².



NWU*

The term pharmaceutical amorphous solid dispersion is often found in literature but for the remainder of this presentation I will refer to ASDs.

Nevirapine is used for the treatment of HIV-1 and AIDS. Nevirapine Form I is poorly soluble in water, with a value of 0.976mg/ml at neutral pH and 37°C although it absorbs well from the gastrointestinal tract.

Biopharmaceutical Classification System



NWU*

The Biopharmaceutical classification system is a scientific framework which classifies a drug based on its aqueous solubility and intestinal permeability. Drug substances may be grouped into one of four categories as illustrated: Class 2 drugs are dissolution rate limited and is therefore our first choice for the preparation of ASDs. Based on the information provided in the previous slide, nevirapine is dissolution rate limited and is classified as a BCS class II drug which makes nevirapine and any other BCS class II drug an excellent candidate for ASD development.

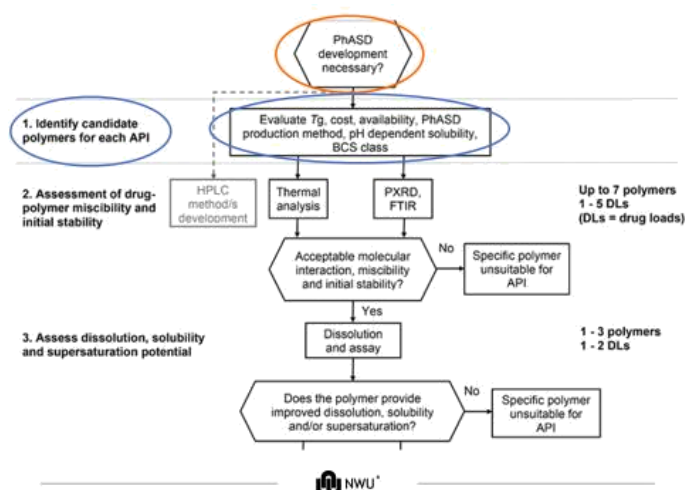
Purpose

- The aim of this study was to improve the aqueous solubility and dissolution rate of nevirapine by creating stable amorphous solid dispersions using a modified screening of polymers for amorphous drug stabilisation (SPADS) process.

NWU*

The aim of this study was to improve the aqueous solubility, and dissolution rate, of nevirapine by creating stable ASDs using a modified screening of polymers for amorphous drug stabilization (SPADS) process.

In order to find the optimal combination and ratio of API:polymer the following SPADS method was used.



This figure is a flow chart indicating the most optimal and economic process which streamlines polymer selection and amorphous solid dispersion production to minimise time and resources spent in a well-equipped academic laboratory setting such as the Pharmatech labs where my experiments took place. The general method is known as SPADS and enables a researcher to find the correct combination and ratios of polymer and API.

This method is designed to eliminate polymer candidates which are unsuitable for stabilising a specific active pharmaceutical ingredient in amorphous solid dispersion form. In the end, only the best polymer and ratio for nevirapine which remained after all the assessments, was deemed suitable for amorphous solid dispersion product formulation. The method begun by identifying the necessity of ASD development for the chosen API – Nevirapine. Secondly, candidate polymers were identified by evaluating cost, availability, ASD production methods and glass transition temperature. Initial screening begun with 64 different ratios of API:polymer.

Glass Transition Temperature (T_g)

- A general proposal of the storage temperature (usually ambient) being less than $T_g - 50^\circ\text{C}$ has been provided as a method to predict whether an ASD will be stable under long-term conditions.
- All the polymers used in this study are GRAS polymers.

A general proposal of the storage temperature being less than 50°C below the glass transition temperature has been provided as a method to predict whether an ASD will be stable under long-term conditions. This general rule assumes that if the glass transition temperature is greater than 50°C above storage temperatures the molecular mobility in the glassy state will be suppressed sufficiently to provide long-term stability

API-Polymer	Ratio	Resulting T _g (°C)
NEV:HPMC	1:1	131.0
NEV:HPMC	1:2	145.7
NEV:HPMC	1:3	153.0
NEV:HPMC	1:4	157.4
NEV:HPMC	4:1	104.6
NEV:HPMC	3:1	109.0
NEV:HPMC	2:1	116.3
NEV:HPMCAS	1:1	100.0
NEV:HPMCAS	1:2	104.3
NEV:HPMCAS	1:3	106.5
NEV:HPMCAS	1:4	107.8
NEV:HPMCAS	4:1	92.2
NEV:HPMCAS	3:1	93.5
NEV:HPMCAS	2:1	95.7



The following table was drawn up after determining the theoretical resulting glass transition temperature for each physical mixture of active pharmaceutical ingredient (API) and polymer in ratios ranging from 1:1 – 1:4.

API-Polymer	Ratio	Resulting T _g (°C)
NEV:PVP 25	1:1	123.5
NEV:PVP 25	1:2	135.7
NEV:PVP 25	1:3	141.8
NEV:PVP 25	1:4	145.4
NEV:PVP 25	4:1	101.6
NEV:PVP 25	3:1	105.3
NEV:PVP 25	2:1	111.3
NEV:PVP 30	1:1	131.0
NEV:PVP 30	1:2	145.7
NEV:PVP 30	1:3	153.0
NEV:PVP 30	1:4	157.4
NEV:PVP 30	4:1	104.6
NEV:PVP 30	3:1	109.0
NEV:PVP 30	2:1	116.3



Only the results conforming to above mentioned “rule of thumb” are included in the table. This served as an initial screening process in order to determine which mixture would, theoretically speaking, be a potential candidate and undergo further investigation.

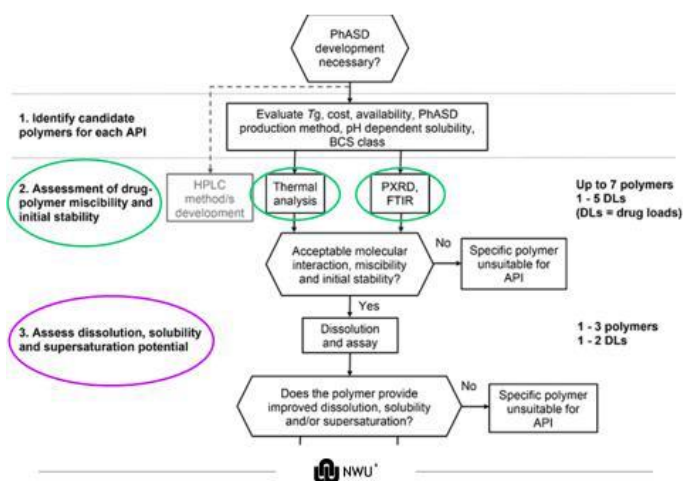
API-Polymer	Ratio	Resulting T _g (°C)
NEV:PVP 90	1:1	138.5
NEV:PVP 90	1:2	155.7
NEV:PVP 90	1:3	164.3
NEV:PVP 90	1:4	169.4
NEV:PVP 90	4:1	107.6
NEV:PVP 90	3:1	112.8
NEV:PVP 90	2:1	121.3
NEV:PVP VA64	1:1	96.5
NEV:PVP VA64	1:2	99.7
NEV:PVP VA64	1:3	101.3
NEV:PVP VA64	1:4	102.2
NEV:PVP VA64	4:1	90.8
NEV:PVP VA64	3:1	91.8
NEV:PVP VA64	2:1	93.3



All polymers which were used during this study are generally regarded as safe as these polymers are commonly used for other purposes as well as in the final production of both food and medicine

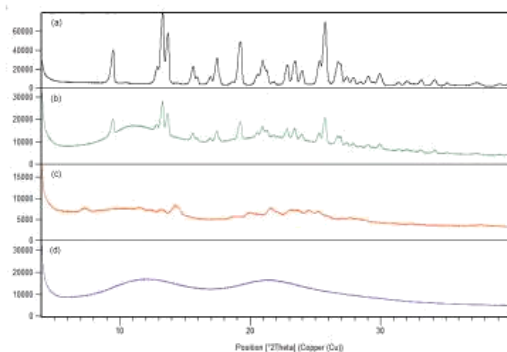
Production Methods

- SPADS indicated that the most successful ASD was produced by rapid solvent evaporation with a specific organic solvent.
- Rapid solvent evaporation was performed with a rotary evaporator as well as a spray-dryer.
- The exact polymer and/or polymer combinations as well as ratio/s may not be disclosed due to IP restrictions.



Various production methods such as physical mixture with a mortar and pestle, hot-melt by heating on a hot plate as well as in an oven under vacuum and rapid solvent evaporation were attempted. SPADS indicated that the most successful ASD was to be produced by rapid solvent evaporation with a specific organic solvent. Rapid solvent evaporation was performed with a rotary evaporator as well as a spray dryer. The exact polymer and/or polymer combinations as well as ratio/s may not be disclosed due to IP restrictions.

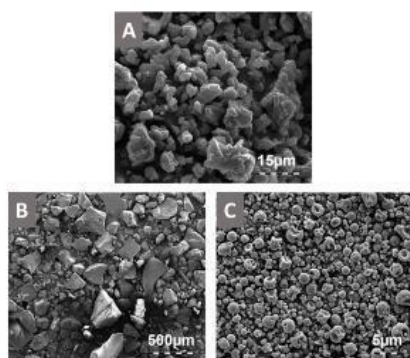
After identifying possible candidates, miscibility and stability was tested for each of the above mentioned combinations by means of Simultaneous Thermal Analysis (STA:TGA/DSC) and X-ray powder diffraction (XRPD). Candidates proving to be amorphous throughout this phase were subjected to dissolution studies, and only those who delivered an improvement in dissolution and apparent solubility remained for further investigation.



An example of the effect of the method of preparation on the amorphous nature of nevirapine:polymer produced by different methods, as determined by XRPD: (a) nevirapine Form I; (b) NEV:polymerX physical mixture; (c) NEV:polymerX solvent evaporation prepared with a rotary evaporator; and (d) NEV:polymerX prepared by spray-drying.



This image is an example of the effect of the method of preparation on the amorphous nature of nevirapine:polymer produced by different methods, as determined by XRPD: (a) is nevirapine Form I which is completely crystalline. (b) Indicates nevirapine:polymerX prepared through physical mixture with a mortar and pestle and proved to be mainly crystalline with a slight amorphous component. The next diffractogram (c) showed the product which was prepared by means of solvent evaporation, rotary evaporator. This product was mainly amorphous with a slight crystalline component. Only the XRPD was able to indicate that a (C) has a slight crystalline component, all the other techniques used did not indicate this. Due to the low peak intensity and peak broadening, it is most likely that C is a nano-crystalline solid dispersion. The ASD prepared with the spray dryer which is (D) in the image proved to be most successful as it presented to be completely amorphous. The spray dried product will be the most stable because C already has a partial crystalline component which can lead to further crystallisation.

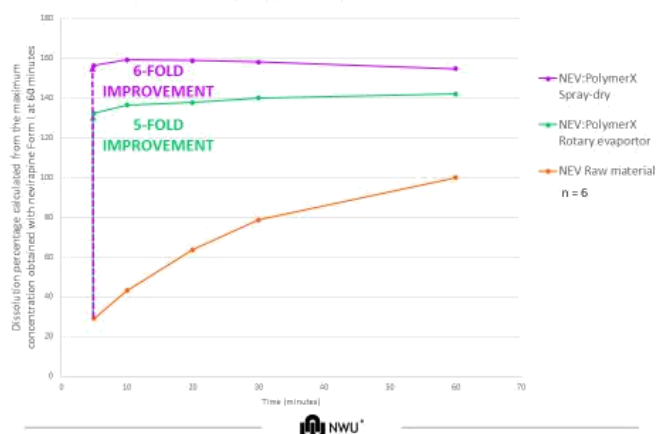


SEM images of: (A) Commercial anhydrous Form I; (B) NEV:polymerX nano-crystalline dispersion prepared by rapid solvent evaporation; (C) NEV:polymerX ASD prepared by spray drying.



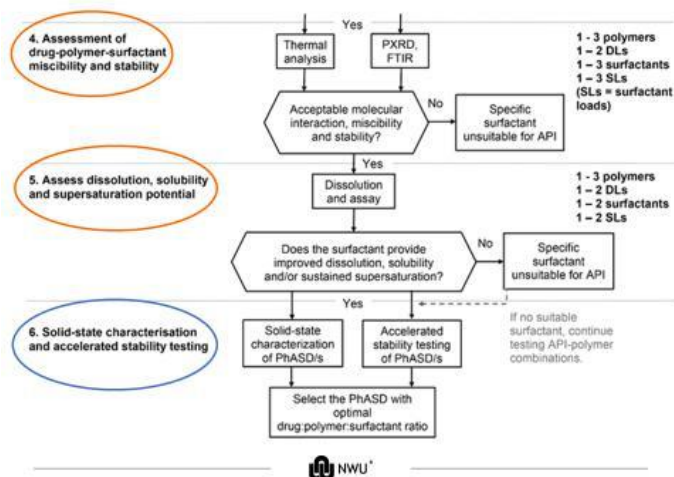
Illustrated here are scanning electron microscopy images of various forms of nevirapine. (a) Being the commercial anhydrous Form I where the particles look as if it has been milled to reduce particle size. (b) Is the nevirapine:polymer X mixture prepared by rapid solvent evaporation. The particles are mostly plate-like, amorphous particles. (c) Illustrates the nevirapine:polymer ASD prepared by spray-drying. The spray-dried particles are much smaller and spherical in size.

Powder Dissolution Profile



Dissolution studies were performed on i) Nevirapine raw material, ii) Solid dispersion prepared by solvent evaporation, rotary evaporator and iii) ASD prepared by solvent evaporation, spray dry. For the nano-crystalline solid dispersion prepared by rotary evaporation, an approximate five-fold improvement in apparent solubility over nevirapine Form I was observed after 5 minutes of powder dissolution testing. The spray dried ASD, with smaller and more uniform particle size performed even better and the improvement in apparent solubility at 5 minutes was six-fold that of Form I. A large solubility advantage was maintained for the 60 minute dissolution testing. There was no pronounced spring and parachute effect due to the presence of the polymer

inhibiting the crystallisation and precipitation of the dissolved nevirapine.



As nevirapine did not present with wettability challenges/problems during the standard powder dissolution test, the fourth and fifth step in this model, assessment of drug-polymer-surfactant miscibility and stability, and the dissolution thereof was not performed. The candidates that delivered an improvement in dissolution and apparent solubility underwent the final part of this study which was accelerated stability testing at 40°C and 75% RH over a period of three months.

Stability

- Samples with enhanced dissolution profiles were exposed to three months accelerated stability tests at 40°C and 75% RH.
- Stability was found to be acceptable, with no crystal growth or chemical degradation detected after 3 months of exposure to extreme conditions.

Stability was found to be acceptable, with no crystal growth or chemical degradation detected after 3 months of exposure to extreme conditions.



Conclusion

- The most successful candidate was thus chosen based on the best balance between dissolution, apparent solubility, sustainability of the solubility benefit over time as well as stability.

I conclude with stating that the use of the modified SPADS method was extremely successful as a solid dispersion presenting with acceptable physico-chemical properties and consequently the best pharmaceutical relevant parameters such as apparent solubility, and outstanding improvement in dissolution and stability was formulated.



Acknowledgements

- This work is based on the research supported in part by the National Research Foundation of South Africa (Grant Number: 112078) and North-West University.



Pharmacien



National
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ANNEXURE B

CERTIFICATE RECEIVED FOR

APSSA YOUNG SCIENTIST RUNNER UP

AT THE FIRST CONFERENCE OF BIOMEDICAL AND

NATURAL SCIENCES AND THEAPEUTICS (CoBNeST 2018)



It is hereby certified that the

**Young Scientist
Runner-Up**

was awarded to

Nadine de Melim

at the First Conference of Biomedical and Natural Sciences and Therapeutics (CoBNeST 2018)

Chair: APSSA

Chair: CoBNeST

CoBNeST



ANNEXURE C

PATENT APPLICATION

D.M. KISCH INC., Johannesburg
Patent Trade Mark & Copyright Attorneys

South Africa
T: 011 461 1111

P55120ZAPO

ASSIGNMENT OF INVENTION

WHEREAS I/we

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2531
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LIEBENSBERG, Wilma
2 Kiewiet Street
Baillie Park
Potchefstroom
2531
South Africa

DE MELM, Nadine
20 Carel Roode Laan
Lichtenburg
2740
South Africa

own the invention(s) of an invention defined:

NEVIRAPINE AMORPHOUS SOLID DISPERSION

AND WHEREAS **NORTH-WEST UNIVERSITY**

is
1 Hoffman Street
Joos van Rooy Building
Potchefstroom
2531
South Africa

has / have acquired the said invention from me/us for all countries

NOW THEREFORE this Deed witnesses that I/we have assigned the said invention to

NORTH-WEST UNIVERSITY



its successors, assigns or legal representatives, for all countries, together with the right to apply for Letters Patent in respect thereof in his / their own name, the assignment taking effect on or before

27 July 2018

I/we agree that when required, I /we will, without charge to the assignee, but at its expense, sign all papers, take all rightful oaths, and do all acts which may be necessary, desirable or convenient for securing and maintaining patents for said invention in any and all countries and for vesting title thereto in said assignee, its successor, assigns, or legal representative.

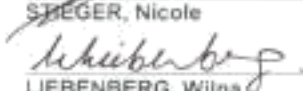
Date: 13-08-2018

Signature:


STEGER, Nicole

Date: 13-08-2018

Signature:


LIEBENBERG, Wilna

Date: 14/08/2018

Signature:


DE MELIM, Nadine