FORMULATION OF A NEVIRAPINE CO-CRYSTAL AS A LIQUID DOSAGE FORM

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A full thesis submitted in partial fulfilment of the requirements for the degree of Magister Pharmaceuticae

Faculty of Natural Science

University of the Western Cape, South Africa

December 2015

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Abstract

Co-crystals are a solid phase phenomena that could enhance the physicochemical properties of an active pharmaceutical ingredient. A co-crystal has never been incorporated into a liquid dosage form with the assurance of maintaining its co-crystal state until absorption under defined conditions. This study aims to develop a liquid formulatiln with a nevirapine co-crystal.

A protocol was developed to investigate all the five co-formers that were used to make the nevirapine co-crystals to-date. The most appropriate co-former was selected for a liquid dosage form to study the integrity and the scaling up of the co-crystal in a suspension formulation. Co-formers used were viz. saccharin, glutaric acid, salicylic acid, rac-tartaric acid and maleic acid. These were characterized according to their physical, chemical, pharmacological and pharmaceutical properties. A grading scale was used to select the most appropriate co-former for a suspension formulation. Comparatively, saccharin produced the best combination of physical, chemical, pharmacological and pharmaceutical properties, especially with regard to the particle size and the specific gravity which proved to be very useful as optimal criteria for suspension formulation. Upon selection of the ideal co-former, scale-up of the nevirapine saccharin co-crystal was performed from a small scale of 350 mg to a large scale of 5 g. Nevirapine-saccharin (NVSC) co-crystals were prepared utilizing the slow evaporation technique, using methanol as the solvent and the percentage yield of the co-crystals were > 80 %. The identity of co-crystals was confirmed using hot stage microscopy (HSM), differential scanning calorimetry (DSC), fourier transform infra-red (FTIR) and thermogravimetric analysis (TGA).

Three co-crystal suspension formulations were prepared using the excipients identified in the branded, Viramune® suspension, with each formulation containing viscosity enhancers such as aerosil 200, carbopol 971G and carbopol 974P. To ascertain the co-crystal integrity in the suspension, it was filtered and the filtrate was identified with DSC and FTIR while the filtered solution was identified with ultraviolet spectroscopy (UV). The co-crystal suspension formulation with optimal pH, viscosity and assurance

of co-crystal integrity was the carbopol 974P formulation. The UV and DSC of the filtrate of the suspension revealed that the co-crystal had not separated into its individual components and remained intact while in suspension form irrespective of the excipients added.

This formulation proceeded to the quality control stage. It was assessed for its pH, viscosity and dissolution according to the USP 32 standards and compared to the branded nevirapine suspension, Viramune ®, presently on the market. The suspension was characterized for particle size, zeta potential and polydispersity index. The dissolution results assayed by High Performance Liquid Chromatography (HPLC) revealed a drug release of 86 % in the Viramune® suspension while the NVSC co-crystal suspension achieved a drug release of 94% within 30 minutes of dissolution.

Acknowledgement

I owe my heartfelt appreciation to the following:

Dr. Samsodien, my supervisor, for her guidance, insight and motivation that enabled me to pursue this project. Thank you for your patience and supervision. To Mr. Rossiter as my co-supervisor for his valuable input and support.

Mr. Kippie for his valuable assistance with the viscometer equipment. Dr. Mbamalu and Mr. Bepe for their gracious assistance with the HPLC.

Fellow post-graduate colleagues and staff in the Discipline of Pharmaceutics, for their encouragement.

To my parents, for being my source of inspiration and for constant encouragement, without which I would not have made it thus far. To my brother, Steven, for helping me to see the lighter side of things.

To all my family members and various friends, both far and near who have always encouraged and supported me.

To the National Research Foundation of South Africa for their financial assistance towards conducting this project.

Most importantly to God, from whom all blessings flow.

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List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
API	Active Pharmaceutical Ingredient
BCS	Biopharmaceutical Classification System
COA	Certificate of Analysis
DSC	Differential Scanning Calorimetry
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
GRAS	Generally Regarded As Safe
HAART	Highly Active Retroviral Treatment
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HSM	Hot Stage Microscopy
IC_{50}	Inhibitory Concentration where 50% of virus is inhibited
LD_{50}	Lethal Dose that kills 50% of test subjects
LDV	Laser Doppler Velocimetry
NNRTI	Non- Nucleoside Reverse Transcriptase Inhibitor
NVGLT	Nevirapine-glutaric acid co-crystal
NVMLE	Nevirapine-maleic acid co-crystal
NVSC	Nevirapine-saccharin co-crystal
NVSLI	Nevirapine-salicylic acid co-crystal
NVTTA	Nevirapine-rac-tartaric acid co-crystal
PXRD	Powder X-ray Diffraction
SCXRD	Single Crystal X-ray Diffraction
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis
USP	United States Pharmacopeia
UV	Ultra Violet Spectroscopy

Dedication

This thesis is dedicated to my parents for all their love and support.

Academic output

Part of this thesis has been presented as an oral podium presentation:

Sahana Injety*, Halima Samsodien and Richard Rossiter, *Formulation of a nevirapine co-crystal as a liquid dosage form.* Presented under the Young Scientist category at the 36th Annual Conference of the Academy of Pharmaceutical Sciences of South Africa, Johannesburg, 17 September 2015.

Chapter 1
Introduction

Chapter 1 Introduction

1.1 Process of drug discovery to drug delivery

The discovery of a potent compound necessary for therapeutic effect is a significant milestone in the drug discovery process. However, this compound can only produce its effect when sufficient quantity reaches the site of action at a suitable rate upon administration. For a drug to reach its site of action it has to be formulated into a dosage form.

To gain approval for public use, the discovered drug has to undergo a well-defined and extensive process laid out by regulatory bodies such as the Food and Drug Administration (FDA) in the United States of America or the Medicines Control Council in South Africa. The proposed drug has to be physically, chemically and biologically characterized for pharmaceutical effects, pharmacological effects, toxicological effects and for potential therapeutic application.[1] Preformulation studies describe the physical and chemical properties of the drug. It deals with the rational, science-based requirements for drug substances, some of which include physicochemical stability, consistency and solid state properties such as structural properties, dimensional properties, chemical, mechanical and electrical properties.[2]

Formulation studies subsequently follow preformulation studies, this segment deals with the final composition of the active drug and functional excipient substances used in the dosage form. It focuses on the stability of the product and drug release properties that are necessary for the product to have an optimal therapeutic effect.[2]

The formulation phase deals with the development of a stable, bioavailable and optimal dosage form for a specific route of administration. The purpose of the formulation phase is to formulate the active drug into a suitable dosage form and to find the most feasible formulation strategy appropriate for scaling up and production in the manufacturing industry.[1] The preparation of an acceptable dosage form should be in accordance with regulatory frameworks such as the United States pharmacopeia, European pharmacopeia, Japanese pharmacopeia or Indian pharmacopeia, a reference

book consisting of directions for identification of medicines, published by the authority of a government.

Formulation involves appropriate consideration of each ingredient and its role in the final product. These considerations include the physical, chemical and biological characteristics of these ingredients together with the compatibility thereof, to create a product that will enhance patient compliance (i.e. easy to administer, palatable and well tolerated) as well as stable and an efficacious drug product.[3]

Thereafter, the drug undergoes a carefully developed program of preclinical and clinical studies to prove the drug's safety and effectiveness for its proposed use.[1]

1.2 Formulation

Formulation studies develop the initial features of the proposed pharmaceutical product or dosage form. It is vital for a drug to be formulated in a suitable dosage form because inappropriate dosage forms or invalidated manufacturing processes can result in disastrous consequences during clinical trials. The final formulation includes excipients which have specialized functions and fashion medicinal agents into efficacious and appealing dosage forms.[4]

1.3 Dosage Forms

Drugs are rarely administered alone because minute quantities are required for therapeutic effect. The primary reason for incorporating a drug substance into a dosage form is for easy handling. Dosage forms are designed to provide the drug in a suitable form for absorption from the selected route of administration.[5]

Most drug substances used today are unpalatable and unattractive in their natural form. An appropriate drug will only have its beneficial effect if the patient adheres to the therapeutic regimen. Modern pharmaceutical formulations aim to satisfy the triple combination of flavour, taste and colour as this triad contributes to patient acceptability.[3]

Information gathered from the preformulation studies of the active pharmaceutical ingredients (API) can be used as a framework in the fabrication of a dosage form. Dosage forms can be designed by alternative delivery routes to maximize therapeutic response. The most common and convenient route of administration is the oral route and one of the ways in which the rate and extent of transportation of the drug into the blood circulation can be controlled is by the addition of excipients.

Dosage forms are classified into solid dosage forms such as tablets, capsules, powders and granules while liquid dosage forms comprises of solutions, syrups, suspensions and emulsions.

For a medicinal agent to be formulated into a dosage form, the age of the patient and the manner in which the disease is treated is considered (local or systemic action). Adults are given dosage forms that require less frequency of administration without compromising the efficacy. Tablets and capsules are usually given to adults because of their simplicity in storage and handling. Oral dosage forms are also most convenient in the self-administration of medication.

Different dosage forms are preferred according to the age of the paediatric patient. Suppositories are preferred for neonates while solutions and syrups for infants. Liquid preparations usually utilize benzyl alcohol as a preservative and this is not suitable when administered to neonates due to immature metabolic function, however it is suitable for infants.[6] Solutions, syrups, suspensions or effervescent dosage forms can be administered for two to five year olds. The taste of a bitter drug can be masked by using these dosage forms hence they are suitable for administration for two to five year olds. A solid dosage form such as tablet or capsule is generally acceptable from six years onwards.[6] At this age, it is generally considered safe to swallow a tablet.[6]

With respect to the paediatric population, dosage forms are challenging to develop for this particular group because the prerequisites such as a measurable dosage form to administer based on body weight and taste masking are unique for paediatric oral formulations.

The formulation philosophy for developing a paediatric dosage form is ‘‘from simple to complex,’’ which involves minimizing the number of excipients and developing the simplest possible formulation and manufacturing process.[6]

Therapeutic situations also affect the selection of a dosage form, for instance in motion sickness a patient may be uncomfortable in taking medication orally hence skin patches can be used instead.[1]

1.3.1 Liquid dosage forms

Liquid dosage forms are typically utilized by geriatric and paediatric patients. In comparison to solid dosage forms, they have an advantage of faster onset of action due to the absence of the disintegration step thus they have a more rapid absorption.[5] Liquid dosage forms also provide greater convenience in administration, where larger doses are required.[7] Many people, particularly the paediatric and elderly groups struggle to swallow solid dosage forms and thus prefer a drug that is dispersed in a liquid.[8]

Challenges involved in developing liquid dosage forms include stability of the drug in solution, solubility of the drug and an acceptable taste. An additional challenge that is observed is that the oral liquid dosage forms have a risk of microbial contamination.

1.3.1.1 Solutions

Solutions are defined as liquid preparations that contain one or more chemical substances dissolved in a suitable solvent or mixture of mutually miscible solvents.[1]

Types of solutions

Solutions can further be classified as oral solutions, otic solutions, ophthalmic solutions or topical solutions. Oral solutions can further be categorized into aqueous solutions and non-aqueous solutions. Aqueous solutions containing sugar are classified as syrups. Solutions containing hydroalcoholic solutions are known as elixirs and solutions prepared from crude drugs are referred to as tinctures.

Preparation of solutions

In formulating a solution, considerations such as the solubility and stability of the solute in the solvent to be employed is important. Upon administration of solutions, the absorption form the gastrointestinal tract into systemic circulation is more rapid than suspensions or solid dosage forms of the same drug. For a drug to be formed as a solution it is necessary for the drug to be soluble in an aqueous system. Solvents that can be employed in the preparation of solutions are ethyl alcohol, ethanol, glycerol, propylene glycol and water.[9]

Solutions can be prepared by simply dissolving the solute in the solvent. A pharmaceutical product that is formulated in this manner is calcium hydroxide solution, where calcium hydroxide is mixed with water. Solutions can also be prepared by reacting two or more solutes in a suitable solvent. An example of this is aluminium subacetate, it is prepared by reacting aluminium sulphate solution with calcium carbonate and acetic acid.[1]

Nearly 80 – 90 % of drugs are poorly soluble.[10] The term 'poorly soluble' is used when 30 to 100 parts of solvent are required for one part of solute. Thus, large amounts of solvent are required to dissolve minute amounts of the drug. In practice, solutions are preferred to be given as 5 ml or 15 ml (equivalent to one teaspoon and tablespoon, respectively). Thus, for poorly soluble drugs in a solution form, doses will have to be increased to more than a teaspoon or tablespoon and this would hamper patient acceptability.

1.3.2 Disperse systems

Dispersed systems consist of particles, known as the dispersed phase which is distributed throughout a medium known as the continuous or dispersion medium.[11] Dispersed systems are classified on the basis of the mean particle diameter of the dispersed matter. Three types of disperse systems exist: molecular dispersions, colloidal dispersions and coarse dispersion systems.

1.3.2.1 Suspensions

Suspensions are a class of dispersed systems in which a finely divided solid material known as the internal phase is dispersed uniformly in a liquid dispersion medium which is known as the suspending medium.[12]

The internal phase consists of insoluble solid particles which has a size of 0.5 to 5 μm and which are maintained uniformly throughout the suspending vehicle with the aid of a single or a combination of suspending agents.

Types of suspensions

Suspensions can be classified according to their size. Colloidal suspensions are those that have a particles size of less than 1 μm, coarse suspensions contain particles that are greater than 1 μm in size and nano suspensions consists of particles smaller than 1 μm. The practical upper limit for individual suspendable solid particles in coarse suspensions is approximately 50 to 75 μm.[12]

Flocculated & Deflocculated suspensions

In a flocculated system, light, fluffy conglomerates that are held together by weak Van der Waals forces called flocs are formed.[11] They settle rapidly but they do not form a hard cake and thus they can be easily resuspended.

Flocs tend to fall together, producing a distinct boundary between the sediment and supernatant liquid. The liquid above the sediment is clear because even the small particles in the system are associated with the flocs. However, from an aesthetic point of view, flocculated suspensions have a supernatant layer which may be unattractive. Floc formation of particles decreases the surface free energy between the particles and liquid medium thus acquiring thermodynamic stability. The structure of flocs contains small amounts of liquid entrapped within the flocs. The entrapment of liquid within the flocs increases the sedimentation volume and the sediment is thus easily redispersed

by a small amount of agitation. To prepare a flocculated suspension, a flocculating agent is used to produce the formation of flocs.[11]

In a deflocculated suspension, larger particles settle more rapidly than smaller particles. There is no clear boundary formed and the supernatant remains turbid for a long period of time. During the initial stages of settling, clear or turbid supernatant is a good indication of whether the system is flocculated or deflocculated, respectively (Fig. 1.1).[11]

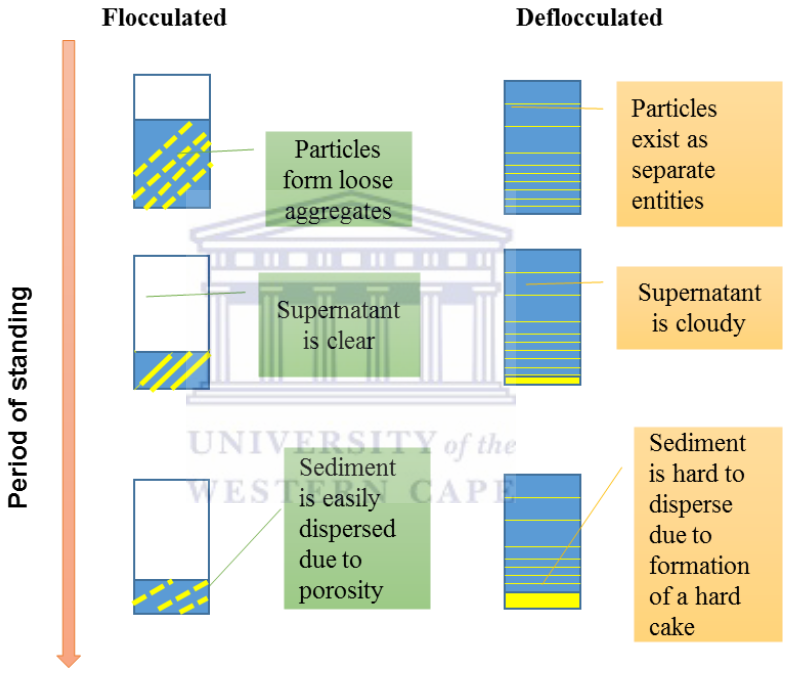

Figure 1.1 Differences between flocculated and deflocculated suspension

Preparation of suspensions

Suspensions can be formed through either precipitation methods or through dispersion methods. Precipitation methods can further be divided into organic solvent precipitation, precipitation effected by changing the pH of the medium and double decomposition. Organic solvent precipitation can be achieved by dissolving drugs in water-miscible organic solvents and then adding the organic phase to distilled water.

Ethanol, methanol, propylene glycol and polyethylene glycol are examples of organic solvents.[13]

The technique of changing the pH of the medium is applicable to the drugs whose solubility is dependent on pH. For example, in the case of the drug estradiol, suspensions can be prepared by changing the pH of its aqueous solution.[14]

The dispersion method consists of a vehicle that is formulated so that the solid phase is easily wetted and dispersed. Glycerin and sorbitol are generally used in cases where a hydrophobic drug is needed to be adequately wet. The use of a surfactant can also be accomplished to improve dispersion. Small quantities should be tested to see which approach works better, the dispersion method or usage of a surfactant.[14]

1.3.2.2 Emulsions

An emulsion is a dispersion in which the dispersed phase is composed of small globules of a liquid distributed throughout a vehicle in which it is immiscible.[1]

Two phases exist in emulsions, the dispersed phase and the dispersion medium. The particle size of the dispersed phase is between 0.1 to 100 μm.[14]

Types of Emulsions

There are two types of emulsions, oil-in-water and water-in-oil emulsions (Fig 1.2). When oil droplets are dispersed throughout the aqueous phase it is referred to as an oil-in-water emulsion. Water soluble drugs are released more quickly from oil-in-water emulsions because of the presence of hydrophilic parts that are attracted to the external phase. The oil is readily absorbed in the gastro intestinal tract due to its high permeability and thus it is suitable to be used internally.[14] This type of emulsion is suitable for medicinal oils that have a bitter and oily taste. An example of this is liquid paraffin emulsion, the water forms a continuous phase and the liquid paraffin is surrounded by a thin film emulgent which masks the bitter and oily taste of the drug.[14]

When water is dispersed as globules in oil, it is referred to as a water-in-oil emulsion. Oil soluble drugs are formulated as water-in-oil emulsions. They have an occlusive effect and greasy in texture, therefore making it suitable for cleaning the skin from oil soluble dirt. This makes it suitable to be used externally. An example of this is cold cream, it is greasy and not water washable and is used externally to prevent evaporation of moisture from the skin.[14]

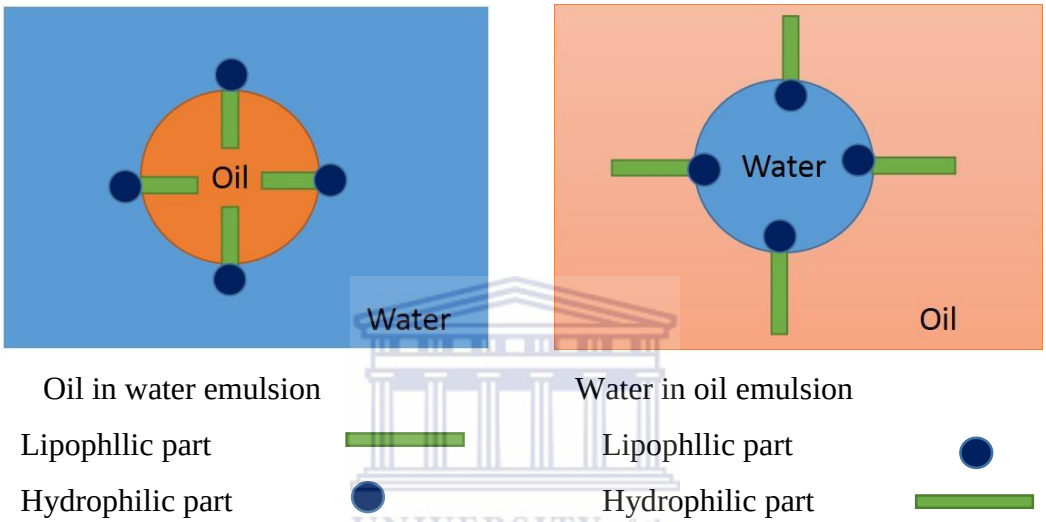

Figure 1.2 Illustration of oil in water emulsion and water in oil emulsion

An important parameter to the formulation of emulsions is stability. Instability of emulsions is shown by flocculation, creaming, coalescence or phase inversion.[14] Flocculation of emulsions is the same as flocculation of suspensions, where small particles aggregate to form a larger particle that is redispersable upon shaking. Creaming is a phenomenon in which the dispersed phase separates on top of the continuous phase. This can also be redispersed upon shaking. Coalescence is presented when the mechanical or electrical barrier is insufficient to prevent the formation of progressively larger droplets. Phase inversion occurs when the dispersed phase becomes the continuous phase, for example when oil-in-water becomes water-in-oil.

Preparation of emulsions

Emulsions can be prepared by mainly two methods, the dry gum method and the wet gum method. Both these methods utilize gum, water and oil. In the dry gum method the oil is triturated with gum and with a little amount of water to form a primary emulsion. The trituration process is continued until a clicking sound is heard. Water is slowly added to form the final emulsion. In the wet gum method, the gum and water are triturated to form a mucilage.[14] Oil is added in small quantities to form the primary emulsion. Thereafter, water is added to make the final emulsion.

The membrane emulsification method is a relatively novel concept, whereby droplets are generated to produce an emulsion. Pressure is applied directly to the dispersed phase which seeps through a porous membrane into the continuous phase. Droplets are formed and remain detached from the surface due to the shear motion between the continuous phase and membrane surface.[15]

1.4 Human Immunodeficiency Virus (HIV)

Since the discovery of HIV in 1983, efforts have been made by researchers towards the discovery of anti-HIV drugs.[16]

One new infection occurs every ten seconds or 7400 new infections occur every day. 33 million people are now living with HIV, 67% of them live in sub-Saharan Africa.[17] Despite these alarming statistics, the use of antiretroviral drugs is useful in limiting the pandemic and prolonging the lives of those infected. The roll out of antiretroviral drugs has a significant reduction in Acquired Immune Deficiency Syndrome (AIDs) related mortality and improved survival. Highly Active Retroviral Treatment (HAART) is a triple drug combination therapy consisting of nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors, each class of the anti-retroviral agent targets a different step in the viral cycle of the HIV thus preventing immune deterioration.[16]

1.5 Nevirapine

Nevirapine (Fig 1.3) is a potent Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI). It was the first approved drug from this class in 1996 by the FDA.[18]

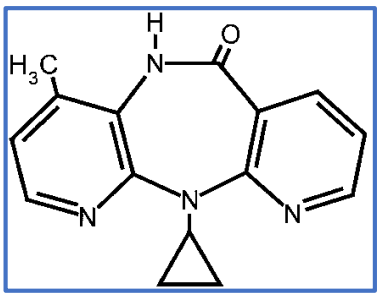

Figure 1.3 Chemical structure of nevirapine

1.5.1 Synthesis of nevirapine

Researchers at Boehringer Ingelheim Pharmaceuticals discovered nevirapine. The synthesis of nevirapine begins with acylation of 3-amino-2-chloro-4-methylpyridine with 2-chloronicotinoyl chloride to form 2,2′-dichloro amide. Thereafter, the 2′-chlorine atom is displaced by cyclopropylamine. Nevirapine synthesis is complete by ring closure/cyclisation which is achieved by heating under reflux.[18]

1.5.2 Physiocochemical properties of nevirapine

Nevirapine is classified as the dipyridodiazepones class of compounds. The chemical name for it is 11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2b:2′,3′*e*] [1,4]diazepin-6-one. The molecular formula is $C_{15}H_{14}N_4O$. The molecular weight is 266.30 g/mol.[19] It is a white, or almost white, crystalline powder. It is a weak base with a pKa value of 2.8 and exhibits pH dependent solubility. Nevirapine is a hydrophobic molecule and is classified as a class II drug according to the Biopharmaceutical Classification System (BCS).

1.5.1 Pharmacological properties

1.5.1.1 Mechanism of action

Nevirapine, an NNRTI, acts by binding directly to the HIV reverse transcriptase enzyme and it blocks the ribonucleic acid dependent and deoxyribonucleic dependent polymerase activities by causing a disruption of the enzyme catalytic site.[20]

1.5.2.2 Use of nevirapine

Nevirapine has demonstrated potent and sustained antiviral activity when used in HAART with drugs of the nucleoside and protease inhibitor classes. Nevirapine is used for the treatment of HIV-1 infection in both young and adult patients. It is the blockbuster drug for prevention of mother-to-child transmission of HIV. Reduction of HIV transmission by 40% is achieved by administration of a single dose of nevirapine to the mother during labour and to the infant after birth.[21]

1.5.2.3 Dosage of nevirapine

Nevirapine is given to adults as 200 mg orally for 14 days followed by 200 mg twice a day. The paediatric dose of nevirapine for 2 months to 8 years old is 4 mg/kg once daily for 2 weeks thereafter 7 mg/kg twice daily. For children over 8 years old 4 mg/kg is given once daily, for two weeks thereafter 4 mg/kg is given twice a day. A maximum of 400 mg/day of nevirapine is advised. In South Africa, it is available under the trade names Viramune®, Aspen Nevirapine®, Auro-Nevirapine®, Cipla Nevirapine®, Sonke-Nevirapine® and Adco-Nevirapine®.

1.5.3 Pharmacokinetics

1.5.3.3 Absorption

Nevirapine is readily absorbed (greater than 90%) after oral administration. Peak plasma nevirapine concentrations of 2 ± 0.4 μg/mL are attained by 4 hours following a

single 200 mg dose.[20] Following multiple doses, nevirapine peak concentrations appear to increase linearly in the dose range of 200 to 400 mg/day.

Upon administration of Viramune® with either a high-fat breakfast or antacid, the extent of absorption is comparable to that observed under fasting conditions. Thus, Viramune® can be administered with or without food or antacid.[20]

1.5.3.2 Distribution

Nevirapine is highly lipophilic and is nonionized at physiologic pH. After intravenous administration to healthy adults, the volume of distribution of nevirapine is 1.21 L/kg, this indicates that nevirapine is widely distributed. Nevirapine readily crosses the placenta and is found in breast milk. Nevirapine has a half-life of 45 hours. A single dose is decreased by auto-induction to about 25 to 30 hours after multiple dosing.[20]

1.5.3.3 Metabolism

Nevirapine is extensively metabolized by P450 enzyme to hydroxylated glucuronides. The multiple dose pharmacokinetics is characterized by metabolic autoinduction of cytochrome P450 isoenzymes, 3A (CYP3A) and 2B6 (CYP2B6) resulting in a 1.5 to 2 fold increase in nevirapine apparent systemic clearance as treatment continues from a single dose to 2 weeks of dosing with 200 mg/day or higher.[20]

1.5.3.4 Excretion

In vivo and *in vitro* trials have shown that more than 80 % of radioactivity in urine is made of glucuronide conjugates of hydroxylated metabolites; this indicates that cytochrome P450 metabolism, glucuronide conjugation, and urinary excretion of glucuronidated metabolites are the main route of nevirapine biotransformation and elimination in humans. Less than 3 % of the dose is excreted in the urine as the parent compound.[20] Renal excretion plays a minor role in elimination of nevirapine.

1.5.3.5 Protein binding

Nevirapine is 60 % bound to plasma proteins in the plasma concentration range of 1-10 μg/mL. The concentration in human cerebrospinal fluid is 45 % of the concentration in plasma; this ratio indicates that it is approximately equal to the fraction not bound to the plasma proteins.[7]

1.5.4 Pharmaceutical aspects

1.5.4.1 Solubility

The solubility of the anhydrous nevirapine is 0.123 mg/mL.[19] It is practically insoluble in water; however it is sparingly soluble or slightly soluble in methylene chloride and slightly soluble in lower alcohols. Interestingly, it is highly soluble at a pH of less than three, due to protonation of its weak basic functional group.[6] The aqueous solubility decreases with an increase in pH to 0.1 mg/mL at neutral pH.

Nevirapine is a hydrophobic drug and at doses of 50 mg and higher it exhibits characteristics of solubility rate-limited absorption (delayed time-to-peak, multiple peak concentrations) which could lead to a slight decrease in bioavailability.[20]

1.5.1.2 Stability

Nevirapine is stable at room temperature.[19] Nevirapine has a high stability in the solid form and it is not susceptible to degradation under light exposure. It has a high melting point (246 °C) and exhibits low hygroscopicity.[22]

1.5.1.3 Dosage form

Nevirapine is currently being used in the form of tablets (200 mg) and a suspension (50 mg/5mL). Nevirapine anhydrous and nevirapine hemihydrate are used in tablets and suspensions, respectively (Fig. 1.4). It is also used in fixed dose combinations together with two other antiretroviral drugs (Lamivudine and Zidovudine).

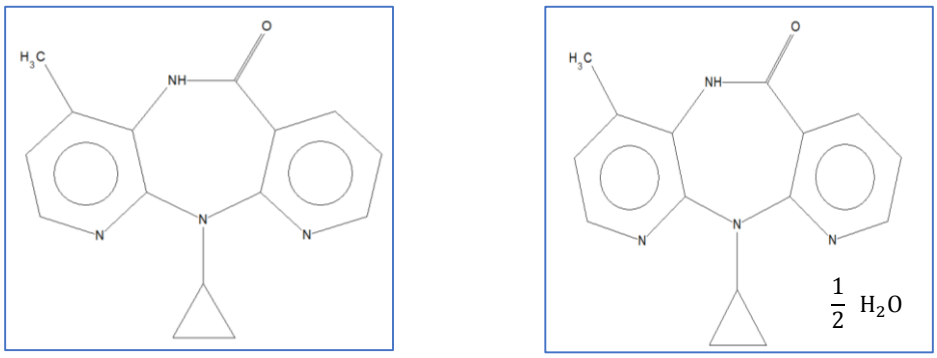

Figure 1.4 Structure of nevirapine and nevirapine hemihydrate (from left to right)

1.6 Classification of solids

Drug substances that are incorporated in dosage forms today are usually solid materials that are pure chemical compounds that are either in crystalline or amorphous form. The selection of the proper form of the drug is of paramount importance to ensure optimal solubility, absorption and stability characteristics.

Solvates, salts, co-crystals, hydrates and cyclodextrin inclusion complexes represent multi-component systems while polymorphs represent single-component systems (Fig. 1.5). Polymorphism is defined as materials with the same chemical composition and different lattice. The multi-component systems are bound together by means of non-covalent bonds viz. hydrogen bonding, π-π interactions or Van der Waals forces.[23]

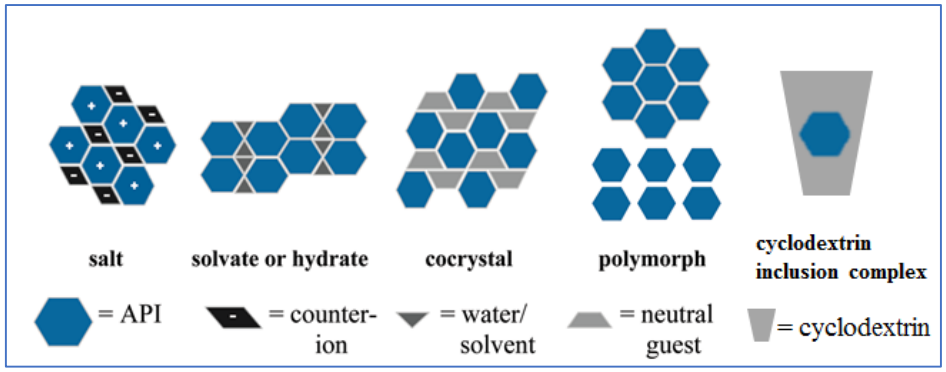

Figure 1.5 Classifications of solids based on internal structure of compounds[24]

Each crystalline form of an API has unique physicochemical properties that can influence the bioavailability, manufacturability, purification, stability and other performance characteristics of the drugs.[25] Thus, selection of the appropriate form is of paramount importance for drug development and formulation.

1.7 Strategies to improve solubility of an API

Solubility is a key determining factor in the formulation of dosage forms, since molecules that are poorly soluble may lead to slow dissolution rates, insufficient and inconsistent systemic exposure and consequent sub-optimal efficacy in patients.[26] About 40 % of drugs that are administered as a tablet have poor solubility. What is more concerning is that 80–90 % of drug candidates in the Research & Development pipeline have a low solubility problem, prohibiting them from advancing into clinical trials.[10] Despite this alarming scenario, solubility problems can be overcome by either the physicochemical modification route or by formulation of the API into special dosage forms (Fig. 1.6).[27] Physicochemical modification can be achieved by application of crystal engineering to form either solvates, hydrates, polymorphs co-crystals or cyclodextrin inclusion complexes, thus ensuring the most appropriate solid form of the drug. Alternatively, special dosage forms such as solid dispersions, self-emulsifying formulations or liquid-filled capsules may be developed to improve the solubility profile of the compound.

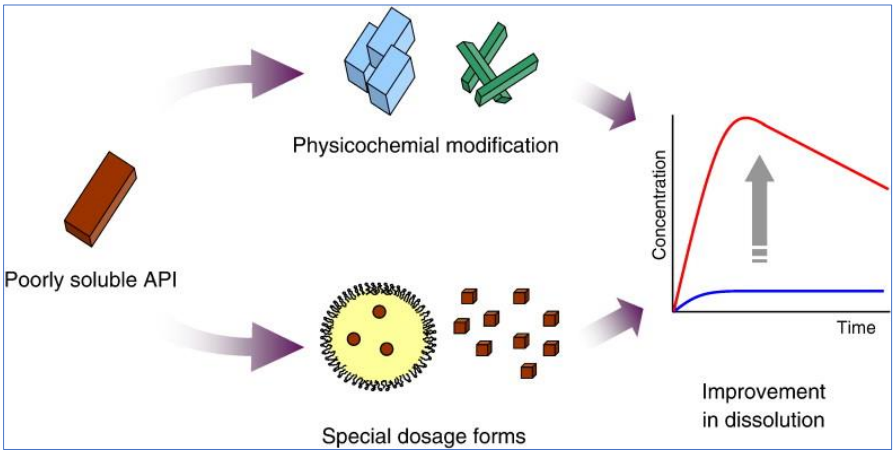

Figure 1.6 Modification of API to improve solubility[27]

1.7.1 Supramolecular derivatives of nevirapine

Nevirapine is a poorly soluble API which has undergone physicochemical modification in an attempt to improve its solubility (Fig. 1.7).

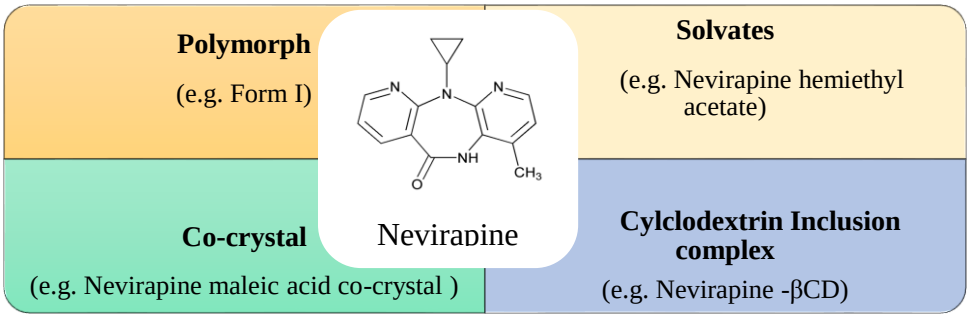

Figure 1.7 Derivatives of nevirapine

In its structure, the amide function (-COHN) of nevirapine makes it a viable candidate for crystal polymorphism or pseudopolymorphism. This can be achieved by interacting the drug with various solvents that present with unique physicochemical properties. Nevirapine has three polymorphic forms (Form I, II, III)[28,29] and it has numerous solvates with (ethanol, acetonitrile, chloroform, tetrahydrafuron, toluene, mixed ethanol-water, ethyl acetate, dichloromethane, 1,4 dioxane and water). [30,31]

To date there are five co-crystals of nevirapine that can be formed with saccharin, glutaric acid, salicylic acid, rac-tartaric acid and maleic acid[32] and a cyclodextrin inclusion complex can be formed with beta cyclodextrin.[33] Table 1.1 shows that the co-crystal and solvate of nevirapine have different structural parameters compared to nevirapine, thus these nevirapine derivatives exhibits unique physicochemical characteristics. As mentioned previously, physicochemical properties are pertinent towards dosage formulation.

	Nevirapine	Nevirapine maleic acid co-crystal	Nevirapine ethyl acetate solvate (2:1)
A (Å)	6.9177(2)	7.4001(5)	7.7744(3)
B (Å)	18.7759(5)	10.4599(7)	8.4447(3)
C (Å)	9.6075(3)	23.256(2)	12.4449(5)
A (deg)	90.0	90	84.610(3)
B (deg)	97.026(1)	98.968(1)	89.455(3)
ϒ (deg)	90.0	90	68.243(2)

Table 1.1 Unit cell dimensions of nevirapine, nevirapine co-crystal and of nevirapine solvate[32, 33]

Formulation of dosage forms have been attempted with two of these nevirapine derivatives, i.e. the cyclodextrin inclusion complex[33] and the nevirapine glutaric acid co-crystal.[34] To further the knowledge base on the formulation of the nevirapine derivatives, a nevirapine co-crystal will once again be used, however this time in a liquid dosage form.

1.8 Choice of liquid dosage form

A formulation scheme to strategize working with early formulations along with enhancement techniques was illustrated by Li et al.[35] The study emphasized the drug's physicochemical properties required prior to development work (Fig 1.8).

It states that for oral dosage forms, a solution formulation is preferred. It commences with aqueous media and buffers. If there is no good solubility, then solution approaches such as co-solvents, cyclodextrins or micelles are utilized.[35] For a suspension formulation, options such as conventional suspensions and micronized suspensions exist. However, the combined pH of the formulation with excipients needs special attention. Thereafter, if no solution vehicles are appropriate for formulation or if there is no adequate solubility, a novel preparation can be explored. Furthermore, the choice of dosage form is based largely on the drug compound used in the study.[35]

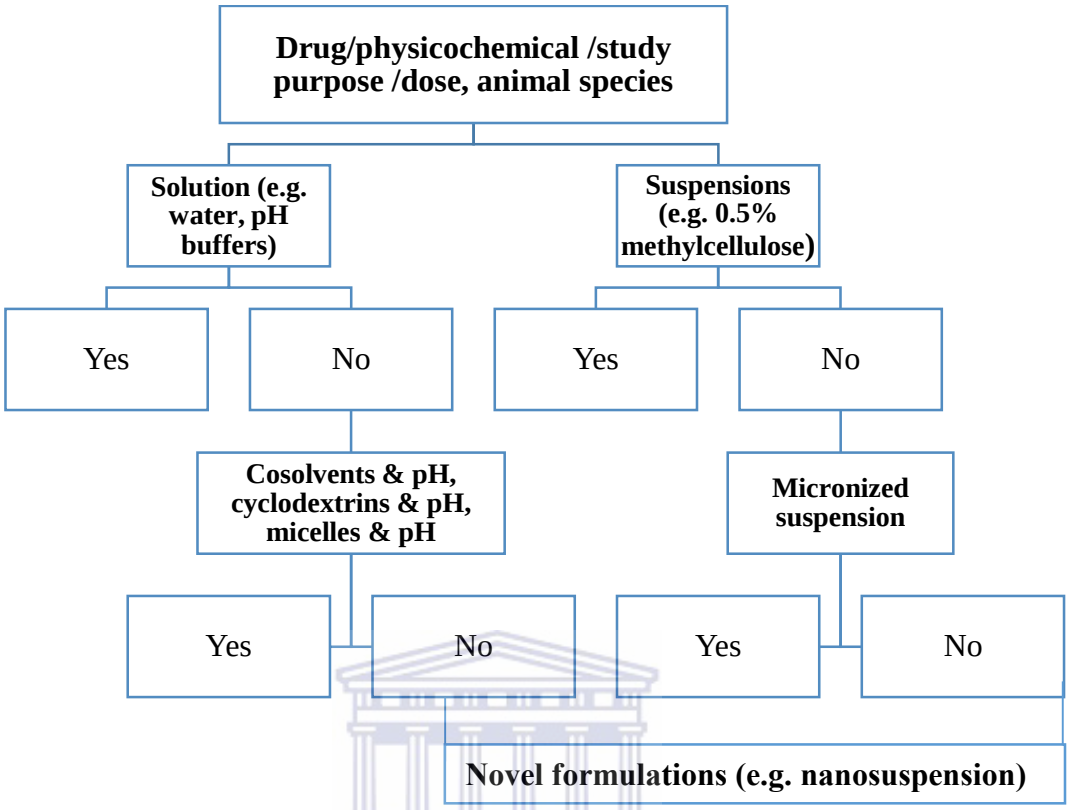

Figure 1.8 Formulation scheme for drugs administered by oral route[35]

The following section discusses the possibility of formulation of nevirapine as a liquid dosage form, applying the formulation scheme suggested by Li et al.[35]

1.8.1 Formulation of nevirapine as a solution

To form a solution, nevirapine has to be suspended in an aqueous or non-aqueous solvent. Nevirapine is a poorly soluble drug. It has an aqueous solubility of 0.123 mg/mL. To get a therapeutic effect for a 12 kg child, a dose of 50 mg is required. If 50 mg were to be dissolved in water, it would require 406 mL. This is not practical because almost half a litre of solution is required for one dose for a child. Therefore, formulation of a solution for nevirapine is not feasible.

Furthermore, since nevirapine exhibits pH dependent solubility, the drug is soluble at acidic pH values and at an acidic pH the drug is not suitable for consumption, hence it is not appropriate to formulate a solution.

1.8.2 Formulation of nevirapine as an emulsion

For a drug to be formulated as an emulsion, the drug has to be in a liquid form. This is not an option for nevirapine since it exists as a solid crystalline powder.

1.8.3 Formulation of nevirapine as a suspension

Suspensions are often selected as a pharmaceutical dosage form when the drug is insoluble in water or aqueous fluids and when attempts to solubilize the drug by the use of co-solvents, non-ionic surface active agents and other solubilizing agents would compromise the stability or safety.[12]

Suspensions are also preferred in contrast to emulsions because they exhibit a higher rate of bioavailability when compared to solid dosage forms (Fig. 1.8).[12]

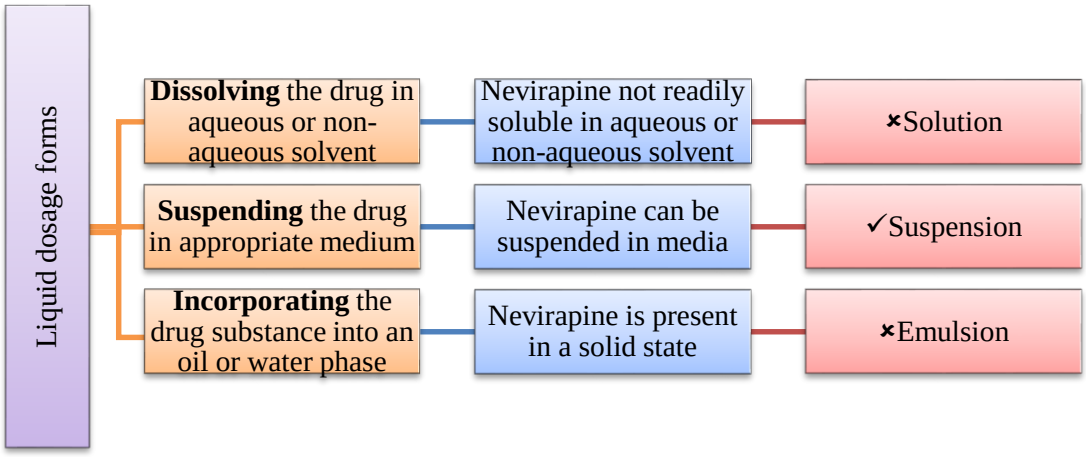

Figure 1.9 Selection process of a liquid dosage form for nevirapine

Suspensions have an advantage in that it can be designed for organoleptic reasons such as to mask the bitter taste of the drug. Paracetamol and chloramphenicol are case in point scenarios, where the drug can be prepared in soluble form, but the bitter taste of the solution would easily prevent the patient from using the correct dose, whereas the dispersed or non-dissolved form is essentially tasteless.[6]

Suspensions can also be designed for patients that have difficulty in swallowing due to certain pathologies particularly in the gastro-intestinal tract.[6] Many patients suffering with HIV present with minor to major oral and throat infections which can cause pain while swallowing tablets.[7] This is applicable in the case of nevirapine, where it can cause mouth ulcerations during the first 14 days.[36] Suspensions would be suitable for these individuals.

Suspensions pose an added advantage in that the dissolution of drug particles and subsequent absorption commence upon dilution into gastrointestinal fluids, unlike tablets and capsules where the dosage form has to first undergo disintegration.[12] The large surface area of the dispersed drug ensures a higher degree of availability for absorption.

1.9 Current production method of Viramune ® suspension

Ingredient	Role	Amount g/100ml
Nevirapine hemihydrate	Active ingredient	0.10 – 50
Carbomer 934P	Viscosity enhancer	0.17 – 0.22
Polysorbate 80	Wetting agent	0.01 – 0.20
Sorbitol solution	Adjust density	5.00 – 30.00
Sucrose	Sweetener	5.00 – 30.00
Methylparaben	Preservative	0.15 – 0.20
Propylparaben	Preservative	0.02 – 0.24
Sodium hydroxide	Buffering agent	q.s. to pH 5.5 – 6
Purified water, USP	Vehicle	q.s. ad 100 ml

Table 1.2 Production ingredients of Viramune® suspension[37]

Currently, nevirapine hemihydrate is used in preparation of the suspension through the pH modification method. It is prepared by heating a portion of purified water to approximately 70 °C and the methylparaben and propylparaben are added while constantly mixing. After the parabens have completely dissolved, the solution is allowed to cool down to less than 35 °C, and then the carbomer 934P is dispersed in the solution while mixing. The pH is adjusted between pH 5.5 - 6 with 20% sodium hydroxide solution. The gel is continually stirred for approximately 20 minutes and the pH is re-measured. The sorbitol solution is added while mixing followed by the addition of sucrose. The solution is mixed for 30 minutes. The polysorbate 80 is then dissolved in a portion of purified water, and the nevirapine is then added to the polysorbate 80 solution. This mixture is homogenized for at least 40 minutes. The nevirapine and polysorbate 80 drug concentrate is thoroughly blended into the carbomer gel. The suspension is adjusted to volume or weight with purified water and blended for 30 minutes.[37]

1.10 Challenges with Viramune® suspension

The anhydrous form of nevirapine when formulated in aqueous suspension, slowly converts to the hemihydrate form, yielding crystals of the hemihydrate which, over time, grows so large as to adversely affect drug dissolution and pharmaceutical performance, hence the nevirapine hemihydrate form is used in the formulation of suspensions.[37] Possible adverse effects of excipients pose a problem in the current nevirapine suspensions. Sucrose can promote the formation of dental caries.[38] The problem with suspensions is that 13 % of the dose remains in the cup; this is due to the viscosity of the suspension. Children are given doses according to weight, thus it might affect therapeutic outcomes.[39]

1.11 Research objectives

Given the major drawback of nevirapine which is poor solubility, nevirapine co-crystals can be formed to counteract this problem. Furthermore, suspensions can be formulated to improve its bioavailability; the hypothesis is therefore to integrate these

two concepts, namely co-crystal formation into a suspension and prove that the preparation will remain stable during its shelf-life and have an enhanced effect upon dissolution.

The objectives of this study are as follows:

1.10.1 To develop a protocol to select an appropriate co-former to form the nevirapine co-crystal that is suitable for formulation of suspensions.

1.10.2 To select an appropriate co-former and formulate the nevirapine co-crystals with the chosen co-former.

1.10.3 To scale-up formulation of the chosen co-crystal.

1.10.4 To establish whether the co-crystal is retained in the suspension during a set shelf-life and attempt to protect the co-crystal in the suspension, if necessary.

1.10.5 To assess the formulated co-crystal suspension according to the particle size, zeta potential, pH, rheology and viscosity according to USP 32 standards.

1.10.6 To perform dissolution tests on the suspension according to USP 32 standards.

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Chapter 2

Literature Review

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A pharmaceutical suspension is a coarse dispersion of finely divided solid particles of a drug dispersed in a liquid medium in which the drug is not readily soluble. An aqueous suspension is a useful formulation system for administering an insoluble drug such as nevirapine. A large surface area of a dispersed drug ensures high availability for dissolution and hence absorption.[1]

2.1 Features of an ideal suspension

Besides therapeutic efficacy, chemical stability of the components of formulation and aesthetic appeal of the preparation, a suspension should have the following features[2]:

- A pharmaceutical suspension should settle slowly and should be readily dispersed upon moderate agitation of the container.
- The particle size should remain constant throughout long periods of undisturbed standing.
- It should be readily and evenly pourable and have no crystal growth.

2.2 Factors affecting formulation of suspensions

Suspensions are biphasic formulations, thus the challenges include both chemical and physical stability. Several factors that govern the formulation of an ideal suspension are depicted below.

2.2.1 Particle size

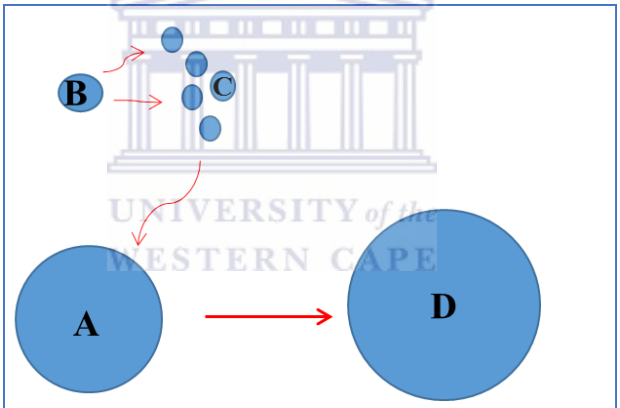
Drug particle size is an important factor that can influence the product appearance, settling rates, drug solubility, *in vivo* absorption, resuspendability and overall stability of the suspension.

Finely divided drug particles dissolve at a greater rate and have higher relative solubilities than similar macroparticles.[3] Particle size has to be fine in nature to ensure the production of a uniform suspension. Larger particles tend to settle faster due to the

gravitational force while smaller particles easily form a hard cake at the bottom of the container.

Knowledge of the particle size distribution provides direction to the formulator. The capability of measuring the particle size distribution is of definite value to the formulator since it is one parameter the formulator has control over, by milling and sieving. Wide distributions in particle size leads to high density suspensions whereas widely differing particle shapes leads to low density slurries.[3]

Crystal growth can occur due to Ostwald's ripening,[3] which is the growth of larger particles at the expense of smaller ones due to a difference in solubility rates of different particle sizes (Fig. 2.1). Change from one polymorphic form to another can also cause crystal growth.



A	Large particle
B	Small particle
C	Smaller particles from particle B
D	C particles join particle A to form an even larger particle

Figure 2.1 Ostwald's ripening process

Particle growth is a destabilizing process which can result from temperature fluctuations. Temperature fluctuations can change the particle size distribution and polymorphic form of the drug thus, altering the absorption rate and consequently bioavailability. Particle growth is of more importance when the solubility of the drug is dependent on temperature. Thus, when the temperature is increased, crystals of the

drug may dissolve and form supersaturated solutions which favour crystal growth. This can be inhibited by the addition of surfactants or polymers.[3]

A particle size range of 50 to 75 μm has good flow properties for a suspension dosage form. The ideal particle size for suspensions is 1 to 50 μm.[2] When the individual particles are smaller than this range, most solids tend to exhibit aggregation in the dry state. Particles in the range of 10-50 μm has increased surface free energy.[3] The powder can become damp if there is a tendency to attract moisture, causing the particles to "ball up."[3] This can occur because the pores between powder particles become smaller with decreasing particle size which causes the surface area to become more accessible to liquid penetration.

2.2.2 Particle shape

Information of the shape of a particle is an important property since the shape can contribute to the understanding of the packing of the sediment and settling characteristics. In liquids, suspended material is seen as spherical shapes. Substances that are used to promote the stabilization of suspensions are termed suspending agents. These agents have rod-shaped or plate like structures. Rod-shaped structures act as suspending agents due to a large surface area, when in contact with water they produce a continuous network of rods that extends throughout the entire vehicle.[4]

Ellipsoid shapes are also seen in colloids. Oval-shaped and spherical shaped powders flow with greater ease than needle-shaped powders and may enable processing of powders into a dosage form.[5]

Shape and size can have an influence on the prospects for reversible change. Attraction between two dispersed particles will be affected by the surface characteristics of the particles. Particles that have the ability to exert attractive forces across a large surface will most likely result in greater attraction and adhesion forces.[5]

2.2.3 pH

A well formulated suspension should exhibit excellent physical stability over a wide range of pH values. For drugs that possess ionizable acidic or basic groups, the pH of the vehicle influences drug stability or solubility. A desired pH value is achieved by the use of a pharmaceutically acceptable buffer. The use of salts and buffers should be cautioned, because small changes in electrolyte concentration will modify the surface charge of suspended particles. A pH of 4-10 indicates stability.[3]

Another consideration that should be made is that the viscosity of some materials changes as a function of pH. This is due to a concept known as the point of zero charge; this is the pH at which the net surface charge is zero. The pH range should be narrow because the magnitude of the charge on the drug particles can differ significantly with pH. Control of pH can prevent large changes during storage. Buffers are utilized to control potential changes in pH. Buffering components are selected on experimental basis so as not to adversely affect the physical stability of the final suspension.[3] The amount of buffer capacity needed is usually between 0.01 and 0.1M. A combination of buffers can be used to get a wider range of pH.[6] Furthermore, pH of the formulation should be considered as it may influence the dissolution of pH dependent drugs like nevirapine.

2.2.4 Temperature

Temperature is another factor that can affect long-term stability of suspensions.[3] Certain suspending agents can tolerate higher temperatures during processing. To bring about solubilisation of one ingredient the preparation may have to be heated and the long-term effect of such a process on the product must be evaluated, this is especially necessary for drugs that degrade at low temperatures. Degradation of the drug will not allow for the necessary therapeutic effect. With regards to physical stability, viscosity of suspensions generally decreases with an increase in temperature and thus, this is an important variable to consider during formulation.

2.2.5 Sedimentation rate

Sedimentation has an effect on both the physical and functional properties of suspensions. To acquire a uniform dosage it is necessary to control sedimentation. Stokes' law is an expression describing the resisting force on a particle moving through a viscous fluid and showing that a maximum velocity is reached in such a case.

Stokes' equation (Equation 2.1) states that (v) is the terminal settling velocity which is equal to the square of the diameter of the settling particle (d) times gravitational acceleration (g) multiplied by the difference in density of settling of solid and density of the liquid ($\rho_\rho - \rho_s$) divided by the viscosity of the dispersion medium(η) (Fig. 2.2).

Equation 2.1 Stokes' equation $v = \frac{d^2 g\,(\rho_\rho - \rho_s)}{18\,\eta}$

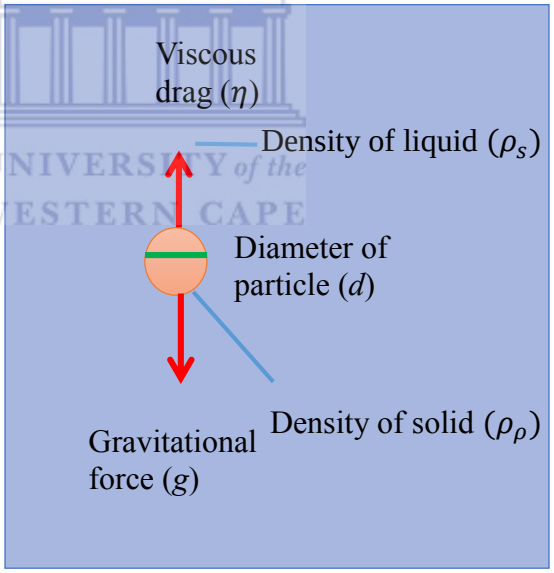


Figure 2.2 Factors affecting particle according to Stokes' law

The various factors that contribute to the rate of settling are embodied in Stokes' law. Stokes' law can be used in an ideal setting, in which uniform, perfectly spherical particles settle without collision or producing turbulence with other particles, and without chemical or physical affinity for the dispersion medium. Stokes' law may not

be applied to a lower size limit of about 0.5 μm. Suspensions with a concentration of 2 g of solids per 100 mL may have conditions that conform to Stokes' law. In suspensions greater than this concentration, particles interfere with one another and Stokes' law can no longer be applied. Stokes' law cannot be applied to the usual pharmaceutical suspension in which the particles are irregularly shaped and are of various diameters, where particles collide and produce turbulence. Yet, the general concepts of the equation give an indication of the factors that are important to the particles in a suspension and give an idea to the possible modifications that can be made to a formulation to decrease the rate of sedimentation.[2]

The velocity of a particle can be reduced by decreasing particle size and minimizing the difference between the densities of the solid and the liquid. The rate of descent of particles is proportional to the density of the particles in a suspension. The density of the solid is constant but changing the density of the liquid so that it is similar to the solid will minimize the difference between the two densities, thus leading to low velocity. Settling velocity is proportional to the second power of the particle diameter, hence agglomerates and flocculates will settle more rapidly than dispersed particles. Gravity and buoyancy affect the particle thus it could either have an upward movement (creaming) or a downward effect (sedimentation).[3]

In pharmaceutical suspensions where aqueous vehicles are used, the density of the particles is generally greater than that of the vehicle. This is a desirable feature because if particles have a lower density than the vehicle, the particles would tend to float and floating particles are difficult to distribute uniformly.

The rate of sedimentation can be reduced by increasing the viscosity of the dispersion medium. Viscosity can be increased by altering the vehicle and the solid particles. As the proportion of solid particles increases, so does the viscosity. A product with too high a viscosity is not desirable because it is difficult to redisperse the suspension. Thus, increase in viscosity should be done within limits of practicality.[3]

2.2.6 Physical stability

Suspensions are thermodynamically unstable systems. At the time of examining the product, it is only the apparent stability that is being assessed.

Suspensions do not form spontaneously. Suspensions may appear to remain stable for a long period of time; however, thermodynamics dictates that it will change to a lower energy state. These changes could either be reversible or irreversible. A large amount of energy would be required to get out of the maximum or minimum energy state. However, if the change is slow enough relative to the anticipated shelf life, then the thermodynamics may have little practical influence.[3]

In a suspended system, particles are thermally mobile and can occasionally collide due to their Brownian motion. As the mobile particles approach each other, both attractive and repulsive forces are at work. If the attractive forces are greater than repulsive forces, agglomerates can grow in the suspension. This phenomenon is termed 'flocculation' or 'coagulation' and it represents an unstable system. If repulsive forces are greater than attractive forces, a more stable suspension will occur. Thus the balance between these forces specifies the overall characteristics of the system.[3]

Physical stability of a suspension can be adjusted by an alteration in the dispersed phase rather than major changes in the dispersion medium. Adjustments that can be done to the dispersed phase are change in particle size, uniformity of particle size and separation of particles so that they do not aggregate and form a solid mass upon standing.[2]

2.2.7 Chemical stability

Insoluble drug materials when suspended in a liquid medium have some intrinsic solubility which may trigger a chemical reaction such as hydrolysis, which leads to degradation.

Particles that are completely insoluble are unlikely to undergo chemical degradation. Decomposition of a suspension is assumed to be solely due to the amount of the drug dissolved in the aqueous phase. The solution is responsible for drug decomposition and the drug will be released from insoluble suspended particles within the range of solubility.[3]

In a zero-order system the rate of a process such as decomposition is independent of the concentration of the reactants.[7] Primarily suspensions behave as a zero order system because the amount of drug in the solution remains constant in spite of the decomposition with time.[3] However, once all the suspended particles have been converted into the drug in solution, the entire system changes from zero order to first-order. A first-order system is defined when the rate of a process is determined by one concentration term.[7] Thus, suspensions are known to have apparent zero-order kinetics. The suspension is stable until the system follows first-order kinetics. If the suspensions are concentrated, the system will require more time to convert from zero- order to first-order. This is why a concentrated suspension is stable enough on the market as compared to the diluted one. Concentrated suspensions affects physical stability of suspensions, thus production should optimize both physical and chemical parameters of the dispersed particles to attain the desired stability of suspensions.[3]

2.2.8 Temperature stability

Increasing the temperature may lead to flocculation of sterically stabilized suspensions (suspensions that are stabilized by non-ionic surfactants). When a suspension is heated, the energy of repulsion between the particles is reduced due to dehydration of the polyoxyethylene groups of the surfactant. Increasing the temperature brings the polyoxyethylene groups closer and consequently reduces the space available for the drugs in this region. The attractive energy is increased and thus, the particles flocculate.

During the freezing process, particles can overcome the repulsive barrier formed by ice. It forces the particles in close proximity to experience strong attractive forces and

form aggregates. For example, when the ice melts, particles remain as aggregates unless work is applied to overcome the attractive force.[3]

2.2.9 Viscosity and rheology

Viscosity is expressed as the resistance of a fluid to flow under an applied stress. Viscosity is a property arising from friction between neighbouring particles moving at different velocities. Fluids with no resistance are termed, ideal fluids. All fluids that have viscosity are termed, viscid. It is termed viscous if it has a viscosity greater than water, if it is less than water it is termed, mobile. The viscosity of a suspension should be high at low shear rates so that the particles settle slowly or remain suspended in the continuous phase.[3]

The rheology of a particular product can affect patient acceptability, physical stability, and even biological availability. Viscosity has been shown to affect absorption rates of drugs from the gastrointestinal tract.[8] When classifying materials according to types of flow and deformation, it is customary to categorize them as Newtonian or non-Newtonian systems.[8] This is dependent on whether or not their flow properties are in accordance with Newton's law of flow.[8] Newton's law of flow states that the higher the viscosity of a liquid, the greater is the force per unit area (shearing stress) required to produce a certain rate of shear.[8]

However, the majority of fluid pharmaceutical products are not simple liquids, thus they do not follow Newton's law of flow. These systems are referred to as non-Newtonian. Non-Newtonian behaviour is generally exhibited by liquid and solid heterogeneous dispersions such as colloidal solutions, emulsions, liquid suspensions, and ointments.[8]

In Newtonian fluids, viscosity is constant over a wide range of shear rates whereas in non-Newtonian fluids, they exhibit a variety of different correlations between shear stress and rate. When non-Newtonian materials are analysed in a viscometer they exhibit four types of flow viz. plastic, pseudoplastic, dilatant and thixotropic.[8]

In plastic or Bingham type of flow, fluids that have linear shear stress/shear strain require a finite yield stress before they begin to flow. As seen in figure 2.3, the graph for plastic flow does not start from the origin, implying that it requires force for it to flow. An example of this type of flow is seen in toothpastes, where a force is required to expel it out of the tube. In pseudoplastic type of flow, apparent viscosity decreases with increased stress. This can be seen in polymers in solution, an example of this is tragacanth. In dilatant type of flow, apparent viscosity decreases with increased stress. An example of this type of flow is seen in oobleck's mixture, where it appears to be runny in nature when no stress is applied and once stress is applied it behaves as a solid (Fig. 2.3).

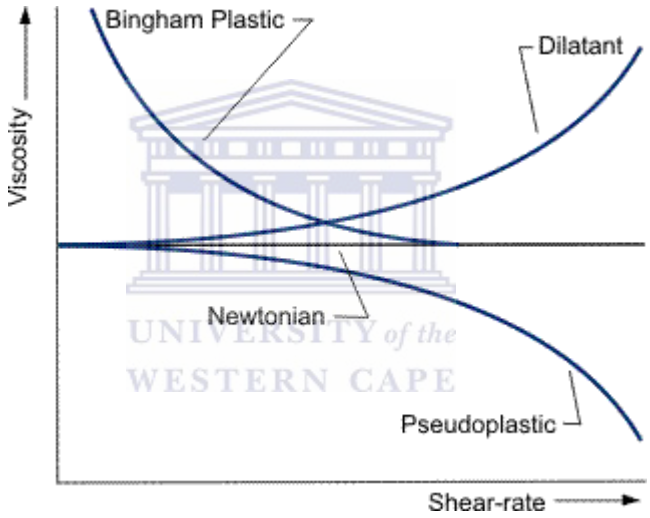


Figure 2.3 Types of flow for Newtonian and non-Newtonian material

A thixotropic fluid is a fluid which takes a finite time to attain equilibrium viscosity when introduced to a step change in shear rate. The longer the fluid undergoes shear stress, the lower its viscosity. Many gels and colloids exhibit a stable form at rest but become a fluid when agitated.[8] An example of this is bentonite calamine lotion. Thixotropy is of particular value for suspensions. During shearing, the yield stress is exceeded and the suspensions flow.[3] The structure begins to re-form after the termination of shear. However, it does not reform immediately. It takes time to rebuild the order or structure that existed when the system was at rest. As long as it rebuilds itself to a point at which sedimentation is avoided or substantially diminished one can

achieve a pharmaceutical suspension of good quality. A thixotropic agent ensures the rate at which the structure is rebuilt.[3] An example of a thixotropic agent is colloidal silicon dioxide.

2.3 Theory of suspensions

The initial rate of settling of flocculated particles is determined by the floc size and the porosity of the aggregated mass. The rate is also dependent on compaction and the rearrangement processes within the sediment. Sediment volume (F) is defined as the ratio of the final volume of sediment (V_u), to the original volume of the suspension (V_o) before settling. Since sedimentation volume is a ratio, it can have values ranging from less than 1 to greater than 1. F is normally less than 1; this means that the ultimate volume of sediment is smaller than the original volume of suspension.[8]

If F=1, the volume of sediment in a flocculated suspension is equal to the original volume of the suspension. The product is then said to be in flocculation equilibrium and shows no supernatant on standing (Fig. 2.4).[8]

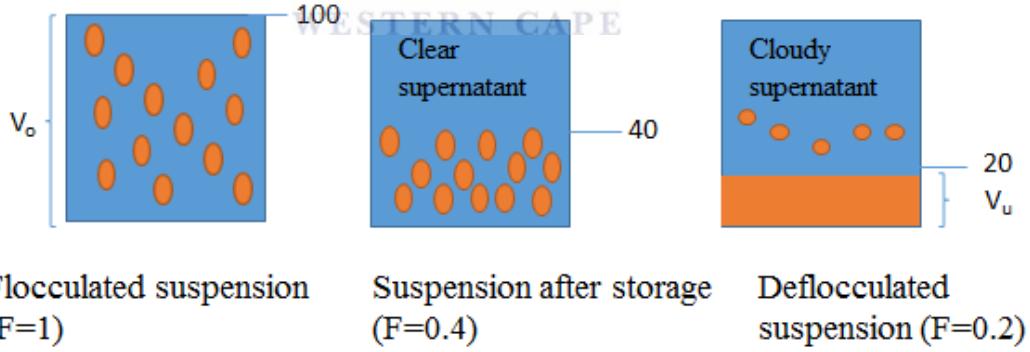


Figure 2.4 Flocculated and deflocculated suspensions

Controlled flocculation can be produced to prevent the formation of compact sediment that is difficult to redisperse. Flocculation can be achieved by use of electrolytes, surfactants and polymers. Optimum concentration of electrolytes, surface active agents or polymers should be used, since change in these concentrations may change a suspension from a flocculated to a deflocculated state.[8]

The controlled flocculation approach is capable of achieving the desired physical and chemical requisites of a pharmaceutical suspension. If the sedimentation volume is not close to or equal to 1, the product may look unsightly. A suspending agent may aid in retarding the sedimentation of flocs.[8]

Electrolytes act as flocculating agents by decreasing the electrical barrier between the particles, thus decreasing the zeta potential and forming a bridge between adjacent particles that link them together in a loosely arranged structure (Fig. 2.5). Non-ionic and ionic surface active agents can also induce flocculation of particles.[8] The zeta potential decays exponentially with increase in the distance from the surface of the particle. The rate of decay is dependent on the electrolyte content.

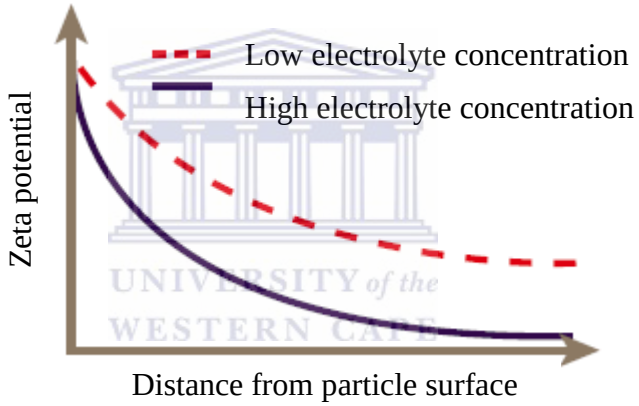


Figure 2.5 Zeta potential versus distance from particle surface for electrolytes

2.3.1 Wetting process

Wetting of suspended particles is important for proper dispersion. Poor wetting leads to poor dissolution and thus, slows down the release of a drug. For the process of wetting to occur, particles must be separated into finely divided particles and each particle should be individually wet.

The first step involves wetting of the solid particles by the liquid medium. A layer of air is adsorbed on the surface of the particle and this makes it difficult to disperse solid particles in liquid. High density particles will float on the surface of the liquid until the

layer of air is displaced completely. Entrapment of air on the particle allows particles to rise to the surface of the dispersion medium causing particle de-aggregation and can lead to instability. A wetting agent or surface active agent serves to remove air from the surface and enable easy penetration of the liquid into the solid.[3] To do this the liquid must displace air at the surface of the solid (Fig. 2.6). An example of a surface active agent is polysorbate 80.

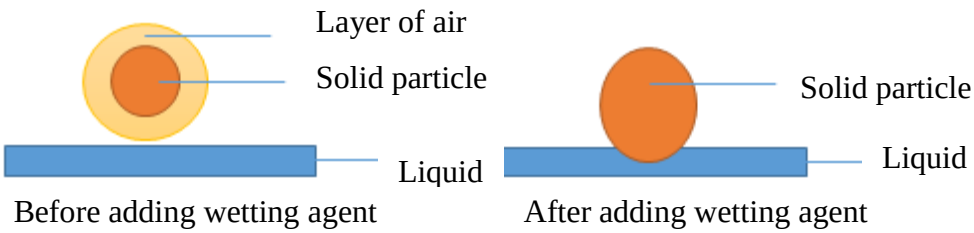


Figure 2.6 Effects of adding a wetting agent to solid particle

The tendency of a solid to be wetted by a liquid is a measure of the interaction of the substances. Hydrophobic particles are referred to as particles that are not easily wetted by an aqueous vehicle even after the removal of adsorbed air. Hydrophilic particles do not require the use of surface active agents because they are easily dispersed in the liquid after the removal of adsorbed air. If the solid is hydrophilic it will be wet more easily by an aqueous medium. If it is hydrophobic, as in the case of nevirapine, it will be more easily wetted by an organic or nonpolar liquid. Use of surface active agents is recommended to promote proper wetting of hydrophobic substances.

The angle that the liquid makes with the solid surface is called the contact angle (Fig. 2.7). If the contact angle is greater than zero the solid is completely wet by the liquid. If the angle is close to 180°, the solid substance would be described as unwettable by the liquid in question. If it is less than 90° wetting is spontaneous.[3]

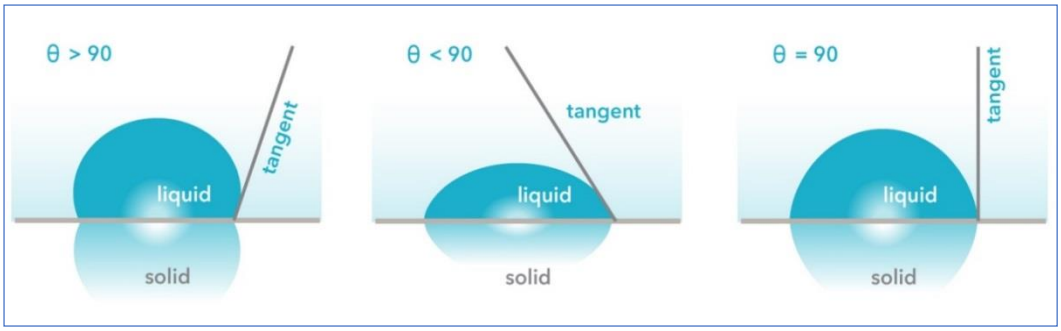


Figure 2.7 Contact angles between solid and liquid

2.3.2 Brownian motion

Brownian motion is the random movement of particles. The erratic motion can be observed microscopically with particles from 2 to 5 μm.[8] This occurs due to the bombardment of particles by the molecules of the dispersion medium. The velocity of the particles increases with a decrease in particle size. Brownian movement offsets sedimentation to a measurable extent at room temperature by keeping the dispersed material in random motion. To decrease the Brownian movement, an increase in the viscosity can be applied. For example, Brownian motion can be counteracted by the addition of 50 % glycerin solution which has a viscosity of about 5 cP.[8]

2.3.3 Electrokinetic properties

Solid particles may acquire a charge due to the presence of a liquid by adsorption of an ionic species present in the liquid or by ionization of functional groups of the solid particles. Addition of electrolytes allows the solid to become positive or negatively charged. In ionization of functional groups of the solid particles, the total charge is a function of the pH of the liquid.

If the solid has a positive charge and anions are present in the liquid, the anions are attracted by positively charged particles and they repel the cations. Adjacent to the surface of a solid is a layer of tightly bound solvent molecules known as counter ions. These are ions of opposite charge to that on the surface layer (Fig 2.8).

Two layers are formed at the interface and this is known as the electrical double layer. The intensity of electrical forces decreases as the distance increases from the surface of the particles. A uniform distribution of ions is achieved and this is known as the zone of electroneutrality.[3]

The DLVO theory named after Derajaguin and Landau, Verwey and Overbeek gives a better understanding for controlling the rate at which particles aggregate depending on the distribution of charge.[7]

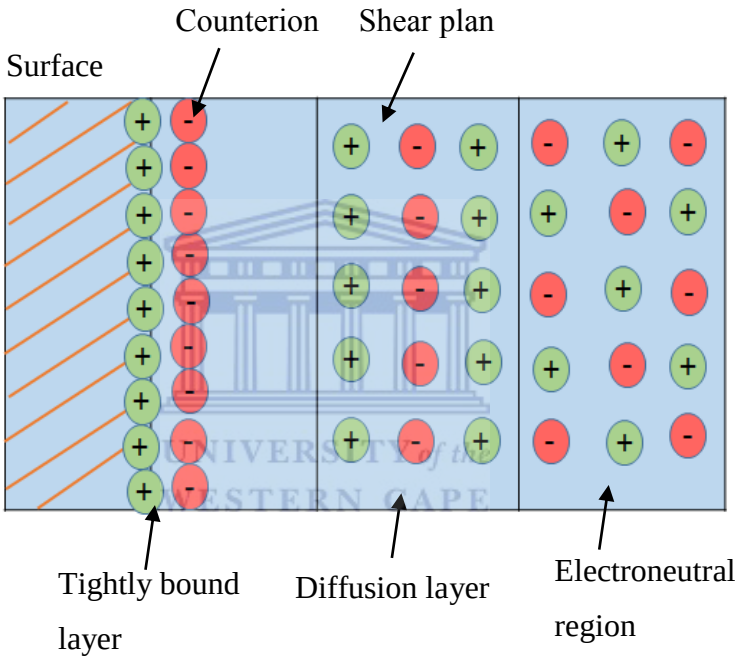


Figure 2.8 Illustration of the electrical double layer

The DLVO theory relates the balance of attractive and repulsive forces. Attractive forces are seen in agglomerated suspensions and repulsive forces are seen in systems that do not show agglomeration, coagulation or flocculation.

Zeta potential is the difference between the potential of the ions at the tightly bound layer and the electroneutral region (Fig 2.9). This plays a significant role in the formulation of suspensions. Zeta potential reflects the future stability of suspensions.

Zeta potential governs the degree of repulsion between adjacent, similar charge and solid particles.[7]

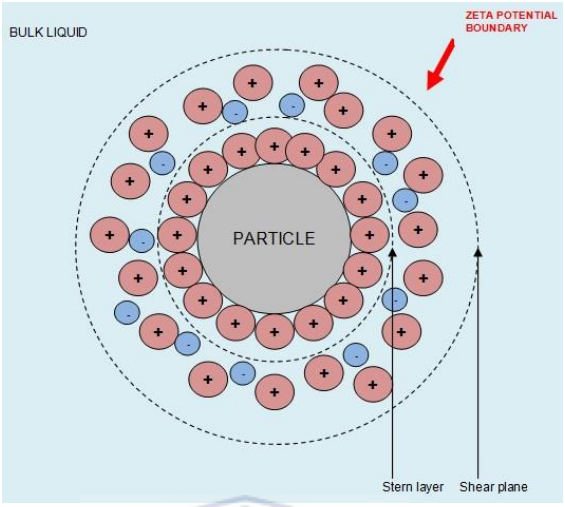


Figure 2.9 Zeta potential

If the zeta potential is reduced below a critical value, the force of attraction is greater than the force of repulsion and particles come together and form a flocculated suspension. Electrolytes reduce zeta potential below critical value and the attractive forces are greater than repulsive forces causing flocculation to occur.[7]

Repulsive forces have a stabilizing influence on suspensions because they work against the aggregation of suspended particles. These forces originate from several sources such as steric hindrance (the hindering of a chemical reaction, as a result of the arrangement in space of the atoms of the reacting molecules) to the close approach of particles and electrostatic repulsion. Stabilization requires the net repulsive term to exceed the net attractive term.[7]

If a polar medium such as water is used, various electrical interactions can occur. Functional groups at the surface of a particle can ionize in the presence of a polar liquid. The pH of the aqueous medium typical of suspensions is important in this instance. Low pH systems will promote a positive charge on the dispersed particle while high pH systems will promote a negative charge.[7]

According to the DLVO theory (Fig 2.10), the forces on colloidal particles in dispersion are due to the electrostatic repulsion and London-type Van der Waals attraction. These forces result in potential energies of repulsion and attraction between particles, V_R and V_A, respectively.[7]

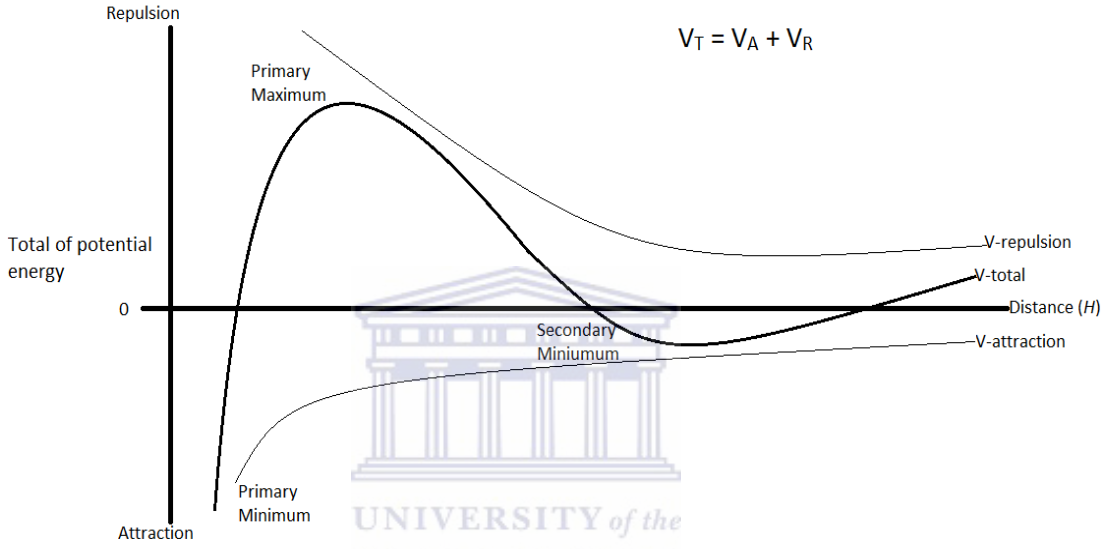


Figure 2.10 DLVO theory

There is a strong potential of attraction near the origin and a high potential barrier of repulsion at moderate distances. A shallow secondary trough (secondary minimum) of attraction can be observed at longer distances of separation. The presence of a secondary minimum is important in controlled flocculation. Addition of electrolytes can cause coagulation of colloidal particles, however, mixing of oppositely charged colloids result in mutual agglomeration.[3]

Highly energetic particles tend to group together, so as to decrease the total area and reduce the surface free energy. Particles in a liquid suspension tend to flocculate. Particles that adhere by stronger forces form aggregates. The formation of any type of agglomerate, either floccules or aggregates, is a measure of the system's tendency to reach a more thermodynamically stable state ($\Delta G = 0$, where 'G' is surface free

energy). To reach a stable state, the system has to reduce the surface free energy and equilibrium has to be achieved. This can be accomplished by reducing interfacial tension or decreasing interfacial area. Interfacial tension can be reduced by addition of a surfactant, however, $\Delta G = 0$ cannot be achieved ordinarily. A suspension with insoluble particles usually possesses a finite positive interfacial tension and particles tend to flocculate.[8]

The forces on the surface of a particle affect the degree of flocculation and agglomeration in a suspension. Repulsive forces arise from the interaction of the electrical double layer surrounding each particle.

When repulsion energy is high, the potential barrier is also high, thus collision of the particles is opposed. The system will remain deflocculated and when sedimentation is complete, a formation of closely packed smaller particles filling the voids between the larger ones is formed. The particles at the bottom of the sediment are gradually pressed together by the weight of the ones above; the barrier is thus overcome by allowing the particles to come into close contact with each other.

To resuspend and redisperse this compact cake, it is necessary to overcome the high energy barrier. However, this is not achieved easily by agitation and particles tend to remain strongly attracted to each other and form a hard cake due to the accumulation of London-type Van der Waals attraction.[8]

When particles are flocculated, the energy barrier is still too large to be surmounted and the approaching particle remains in the secondary energy minimum which is at a distance of separation of 1000 to 2000 Å. This distance is adequate to form loosely structural flocs.[8]

In deflocculated suspension systems, the dispersed particles carry a finite charge on their surface. When particles approach one another, they experience repulsive forces. These forces create a high potential barrier, which prevent the aggregation of the particles. When sedimentation takes place, the particles form a closely packed arrangement.[8]

The lower portion of the sediment gets pressed by the weight of the sediment above. This creates a force that is sufficient to overcome the high energy barrier. After this energy barrier is achieved the particles come in close contact with each other and establish strong attractive forces. This leads to the formation of a hard cake in a deflocculated system.

In the flocculated system, particles are not able to overcome the high potential barrier, they also remain loosely attached with each other forming flocs known as the secondary minimum. Particles at this stage still experience a high energy barrier but allow the suspension to be easily re-dispersed. A secondary minimum flocculation is desirable for a suspension. This is favoured by particles greater than 1 μm.[8]

The deflocculated system provides the apparent stability, while the flocculated system is necessary to achieve the long-term stability. Hence, controlled flocculation represents both properties.

Bearing in mind the above information pertaining to the theory and the factors affecting the dosage from to be formulated i.e. suspensions; the next segment provides a literature review of the type of supramolecular derivative to be used as the active ingredient in the formulation of the aforementioned dosage form i.e. co-crystals, commencing from the broad umbrella of supramolecular chemistry and focusing on crystal engineering.

2.4 Supramolecular chemistry

Supramolecular chemistry is known as the chemistry of the intermolecular bond, covering the structures and functions of the entities formed by association of two or more chemical species.[9] The term supramolecular signifies that which is beyond the molecule. As of present, supramolecular chemistry encompasses the study of molecular crystals applicable to various fields such as solid-state chemistry, crystal engineering and materials science.[10]

The concurrent use of supramolecular chemistry and crystallographic techniques has given rise to a niche research field area known as crystal engineering.[11] Chemistry deals with molecules while crystallography deals with crystals, which are extended, ordered assemblies of molecules. The relationship between chemistry and crystallography is therefore the interplay between the structure and properties of molecules on one hand and those of extended assemblies of molecules on the other.[12] Though it is still considered a young research field, extensive research has already been done in this area and thus it can be considered as a scientific genre on its own.

2.4.1 Crystal engineering

The term crystal engineering was first used by Schmidt,[11] who wrote a full scientific paper in this area. It postulated that under suitable conditions, molecular recognition events could be the major factor leading to crystal formation. Lehn[9] further took this concept and elegantly defined it as the "chemistry beyond the molecule, bearing on the organized entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces." The weak intermolecular interactions that form supramolecules are the same as those that act in the formation of crystals, thus the link between supramolecular chemistry and crystal engineering became obvious.

The original objective of crystal engineering was to design organic molecules which would adopt particular crystal structures within which topochemical reactions could take place, leading to regioselective or stereoselective products. Due to its broadened scope today, Desiraju has defined it as 'the understanding of intermolecular interactions in the context of crystal packing and in the utilization of such understanding is the design of new solids with desired physical and chemical properties.'[10]

Each crystal structure is the result of a delicate balance between a range of intermolecular forces, many of which are weak and non-directional. A small change in the molecular structure can trigger unpredictable changes in the extended crystal

structure. Thus, an improved understanding of the strength, directional behaviour, and structural influence is the very essence of non-covalent forces that is the underlying focus for initial research into crystal engineering.[13]

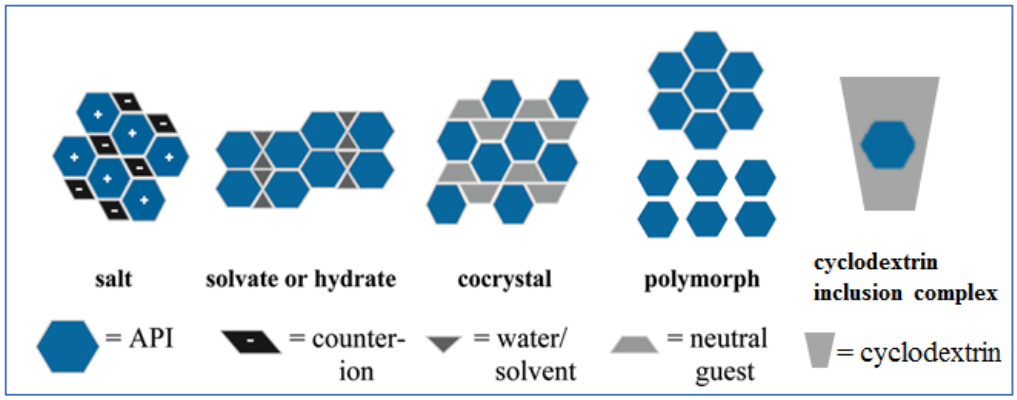


Figure 2.11 Schematic illustrations of salt, solvate, co-crystal and polymorph[14]

Application of crystal engineering of an API can result in a salt, solvate, co-crystal or a polymorph. Figure 2.11 illustrates the various modifications that can be applied to an API. A pharmaceutical drug in the salt form is an ionisable drug that has been combined with a counter-ion to form a complex.[15] A drug that shows an alternative packing arrangement in the crystal lattice than that of the same molecule is known as a polymorph.[16] A solvated drug is formed when a material is crystallised and in the process of crystallisation, solvent becomes trapped in the crystal lattice.[7] If the solvent used is water then the resulting drug is called a hydrate. A co-crystal is a multiple component crystal in which all components are solid under ambient conditions when in their pure form.[17] More information regarding co-crystal follows, as this was the API derivative used in this study.

2.4.2 Supramolecular synthons

Supramolecular synthons are structural units within supermolecules which can be formed or assembled by known or conceivable synthon operations involving intermolecular interactions.[12] Synthons are kinetically defined structural units that convey the crucial features of a crystal structure, and a critical assumption is that the

synthon is a reasonable approximation to the whole crystal. The crystal structure in these cases may be viewed as a sequence of kinetically controlled events. Crystals are built when robust synthons are formed with strong and directional interactions. Once these are formed, they tend not to dissolve.[12] This is followed by new synthons that are formed which involves slightly weaker and slightly less directional interactions. In this manner the building up of a crystal can be rationalized as a series of chemically reasonable and logical steps.[12]

The intermolecular interactions that are used are the hydrogen bond, including its weakest variant, the C−H···π interaction,[18] Van der Waals interactions,[19] dipole−dipole interactions,[20] and more recently the halogen bond.[21] There has also been mention of interactions such as aurophilic[22] and argentophilic[23] forces and the cation···π interaction.[24]

2.4.3 Co-crystals

A co-crystal is a multiple component crystal in which all components are solid under ambient conditions when in their pure form.[17] A pharmaceutical co-crystal consists of an API and one other component known as the co-former.[25]

These components co-exist as a stoichiometric ratio of a target molecule and a neutral molecular co-crystal former(s) (Fig. 2.12).[26]

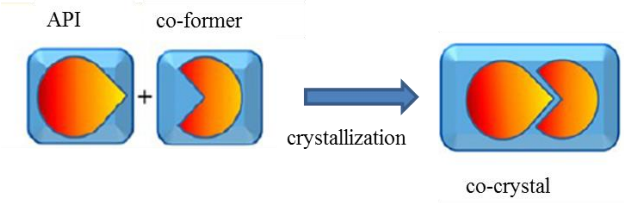
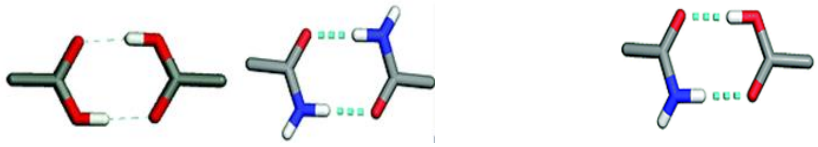


Figure 2.12 Illustration of a co-crystal

Pharmaceutical co-crystals are non-ionic supramolecular complexes that can be used to address solubility, stability and bioavailability issues in pharmaceutical development without changing the chemical composition and biological function of the API.[25]

A. Co-crystal design

The basic principle of supramolecular chemistry is the molecular recognition between complementary molecular fragments that give rise to self-organization of molecules to give a supramolecular function. Co-crystal formation is largely based on supramolecular structural assembly and heterosynthons (Fig 2.13). These can be identified through the Cambridge Structural Database.[27] Examples of such supramolecular synthons include amide-amide dimers, carboxylic acid–amide dimers and pyridine-carboxylic acid dimers (Fig 2.13).



(a) Homosynthons of acid-acid and amide-amide dimers

(b) Heterosynthon of acid-amide dimers

Figure 2.13 Schematic illustration of synthons between acids and amides

The co-former interacts with the API by means of non-covalent interactions such as hydrogen bonds, ionic bonds, π-π or Van der Waals forces.[28] The directing nature of the hydrogen bond in the solid-state, gives control over physical processes apparent in the crystalline form such as optical properties, thermal stability, solubility, colour, conductivity, crystal habit and mechanical strength.[29]

The current advances in this arena have brought about the possibility to produce pharmaceutical materials by design. Thus, formation of co-crystals represents a potential route to achieve pharmaceutical materials with improved properties of interest.[28]

During the design of co-crystals, Δ p*K*a and stereo-hindrance effect should be considered. Regarding Δ p*K*a, it is generally considered that, if the API and the co-former have a ΔpKa (pKa (base) - pKa (acid)) < 0, there will be negligible proton transfer and the molecular complex will be a co-crystal.[28] If the ΔpKa > 3, there will be complete proton transfer resulting in complete ionization and formation of a salt. However, for the region

of ΔpKa between 0 < ΔpKa < 3, the ability to forecast whether the resulting complex will be neutral or charged is inadequate. In these instances, spectroscopic tools are required to probe the extent of proton transfer and ionization states to clarify if it is a salt or a co-crystal.[28]

The utilization of hydrogen bonding rules, synthons and graph sets contribute to the design and analysis of co-crystal systems.[17] The cornerstone of co-crystal synthesis involves the formation of ternary phase diagrams for the equilibria involving the solvent. The ternary phase diagram is dependent on relative solubilies of the two components. The diagram below (Fig. 2.14) represents ternary phase diagrams from similar and different solubilities. The numbers 1 and 2 represent component 1 and component 2, respectively. Region A consists of a component 1 and solvent, region B consist of a mixture of component 1 and co-crystal, region C represents the co-crystal exclusively whilst region D consist of a mixture of component 2 and co-crystal, region E contains component 2 and solvent and region F is a solution. [30]As seen in figure 2.12 in area C, there is a greater chance of forming a co-crystal with components that have similar solubility as opposed to components with different solubilities.

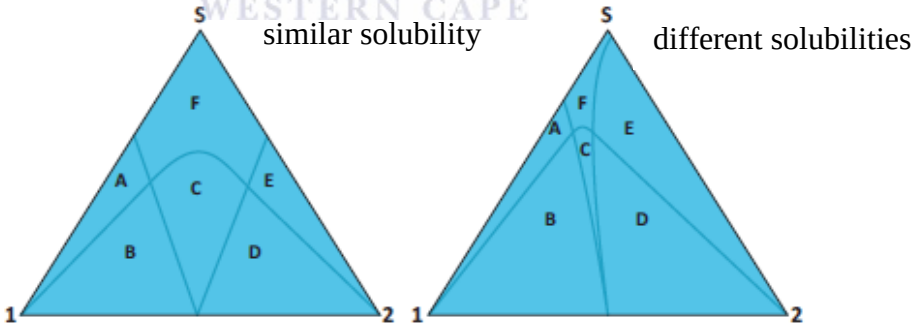


Figure 2.14 Schematic illustration of ternary phase diagrams of two components[30]

Differences in ΔpKa, collaboration of heterosynthons and formation of ternary phase diagrams alone do not dictate the formation of a co-crystal. The ability of an API to form a co-crystal is reliant on a range of variables during the crystallization process

such as the API co-former ratio, solvents, temperature, pressure and crystallization technique.[17]

B. Preparation of co-crystals

Co-crystals can either be prepared by using solvent based methods or solid based methods (Fig 2.15).[17]

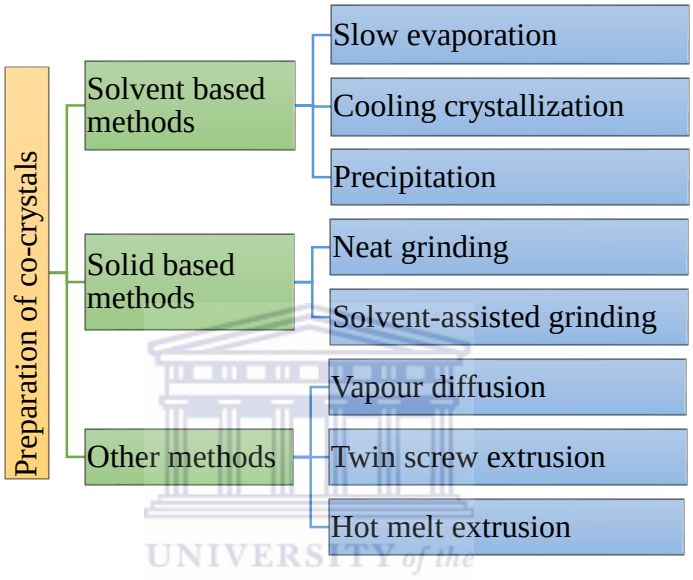


Figure 2.15 Preparation methods of co-crystals

Slow evaporation

Co-crystallization by evaporation of stoichiometric solutions is based on the use of solvents or solvent mixtures where the co-crystal congruently saturates and a pre-requisite is that the components should have similar solubility in the solvent. In the case of components having non-equivalent solubilities, solution co-crystallization of equimolar solution may result in the formation of a single component crystal. However, there is a possibility of crystallizing a single reactant or a mixture of individual reactant and co-crystal. In these instances, reaction co-crystallization approach may be adopted.[17] Nevirapine rac-tartaric acid co-crystals was prepared by this method.[31]

Cooling crystallization

Cooling crystallization method involves manipulating the temperature of the crystallization system. This method has gained attention due to the potential of a large scale of co-crystal production. Large amounts of reactants and solvent are mixed in a reactor containing a jacketed vessel, the system is then heated to a higher temperature to ensure all the solutes are totally dissolved in the solvent and it is then followed by a cooling down step.[17] Co-crystals will precipitate when the solution becomes supersaturated as the temperature drops down. An example of a co-crystal prepared by this method is caffeine and p-hydroxybenzoic acid.[32]

Precipitation

This involves recrystalization of the co-former and another component which is usually an API. Solvents include buffers (pH) and organic solvents. An example of a co-crystal prepared by this method is aceclofenac using chitosan as the solvent.[33]

Neat grinding

Neat grinding may also be referred to as dry grinding. It comprises of mixing stoichiometric amounts of co-former and API by grinding them together either mechanically or using a mortar and pestle.[17] This method requires one or both reactants exhibiting significant vapour pressures in the solid state. An example of a co-crystal formed by this method is the theophylline co-crystals using oxalic acid as the co-former.[34]

Solvent assisted grinding

This method is also referred to as kneading, solvent drop grinding or wet co-grinding. Co-crystal formation by grinding can be achieved by utilizing small amounts of an appropriate solvent. The addition of solvent enables the additional degrees of orientation and conformational freedom and open molecules at the various interfaces thus increasing the opportunities for molecular collisions.[17] This is seen in the caffeine-glutaric acid polymorph.[35]

In comparison to the slow evaporation method, little solvent is used in the former method. Thus, it is a cost-effective and a "greener" method. Co-crystals allows companies to practice green chemistry, by eliminating the need for use of solvent in a chemical reaction and thus reducing the cost of the material as well as the costs of dealing with solvent waste.[36]

Vapour diffusion

A solution containing the compound to be crystallized in a solvent is positioned in an open tube stored in a desiccator containing a small amount of the solvent. This second solvent should be less dense than, and miscible with, the first solvent. As solvent equilibrium is reached, the non-solvent diffuses through the vapor phase into the solution, and saturation or supersaturation can be achieved. An example of a co-crystal formed by vapour diffusion method is diclofenac co-crystals.[37]

Twin screw extrusion

This process is a recommended procedure for scaling up. It is a relatively novel method that was explored. Extrusion can be considered as an efficient, scalable and an environmentally friendly process. Parameters such as screw design, temperature and residence time affect the formation of co-crystals.[38] A high mixing screw design was used to enhance surface contact between co-crystal components in the extrusion barrel. Temperature for the caffeine–oxalic acid co-crystal system was set to 75 °C throughout the screw zones.

Extrusion may be considered as an efficient, scalable, and environmentally friendly process for the manufacture of co-crystals compared to solvent crystallization methods.

Hot melt extrusion

This method uses the application of heat and pressure to melt a substance and force it though an orifice in a continuous process. An example of a co-crystal formed through hot melt extrusion is ibuprofen– nicotinamide co-crystal. A single-step, scalable, solvent-free, continuous co-crystallization and agglomeration technology was developed for this co-crystal using the hot melt extrusion method.

C. Identification of co-crystals

In certain instances, co-crystal formation is visibly apparent due to the changes in physical properties of the new material. This is seen in the formation of a red co-crystal of acetaminophen and 2,4-bipyridine dicarboxylic acid, while the individual components are white in colour.[39] However, it is superior to confirm the formation of co-crystals with a wide array of techniques available.

Single-crystal X-ray diffraction, powder X-ray diffraction and thermal methods such as differential scanning calorimetry, thermogravimetric analysis, hot stage microscopy, vibrational spectroscopy (Infra-red and Raman) and solid state nuclear magnetic resonance encompasses the full battery of techniques that are conventionally used to characterize co-crystals.[30]

Out of these methods, single-crystal X-ray diffraction is a superior method which uniquely determines the crystal form and gives a 3D representation of the structure. Powder X-ray diffraction can determine the structure by Rietveldt refinement methods.

Infra-red and Raman spectra can determine the fingerprint of a particular solid form. If particular bands are sensitive to the solid form, vibrational spectra are useful to distinguish between polymorphs and co-crystals.[30] Differential Scanning calorimetry is useful in detecting phase changes that do not result in a change in mass. It is also useful in giving an accurate temperature for melting and onset. Thermogravimetric analysis is beneficial for determining the onset temperature of co-crystal decomposition and loss of volatile component. The stoichiometry of the co-crystal can also be established. Solid state nuclear magnetic resonance can give information on chemical shift values for particular solid forms.[30]

D. Advantages of co-crystals

Co-crystals are in their crystalline form hence they are more stable than their amorphous form. Co-crystals are stable because both the solids are ambient at room temperature.[17] All types of API molecules, irrespective of their ionization capability

can form co-crystals. There is no need to make or break covalent bonds since they are formed by non-covalent bonding.[17] Additionally, there are no by-products involved.[17]

Co-formers can be chosen based on their potential use as a pharmaceutical excipient.[40] Co-crystals are preferred over solvates for a number of reasons, solvates are volatile and susceptible to desolvation during storage and the solvent loss may revert to the amorphous phase. Whereas, co-formers used are unlikely to evaporate from solid-dosage forms making phase separation and other physical changes unlikely.[41]

Solvents used to make solvates are often at a concentration that is not approved by regulatory authorities and may have toxicological consequences.[42] Whilst, several co-formers used to make co-crystals are approved by the FDA and listed in the Generally Regarded As Safe (GRAS) list.[43]

Co-crystals have favourable properties over salts because, formation of salts requires the presence of ionisable sites while co-crystals can be employed to acidic, basic and even non-ionizable molecules.[17] Moreover there are a limited number of counter ions available for salt formation.[17]

Co-crystals can be patented hence broadening pharmaceutical landscapes. This is particularly advantageous for companies that are dependent on rigorous intellectual property protection for safeguarding the product revenues; this is highly pertinent to an industry which combines extensive regulatory challenges, high research and development costs, and inherent risks. Patents are a mechanism that gives the right to exclude others from practising a patented invention and affords an economic incentive to the inventor.[44] Further information regarding co-crystals and intellectual property are outlined in the concluding section of this chapter.

The ability to modify the solid-state arrangement of molecules provides a way to control the intrinsic mechanical and physical properties of solids.[45] Table 2.1 depicts some examples of the active ingredient that can be enhanced by co-crystal formation.

Active ingredient of co-crystal	Property of drug enhanced
Fluoxetine Hydrochloride	solubility [46]
Benzoquinone	colour[47]
Adefovir Dipivoxil	kinetic stability[48]
Caffeine	hygroscopicity[49]
Theophylline	relative humidity[34]
Paracetamol	compressibility[50]
Nicotinamide	thermal stability [51]
AMG-517	decrease in required dose[52]
Itraconazole	solubility and bioavailability[53]
Ibuprofen	moisture sorption and tableting behaviour[54]
Carbamazepine	dissolution rate and suspension stability[55]
Pyrazinamide –Difunisal (drug-drug co-crystal)	decreased side effects and improved aqueous solubility[56]

Table 2.1 Example of co-crystals and the property enhanced

Hence, there is well established evidence that co-crystals can be developed to improve certain properties of drugs. Nearly ten years ago, Datta et al. mentioned that there has not yet resulted the design of crystals with desired mechanical properties.[57] Presently, there are a number of publications indicating that co-crystals have been able to improve tableting properties. Examples of these are co-crystals of ibuprofen-flurbiprofen and caffeine-oxalic acid.[58] This indicates the progress of pharmaceutical landscapes. To further the progress already made, this study aims to investigate co-crystal's usage in a liquid dosage form.

E. Delivery and integrity of co-crystals

Literature indicates that the behaviour of formulated co-crystals has received minimal attention in the pharmaceutical arena. However, this is on route to change as co-crystals are in the development pipelines of pharmaceutical companies.[59]

Co-crystals have been successfully formulated in solid dosage forms. In 2007 carbamazepine-saccharin co-crystals which has been one of the pioneer prototypes of co-crystals was formulated as a tablet.[55] Another case in point illustration of a co-crystal tablet with superior tabletting properties is theophylline-methyl gallate co-crystal.[34] In 2015 prulifloxcin co-crystals were formulated as an immediate release tablet.[60] Danazol co-crystal has been formulated with controlled supersaturation by preparing aqueous suspensions and capsules.[61] There has been evolvement of formulation of co-crystals from tablets and capsules to most recently immediate release from tablets. Quality control has also been ascertained for these co-crystal tablets. However, for the supersaturation preparations no quality control has been performed.

For successful delivery of co-crystals it is imperative for a co-crystal to be delivered intact. In addition to this, the FDA requires data to show that there is complete dissociation of the API from its excipient prior to reaching the site of action for pharmacological activity.[62] Therefore, co-crystals need to be intact until dissolution of the drug takes place.

Co-crystals by definition are crystalline solids formed by non-covalent bonds and when formulated as a solid dosage form it is exposed to other excipients which are typically solid in nature. Thus, in the case of a solid dosage form the integrity of co-crystals formulated in the solid dosage form is not problematic. However, co-crystal dissociation into the amorphous form and co-former can be detected upon storage under high humidity conditions.[63] Thus, regarding solid dosage forms the integrity of the co-crystal is challenged during storage and not necessarily during the formulation process itself.

Conversely, regarding formulation of co-crystals in a liquid dosage form, co-crystal disproportionation may occur when co-crystals are present in a liquid phase.[64] Due to the physical interaction, in particular the non-covalent bonding between the API and its co-crystal former, these pharmaceutical co-crystals are sensitive to rapid or slow dissociation in an aqueous microenvironment losing their effects prior to oral administration.[65]

The target of pharmaceutical development is to administer pharmaceutical co-crystals in formulations where the integrity of the co-crystal is ensured as much as possible. In a study done by Márta Venczel the physical integrity of the co-crystal during high shear wet granulation process was investigated. In cases where the integrity of co-crystals is compromised, cremophor ELP which is used as a solubiliser may be employed to ensure the integrity of the co-crystals.[65]

The European Medicines Agency also advises that the integrity of the co-crystal during the entire manufacturing process should be experimentally confirmed.[66] Keeping the integrity of a co-crystal as pharmaceutical ingredients after the manufacturing process is essential to ensure advantages like faster dissolution kinetics and higher bioavailability.

The faster solubility and dissolution kinetic of co-crystals is responsible for higher absorption therefore keeping the integrity of the co-crystal as an API is essential to reach the targeted effect and ensure the robustness of the formulation.[65]

F. Scaling up of co-crystals

For co-crystals to be industrially beneficial, the robust preparation of bulk co-crystal powders is a prerequisite. Further research is necessary to develop scale-up co-crystal systems and implement manufacturing of final dosage forms on commercial scale.[67]

Additional developments in screening methodology will further elevate the profile of co-crystals on the pharmaceutical and intellectual property landscapes.[44] Co-crystal screening methods such as slurry conversion, evaporation, and liquid assisted grinding/sonication may not be applicable to co-crystal scale-up.

Reasonably few studies have been reported on co-crystal scale-up. The most common scale-up technique is solution co-crystallization. However, this method requires significant development, which can hinder initial discovery and early development in the pharmaceutical industry.[68]

According to Leung and co-workers[68] scaling up of potential co-crystal leads involves solvent selection followed by determination of the critical region on the ternary phase diagram within which pure co-crystals can be isolated. They developed a general method for selecting solvents for co-crystallization and a method for rapid determination of the region of stability of co-crystals on the ternary phase diagram of API, solvent and co-former. This resulted in a scale-up to 1−2 g of a co-crystal lead.

From an industrial perspective, chemical engineers are concerned with implementation of processes that are developed in the laboratory. As with any other scale-up process, co-crystal scale-up requires a proportional amount of API. However, for some reason API's exhibit differences when produced on a small scale, such as a laboratory scale as opposed to a large scale. Hence, scale-up of an API from a laboratory scale (milligram) quantity to a large scale (kilogram or ton scale) in a plant without changing its optimized properties and reproducibility at an economical level is a major challenge in the pharmaceutical industry. Industrial scale-up processes such as cooling, antisolvent or reactive crystallization are done in batches.[69] The batch method is the most common method, however the issue of batch-to-batch variability is a cause of concern.

On the other hand, continuous processing has advantages over batch processing, they require smaller process equipment and thus the cost of the equipment may be lower.[69] Continuous processing offers enhanced reproducibility of the crystalline material.

In order to implement scale-up processes in a pharmaceutical industry, data from laboratory activities such as purity, size distribution, shape and crystal form will be required.[69] As rightly noted by Chen et al. factors such as time, reactant addition,

mixing, stability, centrifuging, drying, maintenance of temperature, pH, water content, and other related parameters are pertinent in scaling up.

With regards to time, due to larger volumes of materials being crystallized, materials require more time in slurry prior to solid-liquid separation. During the addition of reactants, the magnitude of heat released or absorbed must be observed to ensure it is a controlled process. The author also suggests that thermal stability of raw materials should be pre-determined to detect endothermic or exothermic behaviour.[69]

Mixing of solutions and slurries are a critical part of scale-up process. Solids have to be suspended and minimizing of secondary nucleation, crystal breakage, and growth on surfaces has to be adhered. In the case of antisolvent, the rate and location are crucial factors which must be taken into account along with the agitation rate. Stability of raw data should be constantly monitored to ensure similar conditions are simulated in a pharmaceutical industry.

On a large scale filtration process, the compatibility of a filter cloth and parameters such as time flow, temperature, pressure, weight, pore size and shape should be considered.[69] Albeit these factors, the successful implementation of scale-up is also dependent on financial constraints of the business in question.

G. Regulatory concerns

Compared to other classes of solid forms, co-crystals own particular scientific and regulatory advantages, however together with these advantages are intellectual property issues which confer co-crystals with unique opportunities and challenges.[44] The patent system is at the core of an ongoing policy debate over balancing a productive, economically viable, research-based industry with an equitable system for maximizing citizens' access to effective pharmaceutical therapies.

For a compound to be patented it has to satisfy the triad criteria of novelty, utility and non-obviousness.[45,70] Co-crystals satisfy all three criteria in the following manner: **Novelty:** co-crystals are new and distinct solid state structures.

Utility: co-crystals can offer utility with respect to physical property improvements.

Non-obviousness: selection of co-former is routine unlike salts where it is obvious an acid is required to make a salt from a base.

As definitions are without a doubt important in patents, the FDA draft guidance document provides an analytical framework for defining and classification of co-crystals. According to the draft released in December 2011, the requirement for characterization of a co-crystal is the difference in pKa between the drug and co-former should be within a range of three units, this is to rule out the possibility of proton transfer and furthermore, the co-crystal should dissociate to release the free API before reaching the target site. The FDA proposes that co-crystals be treated as a process intermediate en route to a drug product. The labelling of the final product can remain confined to the original API.[36] The manufacturing of the co-crystal should be in a facility that operates in accordance with current good manufacturing practice.

In the United States, there has been an influx of patent applications in the past decade. Patents regarding co-crystals can be broadly classified into methodologies and composition patents.[36] Methodology patents refer to methodologies related to the process to make pharmaceutical co-crystals. Co-crystals are not the sole subject of the patent but are explicitly named in dependent claims. An illustration of this is the TransForm Pharmaceuticals which claimed a method of producing co-crystals through mechanical grinding.[71]

Whereas, composition patents relate directly to the formation of co-crystals. The first US patent issued in this arena, was in 1999 from Eli Lily & Co. which entailed co-crystals of cephalosporin complexes with parabens which are usually used as preservatives.[72] Thereafter, there have been numerous patents in this growing field. However, the standards used to evaluate proposed patent claims differ between countries. Thus the pace of patent activity in countries outside the United States has been trailing slightly slower. In Europe there has been a limited issuance of patents, this is due to the organizations determining patentability of co-crystals on a case-by-case basis. As this is a growing field of interest in novel research space, it is only a matter of time that patents regarding co-crystals may be seen in other parts of the world.

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Chapter 3

Experimental

Chapter 3 Experimental

3.1 Materials

The active ingredient, nevirapine, was obtained from Aspen (batch number BO24277; expiry date: 01 June 2016) as a donation. The co-formers viz. saccharin (batch no. STBC5795V; Germany), glutaric acid (batch no. STBC348G; China), salicylic acid (batch no. SZBCO450V; France), rac-tartaric acid (batch no. MKBJ6801V; India) and maleic acid (batch no. BCBB8525V; Austria) were obtained from the company, Sigma-Aldrich. The solvent, methanol, was obtained from Merck (batch no. SA1SF61031; South Africa). Sorbitol (batch no. 17816) was obtained from Brunel and polysorbate 80 (batch no. BCBN3690V) was obtained from Sigma-Aldrich. The viscosity inducing agents, viz. aerosil 200 (batch no. 153020513, Germany) was obtained from Evonik industries, carbopol 974 P (0101274748, South Africa) and carbopol 971G (0000021275, South Africa) were obtained from Lubrizol. The preservatives; methylparaben (BCBL6776V, India) and propylparaben (BCBM0184V, Japan) were obtained from Sigma-Aldrich.

3.2 Compound verification

All compounds included in this study were supplied with a Certificate of Analysis (COA) and the purity of each compound was confirmed by differential scanning calorimetry (DSC) and fourier transform infrared spectroscopy (FTIR). Data obtained are analysed in chapter 4.

3.3 Brief overview

To-date, there are five different co-crystals that were prepared with nevirapine.[1] Of these, the most appropriate nevirapine co-crystal was selected for a suspension formulation. A suspension was prepared with the nevirapine co-crystal and if excipients were required, these were selected from the formulation of the branded form of nevirapine (Viramune® suspension) and were added when necessary. Quality

control tests were performed on the suspension and compared to both, the USP standard and the Viramune® branded suspension.

3.4 Choice of co-former

Co-crystals are typically found using screening systems. Technological advancements in screening have increased the chances of selecting the appropriate co-crystalline API form.[2] A series of five nevirapine co-crystals was prepared from different co-formers viz. saccharin, glutaric acid, salicylic, rac-tartaric acid and maleic acid during a thorough screening process.[1] Each co-former exhibits a different physicochemical property which in-turn could affect the formulation of a selected dosage form. Therefore, careful selection of a co-former was required for the methodology of this study prior to experimentation and formulation. To select the best co-former for formulation of a suspension, each co-former had to be reviewed under the following criteria (Table 3.1). This will be expanded upon in chapter 4.

Physical	• dissolution rates as a co-crystal and as a mixture • solubility increase of API as co-crystal • solubility of co-former in water • melting point of co-former • particle size of co-former • particle shape of co-former • specific gravity of co-former
Chemical	• log P of co-former
Pharmacological	• lethal dose in rats of co-former • side effects of co-former • antiviral activity against HIV-1 of co-crystal
Pharmaceutical	• method of preparation of co-crystal • percentage yield of co-crystal • solvent used to prepare co-crystal • taste of co-former • sedimentation ratio of co-formers • excipient use of co-former • status of co-former according to FDA database
Other	• cost of co-former

Table 3.1 Classification of selection criteria for chosen co-former for suspension dosage form

After compilation of properties of co-formers according to table 3.1, co-formers were graded using an ordinal scale (excellent = 5, good = 4, average = 3, fair = 2 and poor = 1). The total score was calculated by adding the scores obtained for each co-former. The co-former with the highest score was considered for formulation.

3.5 Preparation of co-crystals

Following the selection of an ideal co-former for suspension, the selected co-former was then synthesized to form the respective nevirapine co-crystal with either of the following methods: [1]

3.5.1 Slow evaporation

Stoichiometric amounts of co-former and API were individually dissolved in a known volume of solvent. The API and co-former must have a similar solubility in the chosen solvent. If solubilities, are not similar the least soluble component would precipitate out, exclusively.[3]

Samples were stirred with the aid of a magnetic stirrer and placed on a hot plate until the powders were completely dissolved in the solvent. The temperatures were kept below the melting point of the lowest melting component and 10 ^{o}C below the estimated boiling point of the solvent system. The two solutions were mixed together by adding the solution with the greater volume to the smaller volume. The solution was then stirred and extracted with a syringe, filtered through a 0.45 μm micro-filter and placed in a clean glass vial. The vial was sealed with Parafilm® and perforated to facilitate solvent evaporation.[4] The vial was placed in a fume cupboard at room temperature (Fig. 3.1).

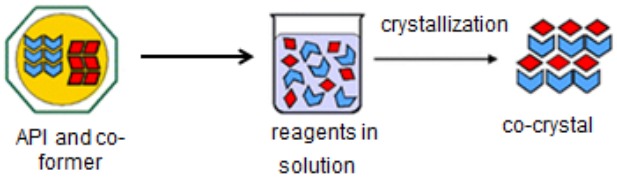

Figure 3.2 Process of slow evaporation[5]

3.5.2 Liquid assisted grinding

A small amount of the appropriate solvent was added to a stoichiometric mixture of the API and the co-former. The mixture undergoes mechanical or manual grinding to reduce the excessive use of the crystallization solvent.[6] The adding of small amounts of solvent during the grinding process had shown to enhance the kinetics and facilitate co-crystal formation.[3] This method is a "greener" approach since it inhibits the use of excessive solvent (Fig. 3.2).

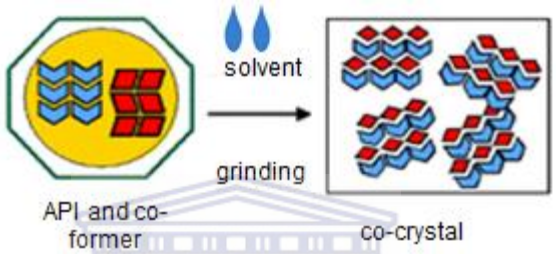

Figure 3.3 Process of liquid assisted grinding[5]

3.6 Identification of co-crystal

Upon preparation of the co-crystal, the preparation had to be identified to ensure the formation of a co-crystal. Techniques such as hot stage microscopy (HSM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), fourier transform infra-red (FTIR), powder X-ray diffraction (PXRD) and single X-ray diffraction (SXRD) were considered to identify the co-crystal, however due to availability only HSM, DSC, TGA and IR were used in this study.

3.6.1 Hot stage microscopy

HSM is a valuable supportive tool when used in conjunction with other techniques. It can be utilized to ascertain the nature of events leading to endotherms or exotherms on differential scanning calorimetry (DSC) traces or weight changes observed in thermogravimetric analysis (TGA).[7]

Decomposition with gas evolution, and especially loss of water of crystallization from hydrates, can be observed by mounting crystals in silicone oil and physically observing the phase changes of the sample during a temperature programme. Gas or vapour bubbles can be observed emanating from crystals at temperatures correlating with decomposition (endotherms) or desolvation (exotherms) in DSC.[7]

The samples are mounted with silicone oil under a microscope slide. This is essential for solvent detection in the sample. In this study, a Linkam TH MS600 Temperature control stage connected to a T95 Linkpad System Controller was used. Heating of the products were controlled at a fixed rate of 10 °C per minute. An Olympus SZX7 stereoscopic microscope connected to Olympus UC30 colour video camera was mounted to capture pictures for the determination of visual observation and characterization. The images were recorded and saved by Stream Essential software®.

3.6.2 Differential scanning calorimetry

Samples were analysed using a Perkin Elmer differential scanning calorimeter DSC7 connected with a Perkin Elmer thermal analysis controller TAC7/DX and Perkin Elmer thermal analysis gas station. The samples were scanned at a fixed heating rate (10 °C/min) under a N_2 gas with a flow rate of 20 mL/min. Samples were removed from the mother liquor, dried on a filter paper, weighed (the mass range was 1-2 mg) and sealed in a crimped, pricked aluminium pan. The reference used was a sealed empty aluminium pan. The calibrations of the DSC was performed by the melting point determination of indium which is 165 °C. The samples were heated over the range of 40-300 °C to see the decomposition. The data were collected and analysed using Pyris software®.

The basic principle of the construction of the DSC cell relies on the sample and reference having separate heaters. Sample and reference are maintained at nominally the same temperature via the system being operated through platinum resistance thermometers and resulting in different amounts of heat being supplied to each

specimen as appropriate. The difference in power output to the heaters is monitored.[7] This technique is used to determine the melting point and decomposition of a sample.

Endothermic and exothermic peaks appearing in the DSC traces are also analysed in terms of their onset temperatures and temperature range of the peak. The peaks observed in the DSC trace were interpreted in combination with HSM and TGA, associated with phase changes and solvent loss respectively.

3.6.3 Thermogravimetric analysis

TGA utilizes a thermobalance, which allows for ongoing monitoring of sample weight as a function of temperature. This may involve a controlled heating or cooling programme or a maintained fixed temperature. The principle of operation is that mass deposited on or lost from the surface of a highly polished crystal results in a shift of an oscillatory frequency.[7]

This method is used to determine small and large weight changes of a sample. It is used to establish the stoichiometry of inclusion compounds and to study the decomposition of desolvated materials.

TGA analyses for all complexes were performed on a Perkin Elmer TGA 4000 instrument under N_2 gas purging at a flow rate of 20 mL/min. The Perkin Elmer TGA instrument was calibrated using alumel (mp = 156.6 ºC), perkalloy (mp = 596.0 ºC) and iron (mp = 780.0 ºC) in an automated process in which temperature calibration, thereafter furnance calibration was done. The cystals were rapidly dried on a filter paper to remove surface solvent. The weighed samples were placed in an open porcelain pan. The programmed TGA analyses were carried out over a temperature range of 30 ºC to 400 ºC at the predetermined linear heating rate of 10 K/min. This technique was primarily used to determine the percentage weight loss.

3.6.4 Fourier Transform Infra-Red

Each molecule has a specific infra-red absorption pattern. FTIR spectral markers that correspond to bonds in a synthon are used to identify supramolecular synthons in co-crystals. All spectra were collected using a Perkin Elmer Spectrum 400 FT-IR Spectrometer. The analysis of the spectra conducted used Spectrum software version 6.3.5. The FTIR spectra of the analysis were compared to the parent compounds spectra, where each molecule has a specific infra-red absorption pattern.

3.6.5 X-ray powder diffraction

PXRD is an instrumental analytical technique, which has been used to identify crystalline materials for almost a century. The technique was originally used to examine the nature of crystal lattices by the diffraction of X-rays through the closely spaced lattice of atoms.

X-ray crystallography techniques are concerned only with the structure analysis. This technique is especially useful in the absence of good quality crystals for single crystal structure determination, it uniquely identifies materials thus allowing for the identification of new compounds. It is considered as the most powerful tool for identity specification.

3.6.6 Single X-ray diffraction

Single X-ray diffraction is the most preferred technique for structure determination. It provides an in depth structure of the crystal.

Single crystals, typically between 0.2 and 0.5 mm in all dimensions, of good quality are selected for their ability to extinguish plane-polarised light uniformly. However, SXRD may not be practicable when the numbers of samples become very large and there are many cases wherein obtaining a single crystal of an appropriate size is problematic.

3.7 Scaling up

Upon preparation and identification of co-crystals, scaling up of the relevant co-crystal was explored. Co-crystals in the development phase and scale-up methods are currently being explored however, relatively few studies have been reported on co-crystal scale-up procedures.[8]

Early development and screening of co-crystals typically rely on slow evaporation and mechanochemical methods, however the main challenge that exists is, "scaling up to a multikilogram scale".[9] Solid state grinding that is achieved through the use of a mortar and pestle in a laboratory scale, can be transformed to a large scale by utilizing the ball-mill grinder. This approach is difficult to scale-up for energetic materials since friction may be caused from the grinding and result in detonation. From the methods existing to prepare co-crystals, solution co-crystallization remains the most commonly used scale-up technique.[10]

In this study, the co-crystal was prepared from milligram scale to a gram scale. Fourteen batches were prepared, batch 1-8 were prepared in a small fume cupboard while batch 9-14 were prepared in a large fume cupboard.

3.8 Preparation of suspension

Following the preparation and identification of the relevant co-crystal, preparation of suspensions was employed. Three formulations were prepared using the same excipients as in the branded version of nevirapine (Viramune®), except that the viscosity inducing agent for these formulations varied. Formulation A, B, C utilised aerosil 200, carbopol 971G, carbopol 974P, respectively. The preparation of suspensions can be divided into two broad categories, the precipitation methods and the dispersion method.[11]

3.8.1 Precipitation methods

Precipitation methods can further be subdivided into organic solvent precipitation, precipitation effected by changing the pH of the medium and double decomposition.[11]

3.8.2 Organic solvent precipitation

Water insoluble drugs can be precipitated by dissolving them in water-miscible organic solvents such as alcohol, acetone, propylene glycol or polyethylene glycol. The organic phase is then added to distilled water under standard conditions and a suspension is produced having a particle size in the 1 to 5 μm range. An example of a suspension made by this method is Prednisolone suspension,[11] it is precipitated from a methanolic solution to produce a suspension in water. This type of preparation is useful in cases of parenteral or inhalation therapy where fine particles are required. However, the disadvantage with this method is that it may be difficult to remove harmful organic solvents.

3.8.3 Precipitation effected by changing the pH of the medium

Some drugs may be readily soluble at a certain pH and precipitate at another pH. This type of drug can first dissolve in a favourable pH and then the solution is poured in another buffer system to change the pH of the medium and the drug will form a suspension in the medium of the second pH. An example of this is Insulin suspension; it has an isoelectric point of 5, when it is mixed with protamine -a basic protein- it precipitates between the isoelectric points of the two components (pH of 6.9 – 7.3).[11]

3.8.4 Double decomposition method

This method requires two water soluble reagents to form a water insoluble product. This is mostly used for topical suspensions. An example of this is White Lotion®,[11] It can be prepared by slowly adding zinc sulfate solution in a solution of sulphurated potash to form a precipitate of zinc polysulphide.

3.8.5 Dispersion methods

This method comprises of first creating a deflocculated suspension. After a deflocculated suspension is made, various techniques are used to alter the suspension so that a flocculated suspension is formed (Fig. 3.3).

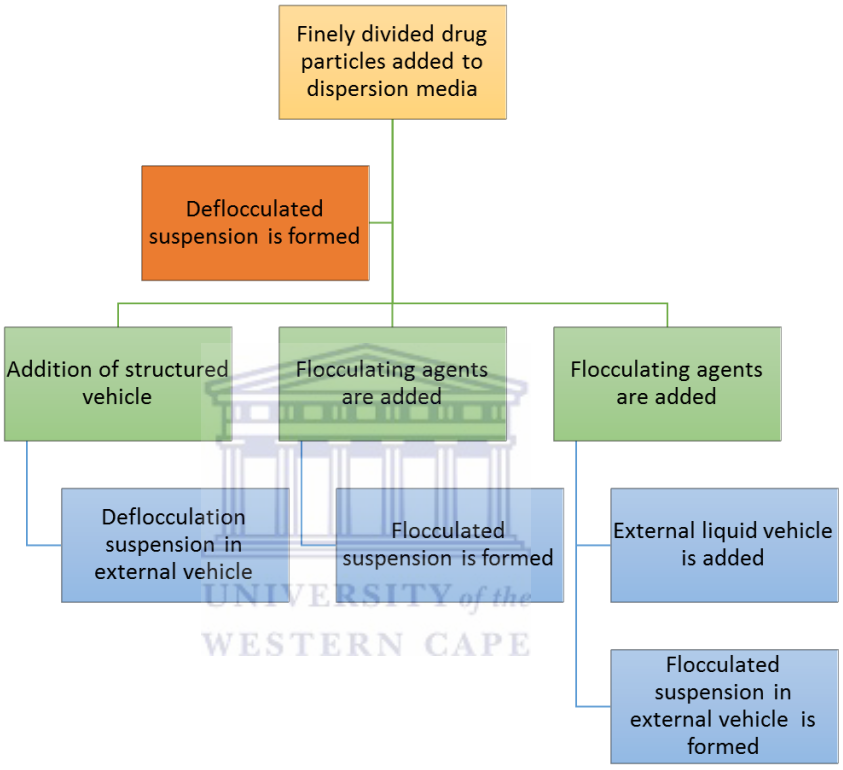

Figure 3.3 Preparation of suspension by dispersion methods[11]

Suspensions are prepared by grinding the insoluble materials in a mortar to a smooth paste with a vehicle containing the wetting agent. This forms a deflocculated suspension. Once a deflocculated suspension is formed, a structured vehicle can be added to convert the deflocculated suspension to a flocculated suspension. Alternatively, flocculating agents are added to the deflocculated suspension to form a flocculated suspension. If this does not work then an external liquid is added together with flocculating agents and a flocculated suspension in an external suspension is formed.

3.9 Identification of co-crystal in suspension during production

According to the European Medicines Agency, in its reflection paper published in (2014)[8], on the use of co-crystals, states that "the integrity of the co-crystal during the entire manufacturing process should be experimentally confirmed".

This study had to ensure the integrity of the co-crystal pursued continually throughout the study. To achieve this target, the formed suspension had to be filtered for analysis.

In a study by Shiraki et al in (2008)[2], transformation behaviour of co-crystal suspensions were examined by PXRD analysis and were observed under polarization microscopy. This is a good technique as it is considered the most powerful tool to characterize a co-crystal.

In this study, a novel dual approach of identifying the integrity of the co-crystal was attempted. The suspension formed with the co-crystal was filtered and the filtrate solution was identified by UV, this is a relatively new ideology where the presence of co-crystal was tested in the filtered suspension. Furthermore, the residue of the filtered suspension of the co-crystal was retained and identified by DSC (Fig 3.4). Analysis of co-crystal tested by UV and DSC is outlined in the following section.

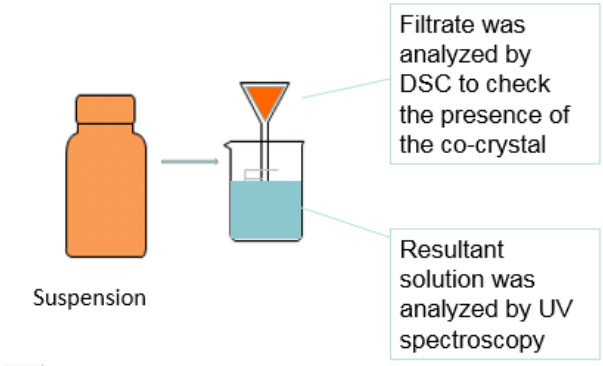

Figure 4.4 Schematic illustration of which component of the formulation was studied to ensure the integrity of co-crystal

3.9.1 Ultraviolet spectroscopy of the suspension

UV spectra were recorded on a Cintra ® 2.2 GBC Scientific Equipment Pty. Ltd. UV/visible spectrophotometer. Samples were filtered through a Whatman® no.41 filter paper as opposed to a membrane filter due to the viscous nature of the sample. It was then placed in 1 mL quartz cuvettes and a wavelength scan was performed between 200-800 nm at a scanning rate of 1200 nm/min. Absence of absorbance at wavelengths where the API and co-former is expected to be seen, indicated that the co-crystal is retained. Conversely, the presence of peaks for the API and the co-former indicated that the co-crystal had fragmented into its two components.

3.9.2 Differential Scanning Calorimetry of suspension filtrate

The suspension was filtered through a filter paper and the filtrate was removed from the filter paper and analysed by DSC using the same procedure that was used to identify the co-crystal as mentioned in section 3.6.2. The presence of an endothermic peak at the melting point of the co-crystal indicated that it was still intact in the suspension. Conversely, the presence of two individual peaks at the melting points of the co-former and the API suggested that the co-crystal had fragmented.

3.10 Quality control of suspensions

Upon assuring the co-crystal integrity in the suspension, the physical characteristics such as particle size, pH, viscosity, zeta potential measurement, and dissolution was performed in accordance with the methods stated in the USP 32.

3.10.1 Particle size analysis

Scanning electron microscopy (SEM)

The morphology of the co-crystal powder was determined by SEM. The model used was LEO1450. A small quantity of co-crystal was placed on carbon adhesive tape and was placed on an aluminium stub, thereafter it was placed in a fumehood until it the

sample was completely dried. The dried co-crystal was then coated with gold palladium using an Emitech K550X (England) sputter coater and viewed using a Auriga F50 HR-Scanning Electron Microscope with working distances of 6.6, 6.7 and 6.8 nm with accelerating voltage of 5 kV.

Zeta sizer

The stability of a suspension depends on the particle size of the dispersed phase. Particle size of particles in suspension was performed by a zeta sizer nano series S90 which utilizes a dynamic light scattering technique that measures the size of particles at the micrometre size range more accurately than particles in the submicron range. Photon correlation spectroscopy was used to measure the size of particles in the nanometer range by placing 1 mL of sample in a cuvette and inserting it in a Zetasizer instrument (Zetasizer nano, Malvern Instruments Ltd., UK). Particle size and size distribution were presented by the Z-average and polydispersity index, respectively.

3.10.2 Determination of pH

The pH of suspensions determines the stability and characteristics of formulations. Therefore, pH of the different vehicles and the pH of different phases of the suspension, have to be monitored to ensure optimum pH environment is being maintained. This was measured by using a pH meter (Eutech Instruments pH 2700 meter). The pH meter was calibrated at room temperature to a pH of 7 initially and further calibarated to a pH of 10 and 4.

3.10.3 Zeta Potential

The Zeta potential has to be measured to establish the stability of disperse systems. The zeta potential was carried out using the Laser Doppler Velocimetry (LDV) principle by using the Zetasizer instrument (Zetasizer nano, Malvern Instruments Ltd., UK). The principle relies on the Doppler shift in a laser beam used to measure the velocity in semi-transparent or transparent fluid flow. The zeta potential was measured by adding

700 μL of the suspension in a zeta capillary cell (DTS1070) and the temperature was set at 25 ºC, with measurements conducted at an angle of 173º. The intensity-weighted mean value was measured and the averages of three measurements were taken.

3.10.4 Viscosity

The Brookfield (Model DVII + Pro) viscometer was used for determining viscosity of suspensions. It was calibrated with silicone oil at three different temperatures, 25 °C, 93.3 °C, 149 °C. The Brookfield's was used because it has an advanced design and special device for reading and recording the speed. A small sample adapter (16 mL) was used due to the size of the sample available. The small sample adapter was connected to a water bath and the temperature was monitored with a thermometer. The suspension was placed in a small sample chamber and was placed into the water jacket. Small sample spindles were placed in the chamber and the viscosity readings were displayed on the digital screen. Rheograms were constructed with the data obtained.

3.10.5 Dissolution method

The USP utilizes Method I basket and Method II paddle apparatus to perform dissolution studies. According to the USP, the paddle II method should be employed for Viramune® suspension. The dissolution media that was used was phosphate buffer (pH=6.2).[12]

To prepare phosphate buffer (pH 6.8), a volume of 50 mL of 0.2 M monobasic potassium phosphate solution was added to a 200 mL volumetric flask. To this 22.4 mL of 0.2 M sodium hydroxide solution was added and distilled water was added to volume. Details of the preparation of the monobasic potassium phosphate solution and sodium hydroxide solutions are listed below.

Preparation of 0.2 M monobasic potassium phosphate solution:

Dissolved 27.22 g of monobasic potassium phosphate in water in a 1000 mL volumetric flask and solution was made up to volume with distilled water.

Preparation of 0.2 M sodium hydroxide solution:

Dissolved 8 g of sodium hydroxide in a 1000 mL of water in a beaker.

The analysis was performed on a Vankel VK 700 (220 V) dissolution apparatus and with the Vankel VK 650 A Heater/Circulator Benchsaver® series. 5 mL of the suspension was introduced to the system through the orifice located on the top cover by means of a syringe, in the dissolution media of 900 mL which was maintained at 37 °C. The rotation speed that is recommended was 25 rpm. A 60 minute run was conducted. Samples were extracted and replaced with 5 mL of dissolution media at 10, 20, 30, 45 and 60 minutes intervals.[12] 1 mL of the dissolution sample was placed in an amber 1.5 mL HPLC vial and labelled accordingly.

The same method above will be employed for the nevirapine co-crystal suspension prepared in this study.

3.10.6 HPLC analysis

A standard curve of nevirapine was constructed before the assays were performed. Phosphate buffer that was prepared was used as the diluent. The stock solution was prepared by adding 60 mg of nevirapine dissolved in 120 mL of diluent. This yielded a concentration of 500 μg/mL. The flask was placed in a sonicator to aid the dissolution of nevirapine. The standard was performed in triplicate to ensure accuracy and reproducibility.[13]

The HPLC system used for the acquiring the chromatograms and UV spectra was an Agilent 1200 series HPLC system, equipped with an in-line degassing system (G1322A, Japan), quaternary pump (G1311A, Germany), auto loading sampler (G1329A, Germany), thermostatted column compartment (G1316A, Germany) and photodiode array detector (G1315B, Germany). Chromatographic separation was obtained using a Phenomenex Luna® C_{18} column (25 cm x 4.6 mm, 5 μm i.d.) with a

compatible guard column, both maintained at 35°C. The following specifications were used for the HPLC analysis

Mobile phase	23 % acetonitrile, 77 % water
Flow rate of mobile phase	1 mL/min
Column	Phenomenex C_{18} column (25 cm x 4.6 mm, 5 μm i.d.)
Injection volume	50 μL
Wavelength	280 nm
Retention time	3 minutes

Table 3.2 HPLC specifications for analysis of nevirapine in suspension

The mobile phase consisted of 23 % acetonitrile and 77 % water was filtered through a 0.45 µm filter and degassed prior to use. The flow rate of the mobile phase was maintained at 1 mL/min with an injection volume was 50 μL, and peaks were separated with an isocratic elution of 23 % acetonitrile. Data acquisition and processing was carried out using the OpenLAB™ CDS ChemStation Edition software.

A reversed phase HPLC separation method combined with DAD detection was employed for nevirapine analysis in the suspension. This method had been validated by Geldenhuys to ensure accuracy, reliability and reproducibility.[13]

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Chapter 4

Results & Discussion

Chapter 4 Results and discussion

To prepare a nevirapine co-crystal liquid dosage form, a co-former had to be selected. To achieve this objective, a protocol was developed to choose the best co-former for the selected liquid dosage form, a suspension formulation. From the known five co-formers available to formulate nevirapine co-crystals viz. saccharin, glutaric acid, salicylic acid, rac-tartaric acid and maleic acid,[1] saccharin was selected for preparation.

4.1 Selection of an ideal co-former for suspension

To develop a protocol to select an ideal co-former, information was gathered from primarily literature and electronic databases about each co-former regarding their physical, chemical, pharmacological and pharmaceutical properties. A similar protocol was established by Sam et al. whereby each formulation was ranked against efficacy, safety and patient access.[2] Their approach was to define scales for scoring the differences for the various criteria in the range from equal to extremely different, followed by a weak point and sensitivity analysis. They also emphasized that the framework should be used on a case-by-case basis and consider the specific product characteristics and medical need.

In this study, the criteria for the selection of the best co-former included physical properties such as dissolution rates as a co-crystal, dissolution rates as a mixture of API and co-former, solubility increase when used as a co-crystal, solubility in water, melting point, particle size, particle shape, taste and specific gravity.
Chemical properties such as Log P were considered. Pharmacological properties such as lethal dose in rats, side effects and antiviral activity against HIV-1 were part of the criteria. Variables relating to pharmaceutical aspects such as method of preparation, percentage yield, solvent used to prepare co-crystal, taste, sedimentation ratio of co-formers and status according to the FDA inactive ingredient database were considered. Another variable that was also considered was the cost.

Thereafter, each of the co-formers was ranked in relation to the other co-formers in a table according to an ordinal measurement scale. For the sake of simplicity and better

comprehension, the numbers were represented with colours and thus a colour coded table was formed. Green represented excellent characteristics, blue represented good, orange signified average, yellow meant fair while red indicated the co-former had performed poor in that particular property. After compilation of the properties of the co-former, the total score for each co-former was calculated by adding the values obtained for each co-former. The co-former with the highest total score indicated that the co-former performed well in the set criteria and hence it is the recommended choice for formulation of the suspension (Table 4.1).

4.2 Validation for selection of variables

The subsequent section justifies why the following variables are considered as part of the protocol in choosing a suitable co-former for a suspension formulation.

4.2.1 Physical properties of co-former

Dissolution rate as a co-crystal

Dissolution can be defined as the transfer of molecules or ions from a solid state into solution.[3] The dissolution rate as a co-crystal provided crucial data for formulation as a suspension. The dissolution rate of the co-crystal was determined by HPLC at 37 ºC at a pH of 7 in six vessels, using water as the dissolution medium.[3]

Dissolution rate as a mixture of the API and co-former

This variable was considered because if the co-crystal was not retained in the suspension, the co-crystal would disintegrate into two components i.e. nevirapine and the relevant co-former, thus the dissolution expected in such a situation would not be the dissolution of the co-crystal but rather the mixture of the API and co-former. The dissolution rate of the mixture was also determined by HPLC at 37 ºC at a pH of 7 in six vessels, using water as the dissolution medium.[3]

Solubility enhancement of the API as a co-crystal

Solubility enhancement parameter can be defined as a ratio of S_{cc}/S_{free}, where S_{cc} is the aqueous solubility of nevirapine from the co-crystal and S_{free} is the aqueous solubility of the pure drug at the same temperature.[1] This ratio was derived from dissolution-time curves. It gives an indication on the solubility improvement of the co-crystal. This variable was chosen because it would ultimately suggest whether or not the API as a co-crystal will dissolve in a specific media, at a specific temperature and pH comparatively to the API alone.

Solubility of co-former in water

The extent to which the dissolution proceeds under a given set of experimental conditions is referred to as the solubility of the solute in the solvent.[3] In this instance, the solubility of the co-former in water was considered, because in cases where the co-former was to be added as a suspension excipient, this parameter would give an indication of whether it would be an aqueous soluble excipient or non-aqueous soluble excipient.

Melting point

This is the temperature at which the crystal lattice breaks down, owing to the molecules having gained sufficient energy from a heating process to overcome the attractive forces that hold the crystal together.[3] The dissolution profile is closely related to the melting point. Low thermal stability implies high water solubility.

Particle size

Particle size influences the subsequent physical performance of the formulation and the pharmacological performance of the drug.[3] A small particle size ensures that the particles form floccules and are large enough to ensure they are suspended in the media to be used. Particle size was determined by SEM.

Particle shape

Particle shape can be classified into avicular, columnar, blade, plate, tabular and equant. However, deviation of crystal habits from the ideal may often be observed due to the alterations in the crystallization conditions.[4] With regards to particle shape, a rod shape is preferred since rod shaped particles are associated with good sedimentation properties. Spherical or ellipsoid shapes are also preferred.[5] The shape was also characterized by SEM.

Taste

This variable contributes to the organoleptic aspects of a suspension. Each co-former had a different taste; this variable was considered to see if the co-former could contribute to the overall taste of the suspension.

Specific gravity

Specific gravity expresses the ratio of the density of a solid or liquid to the density of water at standard temperature and pressure.[5] Specific gravity of the co-former contributes to the density of the co-crystal particle which in turn affects the overall density of the suspension. The density of the particles is one of the factors that affect Stokes' law. The velocity of a suspension is directly proportional to the difference in the density of the particle and density of the dispersion medium.

4.2.2 Chemical property of co-former

Log P

A Log P value indicates that the molecule has sufficient lipid affinity to cross membranes and enough water affinity to diffuse and dissolve in body fluids. The value of Log P is only an estimate of the permeability, which is an important absorption mechanism of drugs.[6] Co-crystals are expanding their territories in not only improving solubility but also in permeability. In a recent study, attempts were made to obtain a higher lipophilicity, where Ibandronate was crystallized with phenyl-β-D-

galactopyranoside, however it was unsuccessful.[6] Hence, choosing a co-former with a good Log P value could possibly influence the permeability of the nevirapine co-crystal.

4.2.3 Pharmacological properties of co-former

Safety

Safety of the co-former refers to the maximum oral consumption for a human per day. This variable was taken into consideration because if it was not safe to be ingested then making a suspension with such a co-former would be futile. It was necessary to ensure that the amount of co-former that would be used was within the stipulated ranges. Along with the maximum consumption, it was necessary to ensure that the co-formers were listed in the GRAS list because the aforementioned list is approved by the FDA and is permitted for use in pharmaceutical formulations.

The European medicines agency advises those applicants of novel co-formers to submit details of manufacture, characterisation controls with cross references to support safety data.[7] This indicates that safety consideration is imperative especially in the instances where the co-former has not yet been classified by the agency.

Lethal dose in rats

The lethal dose (LD_{50}) in rats is the dose at which 50% of the rats are injured.[8] Not many studies have been done with respect to toxicity of the co-formers in humans, hence lethal dose in rats was considered.

Side effects

Side effect is an unintended effect of the drug.[9] This was considered because it was essential to know if the co-former would have any possibility of side effects if it were to be used as a suspension excipient.

Antiviral activity

The antiviral activity against HIV-1 when used as a co-crystal with that specific co-former was considered to see if there was any improvement in inhibitory concentration (IC_{50}) against HIV-1 when compared to pure nevirapine. IC_{50} value is the concentration of the co-crystal where 50% of the virus is inhibited. A viral activity of 0 % indicated complete viral inhibition while that of 100 % indicated no inhibition.[10]

4.2.4 Pharmaceutical properties of the co-former

Pharmaceutical use for suspensions

The pharmaceutical use of the co-former was considered to suggest possibilities of using the co-former as a potential excipient for suspensions. A literature review was done to ascertain if the co-formers could perform as a sweetener, suspending agent, buffering agent, viscosity enhancer or a wetting agent.

Status in FDA Inactive Ingredient Database

The FDA inactive ingredient database gives the approved potency, as a percentage of the inactive to be used in a formulation. This information was pertinent in an instance where the co-former may be employed as a potential excipient in a suspension.

Preparation method of co-crystal

The two options available for preparing the nevirapine co-crystals were slow evaporation and liquid assisted grinding.[1] The slow evaporation method utilizes large amounts of solvent to facilitate the formation of co-crystals. Liquid assisted grinding was preferred in contrast to the slow evaporation method since the former was a "greener pharmaceutical" method of preparation of co-crystals. Liquid assisted grinding was also seen to be more cost-effective due to less solvent usage. Hence, methods that used both the methods were rated as excellent, methods that used liquid assisted grinding was considered as good and co-formers that were prepared by slow evaporation was rated as average.

Percentage yield

Percentage yield is the actual yield obtained over the theoretical yield, expressed as a percentage. This gave an indication of whether scaling up of a co-crystal with the co-former was viable. For this study, a value above 75 % was considered as excellent. Values between 70 % –74 % were considered good, while values between 60 – 69 % were considered as average, 50 – 59 % was considered as fair and values less than 50 % were rated as poor

Solvent

According to the definition of a co-crystal, the solvent is not present in the co-crystal, unless a solvated co-crystal had been prepared. The role of the solvent in this instance was to facilitate the formation of non-covalent bonds between co-former and the API. Technically, there should be no solvent present in the co-crystal but there might be solvent present on the surface of the crystal hence the choice of solvent is of importance. The solvent was chosen based on the safety of solvent for human oral consumption. With respect to solvent choice, the boiling point of the solvent was also considered. A high boiling point meant that it would take a longer time to evaporate from the API-co-former preparation thus consuming more time to form the co-crystal during scaling up.

Sedimentation volume test

The sedimentation volume (F) is defined as the ratio of final or ultimate volume to the suspension volume. If the volume of sedimentation in a flocculated suspension is equal to the original volume of suspension, then F=1 and this is considered as pharmaceutically acceptable. When F=0, it means the total drug has settled and the volume of sediment is negligible. Therefore, the greater the volume of F the more it will be pharmaceutically accepted.[11]

Cost

Information on the cost of the co-former in South African Rands was obtained. This was considered since this project involved scaling up and thus required a greater mass of each ingredient as scaling up progressed.

Co-former	Saccharin	Glutaric acid	Salicylic acid	Rac-Tartaric acid	Maleic acid	
Dissolution [10] of co-crystal at 37 °C in water at pH 7	14% in 45min 16% in 180min [10]	30% in 45 min 59% in 180min[10]	20% in 45 min 32% in 180min[10]	14% in 45 min 41% in 180 min [10]	18% in 45min 39% in 180min[10]	Physical properties
Dissolution[10] of mixture at 37 °C in water at pH 7	26% in 45min 43% in 180min [10]	26% in 45 min 47% in 180 min[10]	49% in 45 min 65% in 180min[10]	24% in 45min 43% in 180min[10]	34% in 45min 54% in 180min[10]	
Solubility increase	1.6 times[12]	3.7 times[12]	2 times[12]	2.7 times	2.4 times	
Solubility in water at 25°C and pH 7[13]	1000mg/290ml = (3.4mg/ml)[13]	430mg/ml[13]	2.240mg/ml[13]	206mg/ml[13]	780mg/ml[13]	
Melting point of co-former[1]	223°C[1]	137°C[1]	203°C[1]	228°C[1]	185°C[1]	
Particle size (*μm*)	169.7 x272	180.1 x 365	410 x 92.4	739 x 493.5	179 x 308.5	
Particle shape	rectangular	irregular with agglomerates	platy	irregular	irregular	
Specific gravity	0.828g/cm^3	1.429g/cm^3	1.40 g/cm^3	1.76g/cm^3	1.59g/cm^3	
Log P[14]	0.91[14]	-0.297[14]	2.21[14]	-0.48[14]	-0.3[14]	Chemical property
Maximum oral consumption (g)	0.175	Not available	0.800	2.100	0.035	Pharmacological properties
Lethal dose in rats (LD_{50})	LD50 oral is 17000mg/kg[15,16]	LD50 oral is 6000mg/kg [16]	LD50 oral 891mg/kg [16]	LD50 oral is 7500mg/kg[7]	LD50 oral is 708mg/kg [16]	
Side-effects[14]	Headaches, diarrhoea, skin problems [14]	None[14]	Gastric ulceration[14]	Nausea, vomiting, chronic toxicity is low[14]	acute toxic skin, eye, respiratory tract irritant[14]	
Antiviral activity IC 50 mM against HIV-1[10]	0.037[10]	0.054[10]	0.037[10]	0.072[10]	0.055[10]	
Method of preparation[1]	slow evaporation [1]	slow evaporation or liquid assisted grinding [1]	slow evaporation or liquid assisted grinding [1]	slow evaporation [1]	liquid assisted grinding [1]	Pharmaceutical properties
Percentage yield while scaling up[10]	78%[10]	73%[10]	76%[10]	60.5%[10]	110%[10]	
Solvent used during preparation[1]	Chloroform, methanol, acetic acid, 1,4-dioxane, n-hexane, n-heptane, diethyl ether and amyl alcohol[1]	chloroform[1]	Chloroform[1]	amyl-alcohol[1]	chloroform [1]	
Taste	sweet (500 times sugar)[16]	sour(acid)[16]	sweetish, acrid [16]	sour /salty[16]	Astringent[16]	
Use	Artificial sweetener, the safest sweetener out of the five sweeteners in FDA database[17]	Surfactant, food supplement and improves protein synthesis[17]	Food preservative, bactericidal and antiseptic properties[17]	Anti-oxidant, acidulant, sequestrating agent, complexing agent, falvouring agent, preservative[17]	Make salts, acidulant, prevents rancidity and buffering agent[17]	
Status according FDA inactive ingredient database[18]	Can be used in oral solutions (1%),suspensions (0.25%), syrup (0.07%) tablets, topical preparations, inhalations and aerosols[18]	Not included in database since it is a not used as an inactive [18]	Not included in database since it can be considered as an active ingredient[18]	Accepted as food additive and use in tablets (15mg), capsules (177.1mg), solutions(0.75%), suspensions (0.2%) IM, IV injections, topical films, rectal and vaginal preparations[18]	Can be used for oral syrups, tablets (4mg), syrup (0.0345%), capsules (2mg) and topical preparations[18]	
Sedimentation volume test * (Ht/Ho ratio at 24 hours)	0.013	0	0	0.020	0	
Cost /100g[15]	R 1 986[15]	R 437[15]	R 914[15]	R 315[15]	R 271[15]	

Table 4.1 Physical, chemical, pharmacological and pharmaceutical properties of nevirapine co-formers

Excellent Good Average Fair Poor

4.3 Selection of an ideal co-former for the nevirapine co-crystal suspension

4.3.1 Physical properties of co-formers

Dissolution rates as a co-crystal at 37 ºC in water (pH =7)

Nevirapine alone shows a dissolution rate of 16%. The release profile of nevirapine is enhanced as co-crystals. Nevirapine-glutaric acid (NVGLT) co-crystal displayed the greatest dissolution rate of 59 % in 180 minutes followed by rac-tartaric acid (NVTTA) with a dissolution rate of 43% in 180 minutes. Nevirapine-maleic acid (NVMLE) displayed 39 % after 180 minutes and nevirapine–salicylic acid (NVSLI) co-crystal had a dissolution rate in the same range of 39 % in 180 minutes. Nevirapine-saccharin (NVSC) co-crystal had the lowest dissolution rate of 26 % in 180 minutes in comparison to the other co-crystals (Fig. 4.1).[12]

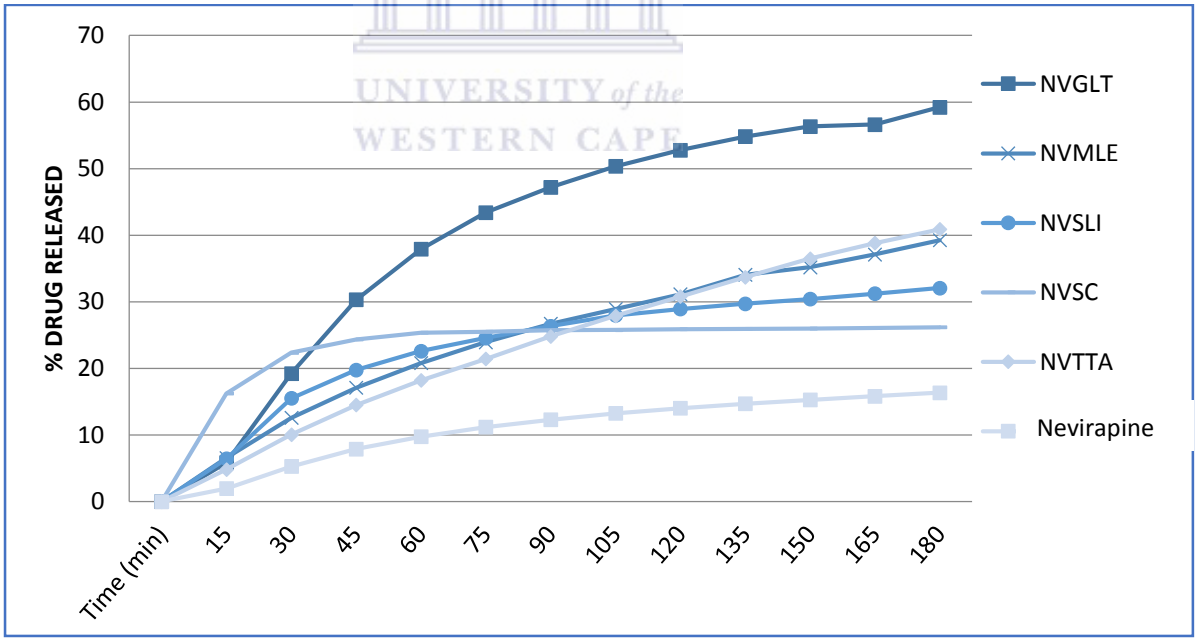


Figure 4.1 Pre-formulation dissolution profiles of pure nevirapine and nevirapine co-crystals at 37 ºC in water at pH 7, determined by HPLC[12]

Dissolution rates as a mixture of API and co-former at 37 ºC in water at pH 7

NV:SLI had the greatest dissolution rate as a mixture, 65 % in 180 minutes, followed by NV:MLE with a dissolution of rate of 54 % in 180 minutes. This was then trailed by NV:GLT exhibiting a dissolution rate of 47 % in 180 minutes. Both NV:TTA and NV:SC physical mixtures had the lowest drug release of 43 % in 180 minutes (Fig.4.2). The dissolution of mixtures is faster than corresponding co-crystals due to the presence of an acidic co-former which lowers the pH significantly, in which nevirapine dissolution is highly favourable.

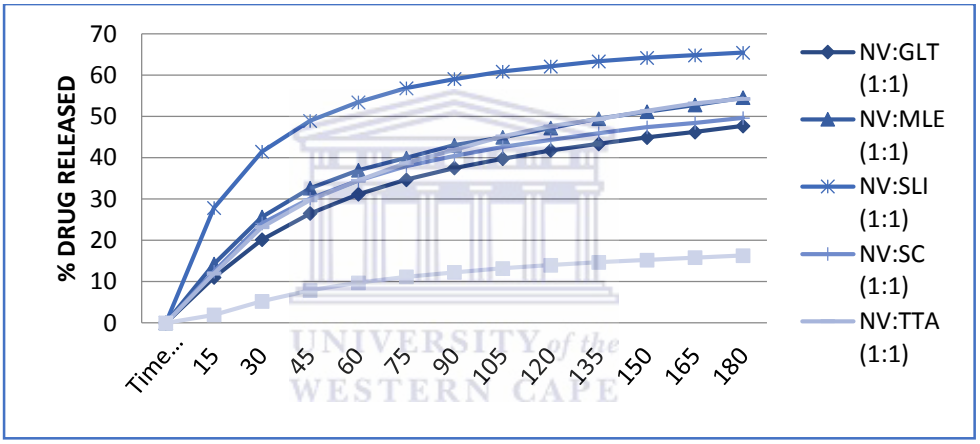


Figure 4.2 Pre-formulation dissolution profiles of pure nevirapine and physical mixtures with co-formers at 37 ºC in water at pH 7, determined by HPLC[12]

Solubility enhancement as a co-crystal

The solubility enhancement theory was applied and it was practically determined using the HPLC analysis technique. It was found that NVGLT had the greatest solubility increase by 3.7 times, followed by NVTTA, NVMLE, NVSLI and lastly NVSC (2.7, 2.4, 2.0, 1.6 times), respectively.[12] The solubility enhancement parameter concurred with the dissolution readings obtained in figure 4.1.

Solubility of co-former alone in water

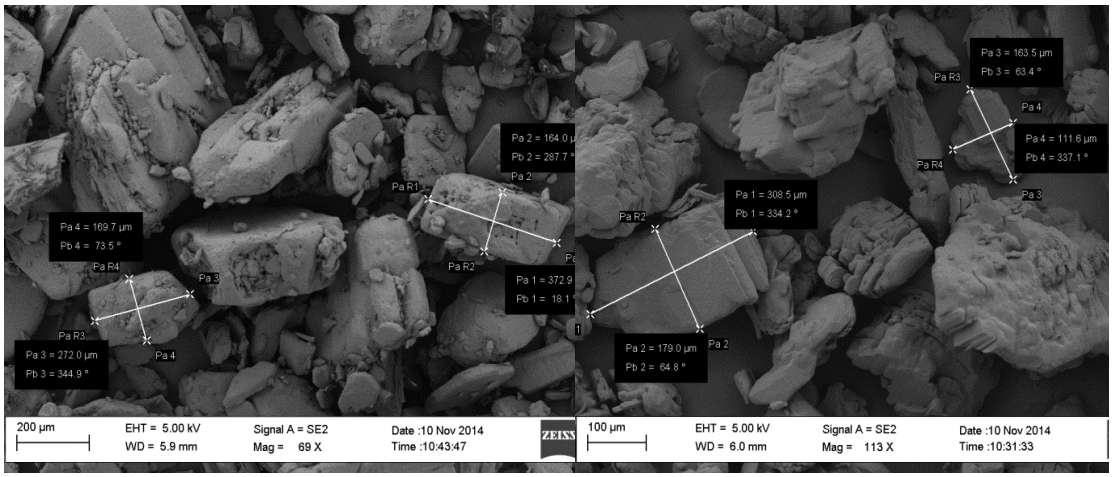
Maleic acid had the greatest solubility at 780 mg/mL but this was marked as red for poor since a co-former which is less soluble is preferable in suspensions. Glutaric acid had a water solubility of 430 mg/mL and this was marked as yellow. Rac-tartaric acid was marked as orange, displaying a solubility of 206 mg/mL. Saccharin was marked as blue since it had a solubility of 3.4 mg/mL. Salicylic acid was rated as green for excellent, exhibiting solubility at 2.240 mg/mL.

Melting point of co-former

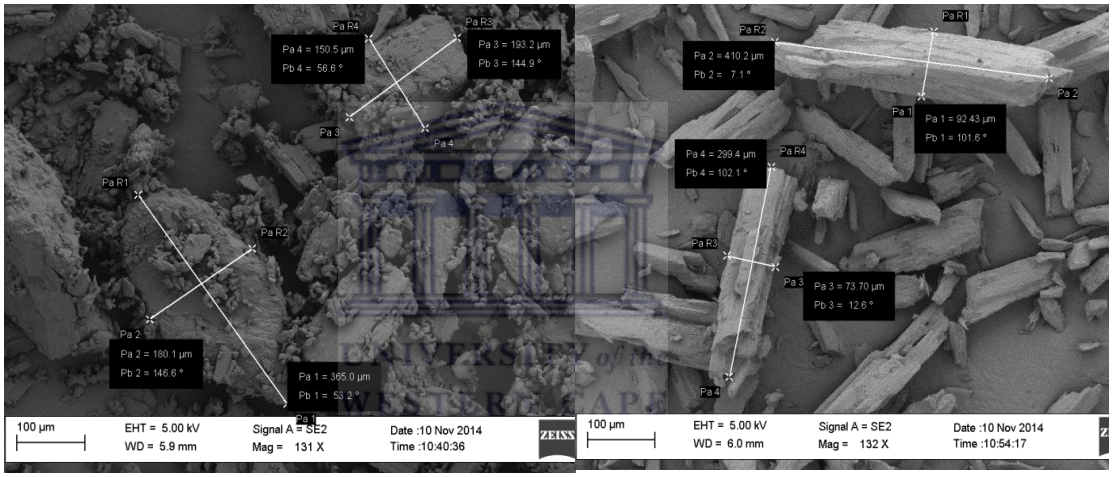
Rac-tartaric acid had the highest melting point of 226 °C, followed by saccharin at 203 °C. Salicylic acid had a melting point of 203 °C while maleic acid had a melting point of 185 °C and glutaric acid had a melting point of 137 °C. A low melting point was not favoured, since a low thermal stability meant that it had high water solubility. In the case of a suspension, a low thermal stability suggests that the co-former will have high water solubility, which is not suitable for suspensions as suspensions are formed when the substance have limited solubility.

Particle size of co-former

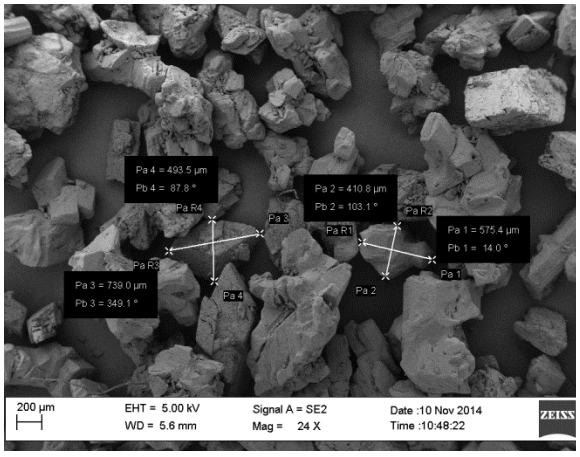
A small particle size was preferred for suspensions as larger particles tend to settle faster due to the gravitational force. Ideally a particle size of 1 to 50 μm is suitable for suspensions, however, particles are rarely in this particle size range and hence a range of 50 to 75 μm is acceptable. Saccharin showed the smallest particle size of 169.7 x 272 μm. Hence, it was rated as excellent. Maleic acid had a slightly larger particle size of 179 x 308.5 μm, hence it was rated as good. This result was followed by glutaric acid which had a particle size of 180.1 x 365 μm. Glutaric acid was rated as average. Salicylic acid had a particle size of 410 x 92.4 μm and thus it was rated as fair. Rac-tartaric acid had the largest particle size of 739 x 493.5 μm therefore it was rated as poor. (figure 4.3 and Table 3.1).



a. Saccharin **b.** Maleic acid



c. Glutaric acid **d.** Salicylic acid



e. Rac-tartaric acid

Figure 4.3 Particle sizes of co-formers determined by SEM

Particle shape of co-former

For a suspension to be prepared, ellipsoid and rectangular shape or rod-shaped structures are preferred. As seen in figure 4.3, saccharin had a rectangular shape thus it was rated as excellent, because a rectangular shape is similar to a rod-shaped structure which exhibits good sedimentation properties. Salicylic acid was rated as good as it appeared platy in nature and this is similar to a rod-shaped structure. Rac-tartaric acid and maleic acid were graded as fair as these co-formers had irregular morphology. Irregular particle shapes are not preferred as they do not have a good particle size distribution and thus surface area for these particles are not large. Glutaric acid was graded as poor as it exhibited irregular particle shape and furthermore it had presence of smaller particles adhering onto larger particles. This attribute of particles adhering to each other alludes to Ostwald's ripening process, which is not favourable process in pharmaceutical suspensions and hence glutaric acid was rated as poor.

Taste of co-former

Taste is a subjective variable; however, generally a sweet taste is acceptable for suspensions. Saccharin was rated as the best since it is sweet (500 times that of sugar)[17], salicylic acid was rated as good, since it had a sweetish and acrid taste.[17] Glutaric acid had a sour taste and this was rated as average, rac-tartaric acid had a salty taste and was rated as fair while maleic acid had an astringent taste[17] and was rated as poor.

Specific gravity of co-former

A particle with low density was preferred in order to minimize the difference between the density of the vehicle and the particle, as this variable was directly proportional to the rate of sedimentation and particle size. Saccharin had the least specific gravity followed by salicylic acid, glutaric acid, maleic acid and then rac-tartaric acid which was predicted due to the order of the particle sizes.

4.3.2 Chemical property of co-former

Log P

An ideal Log P value is within 1 and 4. Log P values within this range are known to have good permeability. Salicylic acid had the best log P value in comparison to the other co-formers with a log P value of 2.21. This was in fact the only co-former within the range desired hence it was classified as an excellent co-former in this respect. Saccharin had a log P value of 0.91 and thus was rated as good. Glutaric acid had a log P value of -0.297 and was rated as average. This result was followed by maleic acid and rac-tartaric acid which had log P values of -0.48 and -0.3, thus they were graded as fair and poor respectively.

4.3.3 Pharmacological properties of co-former

Maximum oral consumption

The maximum amount of oral consumption of the co-former was compared to the amount of co-former that could be included in a dose of suspension. The daily dose required during the first 14 days is 200 mg. Viramune® is available as 50 mg/5mL, thus to meet the required dose, 20 mL per day is administered.

Mass of nevirapine in 20 mL = 200 mg

No. of moles of API in 200 mg = $\frac{0.200}{266.297}$ = 7.510 x 10^{-4} mol

No. of moles of co-former needed for 200 mg API in a 1:1 ratio = 7.510 x 10^{-4} mol

No. of moles of co-former needed for 200 mg API in a 2:1 ratio = 3.755 x 10^{-4} mol

Table 4.2 presents the number of moles as well as the mass needed for each co-former to prepare a co-crystal that consists of 200 mg of API. Maximum consumption of co-former approved per day in grams is also presented.

Co-former	Ratio of API:co-former	Number of moles of co-former (mol)	Molecular Weight of co-former (g/mol)	Mass of co-former = moles X molecular weight (g)	Maximum consumption of co-former approved per day (g)	Maximum limit – mass of co-former (g)
Saccharin	2:1	3.755×10^{-4}	183.18	0.068	0.175	1.070
Rac-Tartaric	1:1	7.510×10^{-4}	150.05	0.112	2.100	1.988
Maleic acid	1:1	7.510×10^{-4}	116.07	0.087	0.035	- 0.052
Glutaric acid	1:1	7.510×10^{-4}	132.11	0.099	Not available	X
Salicylic acid	2:1	3.755×10^{-4}	138.12	0.051	0.800	0.749

Table 4.2 Mass of co-formers needed for one dose of suspension compared to maximum consumption of co-former allowed

The difference between the maximum consumption of co-former allowed per day and the mass of co-former needed to form a co-crystal was calculated. Positive values indicated that the co-former was within the maximum limit whereas negative values meant that the co-former in a daily dose of the co-crystal suspension would exceed the maximum limit allowed by the FDA. From table 4.2, salicylic acid was rated as excellent as only 0.051 g of co-former is required whilst the maximum limit is set at 0.800 g per day. Rac-tartaric acid was rated as good, since 0.112 g is needed to prepare the co-crystal, which fell within the stipulated limit of 2.100g. Saccharin was then rated as average since 0.068 g would be in a daily dose of the co-crystal suspension, while 0.175 g of saccharin is set as the maximum amount of co-former approved per day. Maleic acid followed this result and was rated as poor, as it had a negative value in the

difference between the mass of the co-former required for a daily dose and the mass of the co-former allowed in a daily dose. Glutaric acid was also rated as poor as there was no data available on the maximum consumption of glutaric acid approved per day.

Lethal dose in rats

A high LD_{50} indicates that a greater amount of co-former is required to cause death in 50 % of the test population. Thus, a high LD_{50} value was preferred. Saccharin had the highest LD_{50} of 17000 mg/kg when compared to the other co-formers and thus it was rated as excellent. Rac-tartaric acid had a LD_{50} of 7500 mg/kg and was rated as good. Glutaric acid followed this result with a LD_{50} of 6000 mg/kg and was rated as average. While salicylic acid had a LD_{50} of 891 mg/kg and was rated as fair and maleic acid was graded as poor with a LD_{50} of 708 mg/kg.

Side effects

Glutaric acid had no reported side effects so it was ranked as an excellent co-former, saccharin and rac-tartaric acid was ranked second best with mild to moderate common side effects, maleic acid ranked fair with side effects such as acute toxic skin reactions. Salicylic acid ranked the poorest due to its gastric ulceration side effects.

Antiviral activity of co-crystal

NVSC co-crystal displayed the greatest antiviral activity, followed by NVTTA, NVGLT, NVMLE and then NVSLI.[12] Hence, saccharin was ranked as excellent in this aspect, followed by co-formers rac-tartaric acid, glutaric acid, maleic acid and salicylic acid, respectively (Fig. 4.4).

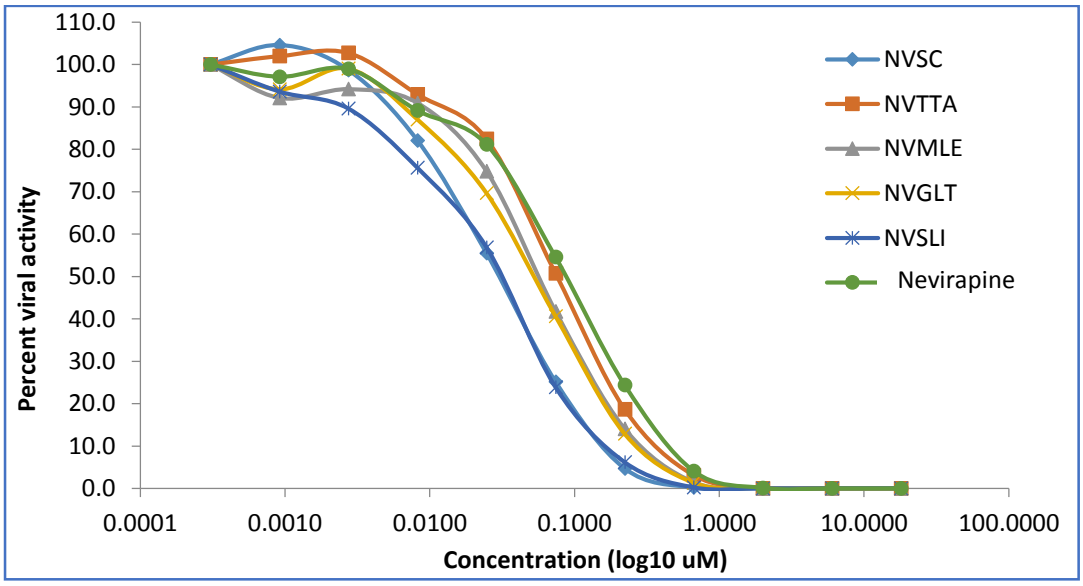


Figure 4.4 Antiviral activities of nevirapine co-crystals against HIV-1, compared to pure nevirapine

4.3.4 Pharmaceutical properties of co-formers

Pharmaceutical use for suspensions

Saccharin was rated as excellent in comparison to the other co-formers since it could be used as a sweetener. It is one of the safest sweeteners out of the five sweeteners approved in the FDA database[17] (acesulfame potassium, advantame, aspartame, neotame). Salicylic acid was rated as good since it can act as a food preservative and has antiseptic properties. Glutaric acid can be used as a surfactant and this was rated as average. Maleic acid was rated as fair since it can be used as a buffering agent. Rac-tartaric acid could be used as a preservative and had other uses which were not relevant to the formulation of suspensions so it was rated as poor.

Status in FDA Inactive Ingredient Database

Saccharin was ranked as excellent since it had the highest percentage of 0.25 % of the total suspension which can consist of saccharin. This value indicated that in an instance where saccharin was to be used as an excipient, 0.25 g may be added to a 100 mL suspension. Rac-tartaric was ranked as good and followed closely with a limit of 0.20

%, meaning that 0.20 g of rac-tartaric acid may be added as an excipient in to a 100 mL suspension. Maleic acid was ranked as average and there was no value stipulated for it in the FDA inactive ingredient database. The other two co-formers viz. glutaric acid and salicylic acid were not included in this database as they are not commonly used as excipients for oral consumption and hence they were rated as fair.

Preparation method of co-crystal

As mentioned previously co-crystals of nevirapine can be formulated by means of liquid assisted grinding or slow evaporation or by both the methods, depending on the co-former.[1] Glutaric acid and salicylic acid were favoured since these co-formers can be formulated as co-crystals by using both the methods. Maleic acid was ranked as good as it can be prepared by liquid assisted grinding only. Saccharin and rac-tartaric acid can only be used in slow evaporation and this was rated as average.

Percentage yield of co-crystal

Saccharin, salicylic acid and maleic acid had a percentage yield of 78 %, 76 %, 110 % respectively.[10] These co-formers were rated as excellent as they had a percentage yield of above 75 %. Glutaric acid had a percentage yield of 73 % and was rated as good. Rac-tartaric acid had the least percentage yield of 60 % and was ranked as average. Saccharin yielded a high percentage inspite of its method of preparation i.e. slow evaporation. Rac-tartaric acid was another example of a co-former made by slow evaporation technique and this co-former showed a yield of only 60%.

Solvent used to prepare the co-crystal

With regards to the solvent used to prepare the co-crystal, saccharin was rated as excellent since the co-crystal formed with saccharin can be formed with a plethora of solvents, thus there are more options of solvents to choose from in the instances where reproducibility of producing the co-crystal is low or where challenges may be encountered during preparation of the co-crystal.[13] Glutaric acid, salicylic acid and maleic acid could only be formed with chloroform, these were ranked as good. Rac-tartaric acid was ranked as fair since it could only be prepared with amyl-alcohol and

when compared to chloroform, it has a higher boiling point, meaning that it took a longer time for the solvent to evaporate during the co-crystal preparation.

Sedimentation volume test

Sorbitol was the chosen vehicle in the co-crystal suspension as it is one of the excipients in the Viramune® formulation hence, to perform the sedimentation volume test the amount of co-former needed in a 100 mL suspension was calculated and suspended in 100 mL of sorbitol.

Volume needed for experiment = 100 mL

Dosage of Viramune: 50 mg/5 mL

Therefore amount of nevirapine in 100 mL = 1 g

No. of moles = $\frac{Mass}{Molecular\ weight} = \frac{1\ g}{266.297\ g/mol} = 3.74\ X\ 10^{-3}\ mol$

Co-former	Ratio of API:co-former	Number of moles (mol)	Molecular Weight (g/mol)	Mass of co-former (g)
Saccharin	2:1	1.87 x 10^{-3}	183.18	0.343
Tartaric	1:1	3.74 x 10^{-3}	150.05	0.561
Maleic	1:1	3.74 x 10^{-3}	116.07	0.434
Glutaric	1:1	3.74 x 10^{-3}	132.11	0.488
Salicylic	2:1	1.87 x 10^{-3}	138.12	0.258

Table 4.3 Mass of co-formers needed for sedimentation height experiment

Five graduated cylinders were placed with 100 mL sorbitol and the mass of each co-former as calculated in table 4.3 was added to each cylinder. The height at time zero (H_o) was measured and the height at 15, 30, 46, 60, 90 minutes and 24 hours was measured (H_t), where t = 15, 30, 46, 60, 90 minutes and 24 hours.

The results are tabulated below in table 4.4:

Co-former	(Ht/Ho) 15 min	(Ht/Ho) 30 min	(Ht/Ho) 45 min	(Ht/Ho) 60 min	(Ht/Ho) 90 min	(Ht/Ho) 24hrs
Saccharin	0.0132	0.0132	0.0132	0.0263	0.0263	0.013
Rac-tartaric	0.0263	0.0263	0.0263	0.0329	0.0329	0.020
Maleic	0.0132	0.0197	0.0329	0.0329	0.0329	0.000
Glutaric	0.0329	0.0329	0.0395	0.0395	0.0395	0.000
Salicylic	0.0000	0.0000	0.0000	0.0000	0.0000	0.000

Table 4.4 Sedimentation height experiment of co-formers

A ratio that is closer to 1 is preferred as this indicates that particles are capable of flocculation. Only two co-formers had a value after 24 hours i.e. saccharin and rac-tartaric acid. The remaining co-formers had settled completely. As seen in table 4.4, rac-tartaric acid had a value of 0.020 which was closer to the value 1, while saccharin had a value of 0.013 which was rated as good. Thus, rac-tartaric was marked as excellent, saccharin as good and the rest of the co-formers as average since all these co-formers settled completely after 24 hours (Fig 4.5).

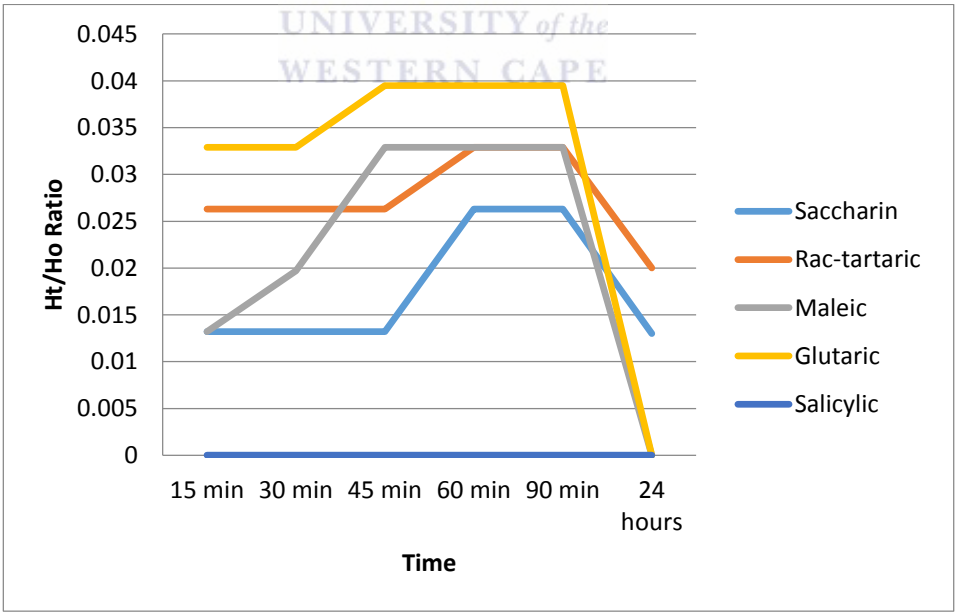


Figure 4.5 Sedimentation height: time ratio of co-formers

Cost

Maleic acid was the cheapest co-former, followed by rac-tartaric acid, glutaric acid, salicylic acid and lastly saccharin.

4.4 Ideal co-former for suspension

After compilation of the properties of the co-formers, the total score was calculated by multiplying the total number of variables for each category according to an ordinal scale (excellent = 5, good = 4, average = 3, fair = 2 and poor = 1). Saccharin had the highest points of 74. Rac-tartaric acid followed this result closely with 72 points, salicylic acid and glutaric acid had 69 and 61 points respectively. Maleic acid had the least points with 58 in total.

	Excellent 5	Good 4	Average 3	Fair 2	Poor 1	Total score*
Saccharin	9	3	3	2	4	74
Glutaric acid	4	3	9	2	3	61
Salicylic acid	5	5	3	7	1	69
Rac-tartaric acid	6	6	3	3	3	72
Maleic acid	2	5	5	4	5	58

**Example for saccharin: (9x5 + 4x3 + 3x3 + 2x2 + 4x1 = 74)*

Table 4.5 Total points of nevirapine co-formers based on physical, chemical, pharmacological and pharmaceutical properties according to table 4.1

According to the total points obtained, saccharin was the most suitable with 74 points. Saccharin was chosen in contrast to other co-formers because it was graded as excellent in nine variables. Saccharin was preferred because of its taste, particle size, specific gravity, and its status according to the FDA inactive ingredient database. In an article review conducted by Changquan Sun, he coined the term "sweet co-crystals".[19] The author mentioned the possibility that saccharin co-crystals exhibit a better taste profile

than the API alone; this is especially true if the API has a low solubility, which is the case with nevirapine.

Furthermore, it was also advised that sweet co-crystals are beneficial for developing chewable tablets, oral suspensions -which is the aim of the project- or solutions, with the potential for new intellectual properties. The reason for preparing sweet co-crystals is to resolve the problem of delivering a bitter API.

Viramune® uses the excipient sucrose as a sweetener in the suspension. Using saccharin in the co-crystal will eliminate the need of using sucrose as an additional excipient in the suspension, as the co-crystal will serve as an API and a sweetener. Consequently, using saccharin as the co-former in the nevirapine co-crystal suspension is advantageous as it will use less excipients.

Therefore, according to the protocol developed, saccharin was the chosen co-former to prepare a nevirapine co-crystal suspension. The nevirapine-saccharin co-crystal will henceforth be referred to as NVSC (Fig. 4.6).

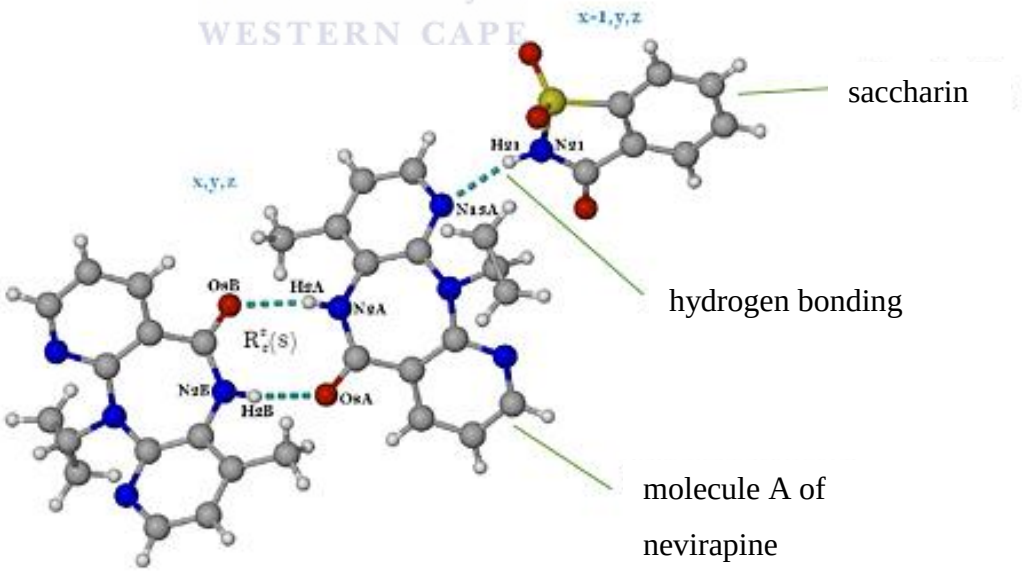


Figure 4.6 Molecular structure of nevirapine-saccharin co-crystal (2:1)[13]

4.4.1 Structure of NVSC co-crystal

The NVSC co-crystal consists of two independent nevirapine molecules and a saccharin molecule. The two molecules of nevirapine form a pseudocentrosymmetric dimer via two N-H•••O hydrogen bonds. The saccharin molecule is linked to a pyridine N atom of molecule A of nevirapine via a hydrogen bond N-H•••N.[13]

pKa is defined as an index to express the acidity of weak acids. A variable used to design a co-crystal is the Δ pKa between the co-former and the API. Nevirapine has a pKa of 2.8 while saccharin has a pKa of 1.8, thus resulting in a Δ pKa [pKa (base) - pKa (acid)] of 1. This confirmed Sekhon's theory that the Δ pKa should be less than three for formation of a co-crystal,[20] which was achieved in the NVSC co-crystal. This alone does not guarantee the formation of a co-crystal and variables in the crystallization process such as API:co-former ratio, temperature, pressure, solvent and crystallization method play a role in the formation of a co-crystal. Details relating to the crystallization process are outlined in the following section.

4.5 Results for preparation and scaling up of the NVSC co-crystal

The NVSC co-crystal was prepared in a 2:1 ratio by the slow evaporation method. Stoichiometric calculations of nevirapine and saccharin were calculated and the amounts were weighed accordingly. Thereafter, two 10 mL glass vials with minimum volumes of methanol were placed on a magnetic hot plate at 54 °C i.e. approximately 10 ºC below the boiling point of the methanol. The co-former and the API were added separately to each of these vials. The solution of greater volume was added to that of smaller volume and the resultant solution was magnetically stirred for 30 minutes. This was filtered through a micro filter of 0.45 μm using a syringe and the resultant solution was placed in a beaker which was covered with a perforated Parafilm® and allowed to crystallise at 20 ºC in a fume cupboard until all the solvent had evaporated.[13]

Najar et al.[21] foresaw the challenges that lied ahead with respect to scaling up the production of the co-crystal, identifying new scale-up methods, and high throughput screening of the possible co-crystal with various co-formers and their polymorphs.[21]

The main challenge with scale-up is scaling up to a multikilogram scale. Solid-state grinding approaches are particularly difficult to scale-up to a multikilogram scale for energetic materials since grinding may present a potential hazard due to friction generated during grinding.[22]

For the purpose of this study, scaling up operations commenced with batches from milligram scale to gram scale. Several batches were made, starting from small laboratory scale progressing to a larger laboratory scale (Table 4.6). The batches were prepared in the same environment but were evaporated in different sized fume cupboards. Batch 1 to 8 evaporated in a small fume cupboard while batch 9 to 14 evaporated in a large fume cupboard. The batches prepared in the smaller fume cupboard took a longer time for the solvent to evaporate, thus the number of days taken for the co-crystal to grow was longer. The batches prepared in the larger fume cupboard took less time for the solvent to evaporate therefore the growth period for the co-crystal was less.

Batch number	Growth period (days)	Volume of solvent used (mL)	Quantity of active (mg)	Quantity of co-former (mg)	Total mass before slow evaporation (mg)	Yield after slow evaporation (mg)	Percentage yield (%)	Type of fume cupboard
1	6	45	350	120	470	400	85	Small fume cupboard
2	13	140	1000	340	1340	1100	82	
3	9	150	1000	340	1340	1100	82	
4	13	140	1000	340	1340	1100	82	
5	23	700	5000	1710	6710	5364	92	
6	20	280	2000	687	2687	2500	96	
7	6	80	500	171	671	660	98	
8	10	80	500	171	671	578	92	

9	2	80	500	171	671	612	91	Large fume cupboard
10	2	80	500	171	671	588	88	
11	2	80	500	171	671	664	98	
12	2	80	500	171	671	625	93	
13	6	140	1000	340	1340	1080	80	
14	3	280	2000	687	2687	2430	90	

Table 4.6 Scaling up of NVSC co-crystal

The percentage yield in the large fume cupboard was 88 to 98 %. The results from the large fume cupboard were reproducible; this can be seen with batch 9 and 10, where it took 2 days each for 500 mg of co-crystal to be reproduced whilst, batch 7 and 8 which were prepared in the small cupboard took 6 and 10 days respectively to produce co-crystals of the same amount. The volume of solvent was evidently directly proportional to the size of the batch prepared (Table 4.6). The results clearly show that co-crystals formed from a larger fume cupboard faired better with respect to percentage yield and the number of days for the co-crystal to form. The percentage yield obtained was in the range of 80 to 98 % across small and large scale laboratory production. This result was in line with the 78 % yield that was noted in literature.[10]

It was notable that the number of days taken for the co-crystal to grow was directly proportional to the size of the batch (Fig. 4.7). This can be substantiated by the recommendations by Chen et al. where it was mentioned that the time required to scale-up is invariably due to larger volume of materials needed to scale-up.[23] Figure 4.7 illustrates that the greater the size of the batch, more quantity of co-former and API are required.

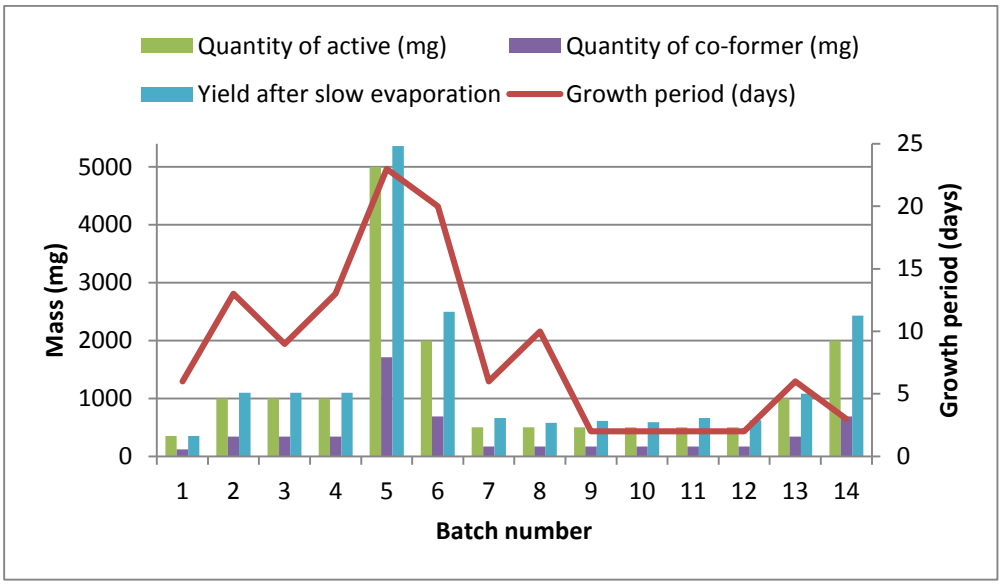


Figure 4.7 Batch size versus the growth period and mass required

4.5.1 Challenges encountered during scaling up

Batch 5 was the largest batch size prepared with 5 g of active ingredient. This batch was left to evaporate in a small fume cupboard. The number of days it took for all the solvent to evaporate was 23 days.

Upon slow evaporation, it was found that not all the powder had crystallized; this was physically seen as clumps on the sides of the beaker. The powder formed on the sides of the beaker was confirmed with DSC as an incomplete crystallisation of the nevirapine and saccharin (Fig. 4.8). The powder at the bottom of the beaker was confirmed as the co-crystal; however decomposition began earlier at 240 °C. This meant that not all the powder had resulted in a co-crystal. The peaks obtained in the DSC did not correspond to the melting point of nevirapine (247- 249 °C) and saccharin (228 - 229 °C). The peak obtained at 210 °C was close to the melting point of NVSC (215 – 230 °C). However, this was not confirmed as a pure co-crystal due to the presence of an endothermic peak at 193 °C. Therefore attempts were made to purify the powder to ensure a pure co-crystal is obtained.

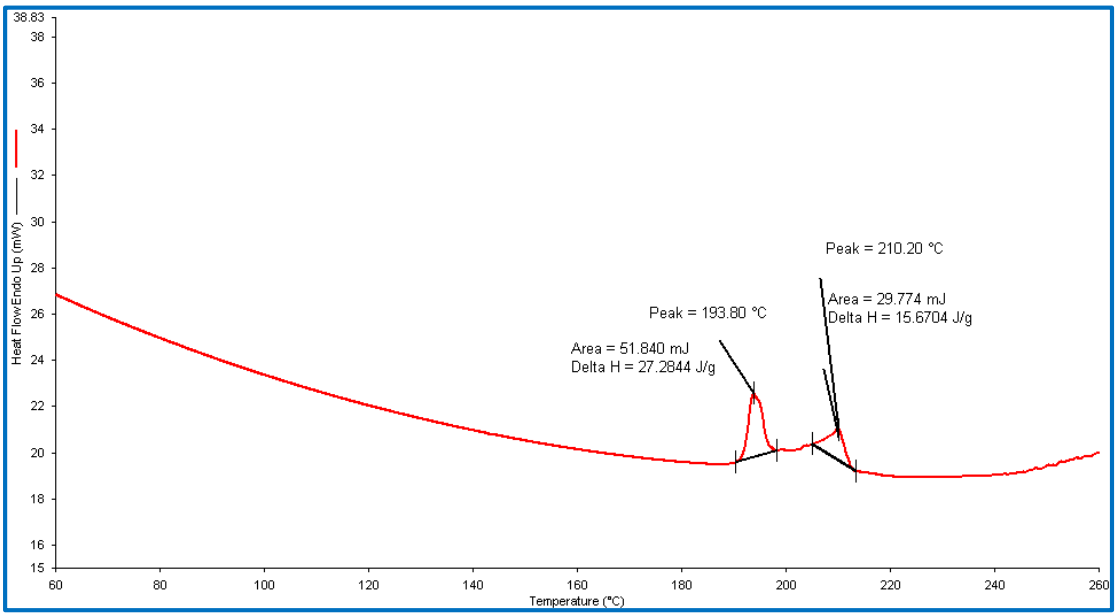


Figure 4.8 DSC of Batch 5

Two methods were attempted to purify the powder. Firstly, a seeding strategy similar to the generic scalable methodology developed by Sheik et al. for carbamazepine-nicotinamide co-crystals was explored.[24] This method involved adding a small quantity of already formed NVSC co-crystal to the powder of nevirapine and saccharin for the purposes of introducing nucleation. However, this strategy proved to be unsuccessful in the case of NVSC co-crystal. The DSC results showed two individual peaks at 212 °C and at 240 °C (Fig. 4.9). The peak obtained at 215 °C can be attributed to the co-crystal while the peak at 240 °C corresponds to the melting point of nevirapine. Thus, the co-crystal obtained was not considered pure as the powder consisted of the API individually and the co-crystal.

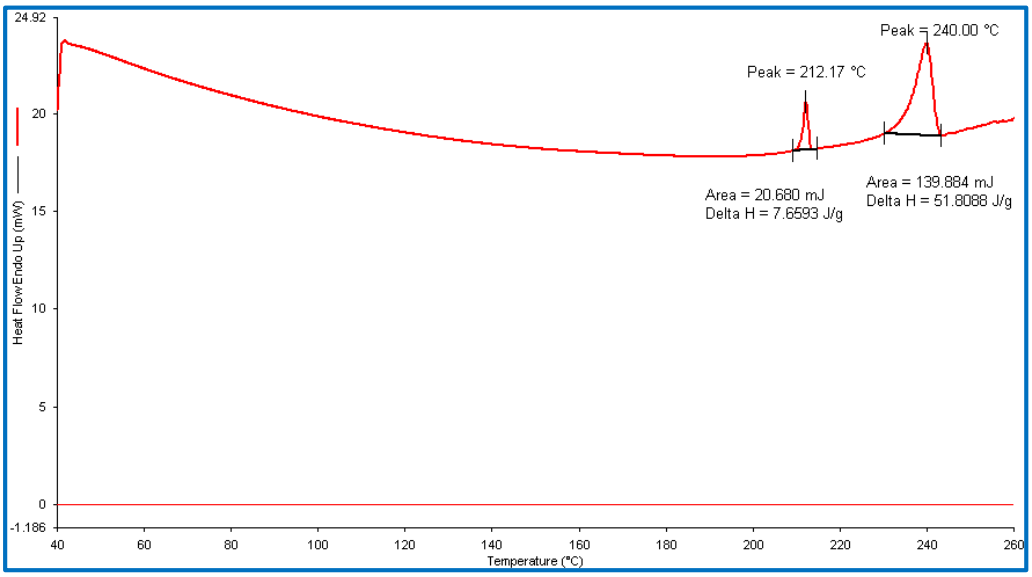


Figure 4.9 Purification of batch 5 through seeding strategy

An alternative method was attempted to purifying the powder. The powder was recrystallized by placing it in methanol, according to the temperature used to prepare the co-crystal; the stirring time was increased to 3 hours, and then filtered in a large 5000 mL beaker. Upon evaporation it was indeed confirmed to be the co-crystal (Fig. 4.10).

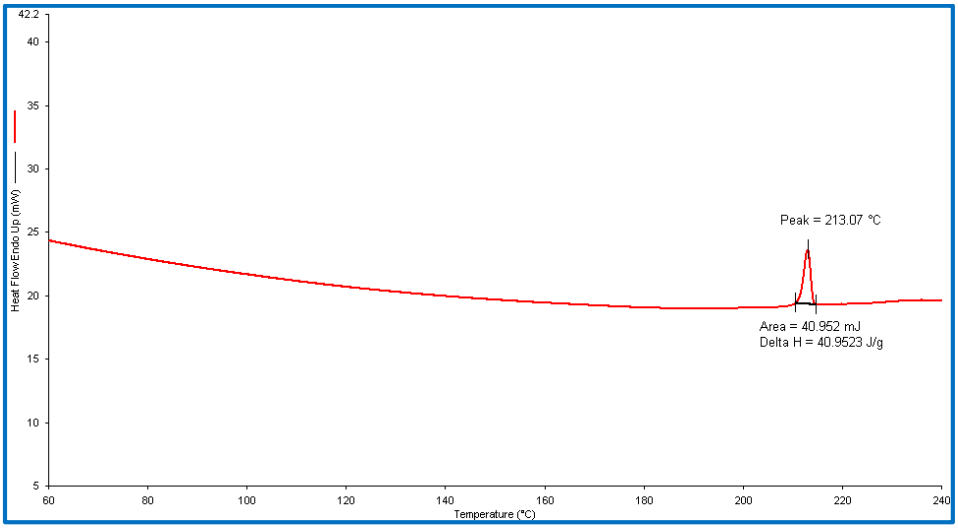


Figure 4.10 Purification of batch 5 through recrystallizing with extended stirring time and larger surface area

The DSC after purifying through recrystallizing with methanol indicated the formation of the NVSC co-crystal at 212 °C. This result indicated that the mixing time in the scale-up process is to be increased as the batch size increases; this was rightly suggested by Chen et al. in their perspective article.[23]

These results suggest that scaling up could be increased to the gram scale; however certain variables directly affecting co-crystal preparation will also have to be adjusted appropriately. These variables include stirring time and surface area available for evaporation.

4.6 Identification of the various batches of NVSC co-crystals

According to the European Medicines Agency, the formation of a co-crystal should be unambiguously demonstrated by means of adequate analytical techniques to rule out the possibility of the formation of a purely physical mixture of two or more crystalline compounds.[7]

This study fully echoes the standpoint of the European Medicines Agency on the identification of co-crystals and thus utilized analytical techniques such as DSC, HSM, FTIR and TGA.

Nevirapine has a melting point of 247 – 249 °C, while saccharin displays a melting point of 228 - 229 °C. According to the original inventor of the NVSC co-crystal, the melting point of the co-crystal was discovered to be in a range of 215-230 °C and subsequently decomposition began at 315 °C.[13] Figure 4.11 displays the DSC endotherms obtained for the NVSC co-crystal, nevirapine the API and saccharin as the co-former.

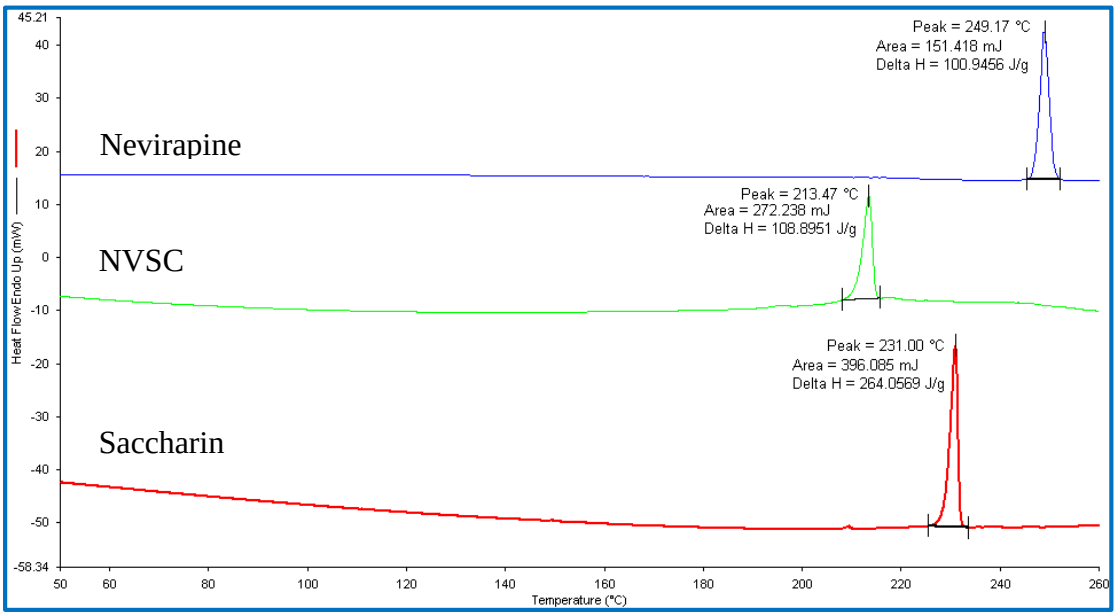


Figure 4.11 DSC of NVSC co-crystal, nevirapine and saccharin

The various batches produced displayed a melting point well within the mentioned range thus, confirming the formation of the NVSC co-crystal (fig 4.12 and 4.13). The hot stage microscopy and the thermogravimetric analysis further attested the formation of a co-crystal by confirming the melting point with no significant mass loss of solvent, respectively.

4.6.1 Differential Scanning Calorimetry of NVSC co-crystal batches

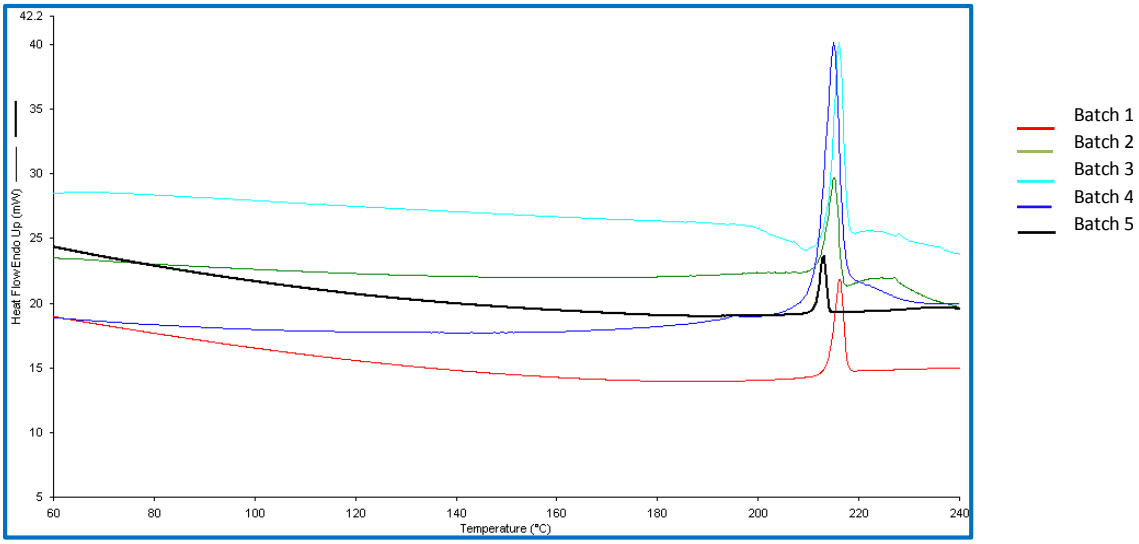


Figure 4.12 DSC of NVSC batches 1-5

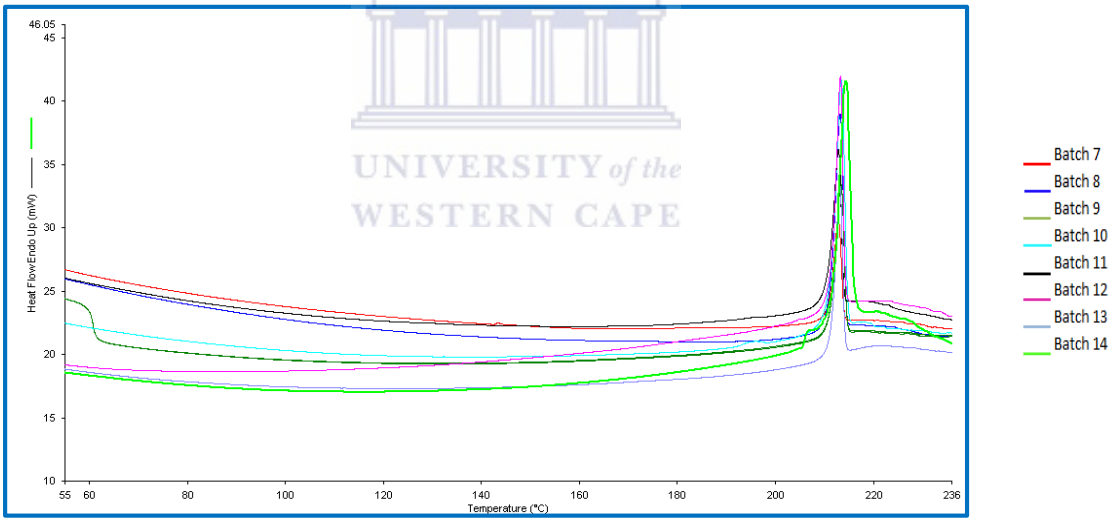


Figure 4.13 DSC of NVSC batches 7-14

Chen et al. suggested that during scale-up, the thermal stability of raw materials should be pre-determined to detect endothermic or exothermic behaviour.[23] Thus, this study followed suite and performed identification tests of raw materials. All the DSC results as seen in figure 4.12 and 4.13 revealed the formation of the NVSC co-crystal by displaying a melting point within the melting point range stipulated in literature.[1]

However, a difference that was consistently observed during scaling up was that decomposition began much earlier (235-240 °C) in comparison to the earlier studies done by Caira et al[1] where decomposition at 315 °C was recorded. This difference in decomposition could be attributed to the increase in batch size.

4.6.2 Hot Stage Microscopy of NVSC co-crystal batches

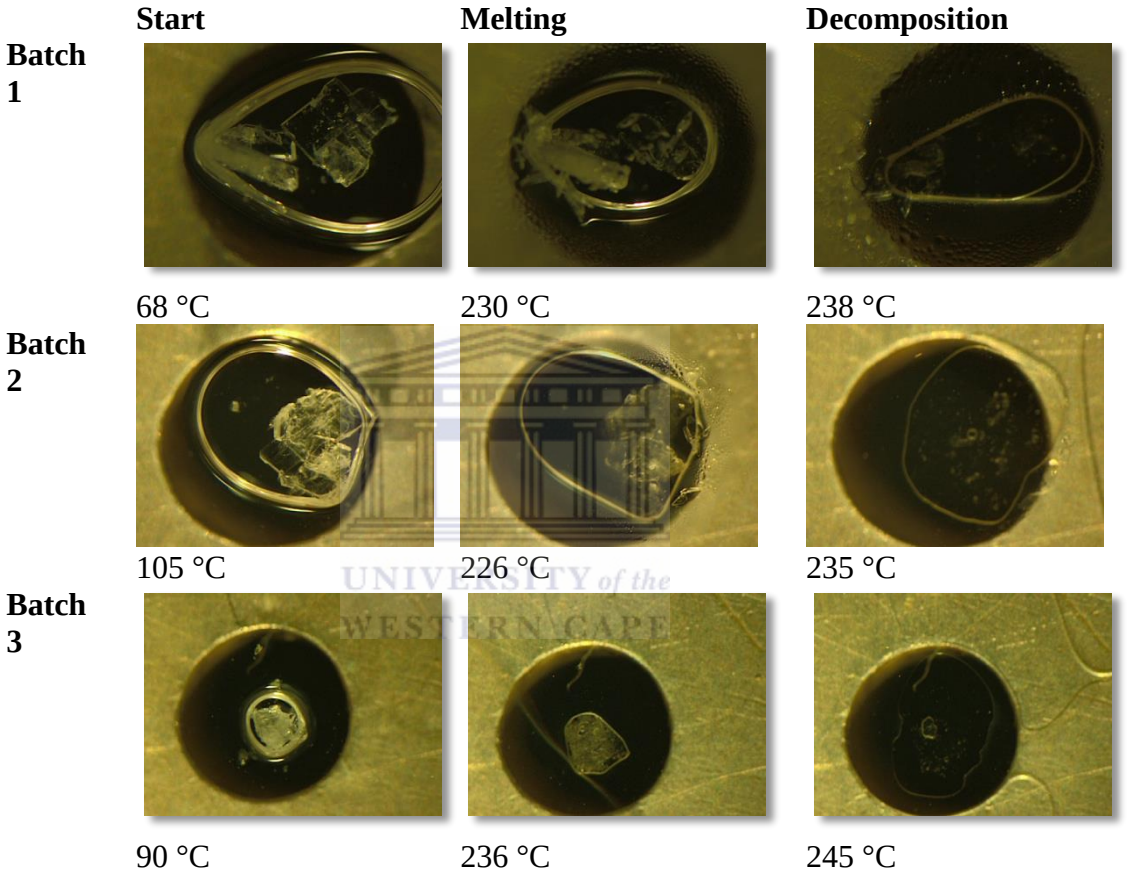


Figure 4.14 HSM of NVSC batches 1-3

HSM results showed a melting point in the range of 230-236 °C reaffirming the formation of the NVSC co-crystal (fig. 4.14). The decomposition results of HSM and DSC were in agreement, with decomposition beginning in a range of 235-250 °C. It can be noted that the appearance of the powder was crystalline in nature.

For HSM of batches 4 to 14 the reader is referred to Appendix A.

4.6.3 Thermogravimetric analysis of NVSC co-crystal batches

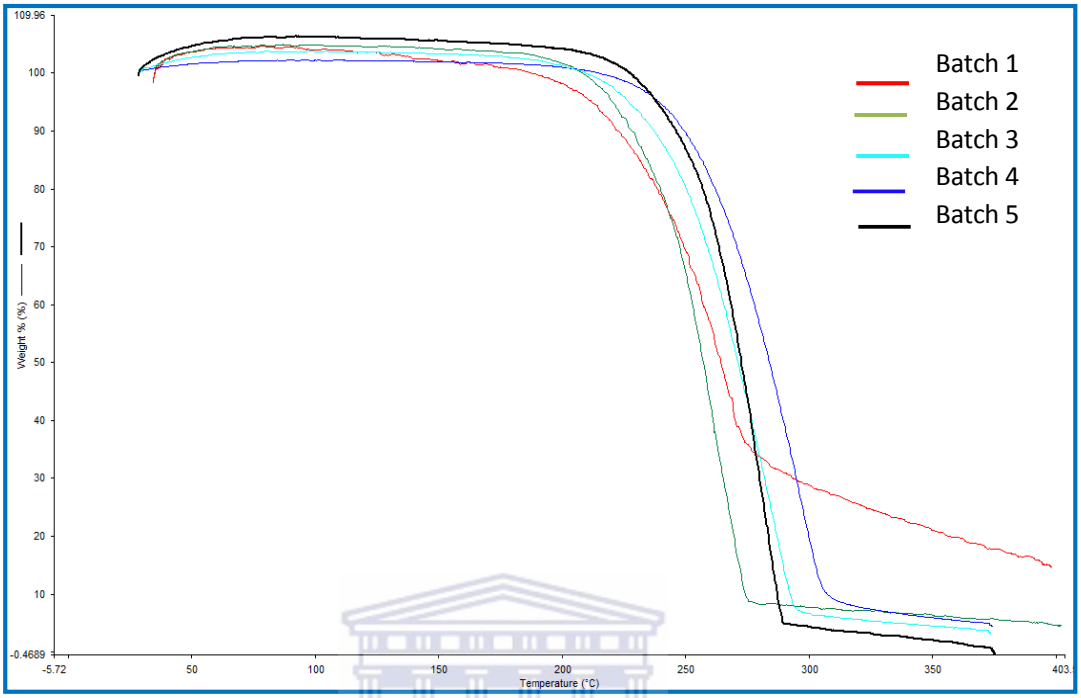


Figure 4.15 TGA of batch 1-5

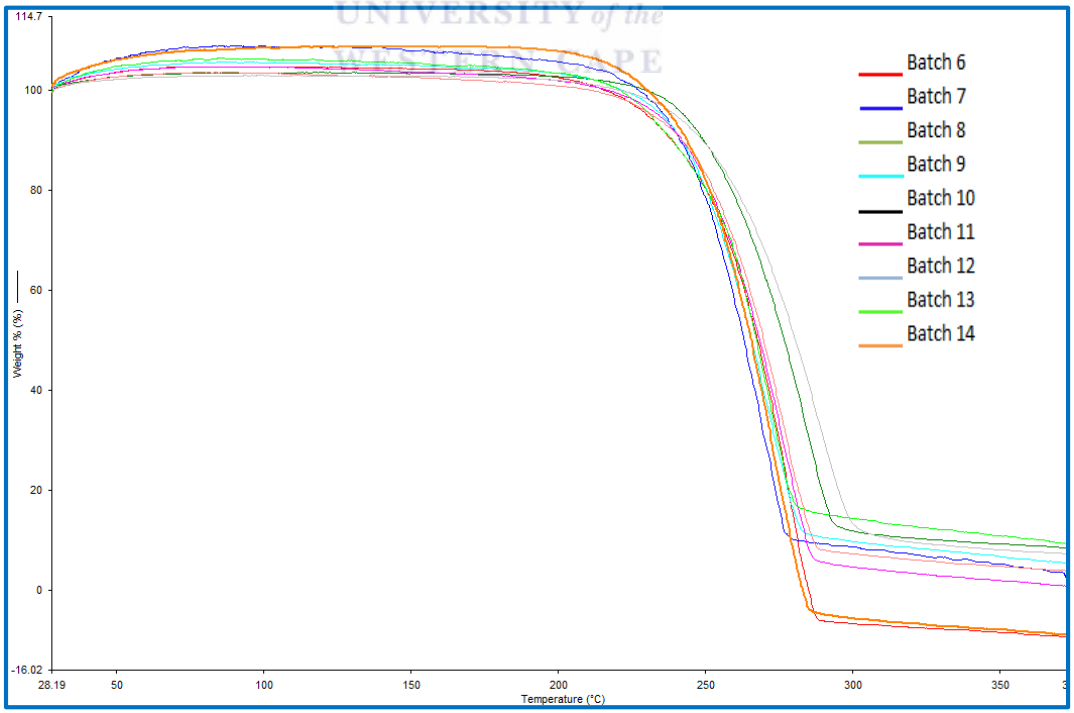


Figure 4.16 TGA of batch 6 - 14

TGA results of all batches recorded a mass loss of less than 2% (fig. 4.15 and 4.16). Mass loss was related to a volatile component which is methanol in this case, the solvent that was used to prepare the sample.[25] The TGA curve presents a melting point in the range of 210 – 230 °C and decomposition at 260 – 275 °C. This is in accordance with the results obtained in the DSC and HSM. Thus, confirming the formation of the NVSC co-crystal.

4.6.4 Fourier Transform Infrared of NVSC co-crystal

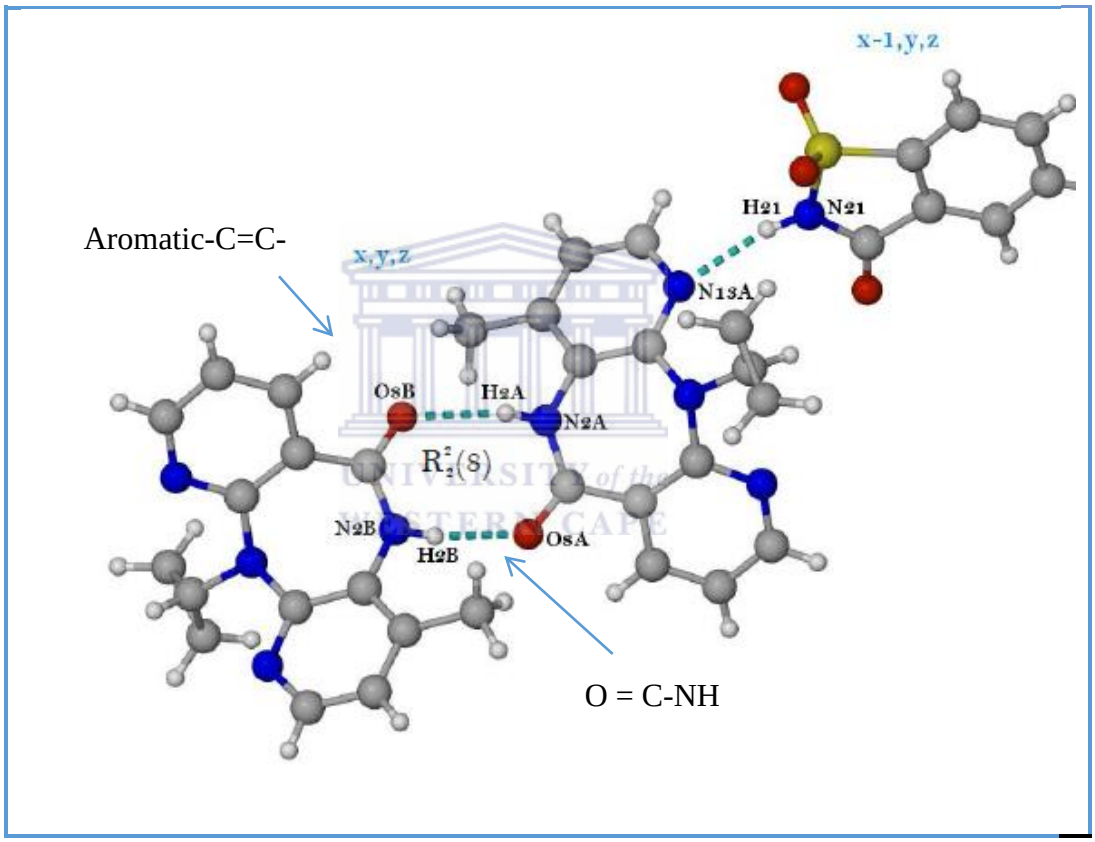


Figure 4.17 Indication of functional groups interacting in the NVSC co-crystal[13]

Figure 4.17 indicates the functional groups of the NVSC co-crystal that are identified in the FTIR spectra. The experimental frequency of the NVSC co-crystal was compared to the standard frequency (Table 4.7). Additionally, it was compared to previous spectra of the NVSC co-crystal (Fig. 4.18).

Bonds for saccharin	Standard frequency	Experimental frequency
S-N-C	975	972
O = C-NH	1722	1715
CO-NH	3100	3093
Aromatic –C = C -	1597	1592
Bonds for nevirapine	**Standard frequency[26]**	**Experimental frequency**
C-O	1646	1643
N-H, C-N	3188	3185
Bonds for NVSC	**Standard frequency[10]**	**Experimental frequency**
S-N-C	975	975
O = C-NH	1722	1644
Aromatic –C = C -	1597	1585
C-O	1646	1643

Table 4.7 Standard frequency versus experimental frequency of saccharin, nevirapine and NVSC

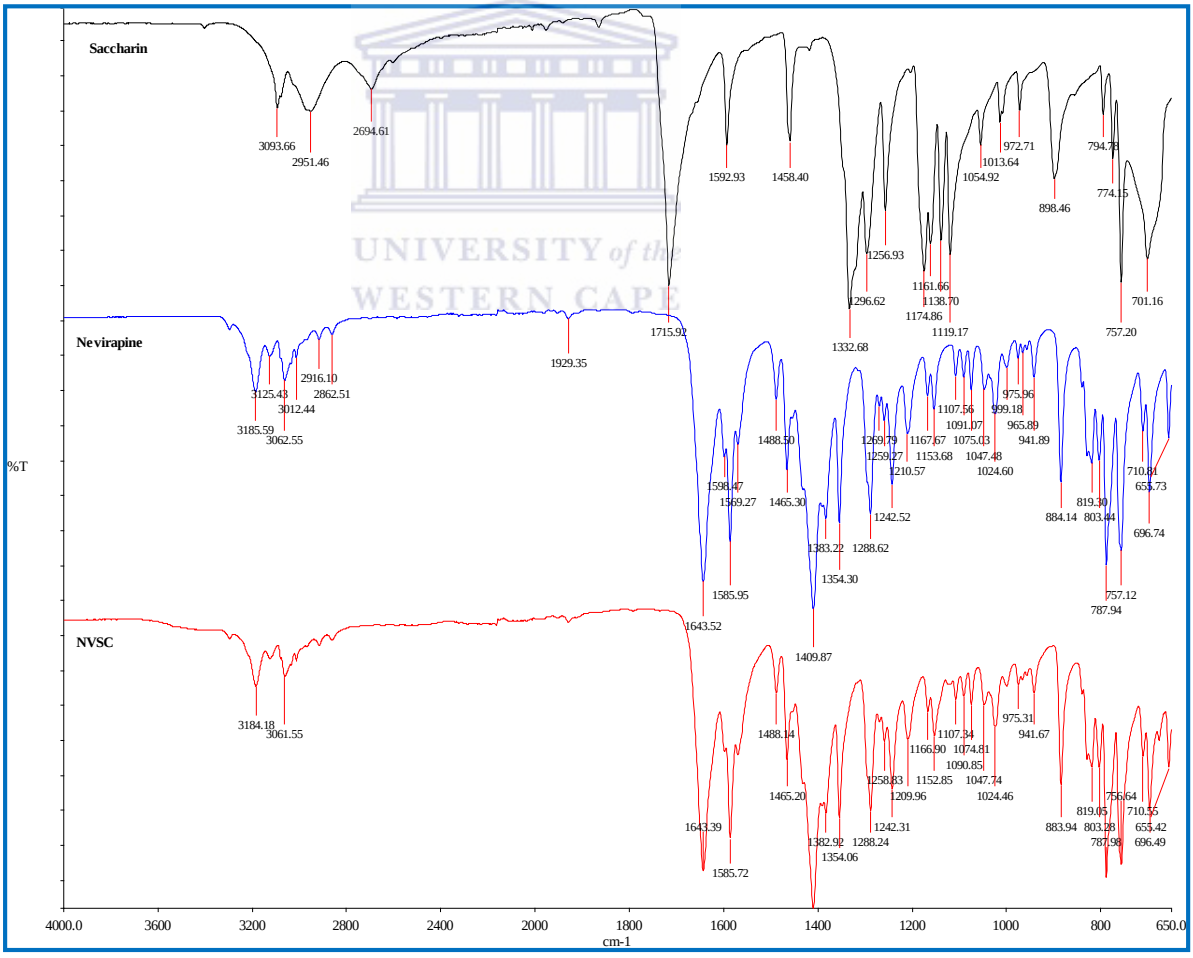


Figure 4.18 FTIR spectra of Saccharin, Nevirapine and NVSC co-crystal

S-N-C bond of the NVSC co-crystal appears at 975 cm^{-1}, which is the exact standard absorbance of this bond. C-O and aromatic-C = C- could be identified at 1643 cm^{-1} and 1586 cm^{-1}respectively; The C-O bond in the NVSC co-crystal is expected to be seen at 1646 cm^{-1}, and the experimental results identified a peak at 1643 cm^{-1}, these bonds signify the formation of NVSC.

4.7 Method for preparation of nevirapine suspension

The branded nevirapine suspension, Viramune®, contains the following excipients: carbomer, polysorbate 80, sorbitol solution, methylparaben, propylparaben, sodium hydroxide and purified water. Viramune ® is prepared by the dispersion technique; the aforementioned technique along with the pH modification technique was utilized to prepare a suspension using the NVSC co-crystals that were described above (Fig. 4.19).

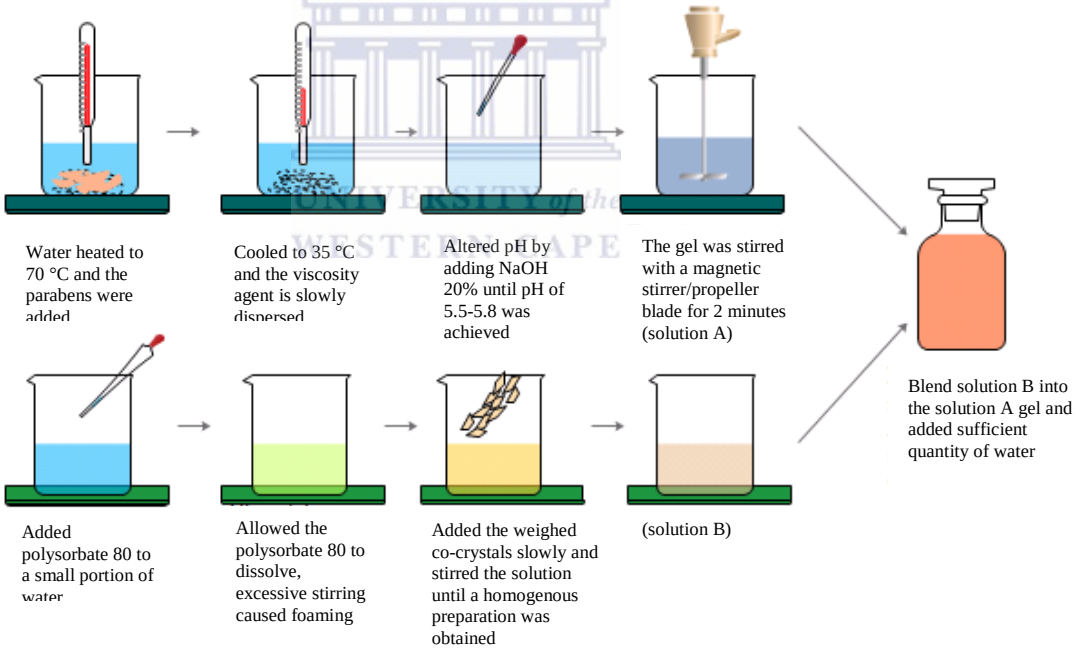


Figure 4.19 Preparation of nevirapine co-crystal suspension as per pH modification method used in Viramune ®

Excipients were added in a stepwise manner and added only if necessary, this was to promote the judicious use of excipients.

Three formulations were investigated with different viscosity inducing agent's viz. aerosil 200, carbopol 971G and carbopol 974P. Furthermore, at each stage the pH and integrity of the co-crystal were deemed as critical variables. pH of suspension was important to the formulation of the suspension to ensure safe consumption of the suspension and to ensure the stability of the preparation while it is on the shelf. Retention of the co-crystal in the suspension formulation was crucial as it is essential for the suspension to have the desired therapeutic effect upon consumption.

4.8 Formulation A

The following combinations were attempted for formulation A, using Aerosil 200 as the viscosity inducing agent.

Formulation A	NVSC co-crystal	Sorbitol	Aerosil 200	NaOH (20 %)	Methylparaben	Propylparaben	Water
A1	250 mg	25 mL					
A2	50 mg		19 mg				
A3	30 mg	1 mL	119 mg	50 μL	1.8 mg	0.72 mg	2 mL

Table 4.8 Variations of formulation A

4.8.1 Formulation A1 (NVSC and sorbitol)

The purpose of this formulation consisting of only NVSC and sorbitol was to identify if the co-crystal was retained in sorbitol alone. To prepare this formulation A1, 250 mg of NVSC co-crystal was added to 25 mL of sorbitol in a beaker. The sample was stirred at 600 rpm on a magnetic hot plate for 24 hours at room temperature. The sample was then filtered through a Whatman® filter paper no. 41 since the sample was too viscous to pass through 0.45μm filter membrane.

4.8.1.1 pH of formulation A1

The pH of the sorbitol and NVSC co-crystal was 2.48 at room temperature. This showed that the co-crystal had significantly reduced the pH of the sorbitol (pH = 8). This was attributed to the acidic nature of the co-crystal.

4.8.1.2 Co-crystal integrity of formulation A1

Filtered sorbitol was used as the blank for UV calibration. The suspension was then placed in a 1 mL glass UV cuvette by means of a syringe. A wavelength scan was performed through ultraviolet spectroscopy from 200 to 800 nm.

A presence of peaks at 234 nm for nevirapine and 260 nm for saccharin indicated that the co-crystal had fragmented into its individual components (Fig. 4.20) and that the co-crystal could not be retained in sorbitol alone.

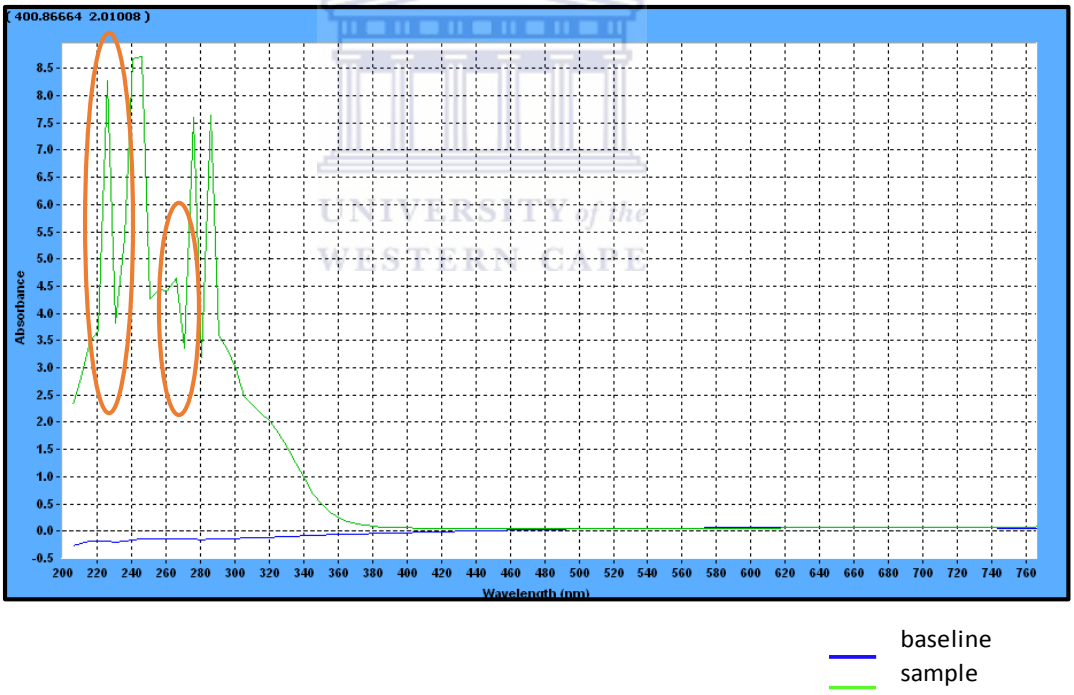


Figure 4.20 UV spectra of formulation A1

The challenge at this juncture became two fold, the first challenge was to retain the co-crystal and the second was to adjust the pH of the co-crystal suspension. Hence, the

next excipient was explored to ascertain if the co-crystal integrity could be maintained and to modify the pH in a range of 6-8.

4.8.2 Formulation A2 (NVSC and aerosil)

This formulation was explored to ascertain if the co-crystal could be retained in an aerosil 200 solution. To prepare this formulation, an aerosil 200 gel was prepared by heating 5 mL of water in a small beaker and adding 0.019 g of aerosil 200 powder. 50 mg of the NVSC co-crystal was added and stirred for 24 hours. The total volume of the aerosil 200 and the water that was added was 5 mL, therefore the amount of co-crystal added was based on the concentration found in Viramune ® 50 mg/5 mL.

4.8.2.1 pH of formulation A2

The pH of aerosil 200 alone was found to be 6.40. The formulation A2, which consisted of aerosil and NVSC co-crystal was measured be 2.32. This once again indicated that the co-crystal reduced the pH of the aerosil 200 suspension. This was again recognized to the acidic nature of the co-crystal.

4.8.2.2 Co-crystal integrity of formulation A2

Since, this preparation was more viscous that formulation A1, it could not be filtered through a 0.45 μm filter membrane nor through filter paper. Hence, DSC was used to determine if the co-crystal was still intact in formulation A2.

A 1 mL of the suspension was filtered “before” stirring the formulation. The DSC of the filtrate indicated that the co-crystal was present (Fig. 4.21). The melting point of the NVSC co-crystal was within the range specified in the pre-formulation studies i.e. (215 – 230 ºC).[13] There was also a broad peak seen between 80 – 120 ºC, this was recognized due to the presence of aerosil 200 and water.

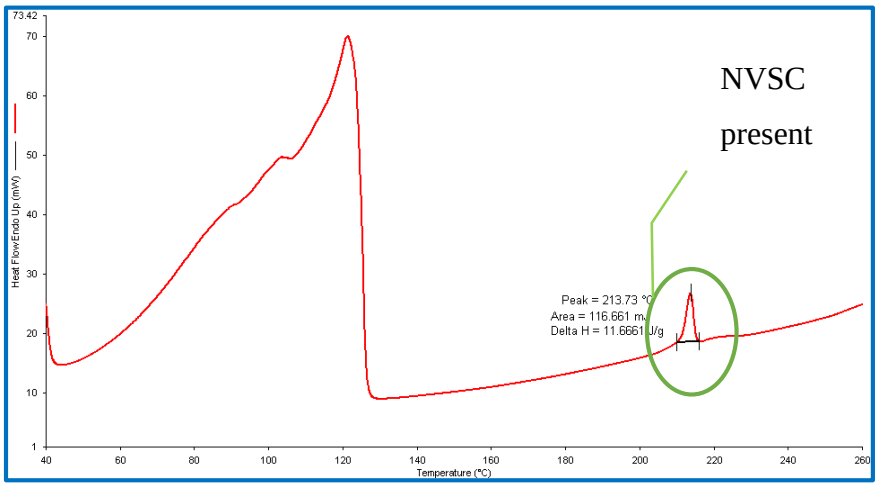


Figure 4.21 DSC of formulation A2 before stirring

However, after stirring for 24 hours, a 1 mL suspension was filtered and the DSC of this filtrate indicated that there was no presence of co-crystal that should have ideally been identified in the range of 215 – 230 ºC, suggesting that the NVSC co-crystal had disintegrated into the aerosil 200 media. This is due to the stirring that took place during preparation of the aerosil suspension. This indicated that this formulation was not viable because it meant that if the suspension is stirred, the co-crystal is fragmented, at the same time, stirring was needed to ensure the preparation is homogenous. Hence, this formulation had failed to retain the co-crystal integrity.

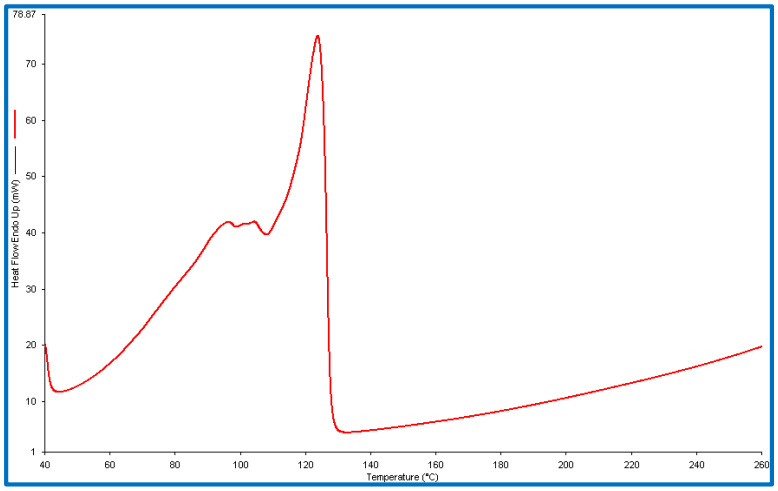


Figure 4.22 DSC of formulation A2 after stirring

4.8.3 Formulation A3 (NVSC, aerosil, sorbitol, sodium hydroxide, and preservatives)

A similar procedure akin to the preparation of Viramune ® was attempted. 5 mL of water was heated to 70 ºC by means of a water bath. Preservatives were added to this solution (0.018 g of methylparaben and 0.024 g of propylparaben). The temperature of the solution was then cooled to a temperature of 35 ºC. Aerosil 200 was added, initially 0.019 g and thereafter additional 0.100 g was added to ensure the preparation was viscous in nature. Upon addition of 1 mL sorbitol, a total volume of 3 mL was formed. Therefore 30 mg of NVSC co-crystal was added and left to stir for 24 hours. 50 μL of sodium hydroxide 20% buffer solution was added to modify the pH. Formulation A3 had good wetting properties, hence usage of polysorbate 80 was omitted.

4.8.3.1 pH of formulation A3

Since this formulation had many excipients, the pH was measured at each stage of the preparation (Table 4.9). After heating the preservatives and adding the aerosil 200 in water, the pH was measured to be 5.82, after stirring the pH increased slightly to 6.43. Upon addition of the co-crystal and the sorbitol and stirring the sample for 24 hours the pH decreased to 3. To modify the pH, sodium hydroxide was added and the pH increased drastically to 12.84.

	pH
Preservatives and aerosil 200	5.82
Preservatives and aerosil 200 (after stirring)	6.43
Preservatives, aerosil 200, NVSC co-crystal	3.00
Preservatives, aerosil 200, NVSC co-crystal, 50 μL sodium hydroxide 20%	12.84

Table 4.12 pH of formulation A3

4.8.3.2 Co-crystal integrity of formulation A3

Despite the failed attempt to modify the pH, the suspension was filtered by means of a Whatman® filter paper. A DSC was performed on the filtrate to identify whether the co-crystal was still present after the addition of various excipients.

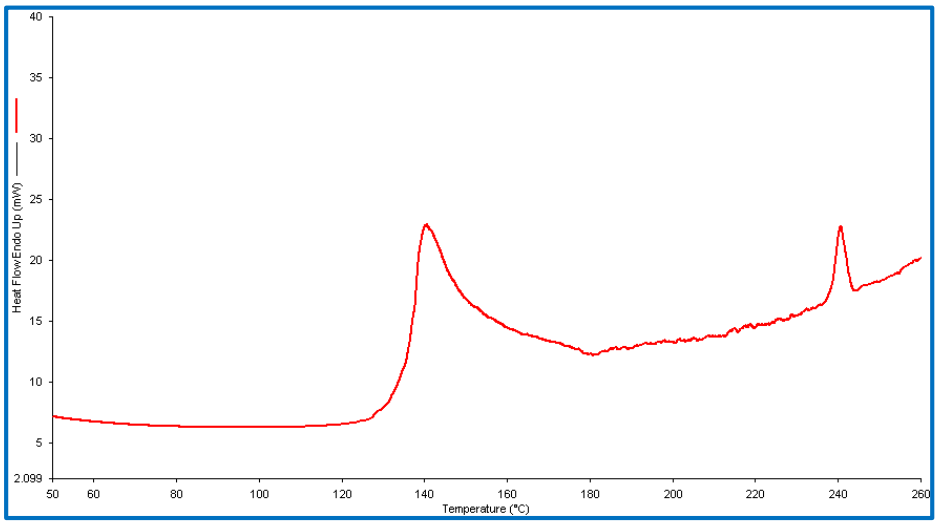


Figure 4.23 DSC of formulation A3

There was a broad peak formed between 120 – 180 ºC. This was attributed to the components such as aerosil 200 and the preservatives. The co-crystal is ideally formed between 215-230 ºC with nevirapine identified at 245-249 ºC and saccharin at 228-229 ºC. The DSC of the filtrate indicated a sharp peak at 240 ºC (Fig. 4.23). This could possibly indicate the presence of the nevirapine, as it is closer to the melting point of nevirapine. Thus, indicating that the NVSC co-crystal had fragmented into its API, nevirapine, and possibly saccharin had dissolved in the preparation.

4.8.4 Comparison of formulation A1 to A3

Formulation A	NVSC co-crystal	Sorbitol	Aerosil 200	Sodium hydroxide (20 %)	Methylparaben	Propylparaben	Water	pH after adding NVSC co-crystal	Integrity of co-crystal in suspension
A1	250 mg	25 mL						2.48	x
A2	50 mg		19 mg					2.32	x
A3	30 mg	1 mL	119 mg	50 µL	1.8 mg	0.72 mg	2 mL	12.84	x

Table 4.23 Comparison of pH and integrity of Formulation A

From table 4.13, it can be concluded that aerosil 200 does not allow for the co-crystal to be intact in a suspension formulation. Furthermore, the pH obtained was not within the desired range. Also, formulation A3 that was prepared was not viscous enough thus causing particles to settle upon storage. The maximum viscosity achieved was 10.35 cP at a shear rate of 50 with a SC4-18 spindle (Fig. 4.24). This viscosity was not sufficient to suspend the NVSC co-crystal particles.

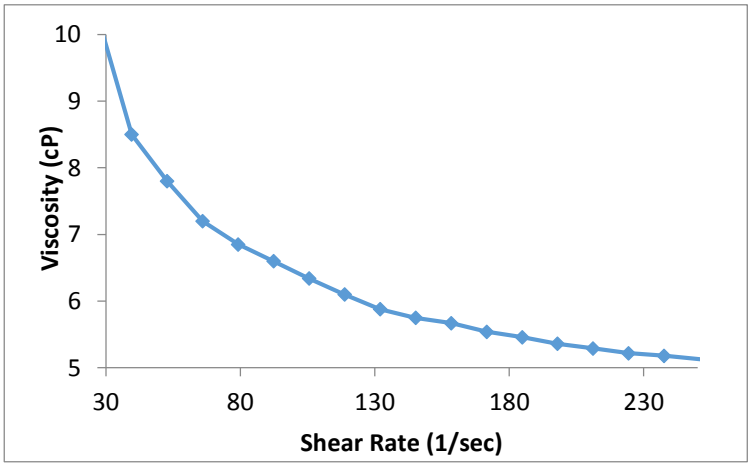


Figure 4.24 Viscosity versus shear rate of formulation A3

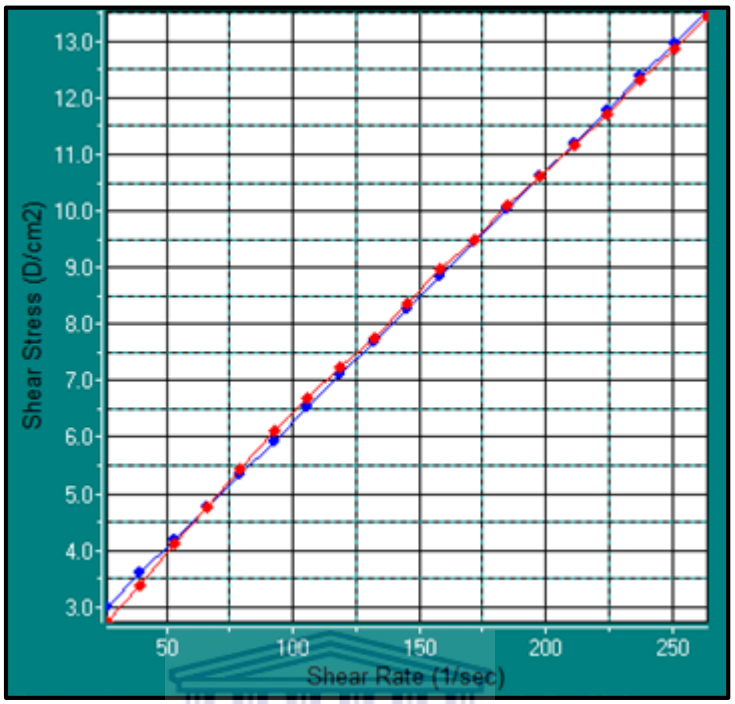


Figure 4.25 Shear stress versus shear rate of Formulation A3

The rheogram (shear stress versus shear rate) of formulation A3 indicated that the suspension was pseudoplastic in nature (Fig. 4.25). Yield stress is the point at which the preparation begins to deform. The yield value obtained was 4.44 cP. On comparison of the yield value to the viscosity obtained (10.35 cP), it showed that the suspension is easily deformed and is not viscous enough as it starts sedimenting instantaneously upon storage.

4.9 Formulation B

Formulation	NVSC co-crystal	Carbopol 971G	Sodium hydroxide 20%	Sorbitol	Polysorbate 80	Methylparaben	Propylparaben	Water
B1	50 mg	10.5 mg						
B2	50 mg	10.5 mg	7 μL					
B3	165 mg	33.6 mg	30 μL	3.7 mL	8 μL	28.8 mg	3.84 mg	q.s

Table 4.14 Variations of formulation B

The above formulations were attempted for formulation B (Table 4.14). The formulation included the same excipients as in Viramune® except that formulation B used carbopol 971G as the viscosity inducing agent. The carbopol 971G is used for oral dosage forms. It is granular in nature and used for tablets and suspensions as well.

4.9.1 Formulation B1 (NVSC co-crystal and carbopol 971G)

The purpose of this formulation was to ascertain if NVSC co-crystal could be retained in carbopol 971G. To prepare formulation B1, 0.0105 g of carbopol was stirred in 5 mL of water. The amount was calculated in accordance to what was stated in the Viramune® patent. The patent required 0.2100 g carbomer in 100 mL, thus for a 5 mL suspension, 0.0105 g carbopol 971G was required. Upon stirring the carbopol 971G in 5 mL of water, a viscous gel was formed and 50 mg of NVSC was added to this. The preparation was stirred together for 24 hours.

4.9.1.1 pH of formulation B1

The pH of formulation B1 consisting of carbopol 971G and the NVSC co-crystal was found to be 3.02.

4.9.1.2 Co-crystal integrity of formulation B1

The suspension was then filtered by means of Whatman® filter paper. The filter paper was dried in a fume cupboard for 3 weeks. DSC was performed on the filtrate (Fig. 4.26).

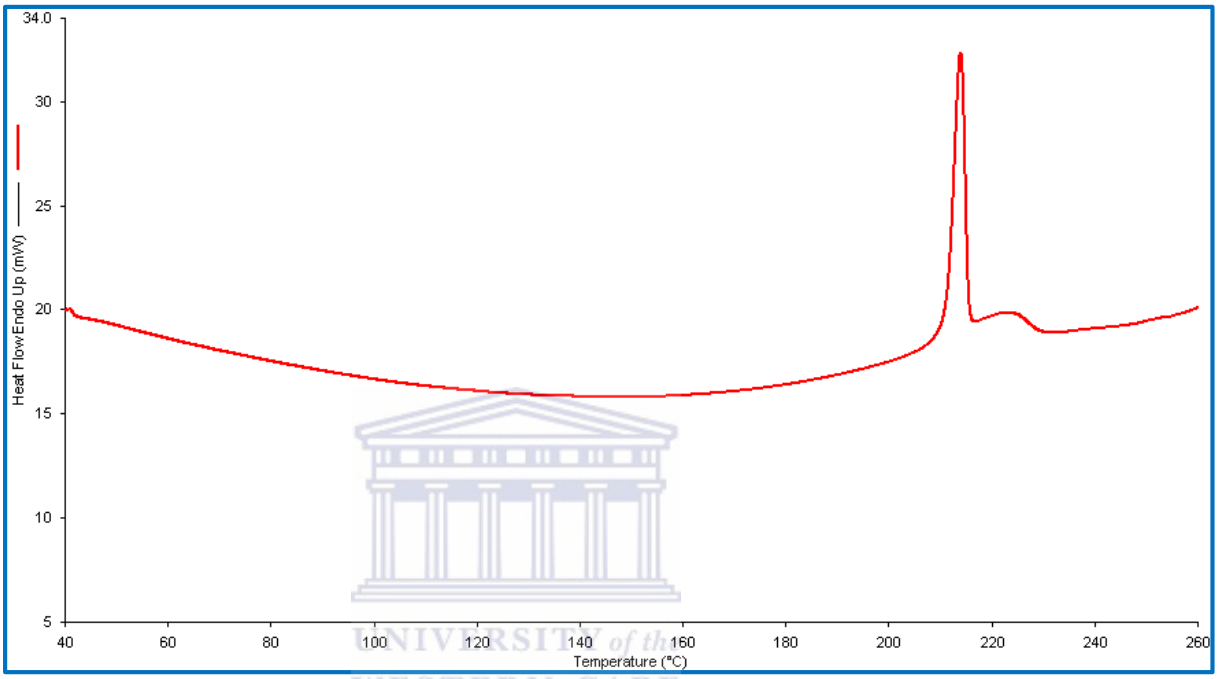


Figure 4.26 DSC of formulation B1

The DSC indicated an endotherm at 215 ºC, which was the exact onset of melting point at which NVSC co-crystal is expected to be seen. Hence, it can be concluded that the NVSC co-crystal is stable in carbopol 971G.

The FTIR of the suspension without filtration was performed. It was compared to the FTIR spectra of the NVSC co-crystal powder alone. The two spectra were superimposed and were found to be identical. Bonds such as C-O and aromatic -C = C- could be identified at 1646 cm^{-1} and 1586 cm^{-1}, respectively; these bonds indicate the presence of NVSC. Therefore, it is conclusive that the NVSC co-crystal can be retained in carbopol 971G.

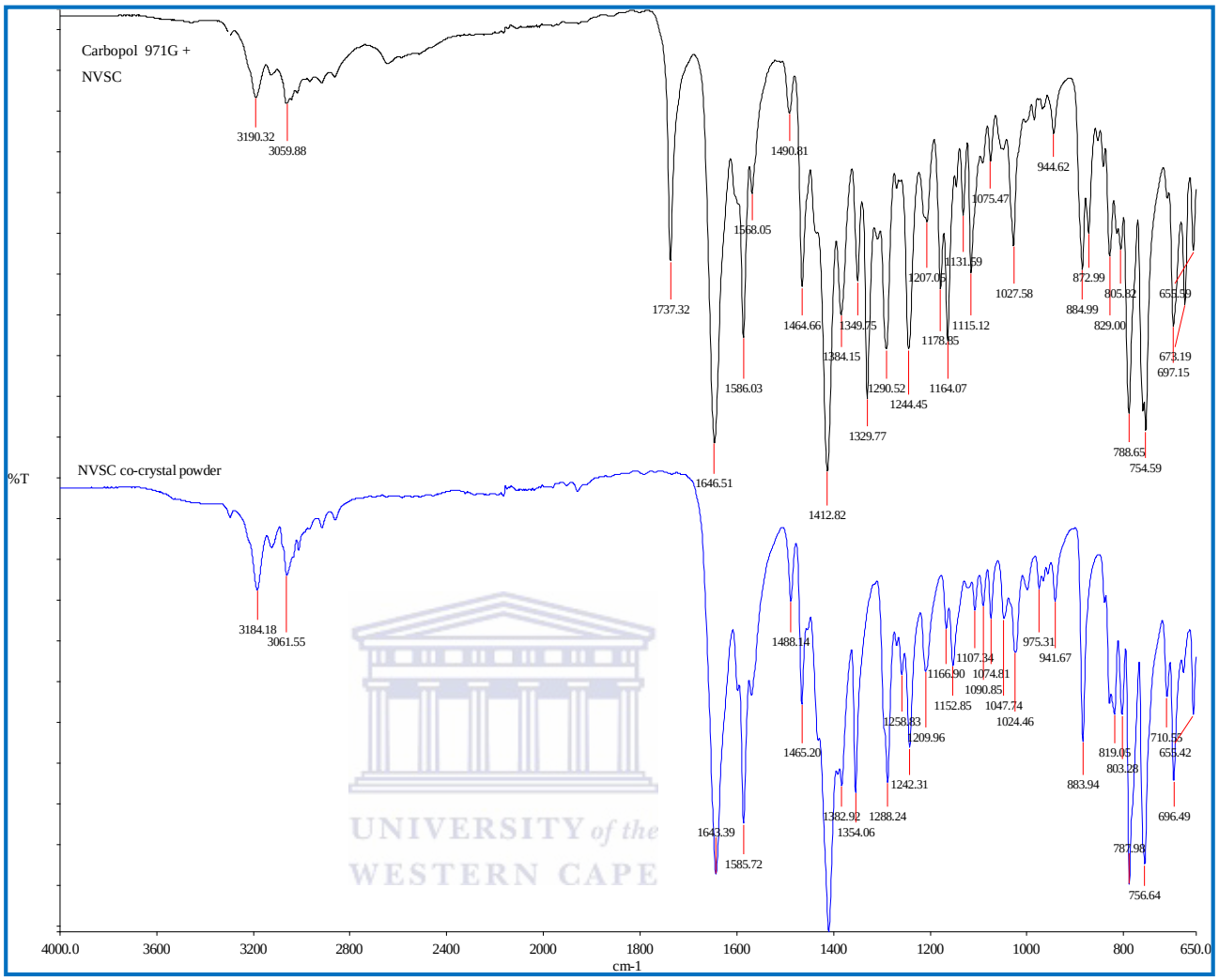


Figure 4.27 FTIR spectra of formulation B1

This formulation was able to retain the co-crystal in the suspension; however, the pH of this formulation was 3.02, which was unsuitable for oral use. Hence, the next formulation aimed to modify the pH suspension whilst retaining the co-crystal in carbopol 971G is formulation B2.

4.9.2 Formulation B2 (NVSC, carbopol 971 G and sodium hydroxide)

Formulation B2 was prepared by adding 0.0105 g of carbopol 971G to 5 mL of water in a beaker. This solution was stirred magnetically and 50 mg of NVSC was added to the suspension.

4.9.2.1 pH of formulation B2

The pH was measured to be 3.02. To modify the pH, 7 μL of sodium hydroxide was added and the pH was re-measured to be 6.95. The addition of sodium hydroxide successfully modified the pH (Table 4.15)

	pH
Carbopol 971G and NVSC co-crystal	3.02
Carbopol 971G, NVSC, sodium hydroxide	6.95

Table 4.15 pH of formulation B2

4.9.2.2 Co-crystal integrity of formulation B2

Formulation B2 was filtered through a Whatman® filter paper and the filter paper was dried in a fume cupboard for 3 weeks. DSC was performed on the filtrate (Fig. 4.28).

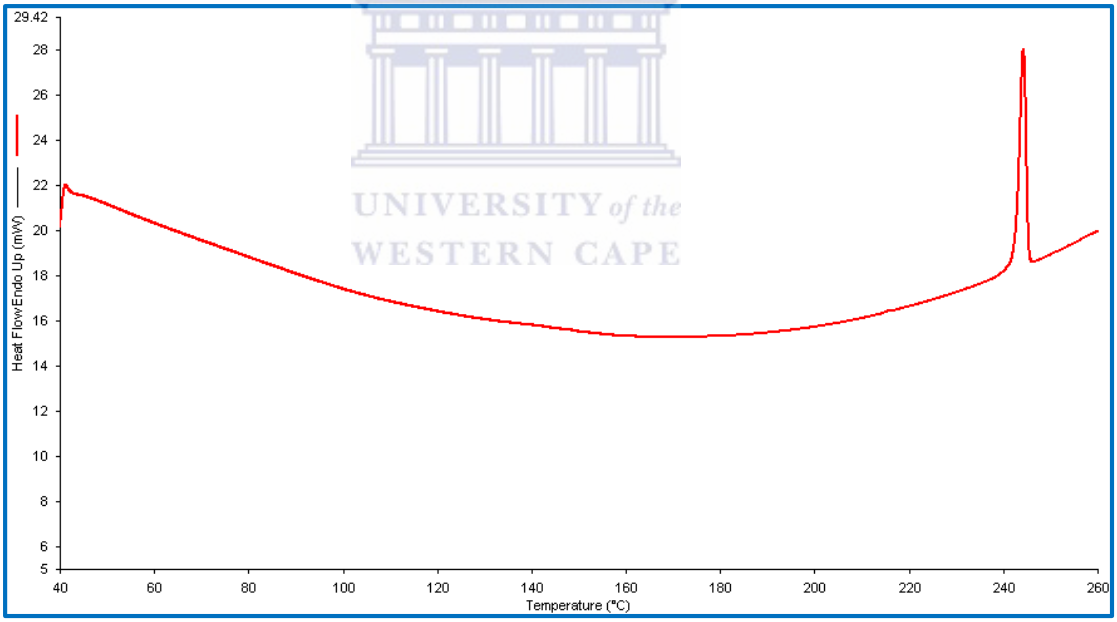


Figure 4.28 DSC of formulation B2

An endotherm was seen at 242 ºC, this was close to the melting point of nevirapine which is at 245 -249 ºC. Hence, FTIR was performed to establish if the co-crystal was still intact or if it had separated into the API and co-former.

The FTIR spectra of the NVSC co-crystal powder was compared to the NVSC co-crystal suspension in carbopol 971G (Fig. 4.29). The C-O bond in the NVSC co-crystal was expected to be seen at 1646 cm^{-1} and the experimental results identified a peak at 1644 cm^{-1}, thus signifying the presence of the co-crystal. S-N-C bond appears at 975 cm^{-1}, which is the exact absorbance of this bond. This signifies the presence of the NVSC co-crystal in the suspension. Hence, this result indicates that the co-crystal was still present in the carbopol 971G medium.

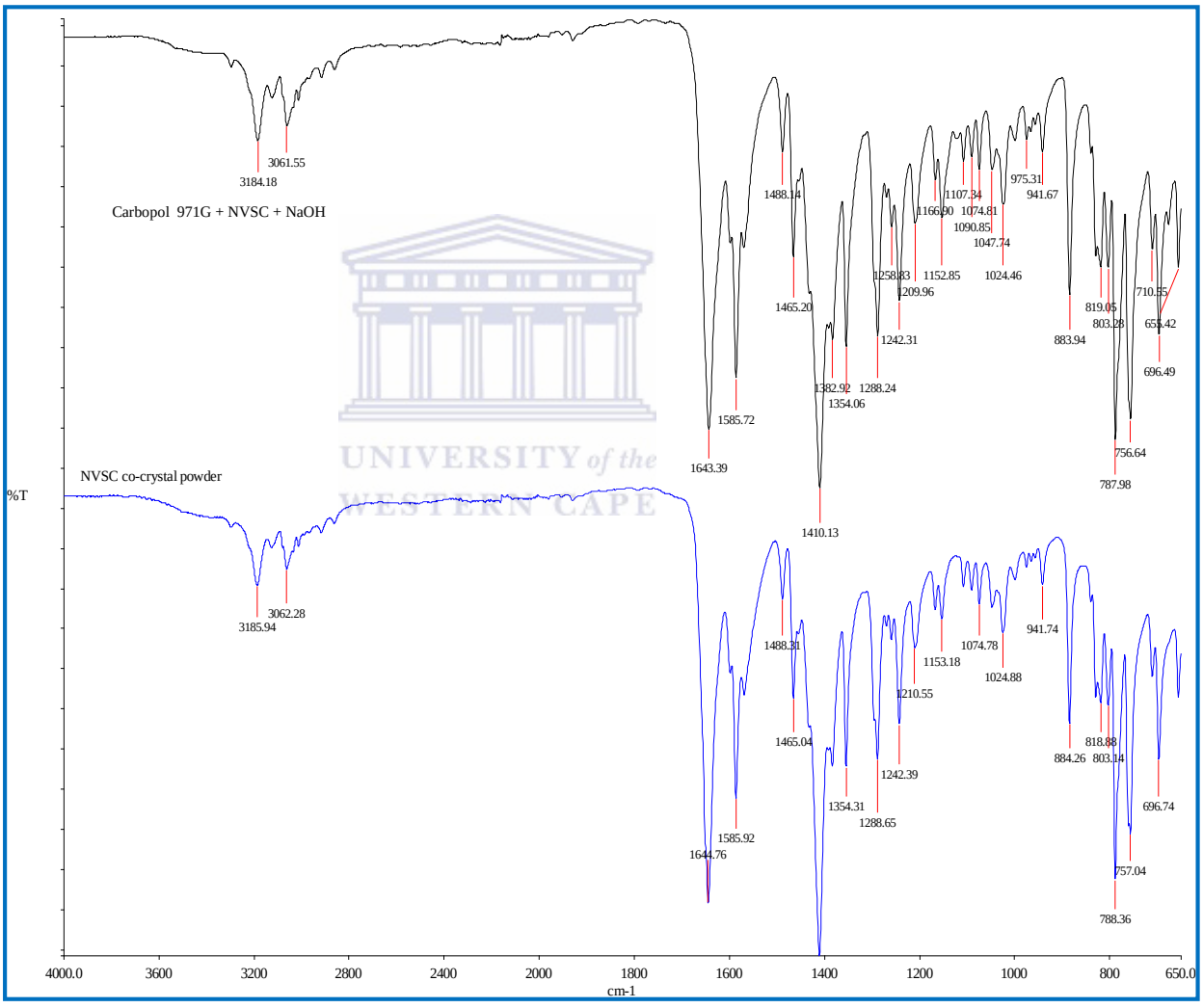


Figure 4.29 FTIR of formulation B2

Formulation B2 had a pH of 6.95, which was within the target pH required, furthermore, the FTIR also indicated that the co-crystal was intact in carbopol 971G and sodium hydroxide. However, co-crystal integrity was not confirmed through DSC. Hence, formulation B3 was developed to ensure that the co-crystal is present.

4.9.3 Formulation B3 (NVSC, carbopol 974P, sorbitol, sodium hydroxide, polysorbate and preservatives)

This formulation was prepared using excipient such as sorbitol to enhance the density of the suspension medium. A 16 mL of formulation B3 was prepared according to the method used to prepare Viramune ® suspension. In a 25 mL beaker, 4 mL of water was heated on a magnetic hot plate at 70 ºC. Preservatives such as methylparaben (0.072 g) and propylparaben (0.0096 g) were added to the beaker and stirred at 600 rpm. The preservative solution was cooled to 35 ºC. Carbopol 971G (0.042 g) was added to the preservative solution. To this solution, sodium hydroxide 20%, 30 μL was added and magnetically stirred until a gel was formed. Sorbitol was added to the carbomer solution.

Polysorbate and water were separately stirred on a magnetic hot plate at room temperature. The NVSC co-crystal was added to this solution.

Both the polysorbate drug concentrate and the carbomer gel were magnetically stirred together and made up with water was to 16 mL.

4.9.3.1 pH of formulation B3

The pH of the preservatives alone in water was 5.54, upon the addition of carbopol 971G to the solution, pH was 3.23. This was attributed to the acidic nature of carbopol 971G. The NVSC co-crystal and polysorbate mixture had a pH of 3.32. When the two solutions were mixed together the pH was 4.52. To achieve a suspension within the target range, sodium hydroxide was added until a pH of 6.42 was achieved (Table 4.16)

	pH
Preservatives, water	5.54
Preservatives, carbopol, water	3.23
NVSC, polysorbate and water	3.32
Sorbitol, carbopol, NVSC, polysorbate , water, preservatives	4.52
Sorbitol, carbopol, NVSC, polysorbate , water, preservatives, sodium hydroxide	6.42

Table 4.16 pH of formulation B3

4.9.3.2 Co-crystal integrity of formulation B3

Once the suspension was prepared it was filtered through Whatman® filter paper. The filter paper was then left to dry in a fume cupboard and DSC was performed on the residue on the filter paper (Fig. 4.30).

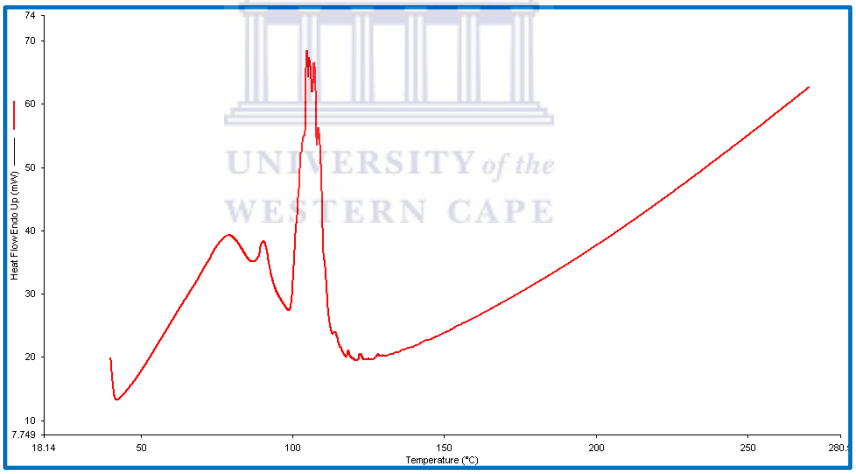


Figure 4.30 DSC of formulation B3

The DSC in figure 4.30 indicated a peak at 100 ºC and this could be attributed to the water present in the suspension. There were no other peaks in the filtrate indicating that the suspension did not contain the NVSC co-crystal.

Furthermore, FTIR in figure 4.29 was performed on the suspension itself and it did not show absorbance of the functional groups such as C-O bond, S-N-C and aromatic-C=C-, that are present in the NVSC co-crystal. This clearly indicated that NVSC co-

crystal was not stable in formulation B3 and that suspension excipients are required to maintain the formulation of a suspension.

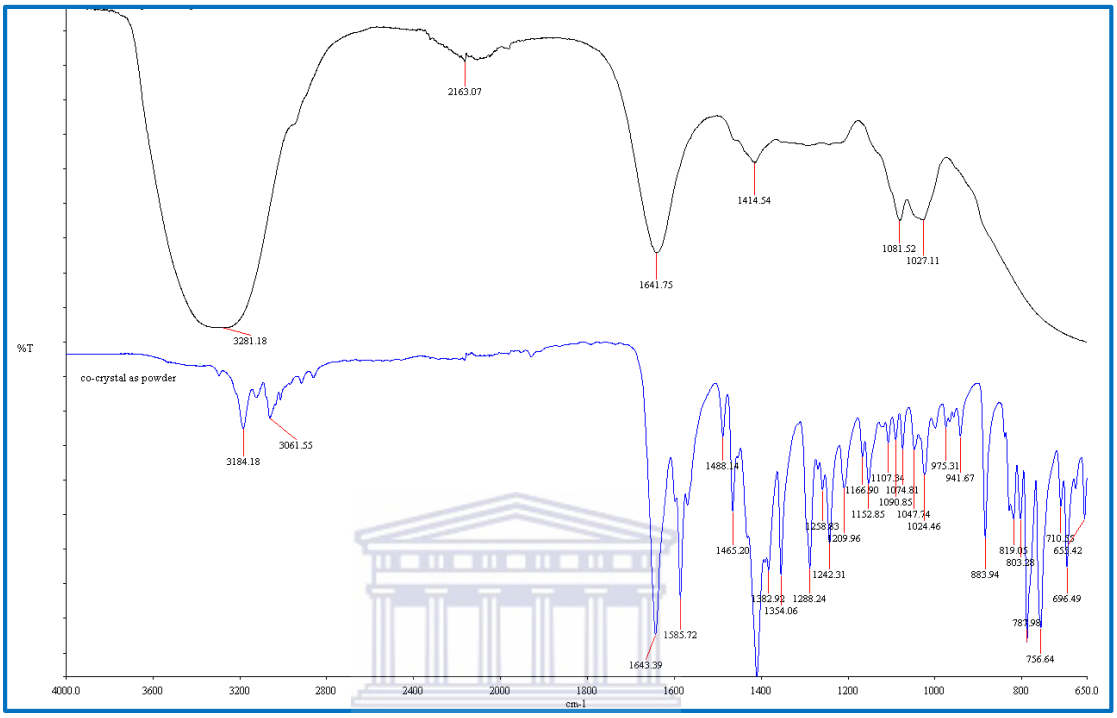


Figure 4.31 FTIR of formulation B3

4.9.4 Comparison of formulation B1 to B3

Formulation B	NVSC co-crystal	Carbopol 971G	Sodium hydroxide 20%	Sorbitol	Polysorbate 80	Methylparaben	Propylparaben	Water	pH of formulation	Integrity of co-crystal (DSC)	Integrity of co-crystal (FTIR)
B1	50 mg	10.5 mg							3.02	✓	✓
B2	50 mg	10.5 mg	7 µL						6.95	x	✓
B3	165 mg	33.6 mg	30 µL	3.7 mL	8 µL	28.8 mg	3.84 mg	q.s	6.42	x	x

Table 4.17 Comparison of pH and integrity of Formulation B1, B2 and B3

In spite of the co-crystal integrity not being maintained in formulation B3, the viscosity of the suspension was measured with a SC4-18 spindle. The viscosity was 94.33 cP with a torque of 62.9%. Torque values between 10-100 % are acceptable.

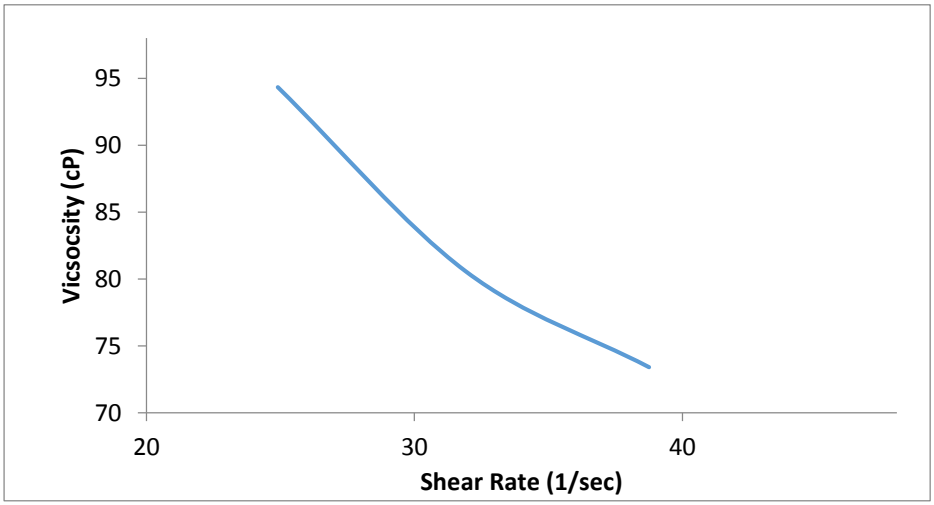


Figure 4.32 Viscosity versus shear rate of formulation B3 suspension

The viscosity of this suspension seemed promising in comparison to formulation A which contained aerosil 200. However, this formulation was not pursued since the integrity of the NVSC co-crystal was compromised.

4.10 Formulation C

Formulation C used carbomer 934P as the viscosity inducing agent. This was the same agent used in the Viramune® suspension. The following combinations were performed for formulation C.

Formulation C	NVSC co-crystal	Carbopol 974P	Sodium hydroxide 20%	Sorbitol	Polysorbate 80	Methylparaben	propylparaben	Water
C1	50 mg	10.5 mg						
C2	50 mg	10.5 mg	7 μL					
C3	165 mg	33.6 mg	30 μL	3.7 mL	8 μL	28.8 mg	3.84 mg	q.s

Table 4.18 Variations of formulation C

4.10.1 Formulation C1 (NVSC and carbopol 974P)

To prepare formulation C1, carbopol 974P (0.0105 g) was added to a beaker with 5 mL of water. The solution was stirred together by means of a magnetic stirrer. 0.0050 g of NVSC co-crystal was added to the solution.

4.10.1.1 pH of formulation C1

The pH of the carbopol 974 P in water was found to be 3.35. Upon addition of the NVSC co-crystal to the carbopol 974 P solution, the pH was recorded as 4.12.

4.10.1.2 Co-crystal integrity of formulation C1

Preparation C1 was then filtered through Whatman® filter paper and it was placed in a fume cupboard where it was allowed to dry. DSC was performed on the residue of the filter paper (Fig 4.33)

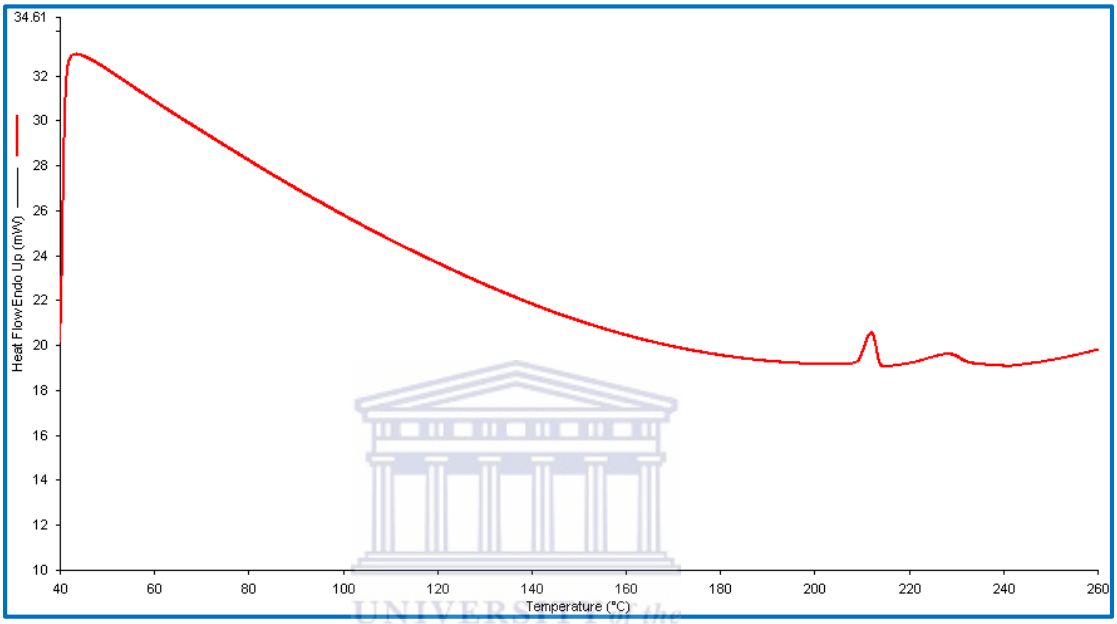


Figure 4.33 DSC of formulation C1

The DSC indicated an endothermic peak at 216 ºC. This was the melting point at which NVSC was earlier reported.1 Hence, the co-crystal was present in carbopol 974P. FTIR analysis was performed on the suspension without filtering to further confirm if the co-crystal was retained in carbopol 974P(Fig.4.34).

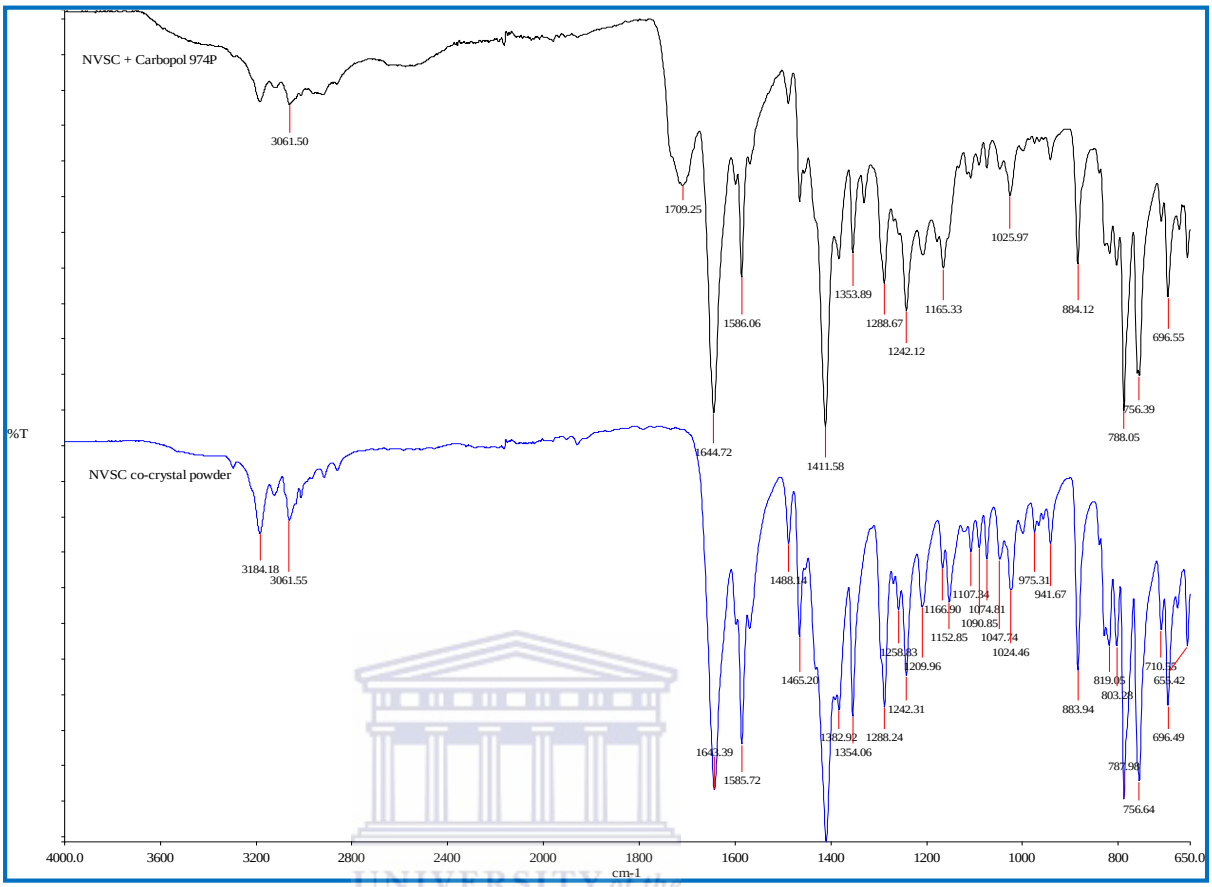


Figure 4.34 FTIR of formulation C1

The FTIR spectra (Fig. 4.34) of the suspension were compared to the NVSC co-crystal powder and were found to be identical. The presence of bonds at 1644 cm^{-1} represent the C-O bond in the NVSC co-crystal while the aromatic -C=C- of the NVSC could be seen at 1586 cm^{-1}. The FTIR together with the DSC results confirmed that the co-crystal was present in carbopol 974P. However, the pH was low and therefore the next formulation used sodium hydroxide to alter the low pH.

4.10.2 Formulation C2 (NVSC, carbopol 974P and sodium hydroxide)

To prepare formulation C2, 0.0105 g of carbopol 974P was placed in a beaker with 5 mL of water and the solution was magnetically stirred. 0.050 g of NVSC co-crystal was added to this solution while stirring. 10 μL of sodium hydroxide through a micropipette was added to modify the pH of the solution.

4.10.2.1 pH of formulation C2

The pH of the solution of carbopol 974P and the NVSC co-crystal was measured to be 2.3. To rectify the pH, 10 μL of sodium hydroxide was added to the solution and the pH was re-measured and found to be 5.8.

4.10.2.2 Co-crystal integrity of formulation C2

Formulation C2 was filtered by means of Whatman® filter paper no. 41 and the filtrate was analysed by DSC (Fig. 4.35).

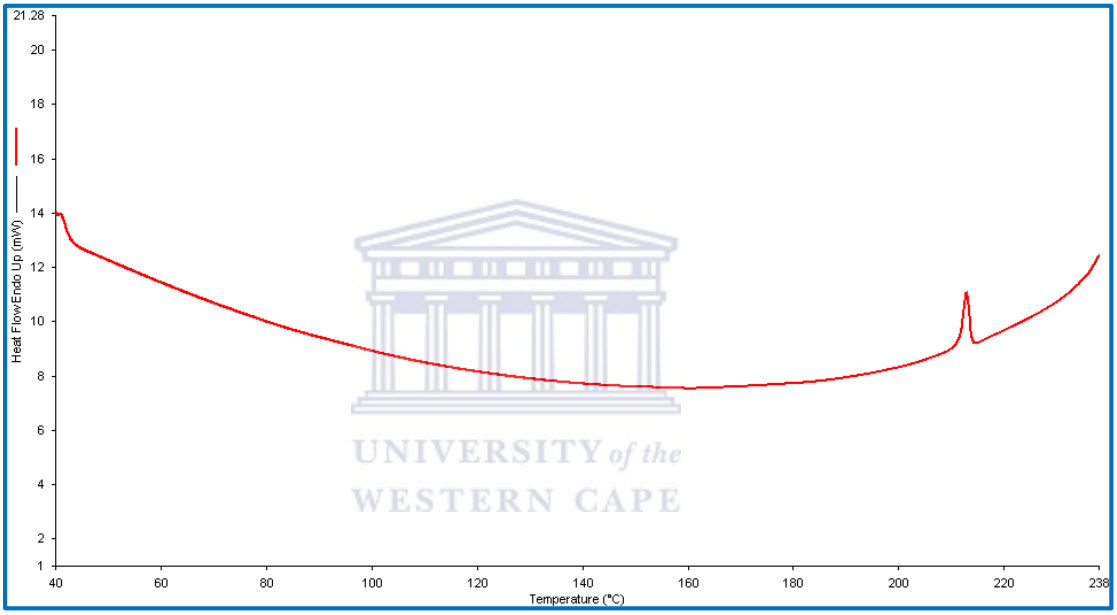


Figure 4.35 DSC of formulation C2

DSC indicated a presence of an endothermic peak at 218 ºC. This melting point was within the range of the melting point of the NVSC co-crystal, thus suggesting that the co-crystal was intact in the carbopol 974P, together with sodium hydroxide at a pH of 5.8.

FTIR analysis was done on the suspension to further attest if the co-crystal was present in the suspension medium.

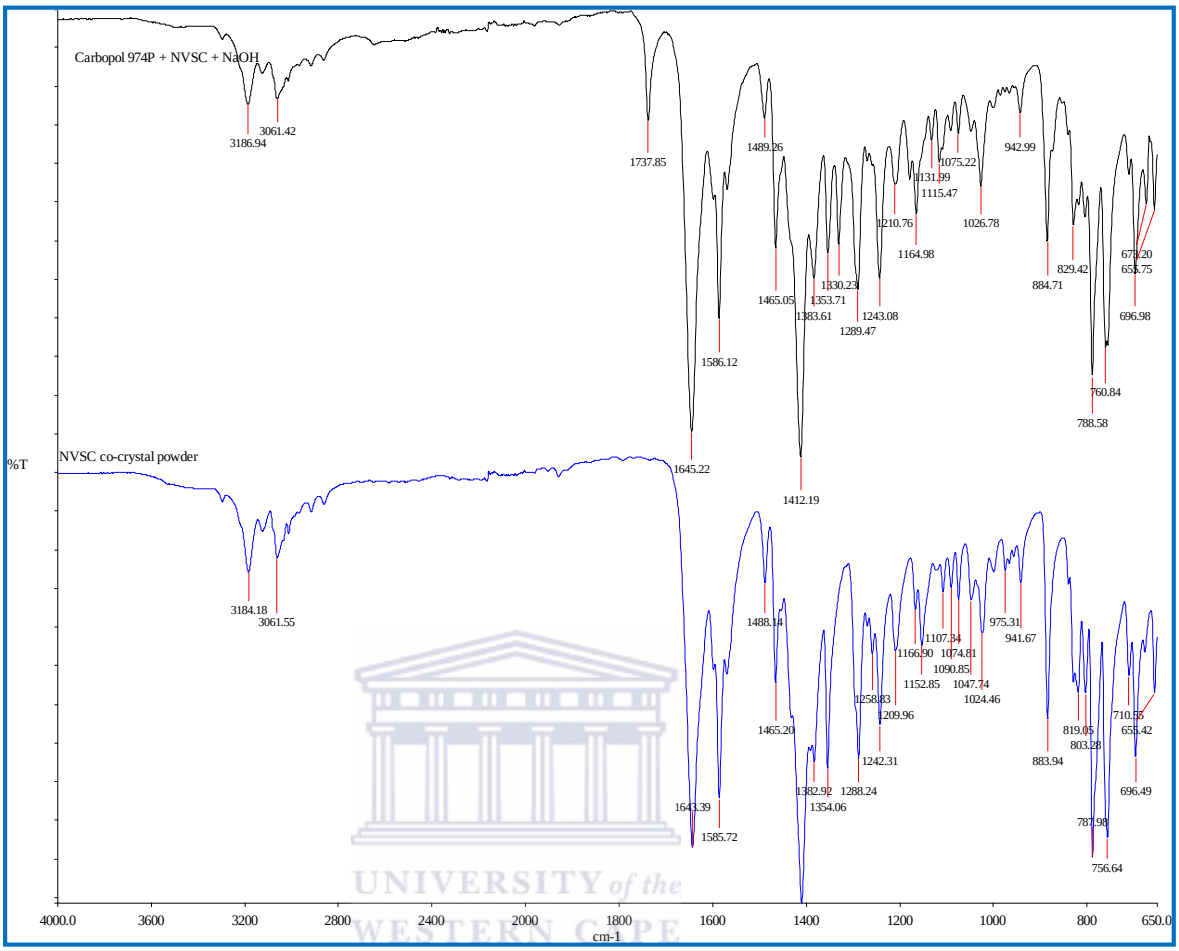


Figure 4.36 FTIR of formulation C2

The C-O bond in the NVSC co-crystal expected at 1646 cm^{-1} was present, signifying the presence of the co-crystal. The aromatic -C = C- could also be identified at 1586 cm^{-1}. This result in conjunction with the DSC result establishes the presence of the NVSC co-crystal in formulation C2. This formulation was suitable, however, since suspensions are prone to microbial contamination, preservatives needed to be added to prevent spoilage. Hence formulation C3 was implemented with preservatives and sorbitol had to be added to improve the density of the suspension.

4.10.3 Formulation C3 (NVSC, carbopol 974P, sorbitol, sodium hydroxide, NVSC, polysorbate and preservatives)

To prepare formulation C3, a beaker with 4 mL of water was heated to 70 ºC on a hot plate and stirred until 200 rpm. Preservatives such as methylparaben (0.027 g) and propylparaben (0.0036 g) were added to the water and mixed with a magnetic stirrer. The solution was then cooled to 35 ºC and carbopol 974P (0.0285 g) was added slowly. The stirring speed was increased to 600 rpm. Thereafter, 10 μL of 20 % sodium hydroxide was added to the solution. The sample was stirred for twenty minutes and subsequently 3.46 mL of sorbitol was added to the solution. In a separate beaker 4 mL water together with 7 μL polysorbate 80 were stirred at room temperature. NVSC co-crystal (0.1552 g) was added to the water-polysorbate mixture. Thereafter, the NVSC/polysorbate 80 concentrate was added to the carbopol 974P gel.

4.10.3.1 pH of formulation C3

Since this formulation contains several excipients, the pH had to be measured at each stage (Table 4.18). The pH of the preservatives in water was 3.16. Upon addition of 10 μL of 20 % sodium hydroxide, the pH was 8.46. To this solution, the NVSC co-crystal and polysorbate 80 was added and this resulted in a pH of 8.08.

	pH
Preservatives, water	3.16
Preservatives, sodium hydroxide	8.46
Preservatives, sodium hydroxide, NVSC co-crystal, polysorbate 80, sorbitol	8.08

Table 4.18 pH of formulation C3

4.10.3.2 Co-crystal integrity of formulation C3

Since this sample was too viscous to be filtered through a vacuum pressure pump, filter paper or through a micro filter, alternate approaches had to be devised to analyse the sample.

To analyse the sample, 5 mL of the prepared sample was diluted in 5 mL of water. The suspension was then slightly stirred with a magnetic stirrer to ensure a homogenous suspension is prepared. The resultant suspension was not as viscous before dilution, hence it could be filtered and it was left to dry overnight in a fume cupboard.

The purpose of dilution was to analyse the suspension through DSC and FTIR. The integrity of the co-crystal, subsequent to dilution was determined; the rationale was that if the co-crystal was present after dilution, it meant that the co-crystal would be present in the viscous state as well. Although the converse may not be true as the co-crystal could be present in the viscous state but upon dilution the co-crystal may disintegrate.

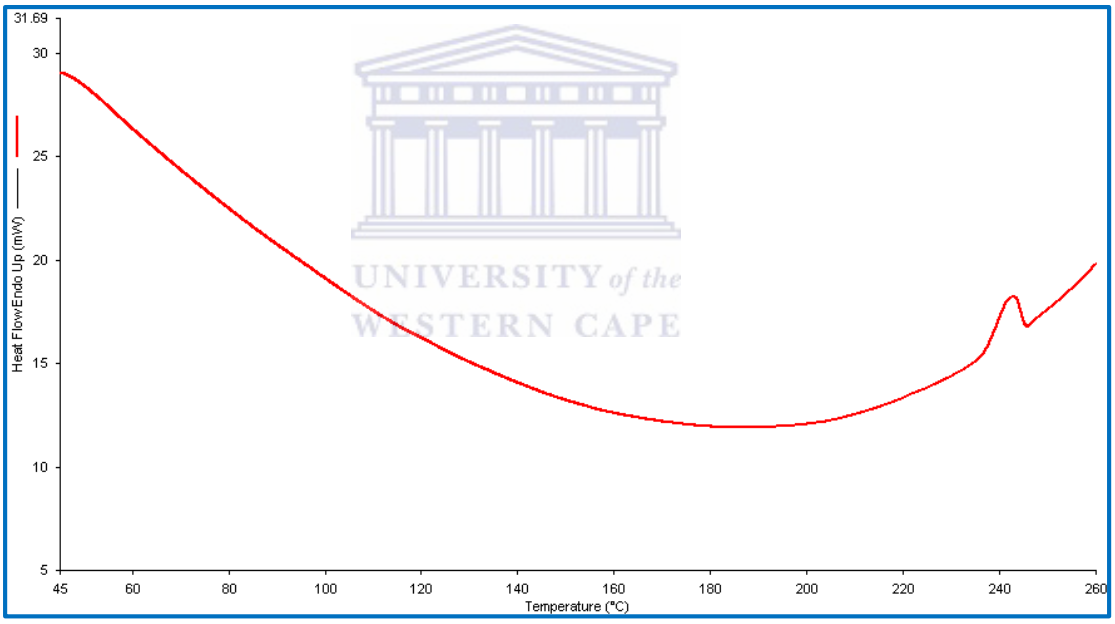


Figure 4.37 DSC of formulation C3

The DSC in figure 4.37 displayed an endothermic peak at 242 ºC. As mentioned previously, the NVSC co-crystal should be ideally identified between 215-230 ºC. The endothermic peak at 242 ºC could represent nevirapine as it had a melting point of 247-249 ºC. To further confirm, if it is nevirapine or if it is the co-crystal at a higher melting point due to the additional excipients , FTIR was performed on the suspension.

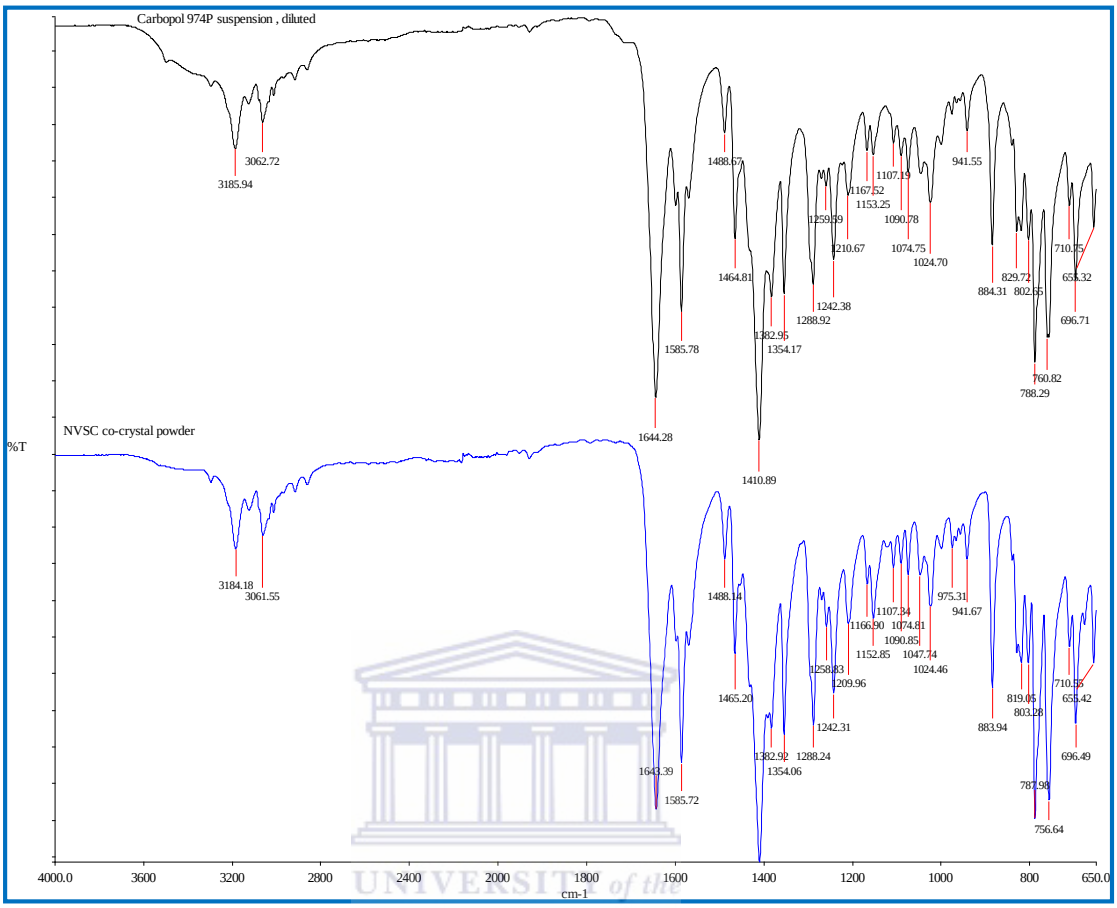


Figure 4.38 FTIR spectra of formulation C3

The FTIR spectrum of the filtrate was compared to that of the NVSC co-crystal powder alone (Fig 4.38). The C-O bond in the NVSC co-crystal was expected to be seen at 1646 cm^{-1}, and the experimental results identified a peak at 1644 cm^{-1}, thus indicating the presence of the co-crystal. The aromatic -C=C- could be identified at 1585 cm^{-1}. It was confirmed that the composition of the suspension was analogous to the NVSC co-crystal, hence it can be concluded that the NVSC co-crystal was retained in formulation C3.

Since, this formulation was diluted, it was less viscous than other preparations, hence UV analysis was performed on it. UV analysis was done by filtering the suspension and utilising the filtered suspension. A blank was prepared in a similar manner as the suspension except that the co-crystal was not added.

Nevirapine is absorbed at a wavelength of 234 nm whilst saccharin is absorbed at a wavelength of 260 nm. The UV analysis shows no peaks at wavelengths of 260 and 234 nm (Fig 4.39). This indicated that the co-crystal did not dissociate into nevirapine and saccharin, hence it is concluded that the NVSC co-crystal is indeed intact in this suspension formulation.

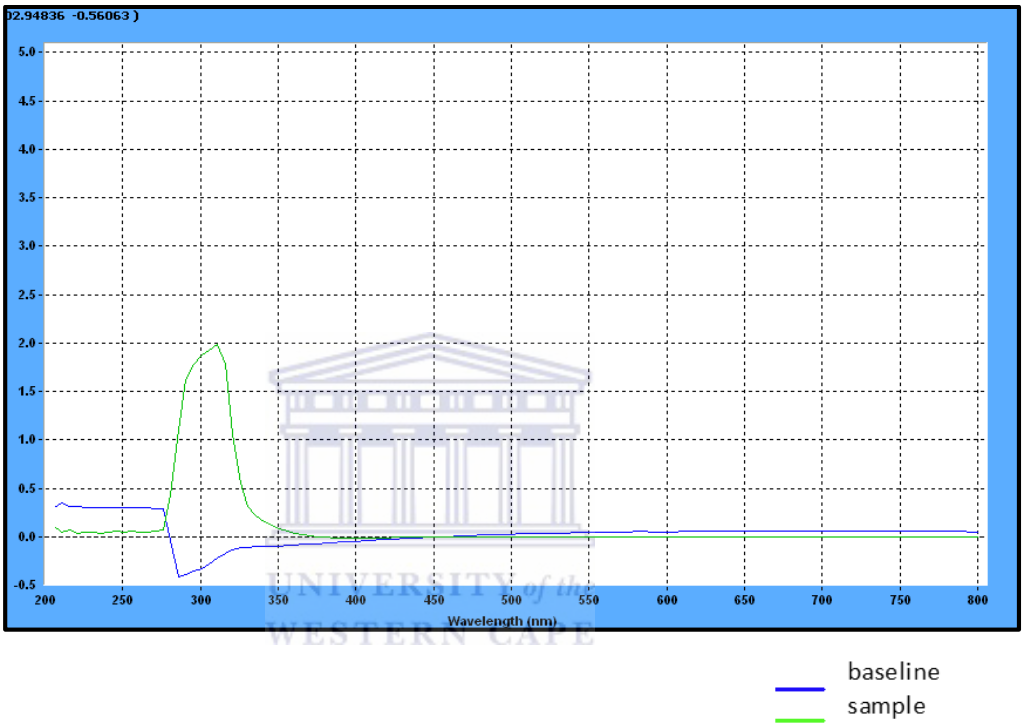


Figure 4.39 UV of formulation C3

4.10.4 Comparison of formulation C1 to C3

Formulation C	NVSC co-crystal	Carbopol 974 P	Sodium hydroxide 20%	Sorbitol	Polysorbate 80	methylparaben	propylparaben	Water	pH of formulation	Integrity of co-crystal (DSC)	Integrity of co-crystal (FTIR)
C1	50 mg	10.5 mg							4.12	✓	✓
C2	50 mg	10.5 mg	7 μL						5.80	✓	✓
C3	165 mg	33.6 mg	30 μL	3.7 mL	8 μL	28.8 mg	3.84 mg	q.s	8.08	✓	✓

Table 4.19 Comparison of pH and integrity of Formulation C1 , C2, C3

From table 4.19, it can be concluded that the integrity of NVSC co-crystal is not compromised in carbopol 974P. This is verified by the results of the DSC, FTIR and UV of the suspension. Furthermore, the pH of formulation C3 was within the target range. Hence, this suspension was scaled up and quality control tests were performed on it.

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Chapter 5

Quality control of suspension

Chapter 5 Quality control of suspension

Formulation C3 proved to be successful in the two criteria that were deemed necessary for co-crystal suspension formulation viz. co-crystal integrity and pH of the suspension. Hence, formulation C3 suspension in a concentration of 50 mg/5 mL was scaled up to 50 mL, characterization and quality control tests of this suspension was pursed. The results obtained were compared to the Viramune® suspension and the standard that was accepted according to literature.

5.1 Quality control of suspensions

5.1.1 Particle size of suspension

The particle size of the insoluble particle should be in a range of 10 to 1000 μm.[1] The particle size of the NVSC co-crystal prior to formulation was measured by SEM. The particle size of NVSC measured was 248.8 μm x 428.0 μm. As seen in figure 5.1, nevirapine had a particle size of 113.2 x 121.6 μm.

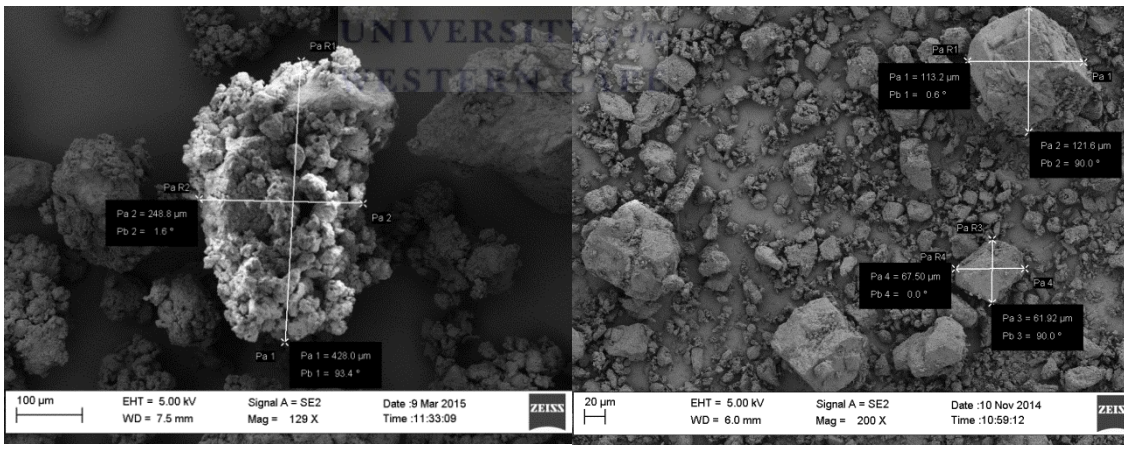


Figure 5.1 SEM of NVSC co-crystal and nevirapine (from left to right)

The particles size of the particles in the formulation was determined by a zeta sizer. The particle size determined for the Viramune® suspension was 935 nm while the NVSC co-crystal formulation revealed a much smaller size of 574.9 nm. The reason for a decrease in particle size after formulation could be due to the agitation the suspension undergoes through during formulation stages.

5.1.2 Polydispersity Index

The polydispersity index gives an indication on the particle size distribution that is measured through a zeta sizer. Suspensions are preferred to have a polydispersity index of 0.1 to 0.3. Viramune® suspension had a polydispersity index of 0.405 while the NVSC co-crystal suspension had a polydispersity index of 0.141. This suggested that the NVSC co-crystal suspension met the criteria of the polydispersity index however, the Viramune® suspension had a slightly higher polydispersity index which meant that the particle size distribution is slightly wider.

5.1.3 Measurement of pH

The ideal pH for a suspension for oral consumption should be between 5–8. Viramune® suspension had a pH of 6.31, whereas the NVSC co-crystal suspension had a pH of 8.01. Thus, both the Viramune® suspension and the NVSC co-crystal suspension were within the desired pH range. The differences in pH range could be attributed to the variation in the formulation process of the two suspensions, as the NVSC co-crystal suspension required more volume of sodium hydroxide than the Viramune® suspension. The NVSC co-crystal required a greater volume of sodium hydroxide because the NVSC co-crystal was acidic in nature, which significantly reduced the pH of the suspension.

5.1.4 Viscosity

For a suspension to have ideal flow properties, it should have high viscosity at low shear rates (during storage) and low viscosity at high shear rates. Viramune® suspension was measured in a small sample adapter at 25 ºC with a SC4-16 spindle and had torque values between 41–99 %. The Viramune® suspension has a maximum viscosity of 2513 cP at a shear rate of 5.8 sec^{-1}. This was in accordance to what was required, as it obtained a high viscosity at a low shear rate. As the speed of the spindle

increases, so does the shear rate, this resultant force causes a decrease in the viscosity of the suspension.

Viscosity (cP)	Speed (RPM)	Torque (%)	Shear stress (D/cm^2)	Shear rate (1/sec)
2513.46	20.00	41.90	145.78	5.80
1939.59	30.00	48.50	168.74	8.70
1616.66	40.00	53.90	187.53	11.60
1406.10	50.00	58.60	203.88	14.50
1253.73	60.00	62.70	218.15	17.40
1144.90	70.00	66.80	232.41	20.30
1057.27	80.00	70.50	245.29	23.20
981.12	90.00	73.60	256.07	26.10
915.40	100.00	76.30	265.47	29.00
868.18	110.00	79.60	276.95	31.90
823.82	120.00	82.40	286.69	34.80
787.22	130.00	85.30	296.78	37.70
750.70	140.00	87.60	304.78	40.60
723.85	150.00	90.50	314.87	43.50
697.35	160.00	93.00	323.57	46.40
676.80	170.00	95.90	333.66	49.30
651.86	180.00	97.80	340.27	52.20
629.55	190.00	99.70	346.88	55.10

Table 5.1 Viscosity of Viramune suspension

As seen in figure 5.1, Viramune exhibits a Bingham's plastic type of flow, where the fluids that have a linear shear stress/shear strain require a finite yield stress before they begin to flow. This is apparent as the graph does not begin from the origin, indicating that the suspension requires force for it to flow.

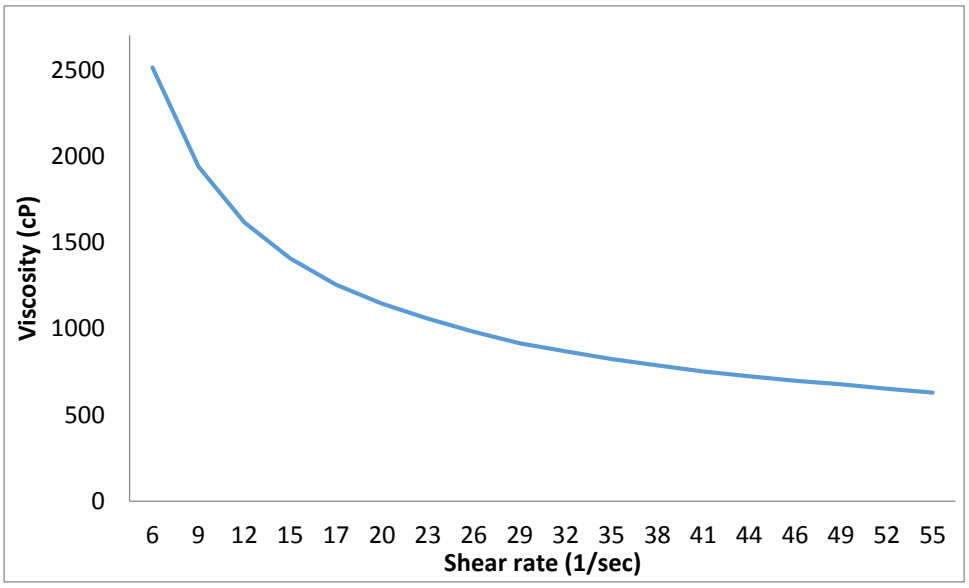


Figure 5.1 Viscosity versus shear rate of Viramune® suspension

The viscosity results of the NVSC co-crystal suspension is outlined in table 5.2. The viscosity of the NVSC co-crystal suspension was also measured in a small sample adapter at 25 ºC with a SC4-18 spindle and obtained torque values between 32 – 82 %. The NVSC co-crystal suspension has a maximum viscosity of 65 cP at a shear rate of 20 sec^{-1}. Although the viscosity of this suspension is significantly lower than Viramune®, it still meets the specification which states that a high viscosity should be obtained at a low shear rate.

Figure 5.2 displays the viscosity of the NVSC co-crystal suspension which exhibits a similar flow to the Viramune® suspension. It exhibits a Bingham's plastic type flow, which requires a finite yield stress before the suspension begins to flow.

Viscosity (cP)	Speed (RPM)	Torque (%)	Shear stress (D/cm^2)	Shear rate (1/sec)
64.59	15.00	32.30	12.79	19.80
47.99	25.00	40.00	15.84	33.00
38.65	35.00	45.10	17.86	46.20
34.13	45.00	51.20	20.27	59.40
31.25	55.00	57.30	22.69	72.60
29.07	65.00	63.00	24.94	85.80
27.23	75.00	68.10	26.96	99.00
25.37	85.00	71.90	28.47	112.20
24.09	95.00	76.30	30.21	125.40
23.45	105.00	82.10	32.50	138.60

Table 5.2 Viscosity of NVSC co-crystal suspension

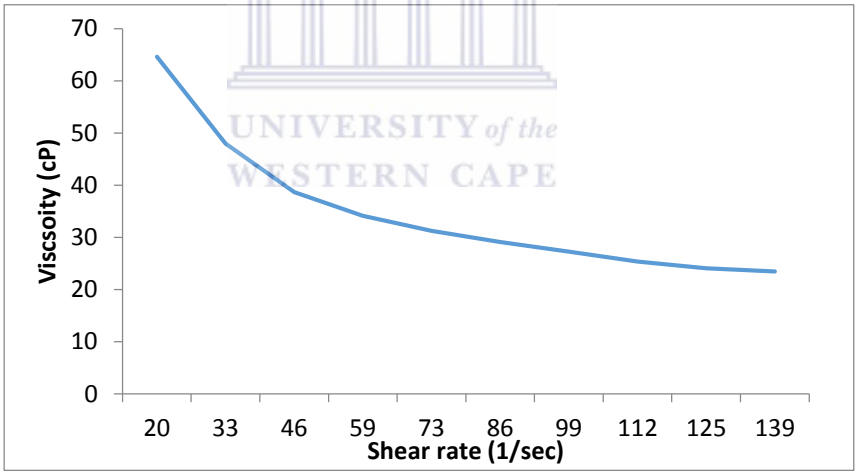


Figure 5.2 Viscosity versus shear rate of NVSC co-crystal suspension

5.1.5 Zeta potential

Zeta potential of the suspension gives an indication of physical stability of the suspension. A value more than +30 mV or less than -30mV is preferred for suspensions. Viramune® obtained a value of -14.2 mV while the NVSC co-crystal suspension obtained a value of -2.37 mV. Both the suspensions did not meet this requirement,

however, the Viramune® suspension was closer to the desired zeta potential range indicating that it was physically more stable. The Viramune® suspension had greater viscosity thus, it was physically more stable as it allowed the particles to be dispersed whereas the NVSC co-crystal had a lower viscosity thus the zeta potential was also significantly lower. Due to the low viscosity of the NVSC co-crystal suspension the particles were not dispersed continually throughout the suspension, hence it had a low zeta potential.

5.1.6 Dissolution

Dissolution tests are an indication of the cumulative amount of API that passes into solution which is studied as a function of time. The test describes the overall rate of all the processes involved in the release of the API into a bioavailable form. Dissolution studies evaluates the potential effect of formulation and process variables on the bioavailability of an API and ensures that preparations comply with product specification. It gives an indication of the performance of the preparation under *in vitro* conditions.[2]

To analyse the concentration of nevirapine in the Viramune® suspension and the co-crystal suspension, a calibration curve of nevirapine was constructed in a phosphate buffer of pH 6.8. A sixteen point calibration curve, of nevirapine ranged from a concentration of 0.3 mg/mL to 0.00234375 mg/mL in triplicate was plotted. A straight line with a regression value of R^2=1 was achieved (Fig 5.3). Nevirapine compound was eluted at approximately 3 minutes at 280 nm. HPLC chromatogram and the peak area observed for the dissolution results can be seen in Appendix B.

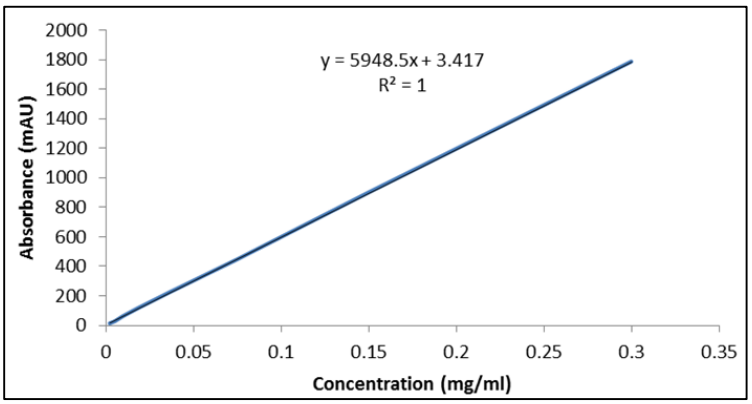


Figure 5.3 Standard curve of nevirapine in phosphate buffer with a pH of 6.8

The dissolution media that was used was phosphate buffer with a pH of 6.8. This is the pH at which nevirapine is absorbed in the body. To simulate *in vivo* conditions the temperature of the dissolution media was 37 °C. Six dissolution vessels were used for the study. 5 mL of the suspension was inserted in the vessel by means of a syringe. 5 mL samples were extracted at 10, 20, 30, 45 and 60 minute intervals and replaced with 5 mL of phosphate buffer. 1 mL of extracted samples was then placed in HPLC vials. Concentration of samples were determined using the peak area of samples obtained at 3 minutes in the equation obtained in the standard curve. Cumulative amount of API was calculated as a percentage for Viramune® suspension and NVSC co-crystal suspension as seen in table 5.3 and 5.4.

Time (min)	Vessel Number					
	1	**2**	**3**	**4**	**5**	**6**
10	40	35	40	45	34	50
20	70	62	69	77	60	69
30	86	78	93	93	79	89
45	98	91	98	98	97	95
60	100	100	100	100	100	100

Table 5.3 Percentage release of nevirapine in Viramune® in phosphate buffer at pH 6.8

Time (min)	Vessel Number					
	1	**2**	**3**	**4**	**5**	**6**
10	76	81	84	87	78	87
20	85	88	92	92	87	93
30	91	92	96	95	93	96
45	97	97	98	100	97	98
60	100	100	100	100	100	100

Table 5.4 Percentage release of nevirapine in NVSC suspension in phosphate buffer at pH 6.8

According to the USP 32 criteria, 81-100 % of drug release must occur within 30 minutes of the dissolution study.[2] Both the branded version, Viramune® and the NVSC co-crystal suspensions met this criteria, with the Viramune® achieving a 86 % drug release while the NVSC co-crystal suspension achieved a drug release of 94 % within 30 minutes of dissolution (table 5.5 and figure 5.4).

Time	**Viramune ® suspension (average % dissolved)**	**NVSC suspension (average % dissolved)**	**Standard deviation**
10	41	82	20
20	68	89	11
30	86	94	4
45	96	98	1
60	100	100	0

Table 5.5 Comparison of average percentage drug release of Viramune ® suspension and NVSC co-crystal suspension

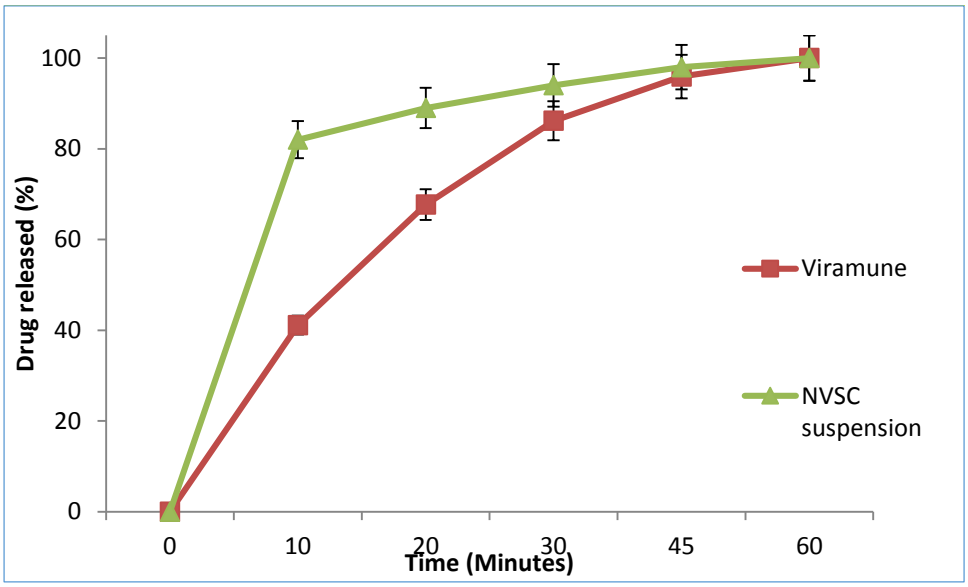
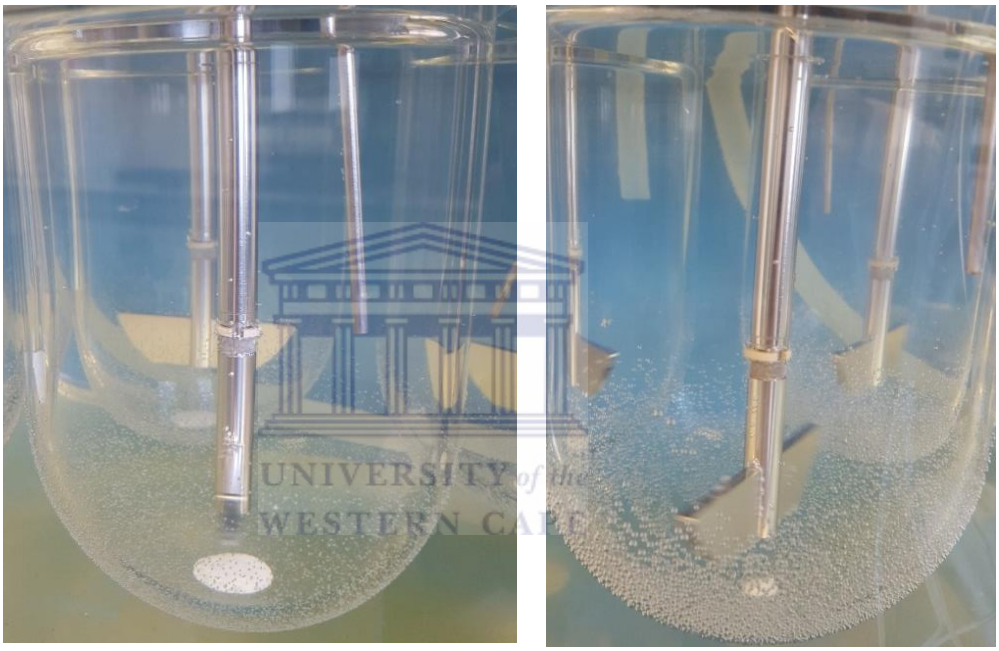


Figure 5.4 Dissolution profiles of Viramune® suspension and NVSC co-crystal suspension

The NVSC co-crystal suspension dissolved more quickly, this is substantiated by the ten minute point, where only 41 % of nevirapine is released in the Viramune® suspension whereas 82 % of nevirapine is released in the NVSC co-crystal suspension within ten minutes of dissolution. This is twice the amount of nevirapine that is released from the Viramune® suspension, indicating that the NVSC co-crystal releases more nevirapine at the ten minute time interval.

The NVSC co-crystal suspension had a consistently higher percentage release than the Viramune® suspension during the dissolution study. At the ten minute time interval, the standard deviation between the Viramune ® suspension and the NVSC co-crystal suspension indicated that the dissolution rate of the NVSC co-crystal suspension is significantly higher. However, at the 45 minute time interval, the standard deviation was low indicating that the dissolution rates of the NVSC co-crystal and the Viramune® suspension is similar. The Viramune® suspension is released gradually over time whereas 82 % of the NVSC co-crystal suspension is released within ten minutes.

Upon placing the suspensions in the vessels, the suspensions are seen as small circular mass, this is illustrated in figure 5.4. Viramune® suspension remains as a circular masses of powder throughout the dissolution study, whereas comparatively the NVSC co-crystal suspension reduces its mass over time. Furthermore, this mass of powder is greater in the Viramune® suspension than the NVSC co-crystal suspension. This can be recognized due to the Viramune® containing an extra excipient -sucrose- which is not in the formulation of the NVSC co-crystal suspension.



Viramune® suspension NVSC co-crystal suspension

Figure 5.4 Dissolution of Viramune suspension and NVSC co-crystal suspension at thirty minutes

Table 5.6 summarizes the quality control tests of Viramune® suspension and NVSC co-crystal suspension. Both the suspensions met the criteria of particle size, polydispersity index, pH and dissolution. Viramune® had a better viscosity while the NVSC co-crystal suspension did not meet this criteria. The zeta potential for both the suspensions did not meet the standard required.

Characteristic	Standard accepted according to literature	Viramune® suspension	NVSC co-crystal suspension
Particle size measured by zeta sizer	The insoluble particle should be between 10 to 1000 μm[1]	935 nm	574.9 nm
Particle size measured by SEM		248.8 x 428.0 μm	113.2 x 121.6 μm
Polydispersity Index	0.1 to 0.3	0.405	0.141
pH	5-8[1]	6.31	8.01
Viscosity	it should have high viscosity at low shear rates (during storage) and low viscosity at high shear rates	2513 cP	64.59 cP
Zeta Potential	More than +30mV or less than -30 mV[1]	-14.2 (average)	-2.37 (average)
Dissolution	81-100% in 30 minutes[1,3]	86 %	94 %

Table 5.6 Comparison of quality control tests of Viramune® suspension and NVSC co-crystal suspension

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Chapter 6

Conclusion

Chapter 6 Conclusion

According to the objectives in chapter 1, development of a protocol to select an appropriate co-former from the five available co-formers with nevirapine was achieved, by clustering variables deemed necessary for suspension formulation according to their physical, chemical, pharmacological and pharmaceutical properties.

A template to select the best co-former was thus created with the variables chosen. Upon completion of the template for the co-formers, an ordinal scale was used to select the most appropriate co-former suspension. Consequently, saccharin had the highest total score and thus it was the chosen co-former for suspension formulation.
NVSC co-crystal was then scaled up accordingly and identified through various analytical techniques. It was found that the NVSC co-crystal had a percentage yield of greater than 75%.

The intention of retaining the co-crystal during formulation was monitored through means of DSC, FTIR and where possible by UV. However, as a recommendation PXRD is better suited for identification purposes if the co-crystal is retained in the suspension.
The formed NVSC co-crystal suspension performed well in the quality control tests i.e. particle size, polydispersity index and pH whilst maintaining the integrity of the co-crystal. However, the zeta potential and the viscosity was not according to the quality desired. The dissolution of the NVSC co-crystal was analysed using HPLC and it revealed that 82 % of nevirapine was released within ten minutes. However, optimization of the final suspension formulation with improvement on viscosity and zeta potential is recommended.

In the pharmaceutical arena, companies are constantly under pressure to practice “greener” approaches during formulation, the NVSC co-crystal suspension had eliminated the use of one less excipient (sucrose) compared to the Viramune®

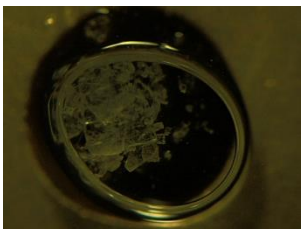
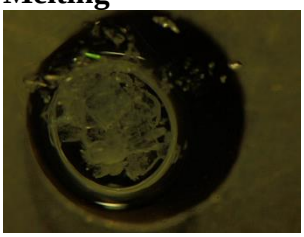
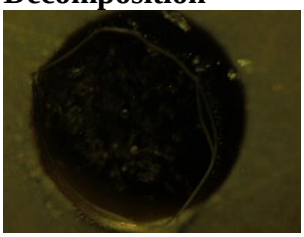
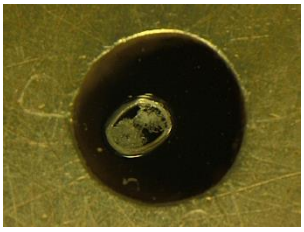
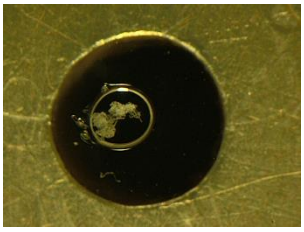
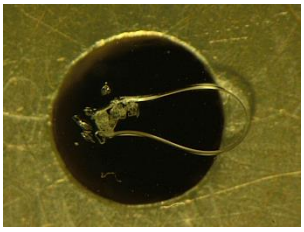
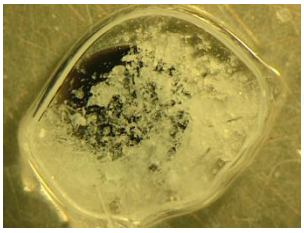
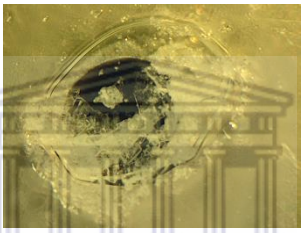
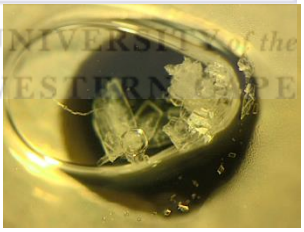
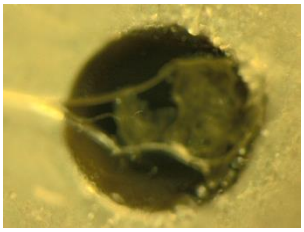
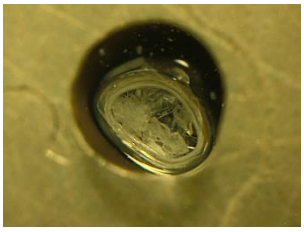
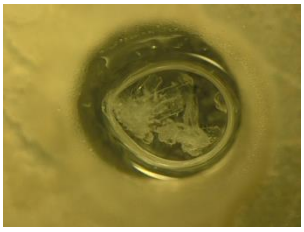
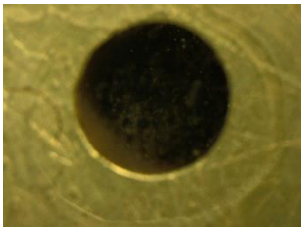
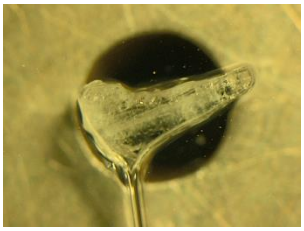
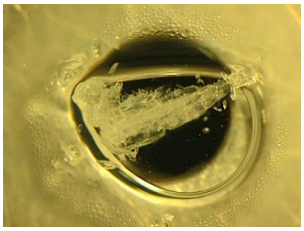
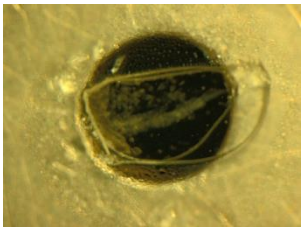
formulation, hence substantiating that co-crystals could be used as a means of promoting judicious use of resources.

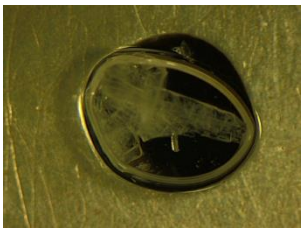
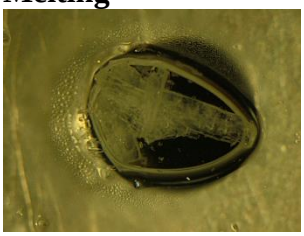
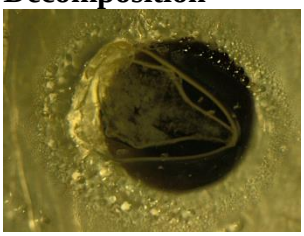
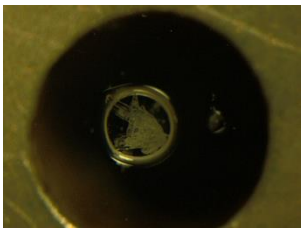
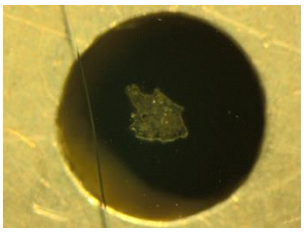
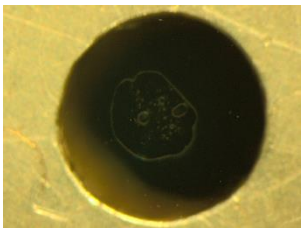
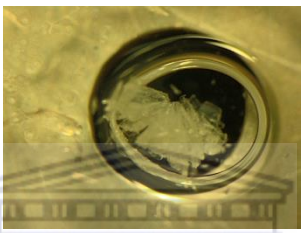
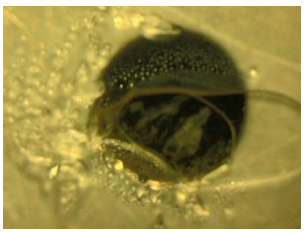
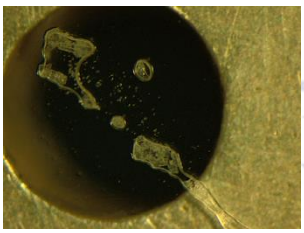
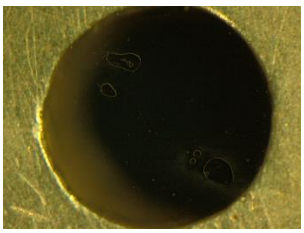
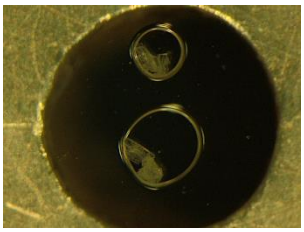
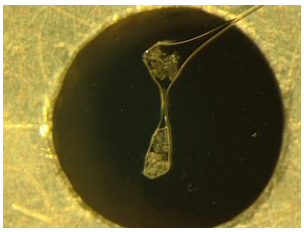
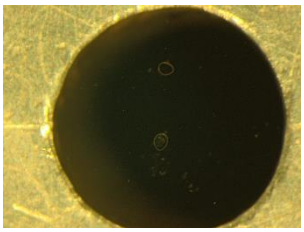
In this study the NVSC suspension as a ready-to-use suspension was explored, an alternative approach to achieve this, could possibly be achieved with extemporaneously prepared suspensions. A suspension of this nature would be worth investigating, if it meets the USP 32 criteria.

Furthermore as a recommendation, once the suspension is optimized with respect to its viscosity, stability testing under accelerated conditions for a period of time would give an indication on the stability of the co-crystal and hence suggest whether the co-crystal is stable in a suspension for a longer duration.

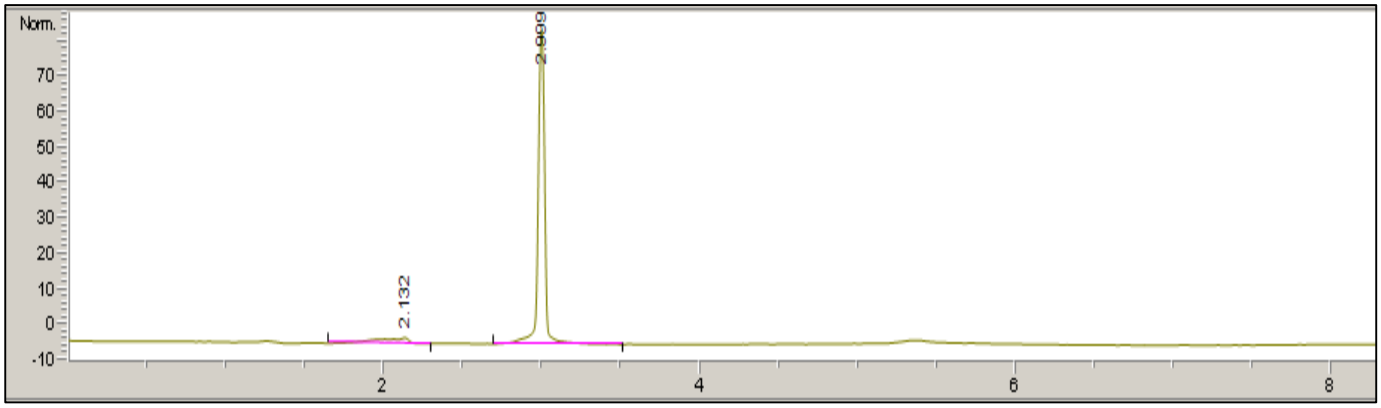
Antiviral testing should also be simultaneously undertaken with stability studies to establish if the antiviral activity of the co-crystal against HIV is compromised over a period of time.

Appendix A

	Start	Melting	Decomposition
Batch 4			
	24°C	230 °C	239 °C
Batch 5			
	25 °C	236 °C	245 °C
Batch 6			
	22 °C	235 °C	247 °C
Batch 7			
	90 °C	215 °C	239 °C
Batch 8			
	25 °C	215 °C	245 °C
Batch 9			
	22 °C	231 °C	237 °C

	Start	Melting	Decomposition
Batch 10			
	24°C	217 °C	239 °C
Batch 11			
	25 °C	232 °C	242 °C
Batch 12			
	63 °C	219 °C	255 °C
Batch 13			
	90 °C	215 °C	249 °C
Batch 14			
	25 °C	224 °C	252 °C

Appendix B



HPLC chromatgram of nevirapine at 280 nm in phosphate buffer

Time (min)	Vessel Number					
	1	**2**	**3**	**4**	**5**	**6**
10	717.2	680.1	660	761.4	578	1162.8
20	1247.8	1207	1124.3	1297.1	1005.9	1596.3
30	1519.2	1521.4	1520.8	1562	1340.3	2052.4
45	1739.3	1779.1	1609.7	1643.6	1628.7	2191.9
60	1772.4	1950.7	1638.4	1674.2	1685.3	2318.5

Peak area of Viramune® suspension at 280 nm

Time (min)	Vessel Number					
	1	**2**	**3**	**4**	**5**	**6**
10	1798.3	2013.3	1228.2	1947.3	1848.2	2238.5
20	2013.5	2200	1334.9	2072.7	2057.4	2396.9
30	2175.2	2299.1	1395.2	2140.0	2207.4	2453.2
45	2315.7	2401.8	1423.6	2240.3	2297.2	2516.5
60	2380.9	2484	1455.8	2242.7	2376.9	2570.7

Peak area of NVSC co-crystal suspension at 280 nm