EFAVIRENZ PRE-FORMULATION STUDY: SELECTION OF A CYCLODEXTRIN INCLUSION COMPLEX OR CO-CRYSTAL COMPLEX FOR TABLETTING

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

Magister Pharmaceuticiae

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Discipline of Pharmaceutics
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December 2015

KEY WORDS

Efavirenz

Co-crystallization

Cyclodextrin inclusion

Scanning electron microscopy

Hot-stage microscopy

Differential scanning calorimetry

Thermogravimetric analysis

Solid dosage form

Tablet

ABSTRACT

EFAVIRENZ PRE-FORMULATION STUDY: SELECTION OF A CYCLODEXTRIN INCLUSION COMPLEX OR CO-CRYSTAL COMPLEX FOR TABLETTING

Efavirenz is a non-nucleoside reverse transcriptase inhibitor used as an anti-retroviral for the treatment of human immunodeficiency virus (HIV) type I. It is classified as a class II drug under the Biopharmaceutical Classification System (BCS) and exhibits a low solubility (aqueous solubility of 9.0 μg/ml) and high permeability (variable oral bioavailability).

This study aims to choose a pre-formulation protocol with the best efavirenz derivative in literature between co-crystals and CD inclusion complexes. Upon selection of the efavirenz derivative, the complications of both small scale and large scale laboratory pre-formulation production is highlighted for formulation of a tablet dosage form.

Numerous variables were selected for the pre-formulation protocol. Physical, chemical, pharmacological, pharmaceutical and economical variables were investigated. Citric acid monohydrate (CTRC) was chosen as the best co-former for a co-crystal while hydroxypropyl-beta-cyclodextrin (HP-β-CD) was selected as a host for an inclusion complex. Pharmaceutically, the angle of repose, Carr's index, Hausner's ratio, moisture content, disintegration time, hardness/resistance to crush, manufacturing process problems and particle size of the CTRC and HP-β-CD were all evaluated. The CTRC was ultimately selected for formulation of a tablet. The preparation of small laboratory scale of EFA/CTRC co-crystal was successfully achieved after several attempts. The large laboratory scale of EFA/CTRC was prepared under various environmental seasons which were indicated as batches 1-6 for purposes of this study. Characterization of the large laboratory scale EFA/CTRC co-crystals was performed by scanning electron microscopy (SEM), hot-stage microscopy (HSM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and by

physical inspection (i.e. season, texture, colour, shape and particle size) of the EFA/CTRC product.

Batch 1 and 2 were prepared during the summer season. The SEM analysis showed that the particles were needle-like shaped. The thermal analysis values of batch 1 by HSM, DSC and TGA results were 123 °C, 119 °C and 1.68 % of mass loss, respectively. In batch 2, morphology results by SEM revealed spikes of irregular and agglomerated particles. Batch 2 melted at 123 °C and a small unmelted quantity was observed at 143 °C. The DSC and TGA (mass loss) analysis were 118 °C and 0.75 % respectively. The hardness test of EFA/CTRC tablet prepared in batch 2 was extremely hard hence failed the disintegration test.

The EFA/CTRC prepared in batches 3, 4 and 5 was during the winter season which is associated with high humidity and wet weather conditions. The SEM, DSC, TGA results were significantly different from the previous batches. The SEM morphology was highly irregular particles for batch 3, clustered and randomly size particle for batch 4 and irregular, needle-like, spikes and spherical shaped particles for batch 5, respectively. The thermal results HSM, DSC and TGA confirmed the presence of moisture in the prepared EFA/CTRC products. The HSM melting point results of batches 3, 4 and 5 were 123 °C, 115 °C and 121 °C, respectively. The DSC results of 110 °C, 105 °C and 118 °C were observed for batches 3, 4 and 5 respectively. The mass loss i.e. TGA results for batches 3, 4 and 5 were 1.178%, 1.5 % and 2.235 % respectively. In batch 6, EFA/CTRC was prepared using a different commercial batch of EFA and CTRC. The SEM results indicated the formation of needle-like and clustered particles. The values obtained from HSM, DSC and TGA results were 124 °C, 114 °C and 0.54 % in mass loss.

The physical appearance of EFA/CTRC prepared from batch 1 and 2 were white in colour while batch 3, 4, 5 and 6 of the prepared EFA/CTRC was pink in colour. The physical appearance of the individual batches differed but the identity of the sample remained intact implying the same pharmacological effects with differing pharmaceutical properties impacting the dosage form preparation.

DECLARATION

I declare that the thesis, *EFAVIRENZ PRE-FORMULATION STUDY: SELECTION OF A CYCLODEXTRIN INCLUSION COMPLEX OR CO-CRYSTAL COMPLEX FOR TABLETTING* is my own work, that it has not been submitted before for any degree examination at any other university and that all the sources I have used or quoted have been indicated and acknowledged by complete reference.

Ali Mohamed Omar Rafieda

December 2015

Signed.....

DEDICATION

THIS THESIS IS DEDICATED TO MY PARENTS; MOHAMED OMAR RAFIEDA, NAJAT ALI SHETWAN

ACKNOWLEDGEMENTS

Firstly, I will like to express my sincere appreciations to my supervisor; Dr. Halima Samsodien and my co-Supervisor: Dr. Naushaad Ibrahim who were instrumental to the successful completion of this project. Thank you so much for your academic input throughout the duration of this project.

Also, Mr. Rafiq Papo is much appreciated for his assistance and technical supports with laboratory experiments.

I will like to express my gratitude to my friends who made my stay in South Africa memorable and awesome.

Lastly but not the least, to my parents and siblings. Thank you so much for the moral, emotional and financial supports during my academic pursuits in South Africa. I am grateful and thankful.

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ABBREVIATIONS

API	Active pharmaceutical ingredient
CDs	Cyclodextrins
CE	Crystal engineering
CGMP	Current good manufacturing practice
CSD	Cambridge Structural Database
CTRC	Citric acid monohydrate
DSC	Differential scanning calorimetry
EFA	Efavirenz
EFA/CTRC	Efavirenz/Citric acid monohydrate
FDA	Food and drug administration
GRAS	Generally Regarded as Safe
HP-*β*-CD	Hydroxypropyl-*β*-cyclodextrin
HSM	Hot stage microscopy
ILAG	Ion-liquid-assisted grinding
IPC	In- process control
IPEC	International Pharmaceutical Excipients Council
JPC	Japanese Pharmaceutical Codex
LAG	liquid-assisted grinding
M*β*-CD	Methyl-*β*-cyclodextrin
Ph.Eur.	European Pharmacopoeia
QC	Quality control
SBE-*β*-CD	Sulfobutylether-*β*-cyclodextrins
SEM	Scanning electron microscopy
TGA	Thermal gravimetric analysis
USA	United States of America
USP/NF	US Pharmacopoeia

CHAPTER 1

INTRODUCTION

1 CHAPTER 1

1.1 Introduction

The vital stages of drug manufacturing are preformulation, biopharmaceutical and formulation studies. During this study, emphasis will be on preformulation for formulation. The need for a concise and consensus approach to drug formulation requires a comprehensive and pragmatic guide including practical studies that need to be undertaken. These practical studies must be designed to adequately address the challenges of drug manufacturing such as solubility and dissolution because they are key stages in the drug development process. The importance of this approach is expected to be beneficial to the pharmaceutical industry and for research and development.

1.2 General criteria of drug selection and development

The criteria for the selection of drugs is often primarily based on their pharmacological properties such as potency, selectivity, duration of action and safety or toxicology assessments. However, in the case of a candidate drug, there is an important difference between selected pharmaceutical properties such as good aqueous solubility, crystallinity, non-hygroscopicity and good stability even though all these factors are satisfactory and similar pharmacologically. This implies that a candidate drug may not indicate an overall suitability with respect to specific pharmaceutical properties.[1]

There are several important studies that must be performed on a candidate drug to investigate and determine their solid-state properties. For instance, solid-state properties such as particle size, powder flow, compression and polymorphism are important to the drug preformulation stage. Consequently, a drug undergoes initial physicochemical testing in order to measure their particle size, true density, bulk density, tapped density, surface area, compression and powder flow properties. In the study by Pe´rez *et al*. (2006)[1], a new expert system for the control of batch powder properties was devised and reported. In general, a candidate drug with preferred pharmaceutical properties stated earlier should be selected to minimize the challenges involved in developing a suitable formulation.[1]

1.3 Pre-formulation

Pre-formulation is a vital process stage of drug development during which the physicochemical and biopharmaceutical properties of a drug are characterized. It is important to understand these physicochemical and biopharmaceutical properties because they are essential for the development of a drug with a distinctive formulation having considerable bioavailability. The commonly evaluated parameters that are investigated during this stage include solubility and dissolution, stability, lipophilicity, permeability and solid-state properties which include crystal form, polymorphs and water-sorption behaviours.[2] One of the primary goals of a preformulation study is the form selection of a selected active pharmaceutical ingredient (API). The use of pre-formulation as a process stage for drug manufacturing dates back to the early 1950s. It was a process developed for supporting the dosage form design of a new drug and its quality control, to enforce scientific principles and also minimise the use of trial and error methods. This process has continuously been improved over the years. There are several studies that have been performed during the preformulation stage and such studies are based on knowledge of physical pharmacy, physical and chemical principles of pharmaceutical science and biopharmaceutics.[3]

One of the main challenges of the pharmaceutical companies is the increase in demand for the development of drugs. These challenges are partly due to the need to replace expiring patent medicines and to counter generic manufacturer competition. However, to develop new medicinal products from a novel synthesized chemical compound from a natural compound is a lengthy and complex process.[1] This is because it is a process that involves different disciplines functioning together to achieve the final formulated dosage form. There are six different stages involved in the discovery and development process of a medicinal product. These stages are; strategic research, exploratory research, candidate drug selection, exploratory development, development and marketing and commercialisation as shown in (Figure 1.1).[1]

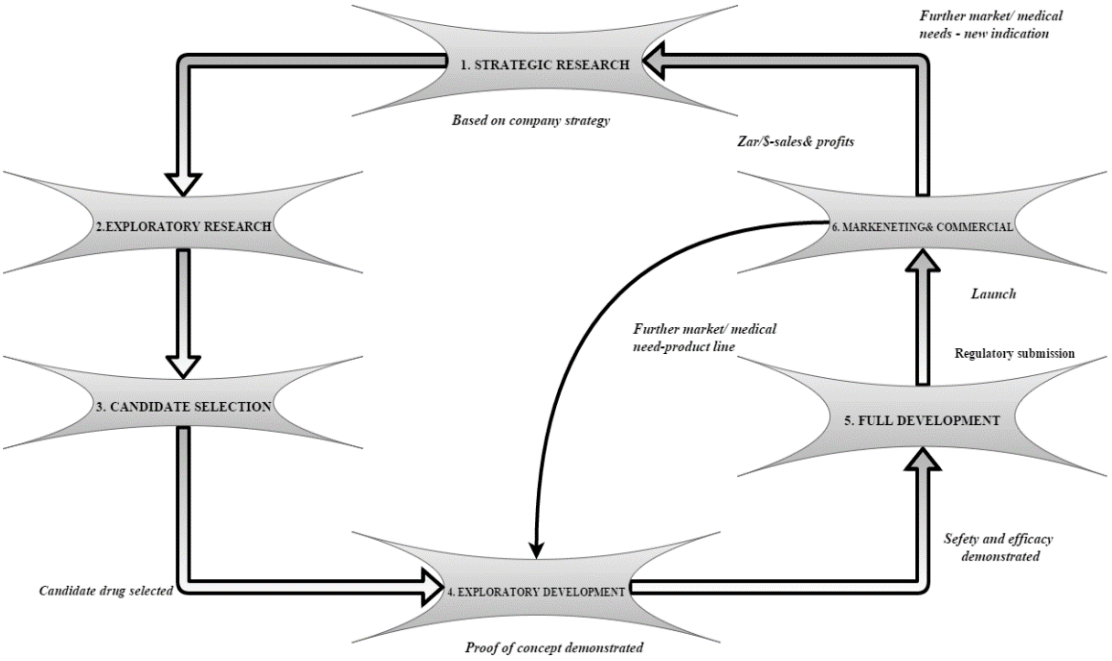

Figure 1-1: Six stages in the discovery and drug development process[1]

The development of any drug product requires good planning and support by adopting a systematic and structured approach. The development process can be broken down into several key stages namely; product design, process design, product optimization, process optimization and scale-up. It is important to emphasise that a good planning stage must consider ethics, safety, clinical and pharmaceutical development, manufacturing operations and regulatory strategies involved to develop the product. In addition, there is a need for one centralized and integrated company project plan that has to be agreed upon by all parties with a vested interest in the project. Another important stage is the experimental protocols. The experimental protocols are useful for defining sequence of tasks, explaining the rationale for the studies, and defining the acceptance criteria. At the completion of experimental activities, results are generated and can be reported with reference to the experimental layout or protocol and acceptance criteria. In some cases, laboratory notebooks are referenced in the protocols and reports so that the raw data can be retrieved in the event of an audit. Lastly, the plan should contain details of activities, timings, responsibilities, milestones, reviews, and decision points. Reviews and decision points are required at the end of a distinct activity to ensure that the project is still meeting its objectives and

should progress to the next stage of development. However, these reviews should not cause any delays to the program but should rather ratify the milestones.[1]

It is important to consider the intrinsic, physical and chemical properties of a drug substance prior to any further pharmaceutical formulation i.e. preformulation studies. These drug properties include solubility, partition coefficient, dissolution rate, physical form and stability.[4] The chemical and physical property enhancements through pharmaceutical co-crystals and cyclodextrin inclusion illustrate the fields of crystal engineering, supramolecular chemistry and the pharmaceutical sciences. Kim *et al.* (2005)[1] discussed the control of the particle properties of a drug substance by the crystal engineering technique and its effect on a drug product. This work showed that controlled crystallization produced large crystals with a narrow distribution which were preserved during the drying process by utilizing low shear in the filter dryer. Drug substances produced by this method was found to have reproducible formulation properties.[1]

1.4 Crystal engineering (supramolecular modification) of pharmaceuticals

Crystal engineering (CE) is a sub-section of supramolecular chemistry and Gerhardt Schmidt propounded this concept by the middle of the 20th century.[5] In 1971, CE was used to describe the photodimerization, packing modes of primary amides and dichlorophenyl derivatives in the solid state.[5] Supramolecular chemistry is dedicated to the chemical systems composite of a discrete number of assembled molecular components and is developed around Lehn's analogy that "Supermolecules are to molecules and the intermolecular bond, what molecules are to atoms and the covalent bond".[6] This implied that if molecules are built by attaching atoms with covalent bonds, then solid-state supermolecules (crystals) are assembled by attaching molecules with intermolecular interactions.[6] The supramolecular modification of pharmaceuticals has been responsible for the coupling of long-range interactions especially with hydrogen bonds for the preparation of new solid forms. Although there are a number of such interactions that can be utilized and they include; hydrogen bonding, metal coordination, hydrophobic or solvatophobic effects, Van Der Waals

forces, electrostatic effects and pi-pi stacking interactions (Table 1.1) ,[7] a term used to describe this process of designing supramolecular assemblies is “crystal engineering”.[8]

CE is a technique that has been in use to design molecular solid-state structures in order to achieve a desired property in the created material. Also, CE has been described as the exploitation of non-covalent interactions between molecular or ionic components for the rational design of solid-state structures that might exhibit interesting electrical, magnetic and optical properties. The use of CE in the pharmaceutical industry is becoming increasingly evident due to its inherent unique characteristics such as the specificity, directionality and predictability of intermolecular hydrogen bonds which can be utilized to assemble supramolecular structures of or at the very least, controlled dimensionality.[6] The synthesis of a wide range of crystalline materials using CE approaches offers an alternative and potentially reliable method for improving drug properties such as solubility, dissolution rate and subsequent bioavailability of poorly soluble drugs. This is because CE can alter the properties of pharmaceutical ingredients without compromising the critical characteristics of such materials. Some of these characteristics include dissolution, physical stability and chemical stability. CE provides a strong incentive for the utilisation of new and existing crystal engineering approaches to drug delivery system design. However, there are challenges associated with drug manufacturing especially in the case of a drug that has a low aqueous solubility. CE therefore provides an ideal situation for the application of CE techniques for improving bioavailability and also in developing stable and robust pharmaceutical products. This study therefore considers the inherent potential of crystal engineering as a viable approach for the design of efficacious dosage forms for poorly soluble drugs. It also reviews the theory, applications, benefits and drawbacks of strategies of this technique.[6]

CE of active pharmaceutical ingredients (APIs) is of significant importance in drug manufacturing. This is because the crystalline form of an API is preferred in the pharmaceutical industry for an efficient delivery system and therapeutic application due to the inherent and thermodynamic stability associated with such forms.[9] According to Desiraju (1989)[10,11], CE was defined as "the understanding of intermolecular interactions in the context of crystal packing and the utilization of such

understanding in the design of new solids with desired physical and chemical properties".

The two main principles are molecular recognition and supramolecular function. This is to make use of the knowledge of hydrogen bond complementarity and common supramolecular synthons,[12] examples of which are illustrated in Figure 1.2.

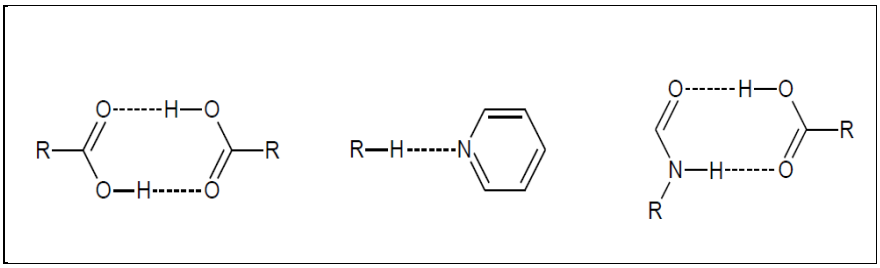

Figure 1-2: Highly prevalent supramolecular synthons

From a CE perspective, the strong directional forces are more useful in the design of target crystal structures. The interaction keynotes for designing crystals are termed supramolecular synthons. The term supramolecular synthons is defined as "structural units within supermolecules which can be formed and or assembled by known or conceivable synthetic operations involving intermolecular interactions".[13] However, API's are inherently predisposed for self-assembly since their utility is generally a consequence of the presence of one or more exofunctional supramolecular synthons.[14] Supramolecular synthons exist in two distinct categories; homosynthons and heterosynthons. These two categories are based on the interacting functional groups. The formation of a supramolecular synthon between the same functional group is called a homosynthon while if it is formed between two different functional groups, it is referred to as a heterosynthon.[15]

Table 1.1 presents the summary of the different categories of supramolecular interactions.

Table 1-1: Summary of supramolecular interactions [16]

Interaction	Strength (kJ mol $^{-1}$)	Example
Ion-ion	200-300	Tetrabutylammonium chloride
Ion-dipole	50-200	Sodium [15] crown-5
Dipole-dipole	5-50	Acetone
Hydrogen bonding	4-120	For example: D-H···A
Cation-π	5-80	K^+ in benzene and graphite
π-π	0-50	Interactions involving π-systems can be found in nature, for example, the weak face-to-face interactions between base-pairs along the length of the double helix are responsible for the shape of DNA.
Van der Waals	< 5 but variable depending on surface area	Argon; packing in molecular crystals
Hydrophobic	Related to solvent-solvent interaction energy	Cyclodextrin inclusion compounds

1.5 Solid forms of API by crystal engineering strategies

Generally, the available pharmaceutical ingredients i.e. active and inactive occur in the solid state forms as amorphous powders or as crystals of various morphological structures. The characteristics of the crystalline and amorphous structures in the solid drug is such that the pure crystalline substances has a definite identifiable shape while the amorphous particles are without definite structure. The amorphous or crystalline identity of a drug substance may be of considerable importance to its ease of formulation handling, chemical stability, and most recently its biologic activity.[4] Also, the delivery of these API as crystalline solids and are commonly delivered as a solid oral dosage form, such as a tablet or capsule.

The main physical and chemical properties of crystalline materials are due to the molecular arrangement within the solid form. However, the alteration of the placement and or interactions between these molecules can directly affect the properties of a particular solid.[17]

There are several strategies in CE and the main aim is to improve the physiochemical properties of the API. These strategies include; formation of salts, polymorphs, host guest complexes (such as cyclodextrin complexes), network solids, hydrates, solvates, and co-crystals[17] (figure 1.3). Currently, most of the commercially available drug medications containing APIs in different drug forms on the South African (SA) medicines market, are formulated as salts. Salts are multi-component solid forms that consist of a stoichiometric ratio of negatively charged anions and a positively charged cations.[18] Examples of drugs on the SA market include diclofenac sodium (Voltaren®) a sodium salt, nevirapine hemihydrate (Viramune® paediatric suspension) a solvate/hydrate and chlorampenicol palmitate (Chloromycetin®) a polymorphic form.[19] Currently, there are also examples of co-crystal drug forms on the market of which there is a co-crystalline salt marketed as Depakote®. Depakote® (divalproex sodium) is sodium valproate co-crystallized with valproic acid that was discovered to exhibit greater characteristics relative to its components.[20] Henceforth, the definition of a co-crystal will be argued to illustrate the possibilities of various co-crystalline forms. Furthermore, the significance of host-guest complexes, more specifically, cyclodextrin inclusion complexes, will be highlighted.

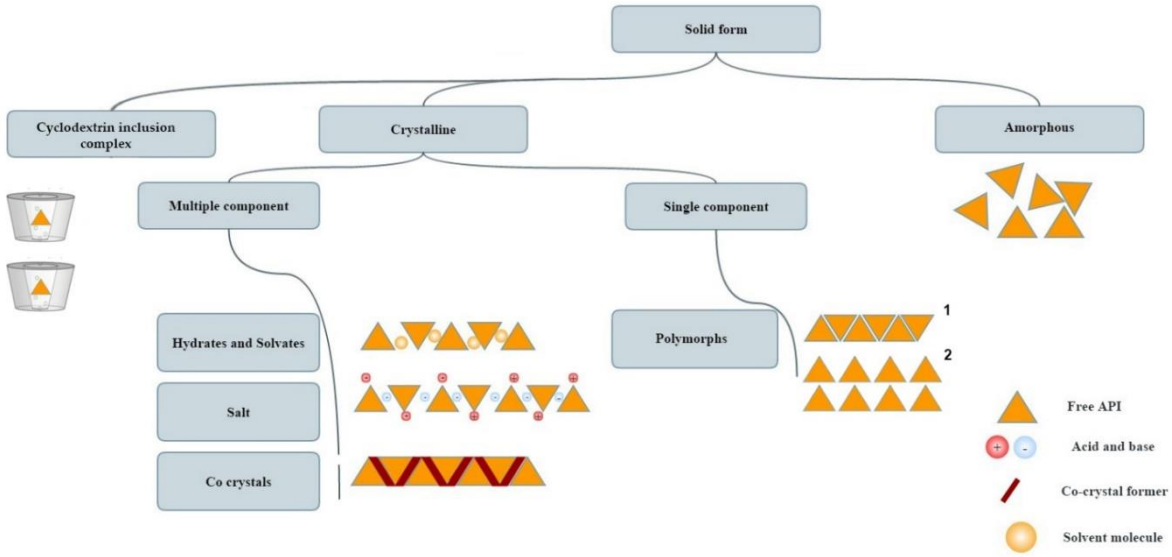

Figure 1-3: API solid form classification based on structure and composition

1.6 Co-crystals

A co-crystal can be defined as a crystalline structure that consists of at least two components which can either be atoms, ions, molecules or solid in its pure form at ambient conditions i.e. room temperature and atmospheric pressure.[21] Co-crystal research in the pharmaceutical industry has been on the increase especially as they are considered to be a highly valuable solid form of the API.[22] Consequently, the use of co-crystals as API formulations in drug manufacturing is due to their ability to provide optimal physical properties and also retain the chemical properties of the individual co-crystal components.[23] In the early 1960s, the term co-crystal was firstly used in a patent where adducts of different phenols and amines were examined as photosensitive compositions to create coloured images.[24]

Co-crystals are constructed by CE which includes the non-covalent intermolecular interactions such as hydrogen bonding, π-π, and Van Der Waals interactions.[25] In co-crystals, the intermolecular interactions between the components confer a unique crystalline structure with distinctive physicochemical properties. These properties include melting points, solubility, chemical stability and mechanical properties. The analysis of co-crystal structures using the Cambridge Structural Database (CSD) indicates that hydrogen bonding is the most prominent route of interaction among co-

crystals and is also responsible for the interaction in co-crystal formation.[26] As a result, better understanding is gained through the investigation of the hydrogen bond patterns in crystalline solids in order to identify hydrogen-bond preferences and reliable synthons which result in co-crystal formation (Table1.2).

The general guidelines for preferred hydrogen bond patterns in crystals are:[27]

- All acidic hydrogens are available in a molecule which may be used in hydrogen bonding in the crystal structure of that compound.
- All good acceptors may be used in hydrogen bonding when there are hydrogen-bond donors available.
- The best hydrogen bond donor and the best hydrogen-bond acceptor may preferentially form hydrogen bonds to one another.

Table 1-2: Hydrogen bond interactions and its properties (A-acceptor; D-donor)[16]

Interaction/property	**Strong**	**Moderate**	**Weak**
D-H···A	Mainly covalent	Mainly electrostatic	Electrostatic
Bond energy (kJ mol $^{-1}$)	60-120	16-60	< 12
Bond length (Å)			
H···A	1.2-1.5	1.5-2.2	2.2-3.2
D···A	2.2-2.5	2.5-3.2	3.2-4.0
Bond angle (degrees)	175-180º	130-180º	90-150º
Example	HF complexes	Acids	C-H···A
	$H_5O_2^+$	Alcohols	D-H···π
	-	DNA/RNA	-

In the design of co-crystals, CE principles can be applied. This typically involves the use of molecules containing complementary functional groups that can form specific heteromeric synthons with an ability to crystallize together to form a co-crystal. Most importantly, co-crystals significantly alter the intermolecular interactions thus modifying crystal packing. As a result, co-crystals can be used to change the physical and pharmaceutical properties of drugs.[24] Co-crystals is only expected to form if it is thermodynamically more stable than the crystals of its components[25] as well as polymorphs of its suggested forms.

Co-crystals also can exist as binary, ternary or quarternary compound molecule systems. However, they can be divided into anhydrates, hydrates (solvates), anhydrates of co-crystals of salts and hydrates (solvates) of co-crystals of salts.[28]

1.7 Regulatory criteria for the recognition of co-crystals

The regulatory requirement for co-crystals as stipulated by USA Food and Drug Administration (FDA) states that co-crystals cannot be regarded as new APIs, but regulators should view the formulation as an innovative way to get an already-approved API to the activity site.[29] This provides a significant driving force for innovative approaches to produce pharmaceutically solid materials with specific physicochemical properties.[30] Also, the FDA requested that sponsors should submit appropriate data showing that the co-crystal formed, can exist in their neutral states and interact via nonionic interactions. This is because it is important for co-crystals to experience complete dissociation of the API from its excipient prior to the API reaching the site in the body where it becomes activated. Consequently, the manufacturing of co-crystals must meet current good manufacturing practice (CGMP) regulations.[29]

1.7.1 Advantages of co-crystals

There are several advantages of co-crystal drugs and they include;[31]

a. Drug molecules lacking easily ionisable functional groups (such as those containing carboxamide, phenol and weakly basic N-heterocyclic groups) can be intermolecularly manipulated by means of co-crystals to alter its physicochemical properties.
b. Drug compounds having particular sensitive groups to treatment of acid and base can be used to make co-crystals.
c. The availability of a large number of neutral Generally Regarded as Safe (GRAS) compounds can be used to make co-crystals.
d. Overcoming problems associated with filterability through co-crystallizing a compound.

e. Co-crystals enable the drug to be delivered to the patient in a safe, efficient and cost-effective manner which depends largely on the physicochemical properties of the API in its solid state.[30]

For the purpose of this study, the project will specifically focus on the subset of co-crystals called pharmaceutical co-crystals. Pharmaceutical co-crystals are significant in the design of new solids, mainly in pharmaceutics as unique dissolution profiles can be achieved through co-crystallization.

1.7.2 Pharmaceutical co-crystals

A pharmaceutical co-crystal is simply defined as a co-crystal in which at least one of the molecular components is an API in combination with a co-crystal former.[32]
In order to be useful, the non-API component should be non-toxic with no adverse side effects.[18] Ideally, the co-crystal former should be included on the USA FDA list as it is the global body responsible for accreditation of drug ingredients. This is to ensure that ethical concerns have been adequately considered and any new drug ingredient has been subjected to rigorous laboratory tests prior to approval.[32] Pharmaceutical co-crystals can be synthesised via a number of traditional mechanochemical methods. Some of these methods are considered to be environmentally friendly techniques of mechanochemistry; liquid-assisted (LAG) and ion-liquid-assisted grinding (ILAG) have both shown to be effective in co-crystallization screening and preparation.[33] The solid-state grinding allows the formation of multi-component forms even with low-solubility components as this would have been difficult to use with the traditional solution techniques. The addition of catalytic amounts of a liquid to the grinding mixture further enhances the efficiency of grinding co-crystallization or traditional solution crystallization methods (like solvent evaporation techniques and slurry techniques).[33] Techniques used for the characterization of co-crystals include single crystal X-ray diffraction, infrared spectroscopy, differential scanning calorimetry, thermogravimetric analysis, hot stage microscopy, and powder X-ray diffraction.[34]

Figure 1.4 presents a co-crystal, which is a stoichiometric molecular complex of a molecule (blue) with a co-former (red) assembled via non-covalent interactions which

is predominantly hydrogen bonds. In a pharmaceutical co-crystal, the molecule is an API and the co-former is a GRAS compound or even a second API. Crystallization of the API gives the reference drug form, whereas co-crystallization leads to multi-component crystal structures (co-crystal).[35]

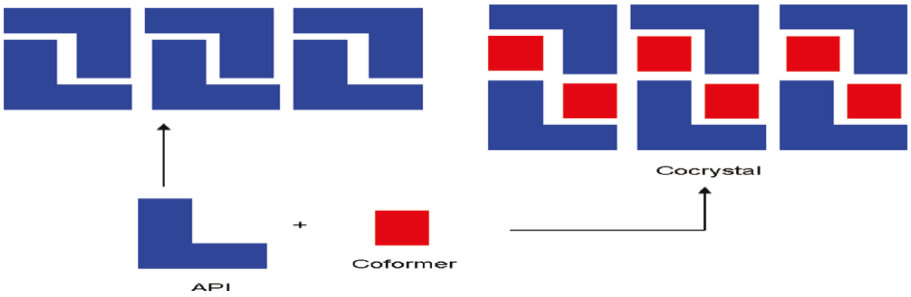

Figure 1-4: Stoichiometric molecular complex[35]

The first recorded application of crystal engineering to the generation of pharmaceutical co-crystals was during a series of studies reported by Zerkowski *et al.*[36] In their study, substituted barbituric acid, including barbital and melamine derivatives were used to generate supramolecular 'linear tape', 'crinkled tape', and 'rosette' motifs by robust supramolecular synthons with three-point hydrogen bonding. Although pharmaceutical co-crystals were successfully formed, the focus of the studies determined the supramolecular functionality of barbitals and its complementarities with melamine and not the physical properties of the resulting co-crystals. However, the study highlighted the potential diversity of forms that can exist for a particular API. As a result, there were more than 60 co-crystals that were structurally characterized, hence the study presented a diversity of co-crystal forms that could offer an exciting opportunity to novel and improved crystalline forms of APIs.[37]

1.7.2.1 Physicochemical properties of pharmaceutical co-crystals

It is important to investigate the physical and chemical properties of a co-crystal drug in the same manner as any other solid state form in order to determine developability into a marketed dosage form. The determination of new compounds of co-crystal physicochemical properties, such as crystallinity, melting point, solubility, dissolution, and stability are important especially during the early development stage i.e.

preformulation stage.[17] Table 1.3 poses these questions to pharmaceutical co-crystals, to determine the pharmaceutical and biological success rate of the product.

Table 1-3: Physical and chemical properties of a co-crystal

Melting point	Does the thermal behaviour (melting point) of a co-crystal change with respect to the individual components and can the melting points be estimated within a series of co-crystals?
Stability	Can physical and chemical stability be enhanced upon co-crystallization of an API?
Solubility	Can the solubility of an API be altered by modifying it into a co-crystal?
Dissolution	Are dissolution rates improved by co-crystalline compounds in comparison to the individual APIs?
Bioavailability	Can the bioavailability of an API be improved using co-crystals?

1.8 History of cyclodextrins (CDs)

Cyclodextrins (CDs) was first reported by a foremost French scientist, A.Villiers in 1891.[38] CDs are formed by reducing dextrins and a small amount of crystalline material obtained from starch digest of *Baccilus amylobacter*. In the early 1950s, precisely 1953, CDs were patented by Freudenberg *et al*., and became available in drug formulations.[39] CDs are known to have unique characteristic such as the ability to form inclusion complexes with many drugs by receiving the drug molecule in whole or part into its cavity.[40] This characteristic results in the significant enhancement of drug properties such as solubility, stability towards drug oxidation and bioavailability. More importantly, CDs are known to aid the decrease in the bitterness and tissue irritation of drugs.[41] In Table 1-4[42] below, the change in drug properties are presented to show the significant improvement in drug properties after the inclusion of cyclodextrin.

Table 1-4: Commercially available CDs in pharmaceutical products that improved after cyclodextrin inclusion[42]

Cyclodextrin	MS[a]	Synonyms	MW (Da)	Oral bioavailability in rates (%)	Solubility in water at room temperature (mg/ml)	Current usage in marketed products
***α*-Cyclodextrin**		Alfadex	973	1	145	Oral and parenteral
***β*-Cyclodextrin**		Betadex	1135	0.6	18.5	Oral, buccal, and topical
2Hydroxypropyl-*β*-Cyclodextrin	0.65	Hydroxypropylbetadex	1400	3	>600	Oral, parenteral, rectal and ophthalmic
Sulfobutylether-*β*-Cyclodextrin sodium salt	0.9		2163	1.6	>500	Parenteral
Methylated-*β*-Cyclodextrin	1.8		1312	≤12	>600	Ophthalmic and nasal
***γ*-Cyclodextrin**		Gammadex	1297	0.02	232	Parenteral
2-Hydroxypropyl-*γ*-Cyclodextrin	0.6		1576	<0.1	>600	Parenteral and ophthalmic

[a] the molar degree of substitution (MS) is defined as the average number of substituents that have reacted with one glucopyranose repeat

Another advantage of CDs is that their derivatives have been successfully adapted to produce supramolecular systems across length scales. CDs are also used to engineer novel functional materials and this is done by taking full advantage of one of their properties such as host-guest interactions which occur between the CD units and guest molecules.[43]

CDs are mainly carbohydrates of cyclic oligosaccharides. They contain six (α-CD), seven (β-CD) or eight (γ-CD) α-1,4 linked glucopyranose units with a hydrophilic outer surface and a hydrophobic cavity (Table 1.5).[42]

In addition, the internal cavity of CDs can include a range of guest molecules and they range from polar compounds such as alcohols, acids, amines and small inorganic anions to apolar compounds such as aliphatic and aromatic hydrocarbons. The hydrophilic exterior assists CDs to be dissolved in water.[44] The use of CDs in drug formulation has shown that there are no covalent bonds that are formed or broken during the drug/cyclodextrin complex formation.[45] This unique attribute is peculiar to CDs and the known controlling factor of the formation of inclusion complexes is from non-covalent interactions such as Van der Waals forces, electronic effects, hydrophobic interactions and steric factors.[44] Consequently, this enables CDs to form non-covalent inclusion complexes by entrapping the drug into the central cavities. It is important to state that these non-covalent inclusion complexes exhibited by CDs in drug formulation offer a variety of comparative physicochemical advantages over non-manipulated drugs.[46]

Table 1-5: Chemical and physical properties of *α, β, γ* and *δ*-Cyclodextrin

Physicochemical properties	***α***	***B***	***γ***	***δ***
No. of glucopyranose units	6	7	8	9
Molecular weight	972	1135	1297	1459
Central cavity diameter (Aº)	4.7-5.3	6.0-6.5	7.5-8.3	10.3-11.2
Water solubility at 25 °C (g/100 mL)	14.5	1.85	23.2	8.19

The naturally occurring CDs have shown limitations especially with their aqueous solubility due to the strong intermolecular hydrogen bonding in the crystal state. However, the substitution of the H-bond forming -OH group has been used to improve its solubility.[46] Although not all the categories of drugs are suitable substrates for CD complexation. This is because the process of complexation with CDs requires that the drug should exhibit some characteristics. For instance, for a drug molecule to be complexed with CDs, they must have the following characteristics;

a. More than five atoms (C, P, S, and N) form the skeleton of the drug molecule.
b. Melting point temperature of the substance is below 250 °C.
c. Solubility in water is less than 10 mg/mL.
d. The guest molecule consists of less than five condensed rings.
e. Molecular weight between 100 and 400 g/mol.

It is important to emphasise that these characteristics are generally favoured for pharmaceutical and medicinal benefits. However, there are certain exceptions that cannot be neglected.[47,48] There are four actively favourable interactions that assist in the shift of equilibrium towards complex formation (Figure 1.5). The process of complexation can thus be summarized as follows:[49]

i. Displacement of polar water molecule from the apolar cyclodextrin cavity.
ii. The increase number of hydrogen bonds formed as the displaced water returns to the larger pool.

iii. A reduction of the repulsive interaction between hydrophobic guest and the aqueous environment.

iv. An increase in hydrophobic interaction as the guest inserts itself into the polar CD cavity.

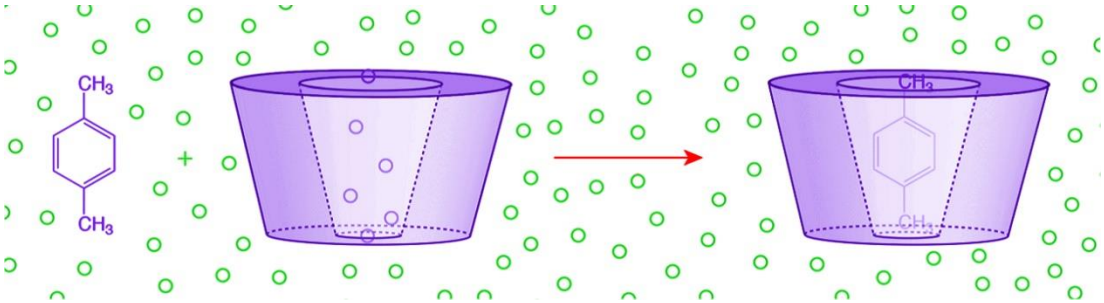

Figure 1-5: Schematic illustration of the complexation process[50]

1.8.1 Cyclodextrins in drug formulations

One of the main challenges of pharmaceutical exploitation of cyclodextrins is the bulk formulation of active pharmaceutical ingredients. This is because CDs in solid dosage forms can be used as solubility enhancers for potent to medium potency drugs and this is possible if these drugs have relatively high complexation efficiency.[51] However, present studies have shown that only α-CD, β-CD and γ-CD, as well as some derivatives have commercial viability out of which there are currently over 30 marketed cyclodextrin-containing pharmaceutical products worldwide.[41] Table 1.6 below describes the oral pharmaceutical products containing CDs that are commercially available.[52]

Table 1-6: Some marketed oral pharmaceutical products containing cyclodextrins[52]

Drug/cyclodextrin	Trade name	Formulation	Company (country)
α-Cyclodextrin (α-CD)			
Cefotiam-hexetil HCl	Pansporin T	Tablet	Takeda (Japan)
OP-1206	Opalmon	Tablet	Ono (Japan)
β-Cyclodextrin (β-CD)			
Benexate HCl	Ulgut, Lonmiel	Capsule	Teikoku (Japan); Shionogi (Japan)
Cephalosporin	Meiact	Tablet	Meiji Seika (Japan)
Cetirizine	Cetrizin	Chewing Tablet	Losan Pharma (Germany)
Chlordiazepoxide	Transillium	Tablet	Gador (Argentina)
Diphenhydramin and Chlortheophyllin	StadaTravel	Chewing Tablet	Stada (Europe)
Meloxicam	Mobitil	Tablet	Medical Union Pharmaceuticals (Egypt)
Nicotine	Nicorette	Sublingual Tablets	Pfizer (Europe)
Nimesulide	Nimedex	Tablets	Novartis (Europe)
Omeprazole	Omebeta	Tablet	Betafarm (Europe)
Tiaprofenic acid	Surgamyl	Tablet	Roussel-Maestrelli (Europe)
2-Hydroxypropyl-β-Cyclodextrin (HP-β-CD)			
Itraconazole	Sporanox	Oral	Janssen (Europe, USA)

The commercial availability of CD-containing pharmaceutical products vary between regions. However, the world's first CD-containing pharmaceutical product is known as prostaglandin E2/*β*-CD (Prostarmon E™sublingual tablets) and was marketed in Japan in 1976.[52] In Europe, CD-based pharmaceutical product piroxicam/*β*-CD (Brexin® tablets) was marketed in 1997 while the first US-approved product was itraconazole/2-HP-*β*-CD oral solution (Sporanox®).[52]

1.9 *β*-Cyclodextrins

From the three types of CDs, *β*-Cyclodextrin (*β*-CD) (figure 1.6) is known to be more suitable for practical use for the following reasons:[49]

a. Its cavity diameter is the best for guest molecules.
b. Its production procedure does not require sophisticated technologies.
c. It is cheaper.

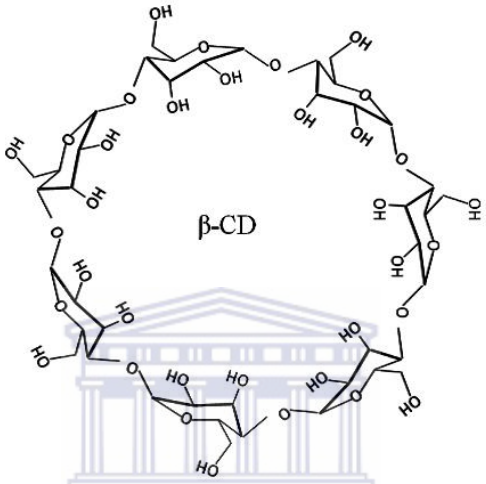

Figure 1-6: Geometry and chemical structure of *β*-CD

β-CDs have 7 glucose units in the ring and their complexes are hydrophilic in nature but have comparably low aqueous solubility because it is associated with a high crystal lattice energy and intramolecular hydrogen bond formation that compromise the interaction with solvation.[38,53] This category of CDs has been studied for pharmaceutical purposes in order to resolve the limitation of low aqueous solubility. The derivatives of the *β*-CDs with higher aqueous solubility have been developed by introducing substituents at specific positions of the glucose rings thereby breaking the intramolecular net of hydrogen bonds and enhancing aqueous solubility.[53] The most commonly used derivatives are methyl-*β*-cyclodextrin (M-*β*-CD), HP-*β*-CD, and sulfobutylether-*β*-cyclodextrins (SBE-*β*-CD).[54] This is because some of the solubility properties of the *β*-CD derivatives are high such as methyl-derivatives, but it's known to have strong systemic toxicity. In the case of the hydroxypropyl and sulfobutylether-*β*-CD derivatives, they have been found to show the lowest haemolytic effect and are

therefore considered suitable for parenteral administration as well as α- and γ-CD derivatives with 6 and 8 glucose units, respectively.[53]

1.9.1 Toxicological and regulatory considerations

CDs are generally known to have a favourable toxicological profile in comparison to other pharmaceutical excipients such as surfactants, water-soluble polymers, and organic solvents. Also, their oral bioavailability is very low which is indicative of their suitability to act as true drug carriers.[51] Toxicological studies of CDs have shown that orally administered cyclodextrins are practically non-toxic. This is because of the low absorption into the systemic blood circulation.[51] The toxicological studies are based on a 52-week toxicity analysis utilising dietary administration. β-CD was shown to be non-toxic as the daily dose in dog and rats was <1800 mg/kg and <600 mg/kg, respectively.[55]

1.9.2 Ethical concerns

Although the use of CDs have been considered to have enormous potential, the regulatory status of CDs is still evolving. As a result, the natural cyclodextrin can be found in a number of pharmaceutical formulations in many countries (Table 1.7). However, under certain conditions it is generally recognised as safe (GRAS) by the FDA and is listed in both the European Pharmacopoeia (Ph.Eur.) and US Pharmacopoeia (USP/NF) as well as in the Japanese Pharmaceutical Codex (JPC).[52]

Table 1-7: The regulatory status of selected pharmaceutically important cyclodextrins

Cyclodextrin	Pharmacopoeia		
	PH.EUR.[(a)]	**USP/NF[(b)]**	**JPC[(c)]**
***α*-Cyclodextrin**	Yes	No	Yes
***β*-Cyclodextrin**	Yes	Yes	Yes
2-hydrohypropyl-*β*-Cyclodextrin	Yes	Yes	No
Sulfobutylether-*β*-Cyclodextrin sodium salt	No	No	No
Randomly methylated-*β*- Cyclodextrin	No	No	No
γ-Cyclodextrin	In progress	Yes	Yes

a. European Pharmacopoeia 5th Edition (2005)

b. United State Pharmacopoeia 28th Edition/National Formulary 23th Edition

c. Japanese Pharmacopoeia Codex (1997)

1.9.3 Pharmaceutical applications of Cyclodextrins

There are a number of applications for CDs in the pharmaceutical field. For example, the addition of α- or β-CD has been shown to increase water solubility of several poorly water-soluble drugs.[50] In view of this, the bioavailability of a drug can be improved as well as increase the pharmacological effect thus allowing for a reduction in drug dosage. Also, the ability of CDs in inclusion complexes can also facilitate the handling of volatile products. This can improve the stability of substances in order to increase its resistance to hydrolysis, oxidation, heat, light and metal salts. The inclusion of volatile products in CDs can also help to protect the gastric mucosa for the oral route, and reduce skin damage for the dermal route. Furthermore, CDs can be used to reduce the effects of bitter tasting and awful odour drugs.[50]

In order to prepare the inclusion complexes for various drugs, there have been a number of methods extensively documented such as grinding, kneading, freeze drying, spray drying and slow evaporation.[56]

1.10 Oral solid dosage formulation

One of the integral core areas of research especially in the pharmaceutical industry is the successful development of solid drug dosage forms. The increase in research focus on solid dosage forms is because of its poor physico-chemical and pharmacokinetic properties which continues to be a challenge. The benefits of solid dosage forms include their prevalence rate due to their unique application and convenience for the administration of drugs.[57] Also, there are other reasons for the continued popularity of the oral solid dosage form. This route of delivery is considered to be the least invasive method of delivering drugs and can be self-administered by patients. In the case of the manufacturer, solid oral dosage forms offer many competitive advantages as it utilises cheap technology, compact, aesthetic values such as appearance can be modified to create brand identification and is considered to be the most stable form of a drug.[1] For instance, the oral solid dosage forms such as tablets and hard gelatine capsules remain the most frequently used dosage forms.[1]

Generally, the majority of the pharmaceutical companies prefer to introduce their new molecule into the market as a tablet or capsule in order to save cost, safety and marketing concerns. Also, approximately 70 % of all drugs administered today exist in solid dosage forms. By implication, the default form of a drug has been suggested to be in solid dosage form. However, there may be a predetermined case of therapeutic proteins or other drugs where such a drug must be administered by parenteral route or other specific routes for the desired activity.[58]

The primary objective of dosage form design is to achieve a predictable therapeutic response to a drug in a formulation with the potential to upscale production and quality. In order to ensure product quality, there are several features that are required. These include the chemical and physical stability, suitable preservation against microbial contamination (if appropriate), uniformity of drug dosage, acceptability to users including prescriber and patient, as well as marketing plans such as packaging, labelling, and validation.[59] Solid dosage forms are mostly manufactured from powders hence it is crucial to understand the unique properties of powder systems for the purpose of rational formulation and manufacture procedures.[1] The typical parameter studies for solid dosage forms often relate to the mixing capacity of the powder and compressibility in manufacturing machines.[58] The two most common types of solid dosage forms are tablets and capsules and they are very versatile with each of these forms having its own unique requirements. The properties that are similar to both forms include flow of the correct weight of material into a specific volume, behaviour of the material under pressure, wetting of the powder for both granulation and subsequent disintegration and dissolution of the dosage form.[1] There are different types of tablets and are often designed to satisfy specific therapeutic needs (table 1.8).[1]

Table 1-8: Types of solid dosage forms[1]

Formulation type	Description
Immediate release	The dosage form is designed to release the drug substance immediately after ingestion.
Delayed release	The drug substance is not released until a physical event has occurred, e.g., time lapsed, change in pH of intestinal fluids, change in gut flora.
Chewable tablets	Strong, hard tablets to give good mouth feel.
Lozenges	Strong, slowly dissolving tablets for local delivery to mouth or throat. Often prepared by a candy moulding process.
Buccal tablets	Tablets designed to be placed in buccal cavity of mouth for rapid action.
Effervescent tablets	Taken in water, the tablet forms an effervescent, often pleasant-tasting drink.
Dispersible tablets	Tablets taken in water, the tablet forms a suspension for ease of swallowing.
Soluble tablets	Tablets taken in water, the tablet forms a solution for ease of swallowing.
Hard gelatine capsules	Two-piece capsule shells, which can be filled with powders, pellets, semisolids, or liquids.
Soft gelatine capsules	One-piece capsules containing a liquid or semisolid fill.
Pastilles	Intended to dissolve in mouth slowly for the treatment of local infections. Usually composed of a base containing gelatine and glycerine.

1.10.1 **The tablet**

The first tabletting machine was patented in 1843 by William Brockedon. It was designed as a tool used "for the shaping of pills, lozenges and black lead by pressure in a die."[60] In the pharmaceutical industry, tablets are one of the most widely prescribed oral solid dosage forms. Tablets are a popular dosage form because they are simple and can be cheaply manufactured. They have relative stability and are easy to package and store. In the case of the patient, the choice of tablets has become popular because of the uniformity of dose, blandness of taste and ease of administration. It has therefore become imperative that the purpose of the formulation and the identification of suitable excipients are of primary importance in the development of a successful drug formulation.[61] Typically, the ingredients that make up the tablet blend include the API and various excipients. The advantages of tablets over other forms of dosages include:[62]

a. The oral route represents a convenient and safe way of drug administration.
b. Compared to liquid dosage forms tablets have general advantages in terms of the chemical and physical stability of the dosage form.
c. The preparation procedure enables accurate dosing of the drug.
d. It is easy to handle and can be prepared in a versatile way with respect to its use and to the delivery of the drug.
e. Mass production with quality-controlled production procedures may results in preparation of consistent quality and low price.

However, one of the major disadvantages of tablets as a dosage form relates to the bioavailability of poorly water-soluble or poorly absorbable drugs. In addition, some drugs may cause local irritant effects or otherwise cause harm to the gastrointestinal mucus lining.[62]

1.10.2 **Tabletting**

As in the case with other pharmaceutical products, tablets comprise a mixture of two main components; active substances and excipients (additives) which are, usually in powder form. They can be pressed or compacted into a solid. The intended use of tablets determines their method of manufacture as well as other physical properties such as size, shape, weight, hardness and thickness. The pharmaceutical properties that are often considered include disintegration and dissolution. The excipients can include binders, glidants (flow aids) and lubricants to ensure efficient tableting. The presence of disintegrants as excipients is to promote tablet disintegration in the digestive tract while sweeteners or flavours are to enhance taste. As for pigments, they make tablets visually appealing. In general, tablets must be strong in order to withstand the rigors of manufacturing processes, transport, and handling, and need to be of acceptable size, taste, and appearance.[61] A typical manufacturing process for a tablet product includes laboratory processes such as weighing, milling, granulation, drying, blending, lubrication, compression and coating. In each step, several process parameters are involved which require extra care. For a given formulation, it is recommended that all the processing steps should be thoroughly evaluated so that a robust manufacturing process can be defined and applied effectively to optimize this process.[61]

1.10.3 **Compressed tablet manufacturing methods**

The simplest tablet formulations are uncoated products that are made by direct compression, wet or dry granulation methods. The compressed tablet method is a versatile drug delivery system and can be intended for local action in the gastrointestinal (GI) tract or for systemic effects. Generally, there are several crucial criteria for tablet formulations. These include; accuracy and uniformity of drug content. Others are the stability of the drug candidate and the formulation, optimal dissolution and availability for absorption, and patient acceptability in terms of organoleptic properties and appearance. The use of flocculent and low density drugs has become challenging due to difficulty to compress and formulate these into tablets and this is often the disadvantage with drugs of low potency. Similarly, other properties that have limited the manufacture of compressed drugs are poor water solubility, poor absorption or highly metabolized drugs. Additionally, compressed

drugs can have local irritation which often have harmful effects on the mucosa of the GI tract of patients.[61]

The direct compression method is a process where powder blends of the drug substance i.e. active ingredients and excipients are compressed by a tablet machine. In this case, there is no mechanical treatment of the powder apart from a mixing process.

Granulation is a generic term used to describe particle enlargement whereby powders are formed into permanent aggregates. The purpose of granulating tablet formulations is to improve the flow and compaction properties prior to compression.[1] There are two types of granulation i.e. dry and wet granulation. In dry granulation, the ingredients are blended followed by compaction and size reduction of the blend to produce a free-flowing granular blend mixture for tableting. On the contrary, wet granulation utilizes the use of a liquid binder to develop granules from the formulation blends.[1]

The physical features of compressed tablets vary from size (large or small in diameter) to shape i.e. round, oblong, flat or convex. Other features include colour and code identification unscored or scored; engraved or imprinted with an identifying symbol and/or code number and one, two, or three layered.[4] In general, these physical features help to determine the type of drug and most importantly the dosage. Specifically, a typical compressed tablet has a diameter range between 6 mm and 11 mm and a height range between 2 mm and 4 mm. However, if the drug is too small or large, it may be difficult to handle or swallow. Similarly, if the required dose exceeds 500 mg, the dose is often equally divided into two tablets.[63]

For the purposes of this study, direct compression will be discussed since this is the first consideration for all drugs when compressing solids.

1.10.4 **Direct compression**

The direct compression technique of drug manufacture is a simple economical process and is also less stressful to ingredients in terms of heat and moisture. However, there are limitations governed by the physical properties of the ingredients. The raw active

pharmaceutical ingredients must be carefully controlled. This is because it is difficult to form directly compressed tablets containing a high dose of poorly compactible drugs.[61]

In early studies, the term direct compression was used to identify the compression of a single crystalline compound (i.e. sodium chloride, potassium chloride, potassium bromide, etc.) into a compact form without the addition of other substances. Consequently, it is a process that does not require pre-treatment as well as the inclusion of additives or excipients. As such, it was only used for inorganic materials such as potassium bromide. However, in present, direct compression is used to define the process by which tablets are compressed directly from the powder blends of active ingredients and suitable excipients without any prior treatment such as wet or dry granulation.[1,64]

The direct compression method for drug manufacturing has not been fully explored as approximately less than 20 % of pharmaceutical materials can be compressed directly into tablets. Other pharmaceutical materials are limited by properties such as poor flow, cohesion or lubricating properties necessary for the production of tablets by direct compression. It has been suggested that the use of directly compressible adjuvants may yield satisfactory tablets for such materials.[64] The use of the granulation technique can be employed to improve the important properties of a powder such as compaction characteristics of the powder, improve flow properties and reduce the tendency for segregation of the mix due to a more even particle size and bulk density. Although the basic mechanical process of producing tablets by compression has not changed, there has been continuous improvement on tabletting technology.[61]

There are several challenges in the formulation of low-dose drugs by the direct compression method. These challenges are mostly related to achieving and maintaining a homogeneous mix of both active ingredients and excipients. Although low-dose drugs can be formulated to form ordered mixes that reduce the risk of segregation. It is therefore important that adequate consideration must be given to the manufacturing process in order to minimize the possibility of segregation.[1] However,

the direct compression process is decidedly influenced by powder characteristics such as flowability, compressibility and dilution potential. This implies that the physico-mechanical properties of active ingredients and excipients of tablets must be considered in order to develop a robust direct compression manufacturing process.[64]

1.10.4.1 **Advantages of direct compression**

The direct compression technique can provide several benefits such as technical and economic and also improve the stability of certain drugs. The use of processes such as wetting and drying can be eliminated especially during the formulation of drugs that are moisture sensitive. Consequently, this type of drug manufacture will require fewer operations and the omission of a drying step results in lower energy consumption. The reduction in processing times will produce savings in total cost of production and the simplicity of validation processes. However, the use of specialized, more expensive, excipients become crucial in order to ensure the quality of drugs produced.[1]

In the process of drug "disintegration," it is generally known that for optimal dissolution, the tablet must disintegrate into its primary particles as quickly as possible. This is highly dependent on the direct compression procedure such that if the drug mixture does not agglomerate the tablets will disintegrate directly to the primary particles.[1]

1.10.4.2 **Disadvantages of direct compression**

One of the major limitations of the direct compression method of drug manufacture is that it cannot be used for all drug substances. This is because the direct compression method depends on the major components of the formulation to have specific properties such as appropriate flow and compaction.[1] Although, it is possible to modify the properties of drug substances by use of particle engineering approach. This technique is often beyond the scope of the formulator as it may require specialized expertise hence each drug formulation must be designed to accommodate the limitations unique to the drug substance. In the case of low-dose drugs, it has been reported that such limitations can be overcome through careful selection of excipients.[1]

1.10.5 **Tablet compression process**

The manufacture of tablets involves the compression of uniform volumes of a blended powder within a confined space. This compression process consists of three steps. These steps are die filling, tablet formation and tablet ejection (figure 1.7).

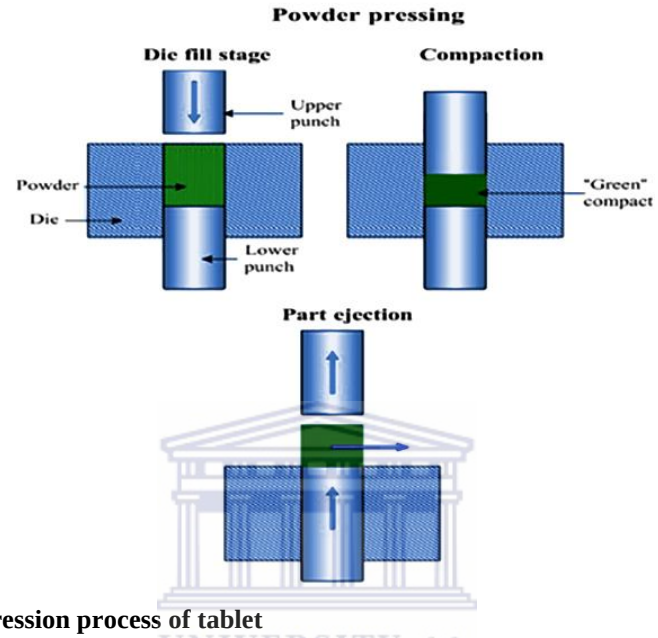

Figure 1-7: Compression process of tablet

1.10.5.1 **Die filling**

This process is generally accomplished by the gravitational flow of the powder from a hopper platform through the die table into the die (although a press based on centrifugal die filling is also used). The die is closed at its lower end by the lower punch.[62]

1.10.5.2 **Tablet formation**

The process of drug formation is such that the upper punch of the tabletting machine descends and enters the die and the powder is compressed until a tablet is formed. As for the compression phase, the lower punch can be kept stationary or allowed to move upwards in the die and after maximum applied force is reached, the upper punch leaves the powder i.e. the decompression phase.[62]

1.10.5.3 **Tablet ejection**

After the formation of the tablet, the next phase is known as the tablet ejection phase. At this phase, the lower punch rises until its tip reaches the level of the top of the die. Thereafter, the tablet is removed from the die and dies table by an automated or mechanical pushing device.[62]

1.10.6 **Tabletting machines**

There are several tabletting machines that exist and they operate with different production capacities ranging between a single and a million tablets per hour. The type of tabletting machine to be used is determined by the material and other factors such as alignment of the particles in the machine, densification and deformation of pharmaceutical material.[61] There are two types of press that are commonly used during tablet production. They are the single-punch press and rotary press. In addition to these two types of presses, the advanced tabletting machines can use other types of presses such as hydraulic presses which are suitable for the evaluation of the tabletting properties of powders and the prediction of scale-up on the properties of the formed tablets. Scale-up refers to the change to a larger apparatus for performing a certain operation on a larger scale.[62] The "Station" in a tabletting machine refers to the basic unit of both presses which consists of a die and an upper and lower pair of punches. The particulate solid is compressed within the die by a collective pressing action of the two punches. However, the direction of movement of the upper punch, the compaction process, can be divided into two phases: compression and decompression. In the compression phase, the upper punch moves towards the bottom punch and this allows the powder bed to experience intensive densification. Finally, the powder particles move together to form aggregates with appreciable cohesive strength due to Van Der Waals forces.[65]

Also, the die and the punches of the tabletting machine determine the shape which can be round, spherical, oval, square, triangular and rectangular with diameters such as (3/16, 7/32, 1/4, 9/32, 5/16,11/32, 7/16, 1/2, 9/16, 5/8, 11/16, 3/4 in) of the tablet. Lastly, the tablet weight is determined by the volume of the die cavity.[66]

1.10.6.1 **Single-punch tabletting machines**

The single-punch machines were reported as the first tabletting machines and were used at the end of the nineteenth century. They consist of the upper punch which is lowered by a lever arm on the powder bed, in the die and the procedure is reciprocated to produce single tablets. However, the single-punch tabletting machines are manually operated and their use has been restricted to drug formulation development, small scale-up and clinical trials.[61] The production output from a single-punch press is about 200 tablets per minute.[62]

1.10.6.2 **Rotary tabletting machines**

These types of tabletting machines are commonly used for tablet production. The rotary press (also referred to as a multi-station press) was primarily developed to increase the output of tablets. They are also important in the pharmaceutical industry because they are used to scale-up in the final part of the drug formulation and also for their operational capacity for large scale-up production. The rotary tabletting machine has a capacity production output of over 10 000 tablets per minute.[62] Also, the rotary tableting machines work with a number of punches and die sets which moves in a circular motion whereas the dies are fixed into a round die table. Collectively, the dies, lower and upper punches circulate on tracks. The densification process is two-sided because both punches pass the compression wheels and the same force is developed on the upper and lower side of the powder bed. Consequently, the produced tablets show the same hardness on the upper and lower surfaces.[61]

1.11 **Tablet components**

Tablets are known to mainly contain active ingredients. In addition to the active ingredients, solid oral dosage forms also contain other substances called excipients. Excipients are essential components of tablets because they confer specific properties and also ensure that the manufacturing process is successful along with quality assurance of the resultant formulation. Therefore, it is important to select appropriate excipients as their relative concentrations in the formulation are crucial in the development of a successful product.[61]

1.11.1 **Excipients in solid dose formulations**

Excipients are known to contribute significantly to a drug formulation's functionality and processability and play different roles in drug production including manufacturability, administration, formulation stability, safety and aesthetics. All of these characteristics are important in the pharmaceutical industry. Also, the correct combination of excipients during the tablet development stage is highly important and it is imperative to understand the behaviour of excipients in order to avoid potential problems during manufacturing.[67] In most formulations, the excipients often contain higher concentrations than the active ingredients which are approximately 70 – 80 %.[64]

The International Pharmaceutical Excipients Council (IPEC) has defined a pharmaceutical excipient 'as any substance other than the active drug or prodrug that has been appropriately evaluated for safety.' According to the IPEC, excipients are included in a drug delivery system because of the following factors:[1]

- Facilitate processing of the system during manufacture.
- It protects, supports, or enhances stability, bioavailability, or patient acceptability.
- Assist in product identification.
- Provides additional characteristics or enhancement that can be attributed to the overall safety and effectiveness of the drug product during storage or use.

The harmonization of acceptable standards is necessary for regulatory authorities and pharmaceutical formulators. This approach is useful especially for the standardization of raw materials in drug formulation in order to manufacture a finished product with consistent quality. However, these performance standards have not been included in pharmacopoeia. This is primarily due to the specifications assessment of raw materials which have always been based on chemical purity and also due to the complications associated with standardized performance criteria.[64,68]

The standardization procedure of Pharmacopoeia is such that particle characteristics or powder properties are not considered even though they determine the functionality

of excipients. It is important to emphasise that functionality control is crucial as it is responsible for the control of identity and purity due to the following reasons:[64]

- Many excipients such as microcrystalline cellulose and starch are known to have multiple functions.
- There is lack of awareness that excipients behave differently, depending upon the vendor (i.e. microcrystalline cellulose).

As a result, excipients with optimal functionality are needed to ensure smooth tablet production on modern machines. The introduction of a special force feeder to improve the flow of granules from the hopper marked a significant advancement in direct compression technology.

One of the major limitations is that only a few excipients can be directly compressed into tablets. This is due to their poor physical properties such as compatibility, flowability and compressibility.[67]

The functionality of excipients is a criterion used to categorize them into groups even though multifunctional excipients exist. Some of the excipients such as diluents, binders, disintegrants, lubricants, glidants, colourings and flavours are used to enhance the quality factors of a drug. Table 1.9 below presents examples of common excipients used in the manufacture of tablets and capsules.[63]

Table 1-9: Common excipients used in the manufacture of tablets, capsules and powders

Excipient*	Desired Function	Typical Examples	Typical Amount
Diluents/Filler (T, H, P)	Make up tablet size, capsule size or the required powder dosage	Microcrystalline cellulose Lactose Starch	20-90% 5-85% 5-75%
Binder (T, H, P)	Increase cohesiveness of powder and hold them together to form granules	Sucrose (Solvent: Water). Microcrystalline cellulose Povidone (Solvent: Water or Water-alcohol solution).	2-25% 5-20% 2-5% 5-10%†.
Filler-Binder (T)	Use in direct compression	Spray-dried Lactose Microcrystalline cellulose	> 80% 10-25%
Disintegrant (T, H, P)	To facilitate the breakup of tablet or granule	Starch Microcrystalline cellulose Cross-linked Povidone Alginic acid	5-20% 5-15% 0.5-5% 5-10%
Lubricant (T, H, P)	Reduce friction during tablet ejection or facilitate drug transport to filling machine	Magnesium stearate (I). ‡ Talc (I). Starch (I). Polyethylene glycol (S).	0.25-2% 5-10% 5-10% 2-10%
Anti-adherent (T)	Reduce sticking of tablets to the punches or die wall	Talc Magnesium stearate Microcrystalline cellulose	1-5% 0.25-1% 5-10%
Glidant (T, H, P)	Promote flow of granules or powders by reducing friction between them	Calcium silicate Silicon dioxide Magnesium stearate Starch	0.5-2% 0.1-0.5% 0.2-2% 1-10%
Pigment (T, P).	Add colour to tablet or powder	Titanium oxide	q.s.

*T = tablet, H = hard gelatin capsule, P= powder.

‡I = water insoluble, S = water-soluble.

1.11.2 Types of excipients

1.11.2.1 Diluents

Diluents which are also known as fillers form an important component of a tablet particularly for low dose drugs. In order to achieve content i.e. active ingredients and diluents uniformity, a tablet size should at least be 3 mm and the corresponding weight should be greater than 50 mg.[69] Fillers are used to complete the size of a tablet or capsule thereby making it practical to produce and also convenient for usage. By increasing the volume, fillers make it easier for the final product to have the proper volume for patient handling.[70] A good filler must possess some unique characteristics such inertness, compatibility with the other components of the formulation, non-hygroscopicity, solubility, be relatively cheap, compactable and be preferably tasteless or pleasant tasting.[70] The physical properties of a drug are combined with reverse filler of opposite properties in order to compensate for the limitation of the drug. For instance, a soluble drug is normally formulated with an insoluble filler in order to optimize the disintegration and dissolution process. The hydrophilic excipients added in the formulation may also change drug solubility.[61]

The use of plant cellulose (pure plant filler) is a prominent filler present in tablets or hard gelatine capsules. Also, another widely used filler used in tablets is dibasic calcium phosphate. In soft gelatine capsules, a range of vegetable fats and oils can be used as fillers. Other examples of fillers include lactose, sucrose, glucose, mannitol, sorbitol, calcium carbonate and magnesium stearate.[70]

A direct compression formulation will require fillers and such fillers must have the following requirements:[1]

- Excellent flow properties.
- Good compaction properties.
- High capacity i.e. ability to retain their compaction properties when mixed with drugs substances.

- Appropriate particle size distribution in order to minimize the segregation potential.
- High bulk density.
- Reproducibility potential in order to minimize batch-to-batch variation.

1.11.2.2 **Binders**

The use of binders is to impart cohesive qualities to the powdered material during the production of tablets. This is to ensure that the tablet remains intact i.e. does not disintegrate after compression as well as to improve the free flowing quality.[71] It is therefore imperative that the selection of a suitable binder for a tablet formulation requires prior and extensive knowledge of its properties in order to enhance the strength of the tablet and also not to compromise the drug qualities especially in cases where there is possibility of interaction of the various drug components.[72] Generally, the addition of binders is to ensure that tablets and granules can be formed with required mechanical strength as well as give volume to low active dose tablets. The constituents of binders are usually carbohydrates such as starches, sugars, cellulose or modified cellulose such as microcrystalline cellulose, hydroxypropyl cellulose, lactose, or sugar alcohols like xylitol, sorbitol or maltitol. There are different types of binders and they are classified based on their application.[70] For instance, solution binders can be dissolved in appropriate solvents such as water or alcohol and are mostly used in wet granulation processes. These types of binders include gelatine, cellulose, cellulose derivatives, polyvinylpyrrolidone, starch, sucrose and polyethylene glycol. As for dry binders, they can be added to the powder blend after a wet granulation step during the direct powder compression formula process. The dry binders include; cellulose, methyl cellulose, polyvinylpyrrolidone and polyethylene glycol.

1.11.2.3 **Lubricants**

Lubricants assist in the reduction of friction between the powder bed and the die wall during compression and ejection. This is done by interposing a film of low shear strength between them. Thus, lubricants facilitate the tabletting process of tablet formulation and ejection of the formed tablets. Essentially, the lubricant can be used

to improve the properties such as fluidity, filling properties and plasticity and ultimately improve the powder processing properties of formulations.[73] Also some lubricants can also act as an anti-adherent thereby preventing the agglomeration of powder to the punches and die. More importantly, lubricants are known to influence disintegration time and hardness with the drug dissolution test being crucial to optimize the concentration of lubricant in the formulation.[74] The amount of pharmaceutical lubricants in tablet and capsule formulations are often in small quantities which is usually 0.25%-5.0 %, w/w. Magnesium stearate and stearic acid are the most frequently used lubricants in the pharmaceutical industry.[73]

1.11.2.4 **Glidants**

Glidants are used to promote powder flow by reducing inter-particle friction and cohesion. This type of excipients are used in combination with lubricants because it has the ability to reduce die wall friction. Examples include colloidal silicon dioxide and talc.[70] In drug manufacture or formulations, glidants are incorporated into solid dosage forms in order to improve the flow properties of cohesive powders and granulates. The main purpose of introducing glidants during tabletting operations is to improve flow into the hopper and die cavities of the tablet press. Also, glidants can increase the tablet weight, decrease the weight variation and minimize the possibility of powder or granule components to separate due to excessive vibrations.[75]

1.11.2.5 **Anti-adherents**

The purpose of anti-adherent fillers is to prevent or reduce the adhesion of tablet granulation or powder to the surface of the punches or to the die wall.[76]

1.11.2.6 **Disintegrants**

Disintegrants are responsible for the dissolution of tablets once they arrive at their target site in the body. They are added to tablet formulations to facilitate disintegration of the tablet especially when it contacts water in the gastrointestinal tract. Also, disintegrants have the ability to attract water into the tablet thereby cause tablet swelling and finally initiate the tablet to disintegrate. The fragmentation of tablet can be critical to the subsequent dissolution of the drug and also to the attainment of a

satisfactory drug bioavailability.[76] The types of disintegrants are mainly water uptake facilitators and tablet rupture promoters. The examples of disintegrants are; cross linked polyvinyl pyrrolidone, sodium starch glycolate and cross linked sodium carboxymethyl cellulose (crosscarmellose).[70] Disintegrants are hygroscopic materials, hence can absorb moisture from the atmosphere. This property can negatively affect the stability of moisture-sensitive drugs thus extra care must be considered especially with packaging. Finally, the activity of disintegrants in a drug can be affected by the presence of hydrophobic lubricants, therefore care must be taken to optimize the manufacturing thereof.[61]

1.11.3 **Technical tablet defects**

In the manufacture of tablets, the most repeated problems include high tablet weight variation, capping and lamination, and further picking and sticking at punches and dies. Also, there could be low product yield, low crushing force including tablet yams and chipping.[61] These problems must be adequately addressed during drug manufacture in order to ensure quality and maximum yield of drug products.

1.11.3.1 **Capping and lamination**

Capping and lamination are some of the defects in tablets. Capping describes the partial or complete separation of the top or bottom crowns of a tablet from its main body. On the contrary, lamination is the separation of a tablet into two or more distinct layers. These two problems often occur after the compression phase. However, capping and lamination may occur several hours or even days after drug production.[76]

1.11.3.2 **Picking and Sticking**

Picking describes the attachment of surface to a tablet and this can be removed from the tablets surface by a punch. Picking is of particular concern especially when punch tips of the tabletting machine has engraving or embossing.[76]

1.11.3.3 **Mottling**

Mottling is a phenomenon that describes an unequal distribution of colour on a tablet. This occurs when the contrast of regions on a tablet differ significantly such that light

or dark areas are obvious on a supposedly uniform surface.[76] One of the causes of mottling in a drug can be due to differences in degradation of excipients and final product.[76]

1.11.3.4 **Weight variation**

The final weight of a tablet is mainly affected by constituents of raw pharmaceutical material such as powder variation and tablet manufacturing processes such as tablet press conditions, tooling and the flow of powder in the tablet press.[61] The design of a tablet often involves the use of a specific amount of drug in a specific tablet formula. The weight of the tablet is routinely measured in order to ensure that the tablet contains the correct amount of drug. During drug manufacture, the quantity of fill placed in the die of a tabletting press determines the weight of the resulting tablet. Also, the volume of fill can be adjusted to yield tablets of a desired weight and content. In addition, the depth of fill in the tablet die must be adjusted to hold a predetermined volume of powder or granulation. It is however important that the tablet weight must be calculated after the amount of drug and other excipients have been decided.

During drug production, sample tablets are randomly selected and periodically removed for visual inspection for automated physical measurement known as in-process control (IPC). Consequently, high tablet weight variation can be reduced by using the weight control systems. In addition, further de-mixing of the tabletted material must be avoided because de-mixing results in higher tablet weight variation and the content uniformity may be compromised. Nevertheless, several techniques such as paddle feeders can be used to achieve homogeneity for problematic products and further spectroscopic techniques can be used for control.[61]

1.12 **Quality standards and compendial requirements**

The final drug products must meet all specifications and quality standards. . These standards include; weight criteria and variation, content uniformity, thickness, hardness and friability, disintegration and dissolution. These quality control (QC) tests will be discussed in Chapter 2. The common practice is that the standards must be controlled during production and each batch produced must be verified after the

production in order to ensure validation quality of product.[4] The desirable properties of tablets are listed as follows:[62]

a. The tablet must contain the correct dose of the drug.
b. The appearance of the tablet should be elegant and its weight, size and appearance should be consistent.
c. The drug should be released from the tablet in a controlled and reproducible way.
d. The tablet should be biocompatible, i.e. not include excipients, contaminants and microorganisms that could cause harm to patients.
e. The tablet should be of sufficient mechanical strength to withstand fracture and erosion during handling.
f. The tablet should be chemically, physically and microbiologically stable during the lifetime of the product.
g. The tablet should be formulated into a pharmace.
h. The tablet should be packed in a safe manner.

1.13 **Efavirenz**

Globally, Human immunodeficiency virus (HIV) is considered as one of the most deadly infections and it has been reported to affect approximately 35-40 million people worldwide. The virus has been over a decade since the discovery of human immunodeficiency virus type 1 (HIV-1) and as the major cause of AIDS.[77] However, even with the discovery of the HIV-1, the development of drugs that can stop or significantly reduce the HIV-1 infection has been modest. The severity of the HIV-1 infection in most infected individuals is specifically by depleting CD4-positive T lymphocytes. These lymphocytes are known to be the major viral reservoirs in lymphoid organs and in the peripheral blood. However, due to the extremely complex life cycle of HIV-1, it has been difficult to develop appropriate therapeutic modalities that can significantly interrupt HIV-1 replication.[77]

Efavirenz (EFA) is a non-nucleoside reverse transcriptase inhibitor (NNRIT) and is used as highly active anti-retroviral therapy (HAART) for the treatment of Human Immunodeficiency Virus (HIV) type I. EFA is used in combination with other anti-

retroviral agents such as reverse transcriptase inhibitors (RTI). Efavirenz was formerly known as DMP-266 and is marketed under the brand name Sustiva®.[78] EFA was approved by the FDA thereby making it the 14th approved anti-retroviral drug.[79] In the United States, Sustiva® 600 mg tablet has been on the market since 2002, while both 300 mg and 600 mg Sustiva® tablets are available in other countries.[80]

1.13.1 **Physico-chemical properties of Efavirenz**

The chemical name of EFA is (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3, 1-benzoxazin-2-one with an empirical formula of $C_{14}H_9ClF_3NO_2$ and a molecular mass of 315.68 g/mol. The chemical structure of EFA is illustrated in Figure 1.8

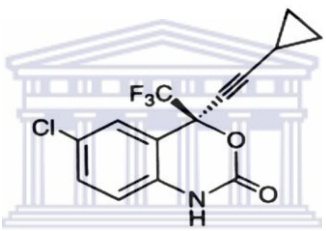

Figure 1-8: The chemical structure of EFA

The FDA classified EFA as a class II drug using the biopharmaceutical classification system guidance (figure 1.9). Class II drugs are characterised with low solubility and high permeability.[81] EFA has similar properties like the so-called "brick dust drugs". These drugs are known to have a melting points ranging from 136 ˚C - 141 ˚C and stable crystalline structure in which strong intramolecular bonds exist with significantly limited dissolution and bioavailability of their pharmaceutical forms.[82] It exists as a crystalline powdery form and has a number of distinct characteristics with a white or slightly yellowish appearance. It is practically insoluble in water (9.0 μg/ml) (Table 1.10) but highly soluble in methanol and dichloromethane.[81,83] It is a crystalline lipophilic solid with a log octanol water partition coefficient of 5.4.

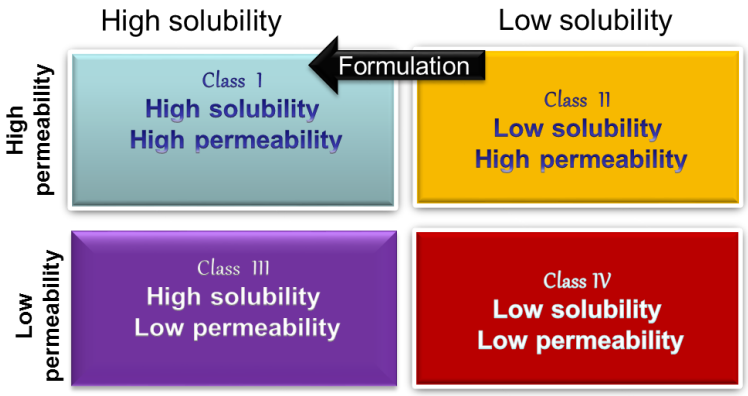

Figure 1-9: Biopharmaceutical classification system guidance

Table 1-10: Solubility of Efavirenz in different buffered media at 37°C and the corresponding dose/solubility (D/S) ratio based on the maximum dose strength of 600 mg[84]

Medium	pH	Solubility (mg/mL)	*D*/*S* ratio (mL)
Water	6.4	0.0115	52,173
SGF$^{a}_{sp}$	1.2	0.0117	51,282
Acetate buffer	4.5	0.0089	67,415
SIFsp	6.8	0.0093	64,516
SIF$^{b}_{sp}$	7.5	0.0107	56,074

The temperature at which the tests were performed was not stated.

SGFsp, simulated gastric fluid *sine* pepsin; SIFsp, simulated intestinal fluid *sine* pancreatin.

*a*Same composition as SGFsp with pH adjusted to 1.0.

*b*Same composition as SIFsp with pH adjusted to 7.0 and 7.5.

Furthermore, EFA has a considerably low intrinsic dissolution rate of 0.037 mg/cm^2/min. This suggests that EFA dissolution rate affects absorption of the drug and this has been one of the problems associated with this drug. This is because a drug with an intrinsic dissolution rate less than 0.1 mg/ cm^2/ min could be a rate-limiting factor for oral absorption of the given drug.[81] The drug-protein binding and its bioavailability have been reported to occur in the range of 99.5 % - 99.75 % and 42 % respectively.[79]

1.13.2 **Dosage and administration**

Stocrin® (figure 1.10) is another trade name for EFA which is currently marketed in tablet form at 50, 200, 300 or 600 mg of EFA. The capsule form has a slight variation in composition containing 50, 100 and 200 mg of EFA. The drug exists in crystalline form with controlled particle size.[39] The available oral solution of EFA contains 30 mg efavirenz per mL. However, the highest dose strength of EFA recommended in the 17th edition of the World Health Organization (WHO) Model List of Essential Medicine is 600 mg for tablets and 200 mg for capsules.[84]

Figure 1-10: Stocrin® formulation - 200 mg EFA tablet formulation

1.14 **Motivation**

EFA belongs to the class II drugs of the BCS. Previous studies on EFA dissolution have shown it has a rate-limiting absorption factor, in addition to its poor palatability. For instance, EFA has an oral dose of 600 mg/day and bioavailability of 40 %. This high dose of EFA has been reported to cause serious side effects like insomnia, loss of memory and suicidal tendencies in human. These associated problems of EFA therefore captures the need to enhance its solubility and bioavailability to reduce dose, the aforementioned side effects.[85] As a result, this study will develop a protocol for EFA using co-crystal engineering and cyclodextrin inclusion complexation to improve its solubility, bioavailability and subsequently reduce the dose of tablet.[85]

1.15 Objectives

The objectives of this study are summarized below and they include;

i. To review co-crystals and cyclodextrin inclusion complexes of EFA as per literature review.
ii. To prepare a protocol considering all efavirenz co-crystal and EFA cyclodextrin inclusion complexes based on selected variables.
iii. To select the best EFA co-crystal and EFA cyclodextrin inclusion complex and compare the two complexes.
iv. To select the best complex from (iii) based on the overall motivation of the study.
v. To prepare the EFA complex selected.
vi. Scale up of the EFA complex.
vii. To formulate the efavirenz complex tablet formulation based and include the excipients present in the proprietor brand on the market called Stocrin,® if necessary.
viii. To conduct the quality control tests on formulation and compare it with the proprietor brand on the market called Stocrin® according to the United States Pharmacopieal (USP) standards.

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CHAPTER 2

EXPERIMENTAL MATERIALS AND METHODS

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2 CHAPTER 2

2.1 Introduction

This chapter contains a full description of the methodology and experimental procedures of this study. This include, a pre-formulation protocol for selecting the most suitable efavirenz co-crystal and cyclodextrin complex, formulation of a tablet dosage form, as well as quality control (QC) tests according to the USP for tablets. Lastly, the chapter presents the materials used in this study and describes the analytical techniques used to characterize, identify and analyse the synthesized compounds.

2.2 Materials

The active pharmaceutical ingredient efavirenz (EFA) was donated from Aspen (South Africa) with batch number B030675 and an expiry date 1/7/2017. Citric acid monohydrate (CTRC) was purchased from Merck Chemicals (South Africa), and the solvent used in the preparation of the EFA co-crystal, ethanol 99.9% was obtained from Sigma-Aldrich Chimie GmbH, USA.

The different excipients used to prepare the tablet dosage form included; Avicel PH-101 (~ 50 μm particle size) and magnesium stearate which was purchased from Sigma-Aldrich Chimie GmbH, USA. The proprietor brand tablet, STOCRIN® 600 mg was purchased from MSD (South Africa) and used to complete all quality control tests. The STOCRIN® 600 mg had a lot number W078970 and an expiry date 1/2016. Finally, distilled water was prepared using a Purite Select Analyst HP water purification system.

2.3 Compound identification

It was important to establish the identity of the active pharmaceutical ingredient, co-former (CTRC) and the EFA co-crystals using appropriate analytical techniques. In addition, the use of these analytical techniques was to determine the physical, thermal and chemical properties of the compound. The detailed explanations of these techniques follows:

2.4 Analytical methods

The use of appropriate analytical methods was useful in order to characterize the physical, chemical, mechanical and thermal properties of the starting materials and final product. This section provides detailed descriptions of the analytical techniques in the identification of specific properties.

2.4.1 Hot stage microscopy (HSM)

In pharmaceutical industry, hot-stage microscopy (HSM) is used in several ways as a complimentary technique to confirm the sample transitions from one phase to another. The solid-state characterization of bulk drugs, evaluation of crystal forms and hydrates and other physicochemical properties like melting point, decomposition, and shape of sample are some of the known uses of HSM.[1] HSM was performed using a Linkam® TH MS600 Temperature control stage connected to a T95 Linkpad System Controller to heat a small amount of sample placed on the sample stage at a controlled rate of 10 °C/min. The visual appearances of thermal events of samples were recorded using a real-time Sony Digital Hyper HAD colour video camera fitted to a Nikon SMZ-10 stereoscopic microscope. The recorded images were analysed by Stream essentials software.® The HSM analysis was performed at the University of the Western Cape, School of Pharmacy, Discipline of Pharmaceutics.

2.4.2 Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was used to determine the melting point, phase transitions and decomposition of samples. The DSC (PerkinElmer DSC7) analyzer utilizing the Pyris® software program was used at heating rate of 10 °C/min under N_2 gas purged with a flow rate of 20 ml/min. In the sample preparation for DSC, a dried, crushed sample was collected into a calibrated aluminium pan and sealed. The sample size was between 1-4 mg and the heating temperature was adjusted between 40 °C to 250 °C. An empty aluminium sealed pan was used as reference. Prior to DSC measurement, the instrument was calibrated with an indium pan (m.p. 156.6 °C and a heat flow of 28.45 J/g) to ensure the reliability of result. The DSC analysis was performed at the University of the Western Cape, School of Pharmacy, Discipline of Pharmaceutics.

2.4.3 Thermogravimetry analysis (TGA)

Thermogravimetry analysis (TGA) is a technique used to determine the thermal profile as well as physical and chemical change of material. This technique measures the mass loss of a sample as a function of time and temperature. TGA was performed on a Perkin-Elmer TGA 4000 instrument connected to a PolyScience digital temperature controller under N_2 gas purged at a flow rate of 20 ml per minute. The calibration of the instrument was performed using indium (m.p.156.6 °C) and aluminium (m.p. 660 °C). Small samples (1−4 mg) were weighed in a porceline crucible and the temperature range was adjusted between 30-300 °C at a constant heating rate of 10 °C/min. The data was collected and analysed using Pyris software.® This technique was primarily used to determine the solvent stoichiometry of the compounds from the percentage weight loss. The TGA analysis was performed at the University of the Western Cape, School of Pharmacy, Discipline of Pharmaceutics. DSC, TGA and HSM analysis were used to confirm the identity and integrity of the co-crystal.

2.4.4 Particle size analysis by scanning electron microscopy (SEM)

In order to determine the shape and size of the co-crystal, scanning electron microscopy (SEM) LEO1450 model was used. A small quantity of co-crystal was placed on carbon adhesive tape; the latter applied onto an aluminum stub and then dried completely in a fume hood. Thereafter the dried co-crystal was 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 mm and accelerating voltage of 5 KV as the instrument operating parameters.

2.5 Pre-formulation protocol for selecting the most suitable efavirenz co-crystal and cyclodextrin complex

The importance of using valuable information for effective planning in the organization and experimental procedures is highlighted in this section. Firstly, it is

important to determine the most suitable method or technique from the available experimental data reported in literature especially in the selection of pre-formulation protocols for cyclodextrin (CD) and co-crystal complexing. This was one of the challenges as there are several methods that have been reported. Also, there is no standardized approach or protocol used in the development of compounds hence the selection criteria from the exhaustive list depends on the prevailing properties and final application as well as the overall objective of the study. Although crystal engineering techniques are used to improve, physicochemical properties of APIs, this study aims to choose a pre-formulation protocol with the best derivative between the in co-crystals and CD inclusion complexes; and also to select the best method that can be used to obtain a good tablet dosage form from the numerous variables associated to prepare the tablet. It is important to emphasise that the choice of the co-former variable will depend on its pharmacological, physicochemical properties and manufacturing process. The details of the variables investigated are presented in chapter 3.

2.6 Preparation methods

There are two main procedures for the preparation of EFA co-crystals; the solvent drop grinding method and the solvothermal method.[2,3]

2.6.1 Solvent-drop grinding method

In the solvent-drop grinding method, two materials i.e. API and co-former are used in stoichiometric amounts in a 1:1 ratio. The material was carefully weighed and grinded into a fine powder with the aid of a mortar with pestle. During grinding, a small quantity of suitable solvent was slowly added to the powders in a drop-wise manner. The added solvent acts as a catalyst as a result of its small quantities and also not a component of the final co-crystal product.[4] The mixture was grinded at room temperature over an extended period in order to allow the solvent to evaporate. For instance, Jones *et al*., reported that grinding of cyclohexane-1,3-cis-5-cis-tricarboxylic acid and 4,4′ -bipyridine in the dry state does not affect a co-crystal whereas grinding the two components with a few drops of MeOH facilitates complete conversion within minutes. Also, the solvent-drop grinding process prevents excessive use of crystallization solvent, hence it can be regarded as a ‘‘green’’ process.[5]

2.6.2 Solvothermal methods

The solvothermal method of preparing co-crystals utilizes fast evaporation of solvent under reduced pressure. In this method, the API and co-former are separately dissolved in solvent and mixed together in a stoichiometric ratio 1:1. The two solutions are mixed together over a specific period of time and transferred to a rota-vapour for drying at specified temperature for the synthesis of the co-crystal.

2.7 Preparation and manufacture of the co-crystal tablet

2.7.1 Direct compression method (Tablet compression)

The simplest tablet formulations are uncoated products that are made by direct compression after wet or dry granulation. In this study, the co-crystal tablet was prepared by direct compression. This is because direct compression can provide technical as well as economic benefits as it requires less processing steps compared with other manufacture techniques. The process of direct drug formulation is illustrated below;

Drug, excipient → blending → compression

2.7.1.1 Blending powders

The process of blending powders (two or more) requires homogeneity of the mixing substances hence they must be reduced to appropriate particle size in order to ensure, uniform and optimal combination. However, there are several factors that must be considered such as nature of ingredients, amount of powder and equipment. In addition, powders can be blended by methods such as spatulation, trituration, sifting and tumbling. Also, the powders can be mixed by passing them through sifters like those used in the kitchen to sift flour. This method is known to produce powders with light, fluffy characteristics.[6] The mixing of powders is a crucial step during the manufacture of almost all solid dosage forms. This implies that a mixture of two particles is considered perfect if any group of particles taken from any position within a mix contains the same proportions of each particle in relation to the whole mixture.[7] In this study, the procedure of blending of the co-crystals particles used is explained;

after drying the co-crystal by using rotor evaporator (V-700/710 (Buchi, Switzerland), the dried sample (co-crystal) was grinded by a mortar and pestle to reduce the particle size and achieve homogeneity of co-crystal particles. A sieve (No. 18) was used to mix and sieve the co-crystals and the excipients.

2.7.1.2 **Compression**

Tablet compression was performed by using a Single Punch Press (Manesty machine LTD, Type F3, No 1 L188, UK) machine. In this procedure, a quantity of co-crystal tabletting material flowed into the die. Afterwards, the upper and lower punches of the tablet machine compressed the material under a high pressure. The depth of the descent was controlled and this was determined by the tablet weight.[7] The die volume was adjusted to produce a tablet of theoretical weight of 250 mg each which was equipped with an 8 mm concave punch. The excipients of the branded formulation were selected and mixed with the co-crystal to improve tablet characterisations were necessary.

2.8 **Quality control (QC)**

The use of quality control (QC) on products is to validate and certify the safety of the products. In the case of tablets, like other dosage forms, they have to adhere to certain acceptable quality standards. In this study, the co-crystal tablets were manufactured by direct compression. Consequently, in order to determine the quality of the manufactured co-crystal tablets they were compared to the proprietor brand tablet and the following tests were performed during this study according to the USP.[8]

2.8.1 **Powder characterisation**

The physical properties of the co-crystal were determined by several methods such as angle of repose, moisture content, bulk density and tapped density. These tests are crucial in determining the flowability of the powder. The importance of flowability of powder helps to indicate whether the powder can be compressed directly with none or few complications with respect to tablet defects.

2.8.1.1 **Flowability-Angle of repose**

The angle of repose is a method used to determine the flowability of the granules. In this method, a vertically inclined glass cylinder is attached to a stand. The lower end of a glass cylinder causes a flush with a flat surface and a sheet of graph paper under the glass cylinder prior to lowering it. The sample was filled into the cylinder top and the cylinder was steadily and slowly raised thus allowing the powder to fall under the effect of gravity. If the sample is not disturbed, the spreading of the powder can be measured by marking the diameter of the powder sample and the height of the pile from the base to the apex. The values obtained are used to calculate the angle of repose, using the following equation 1.

$$\text{Tan } (Ø) = \text{height} / 0.5 \text{ base.} \qquad \text{equation 1.}$$

As a guide, if the angle is less than 30 °C, this is usually indicative of good flow while powders with angles greater than 40 °C are considered to be problematic.[7]

2.8.1.2 **Bulk density**

This is defined as the mass of a powder divided by the bulk volume and can be determined by the following method.[9] A sufficient quantity of powder is passed through a 1 mm pore size (No.18) sieve to break up agglomerated particles that might have formed during storage. 10 g of the test powder is slowly poured into a 10 mL cylinder. The powder is carefully smoothed in order to prevent compaction. The bulk density can be calculated using the following equation 2.

$$\text{Bulk density} = M \text{ (Mass of sample)} / Vo \text{ (Volume of cylinder)} \qquad \text{equation 2.}$$

2.8.1.3 **Tapped density**

The tapped density of powder samples can be determined by tapping a powder glass cylinder containing a known weight of powder sample. The tapping rate was set at 100 Reus/minute for a duration of 12 minutes and the tapped density was obtained by dividing the weight of powder by the total volume of powder attained after tapping. The mean value tapping density can be obtained after replicating the tapping

experiment for a minimum of 3 times and the mean of three determined. The values of the bulk density (section 2.8.1.2) and tapped density (section 2.8.1.3) can be used to calculate the Hausner's ratio (Equation 3) and Carr's index using equation 4.

$$Hausner's\ ratio = \frac{Tapped\ density\ (\rho_B\ \max)}{poured\ density\ (\rho_B\ \min)}\ x\ 100 \quad \text{equation } 3^{10}$$

$$\%\ compressibility = \frac{\rho_B\ \max - \rho_B\ \min}{\rho_B\ \max}\ x\ 100 \quad \text{equation } 4$$

2.8.1.4 Moisture content

A Mettler Toledo HR 73 Halogen moisture analyser was used to determine the moisture content of the samples. This test can also be determined by using the TGA analytical technique. In the sample analysis for moisture content, the Mettler Toledo HR 73 was set at 105 °C and switched on. 1 g of the sample is placed on a disposable aluminium pan and thereafter placed on the designed space on the moisture balance and weighed. The process of heating is started by activating the heating element and once the heating element (Al pan) goes off, the weight of the samples is displayed. The moisture content is recorded as percentage of the original samples used.

2.9 Evaluation of the tablet

After batch manufacturing of tablets, the next stage involves evaluation of tablet in order to ensure that the expected physical, chemical, aesthetic and mechanical properties are not compromised. The following tests were performed on the manufactured tablet.

2.9.1 Uniformity of mass

A METTLER DE series AJ 100 weighing balance was used to weigh twenty (20) tablets that were randomly selected. The selected tablets were dusted in order to remove all the small particles such as dust. Thereafter, each tablet was weighed individually in a weighing boat on a scale to two decimals and the displayed weights were recorded. The average was determined and the tablets were placed into one of 3 categories according to mass obtained per tablet.

2.9.2 Resistance to Crush

The tablets resistance to crush is an important mechanical test. This is because the acceptability of tablets by the consumer requires that tablets must enjoy certain amounts of strength or hardness and resistance to friability. Also, the tablets must be able to withstand mechanical shocks of handling in manufacture, packaging and shopping. The hardness of the tablets was measured using a Pharma test PTB 301. Ten (10) tablets were randomly selected of the brand and co-crystal formulation and resistance to crush measured. The machine consists of a pair of jaws facing each other. One jaw kept motionless while the other jaw is moved toward the tablet (i.e. forced was applied to the tablet). The maximum, minimum and the mean force were recorded in Newtons.

2.9.3 Friability

The friability test was performed as per United States Pharmacopeia (USP) guidelines using an Erweka TAD Gmbh friabilator. The friabilitator's plexi-glass drum was thoroughly dusted. Ten (10) tablets were dusted and weighed and their mass was recorded as Y1X. The tablets were placed into the drum and closed. The drum is attached on friabilitor and rotated at 100 revolutions (25/rpm for 4 min). The weight of tablets after the test was taken and recorded as Y2X. The difference in weight is expressed as a percentage of the initial weight. The acceptable mass loss is 1 %.

2.9.4 Durability

The Erweka TAD Gmbh was used to determine the percentage mass loss in durability. This is similar to the friability test. However, the plexidrum is rotated for 15 minutes and a loss is calculated in terms of percentage and the acceptable mass loss for the durability test is 2 % loss. According to the USP specifications, durability is non-official parameter.[8]

2.9.5 Disintegration time

The disintegration test was carried out in HCl (0.1 %) set at 37 °C ± 1 °C using the Apparatus Veego®. This machine consists of a basket and rack assembly containing six open-ended transparent tubes of USP-specified dimensions. A total of six (6)

tablets were placed in humidity conditions to mimic to the human stomach (37 °C ± 1 °C) and held vertically upon a 10-mesh stainless steel wire screen. During the disintegration testing, a tablet is manually placed in each of the six tubes of the basket. The basket is raised and lowered in the immersion fluid at 29 to 32 cycles per minute and the wire screens always below the level of the fluid. If one or two tablets from 6 tablets failed to disintegrate completely within 30 minutes, it is recommended that the disintegration test should be repeated with another 12 tablets. If less than 16 tablets did not disintegrate then the batch had failed the test. The disintegration time specification was set for an uncoated tablet (core).[6]

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CHAPTER 3

SELECTION RATIONALE OF THE BEST CO-CRYSTAL OR CYCLODEXTRIN COMPLEX FOR THE FORMULATION OF THE EFAVIRENZ TABLET DOSAGE FORM

3 CHAPTER 3

3.1 Introduction

In this chapter, the various techniques used in pharmaceutical preformulation by the crystal-engineering approach will be presented. In addition, this chapter will describe the various co-formers of efavirenz (EFA) co-crystals and EFA cyclodextrin (CD) inclusion complexes including the different methods for the improvement of EFA and its physicochemical properties. Also, chapter 3 will discuss the selection criteria for the EFA co-former and CD. Lastly, this chapter will briefly discuss the different variables that were investigated in the selection of the EFV co-former and CD.

3.2 Techniques used to improve the physicochemical properties of active pharmaceutical ingredients (APIs)

One of the challenges associated with the physicochemical properties of APIs is to select the best technique that can improve these properties. Crystal engineering provides an alternative route to physical and chemical modification of the drug substance including salts, amorphs, solvates, polymorphs, co-crystals and inclusion complexes (e.g. cyclodextrin inclusion complexes). However, all of these drug substances have limitations in their utility.[1] Co-crystals for example, are widely known for their usefulness especially with APIs that are non-ionisable and whose salt cannot be produced. In the case of ionisable drugs, co-crystals have the ability to form large numbers of suitable ligands that can exceed the number of its parent drug.[1] Also, another benefit of co-crystals is that non-toxic co-crystal formers (or co-formers) can be incorporated into a co-crystalline reaction.[2] In principle, a co-crystal is only anticipated to form if it is thermodynamically more stable than the crystals of its individual components.[3] CDs are also known to exhibit unique characteristics that have been identified to improve the physicochemical properties of APIs. These characteristics have been extensively discussed in chapter 1, section 1.8, pp 16-23. The combination of the unique properties of co-crystals and CD inclusion complexes have significant effects on the physicochemical properties of APIs. For instance, dissolution rate and solubility can be improved irrespective of the toxicity level of the second component (i.e. guest compound).[4] In effect, some of the co-crystals and CD

complexes can be considered as pharmaceutically acceptable solids for dosage form development during the pre-formulation stage of a selected API.

3.3 Selection rationale of the best co-crystal or cyclodextrin complex for the formulation of the Efavirenz tablet dosage form

The selection parameters for the best co-crystal or CD complex for the formulation of an EFA tablet was crucial considering the overall objectives of this study. The selection criteria was performed based on the available literature reports on co-crystals and CD inclusion complexes of EFA. Firstly, one of the important selection considerations was the method of preparing each of the co-crystals and CD inclusion complexes. Co-crystal or CD inclusion complexes of EFA could be prepared by several methods. The solvent-drop grinding method was used to prepare co-crystals from different co-formers such as 4,4-bipyridyl, 1,4-cyclohexanedione and oxalic acid dihydrate.[1,5] As for the solvothermal methods, co-crystals were prepared by using citric acid monohydrate (CTRC) as its co-former.[1] In the preparation of CD inclusion complexes, kneading, freeze drying and co-precipitation with different types of CDs was used to prepare the complexes of EFA.[6]

In Table 3.1, co-crystals of EFA found in literature was evaluated in order to choose an ideal co-former for the tablet formulation. Table 3.2 considered the cyclodextrin complex of EFA and its respective CD hosts found in literature. The variables investigated in Tables 3.1 and 3.2 are; safety/toxicity, melting point, solubility, stability, cost, side effects, uses and pka value.

Table 3-1: Variables selected to identify an ideal co-former of an EFA co-crystal

	Citric acid monohydrate	*Oxalic acid dehydrate*	*4,4-Bipyridyl*	*1,4-Cyclohexanedione*
Molecular Weight	210.14 g/mol	126.07 g/mol	156,18 g/mol	112.13 g/mol
Molecular Formula	$C_6H_{10}O_8$	$C_2O_4H_2 \cdot 2H_2O$	$C_{10}H_8N_2$	$C_6H_8O_2$
Appearance	White crystals or powder	Colourless crystals, crystalline powder	Crystalline off-white to tan powder	Yellow crystalline
Water solubility	Soluble in water, 1630 g/L at 20 °C	Soluble in water 138 g/L at 20 °C	Not soluble	Very soluble.
pKa value at 25 °C	pK_a: pK_1: 3.13, pK_2: 4.76, pK_3: 6.4	pK_1: 1.23, pK_2: 4.19	10.73 (pKb) pH: 11	NA
Stability	Highly stable	Highly stable	Stable at recommended storage condition	Stable under ordinary conditions
Melting point	135 °C	104-106 °C	109 - 112 °C	77-79 °C
Use	Acidulant in beverages, confectionery, cheese, effervescent salts, pharmaceutical syrups, elixirs, effervescent powders, and tablets. As a natural preservative, flavouring and antistaling agent in food	Purifying agent in pharmaceutical industry especially in antibiotic medication, such as oxytetracycline, chloramphenicol, etc	In luminescence chemistry and used in spectrophotometric analysis. Organic synthesis and polymerization	As an intermediate for pharmaceuticals, herbicides, plant growth regulator and other organic products.
Cost	500g - 224.76 R	500g -741.52 R	500g -149365.00 R	500g -14436.50 R
Safety/ Toxicity	It has non-toxic characteristics when orally ingested and used as an excipient. Acute oral toxicity (LD50) in rats is 6730 mg/kg. It is endorsed by professional and regulatory bodies e.g. US Food and Drug administration.	LD50 in rats is 375 mg/kg. it is not in the GRAS list Oxalic acid is toxic because of its acidic and chelating properties	The LD50 Oral - rat is 172 mg/kg hence considered toxic if swallowed.	*EC50 - Daphnia magna (Water flea) - > 100 mg/l - 48 h
Side-effects	May be harmful if swallowed. Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhoea.	The side effect of oxalic acid occurs via ingestion and during contact with the body causing gastrointestinal tract burns, ulcerations of the mouth, vomiting of blood, and rapid appearance of shock, convulsions, twitching, tetany, cardiovascular impairment, kidney and brain damage.	It is harmful and toxic after inhalation, swallowing and when in contact with skin. The following symptoms have been reported or observed; lacrimation, eye behavioural somnolence (general depressed activity), lungs, thorax or respiration and dyspnoea.	The toxicological properties have not been fully investigated.

Table 3-2: Variables selected to identify an ideal CD inclusion complex of EFA

	***β*-CD**	**Hydroxypropyl *β*-CD**	**Randomly ethylated *β*-CD**
Molecular Weight	1134.98 g/mol	1400 g/mol	1312 (1135 + n·(14.0)) g/mol
Molecular Formula	$C_{42}H_{70}O_{35}$	$C_{42}H_{70}O_{35}(C_3H_6O)x$ (where x = 7 molar substitution)	$C_{42}H_{70}$-nO_{35} · $(CH_3)n$
Appearance	White fine crystalline powder	White or almost white, amorphous or crystalline powder	white to slight yellow powder
Water solubility at 25 °C	Sparingly soluble 1.85 (g/100 mL)	Very soluble (> 600) (g/100 mL)	Highly soluble (>500) (g/100 mL)
pKa value at 25 °C	12.202	12.202	12.202
Stability	*β*-CD and other CDs are stable in the solid state if protected from high humidity.		
Melting point	255–265 °C 290 °C (decomposes)	278 °C	180-182 °C
Use	Solubilizing and stabilizing agent	Complexing agent, dissolution enhancer, release-modifying agent, sequestering agent, solubilizing agent, stabilizing agent, tonicity agent.	Complexing agent
Cost	3,486.16 R (250 mg)	24.94 R (250 mg)	25 R (250 mg)
Safety/ Toxicity	Considered not toxic since LD50 (rat and oral) is 18.8 g/kg	Low toxicity with LD50 (rat) >2 grams/kg It has been suggested that hydroxypropyl betadex may have a synergistic toxic effect with carcinogens especially in the increase of their solubility and hence bioavailability	Not toxic since LD50 (rat) is >8 g/kg

3.3.1 Safety and toxicological consideration

To ensure that manufactured drugs are fit for human consumption, safety and toxicological evaluations of these drug substances are crucial. When using co-formers and CDs, safety and toxicological analysis must not be compromised in order to guarantee their approval for human consumption. The acceptability of pharmaceutical products is such that the non-API component (co-former) must be non-toxic and without any known adverse side effects to humans. Therefore, these properties i.e. non-toxicity and lack of side effects limit their ability to co-crystallize compared with those that have been approved for human consumption. It is imperative that the safety of any manufactured drug be guaranteed after the drug has successfully undergone certification by appropriate professional bodies such as the US Food and Drug Administration (FDA). Consequently, for it to be globally accepted as fit for human consumption, a co-crystal former must be on the US FDA "Everything Added to Food in the United States" (EAFUS) list. This list is known to consist of more than 3000 substances which can be considered suitable as food additives or approved as Generally Regarded as Safe (GRAS).[7]

According to available literature, there were four (4) co-crystals prepared with EFA (Figure 3.1). Powder X-ray diffraction (PXRD) studies revealed that these co-crystals have different crystalline identities compared with the API and co-former used.[1] However, the 4,4-bipyridyl and 1,4-cyclohexanedione are non-pharmaceutical co-crystals while the oxalic acid dihydrate co-former no longer appears on the GRAS list whereas the citric acid monohydrate co-former is a non-toxic material included on the GRAS list. The implication of this is that the majority of national and international food regulatory agencies including the USFDA and GRAS have approved citric acid as a safe food additive.[8]

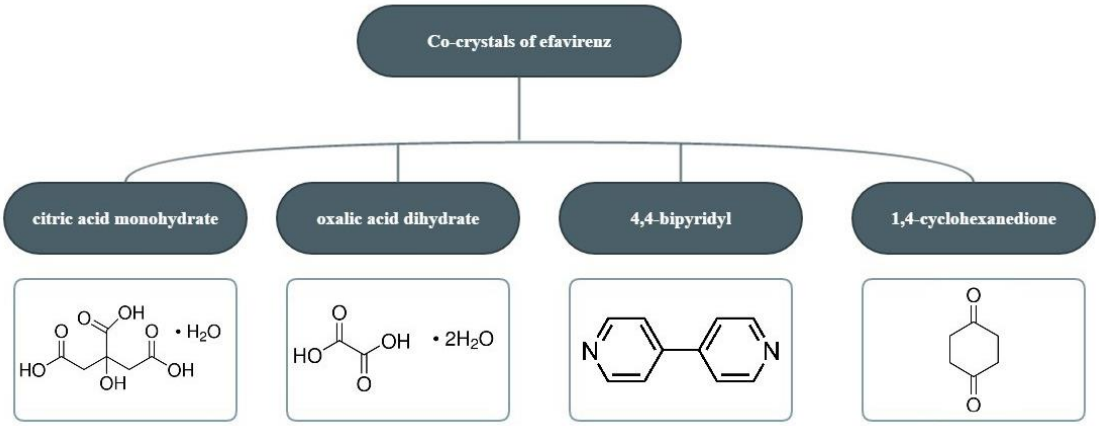


Figure 3-1: Efavirenz co-crystal with different co-formers illustrated

The lethal dose$_{50}$ (LD50) is a dose that is harmful to 50 % of a given population. The materials safety data sheet (Merck & Co) indicated that the LD50 for rats via oral administration was 6730 mg/kg for citric acid monohydrate while 375 mg/kg for oxalic acid dihydrate (Sigma-Aldrich). The LD50 for non-pharmaceutical co-formers, 4,4-bipyridyl, was 172 mg/kg (Sigma-Aldrich) and 1,4-cyclohexanedione was not reported in any of the available data references.

The regulatory status of CDs continues to evolve due to the lack of consensus and continuous research towards understanding their beneficial pharmaceutical properties.[9] For instance, the naturally occurring CDs can be found in several pharmaceutical formulations in many countries. The naturally occurring CDs under certain conditions are GRAS by the FDA. Also, they are listed in both the European Pharmacopoeia (Ph.Eur.) and US Pharmacopoeia (USP/NF) as well as in the Japanese Pharmaceutical Codex (JPC).[9] From a regulatory point of view, a profile for *β*-CDs already exists and is available in both the US Pharmacopoeia/National Formulary (USP 23/NF 18, 1995) and the European Pharmacopoeia (3rd ed., 1997).[9] For instance, a profile for the preparation of 2-HP-*β*-CD can be found in the US Pharmacopoeia/National Formulary. Also there are various monographs for CDs that have been included in compendia sources e.g. the *Handbook of Pharmaceutical Excipients*.[8] Interestingly, after more than one century after the discovery of CDs, they are finally and rapidly being accepted as 'new' pharmaceutical excipients.[10] The water-

soluble CD derivatives have been listed in the Pharmaceutical Excipient Handbook and categorized as a "related substance" of the *β*-CD. The handbook provides details on several water-soluble CD-derivatives such as dimethyl-*β*-CD, 2-hydroxyethyl-*β*-CD, 2-HP-*β*-CD, 3-hydroxypropyl-*β*-CD and the trimethyl-*β*-CD. However, there are no water soluble CD derivatives that are known to be listed in any Pharmacopoeia to date.[11] Although there are reports of CD inclusion complexes with efavirenz especially with the *β*-CD and derivatized CDs. Sathigari *et al*., (2009)[6] described complexes of EFA with *β*-CD, HP-*β*-CD and randomly methylated-*β*-CD (Figure 3.2).[6] The LD50 for both rat and oral was reported as 18.8 g/kg of *β*-CD,[12] oral rat: LD50: >2 g/kg (United States Pharmacopeia. Material safety datasheet: Hydroxypropyl betadex, October 2002) of HP-*β*-CD. The toxicity of hydroxypropyl-*β*-CD material was found to be low while the randomly methylated *β*-CD has a higher LD50 rat (g/kg) > 8.[13]

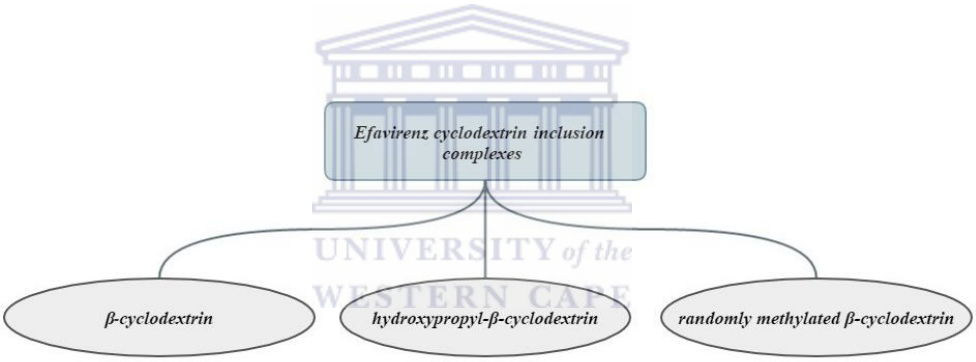


Figure 3-2: Efavirenz cyclodextrin inclusion complexes.[6]

3.3.2 Melting point

The melting point of a substance is the temperature at which the solid phase and the liquid phase are in equilibrium. Melting point determination is one of the earliest practices used to assess the purity of a compound. However, this method is an orthodox practice. In pharmaceutical sciences, the melting point is considered as an important factor as a result of its relationship with aqueous solubility and vapour pressure of a substance. Specifically, the melting point of a substance has been directly associated to the logarithm of solubility. It has been established that there is a direct proportionality relationship between the melting point of the co-crystal and co-formers.[2] For instance, the API AMG517 (analgesic) was co-crystallized with 10 co-formers by taking advantage of the relationship in their melting points where the

melting point of the co-crystal was attributed to the variability of co-formers melting point. In this study[7] it was shown that the melting points of a set of AMG517 co-crystals can be modified depending on the co-former that was selected i.e. a co-crystal with a higher melting range translates to a co-former with similar properties.[2] The study surveyed the analysis of 50 co-crystalline samples and reported that 51 % co-crystals had melting points between those of the API and co-former and 39 % were lower than either the API or co-former. Also, 6 % were found to be higher while 4 % had the same melting point as either the API or co-former. The study concluded that the melting point of an API can be altered through forming co-crystals and the final product will often have a melting point that is higher or in between that of the API and co-former and may be lower than the API or co-former. Also, if a lower melting solid is required and covalent modifications to the API cannot be attained, then another viable option is to co-crystallize the API.[2] The descending order of melting point among the co-formers for EFA is such that citric acid monohydrate has the highest melting point (135-152 °C) followed by other co-formers such as oxalic acid dihydrate (104-106 °C), 4,4-bipyridyl (104-106 °C) and 1,4-cyclohexanedione (77-79 °C). For the EFA-CD complexes, HP-β-CD has the highest melting point 278 °C, then β-CD with 255–265 °C, followed by randomly methylated-β-CD at 180-182 °C.

3.3.3 **Solubility**

Co-crystal and CD inclusion modification can be used to improve the solubility of EFA. There are several applications for CDs especially in the pharmaceutical field and specifically in the improvement of water solubility of pharmaceutical drugs. For instance, the addition of α- or β-CD to poor water soluble drug substances results in the increase of their water solubility properties. Similarly, these results can be used to improve bioavailability and increase the pharmacological effect, thereby allowing a reduction in the dose of the drug to be administered.[10] In the early application of pharmaceutical CDs, the β-CD was the most commonly used because of its availability. Also, β-CD has pharmaceutically useful complexation characteristics with a wide range of drugs such as Nimedex and Omebeta.[11,14] According to experimental data from literature, HP-β-CD was reported to be more soluble than randomly methylated and β-CD with solubility values at 25 °C to be > 600, >500 and 1.85

respectively.[14] For co-crystals, several studies on the solubility properties of co-crystals have focused on kinetic measurements of dissolution. However, the kinetic dissolution results are influenced by phase transformations, surface area, and particle size distribution of the drug as well as fluid dynamics and experimental apparatus. Another limitation associated with the factors that influence kinetic dissolution is that they are difficult to quantify and not easily reproducible. In the characterization of drugs, the kinetic and equilibrium solubility analysis can serve as an alternative and complimentary analytical method and they are useful for addressing oral absorption limitations highlighted in the Biopharmaceutical Classification System (BCS).[4] For instance, kinetic solubility values are often approximate values that are based on single measurement per time point whereas in equilibrium solubility, a series of time points and measurement are recorded to confirm that the solution has reached equilibrium and this is validated by a plateau in the concentration data. The study by Good, D. J.and Rodríguez-Hornedo, N (2009)[4] showed that the co-crystal solubility was dependent on the concentration of the ligand i.e. co-formers in solution. As a result, the dissociation of the co-crystal in solution was described by the solubility product (Ksp). The Ksp is defined as a product of drug and ligand solution concentrations and is analogous to the Ksp of salt forms defined by the product of ionized drug and counterion concentrations. The Ksp value is a constant that reflects the strength of co-crystal solid-state interactions of drug and ligand in relation to interactions with the solvent. Also, the co-crystal solubility product behaviour has shown that high ligand concentrations are associated with low solution drug levels.[4]

Solubility is a thermodynamic parameter, while dissolution is a kinetic phenomenon and these two factors are important for pharmaceutical solids. This is because a drug only delivers its therapeutic effect provided there is a sufficient amount of the effective dose rapidly absorbed in the stomach and gastrointestinal tract. In this case, the equilibrium method of solubility measurement is suited for those drugs especially when they do not undergo transformation in the biological medium which is often aqueous with pH range 1-7 and also for an extended period i.e. 24 and 48 hours.[15] At 20 °C, the water solubility of citric acid monohydrate and oxalic acid dihydrate was 1630 g/L[16] and 138 g/L[17], respectively whereas 1,4-cyclohexanedione was reported to

be very soluble while 4,4-bipyridyl was not soluble. The equilibrium solubility study of citric acid monohydrate was observed to increase as EFA/CTRC co-crystal compared with EFA/OXA co-crystal. Also, the equilibrium solubility studies showed that there was 2.7 and 1.8 fold enhancement solubility for the co-crystal of EFA/CTRC and EFA/OXA, respectively.[1]

3.3.4 **Stability**

In the pharmaceutical industry, the stability of a solid drug material is important but this depends on the atmospheric conditions such as moisture content. This is due to the practical implications of possible hydrate formation upon processing, formulation, storage and packaging of a drug substance.[18] Stability is the ability of a drug substance or drug product to stay within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods.

As a result, the stability of a drug substance is a crucial parameter that must be carefully studied especially during the development of a new chemical entity. Consequently, the different type of stability that must be considered largely depends on the structure and characteristics of the molecule. In effect, the chemical and physical stability data of a drug substance are commonly obtained at accelerated stability conditions in order to determine their developability and shelf- life. The water uptake capacity of a drug substance is included from both handling and packaging point of view. This is because the amount of water present in a drug substance can lead to form changes, degradation and may become worse if the effect of the water uptake is not investigated at the early stage during drug manufacturing processes.[2] In the case of EFA, all the co-formers such as citric acid monohydrate, oxalic acid dihydrate, 4,4-bipyridyl and 1, 4-cyclohexanedione used to prepare EFA co-crystals are stable under recommended storage conditions according to Sigma-Aldrich.

CDs are used to improve the stability of substances due to their ability to increase drug substance resistance to hydrolysis, oxidation, heat, light and metal salts. Also, the inclusion of irritating products in CDs can as well serve as protection to the gastric mucosa especially for drug administration through the oral route and reduce skin

damage for the dermal route.[10] CD interaction with labile compounds can result in several outcomes such as ability to retard degradation, no effect on reactivity or accelerate drug degradation.[19] *β*-CD and other CDs are known to be stable in the solid state provided they are protected from high humidity.

3.3.5 **Cost effectiveness**

In every industrial manufacturing process, cost-effectiveness is an underlining factor that should be considered especially in the design of strategies for preparation of drugs and in the choice of co-former and CD. For instance, the unit price of commercial co-formers used in this study from the company, Sigma-Aldrich were R 224.74 for 500 g of citric acid monohydrate, R 741.52 for 500 g of oxalic acid dihydrate, R 144365 for 500 g of 4,4-bipyridyl and R 14436.50 for 500g of 1,4-cyclohexanedione. The cost of *β*-CD, HP-*β*-CD and randomly ethylated *β*-CD was R 3,486.16, R 24, 94 and R 25 for 250 mg, respectively.

Based on the review of the selected variables, i.e. safety/toxicity, melting point, solubility, stability, cost and side effects, CTRC and HP-*β*-CD proved to be the most ideal co-former and CD for EFA.

In the next section (section 3.4), the following variables i.e. angle of repose, Carr's index, Hausner's ratio, moisture content, disintegration time, Hardness/ Resistance to crush, manufacturing process problem and particle size (table 3.3) were investigated for the chosen co-former (CTRC) and cyclodextrin inclusion complex (HP-β-CD) to determine their suitability in the preparation of a tablet dosage form.

Table 3-3: Selected variables for the EFA co-crystal with citric acid monohydrate as co-former and the EFA CD complex with hydroxypropyl-*β*-CD

Co-former	*Citric acid monohydrate*	*Hydroxypropyl β- CD*
Molecular Weight	210.1 g/mol	1400.0 g/mol
Molecular Formula	$C_6H_{10}O_8$	$C_{42}H_{70}O_{35}(C_3H_6O)x$ (where x = 7 molar substitution)
Appearance	White crystals or powder	White or almost white, amorphous or crystalline powder
Water solubility	Soluble in water 1630.0 g/L at 20 ºC, Soluble in water (59.2 g/100 ml) at 20 °C	Very soluble (> 600.0 mg/ml) at 25 ºC
pKa value at 25°C	pK_a: pK_1: 3.1, pK_2: 4.7, pK_3: 6.4	12.2
Stability	Highly stable. Citric acid monohydrate loses water of crystallization in dry air or when heated to about 40 °C. It is slightly deliquescent in moist air. Dilute aqueous solutions of citric acid may ferment on standing. The bulk monohydrate or anhydrous material should be stored in airtight containers in a cool, dry place.	*β*-CD and other CDs are stable in the solid state if protected from high humidity.
Melting point	Melts completely at 135-152 °C.	278 °C
Use	Acidulant in beverages, confectionery, cheese, effervescent salts, pharmaceutical syrups, elixirs, effervescent powders and tablets. As a natural preservative, flavouring and antistaling agent in food.	Complexing agent, dissolution enhancer, release-modifying agent, sequestering agent, solubilizing agent, stabilizing agent, tonicity agent.
Cost	500 g-224.8 R	500 g-49873.0 R
Safety	It has non-toxic characteristics when orally ingested and used as excipient. Acute oral toxicity (LD50) in rat is 6730 mg/kg. It is endorsed by professional and regulatory bodies e.g. US Food and Drug administration.	Low toxicity with LD50 (Rat) >2 g/kg It has been suggested that hydroxypropyl betadex may have a synergistic toxic effect with carcinogens especially in the increase of their solubility and thus bioavailability.
Side-effects	May be harmful if swallowed. Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhoea.	NA
Ratio and solubility increase	1:1 and 2.7 fold	1:2 and 2.9 fold
Hardness/ Resistance to crush	35 N	60-101 N

moisture content	1.5-2 %	6.5 %
Disintegration time	110 sec.	300 sec.
Angle of repose	28.07°	20.79°
Carr's index	37.59	22.41
Hausner's ratio	1.60	1.28
Manufacturing process problem	None observed	Capping
Particle size	671.0 x 738.5 μm	166.4 x 159.1 μm

3.4 Investigated variables suitable for tabletting

3.4.1 Angle of repose

The successful manufacturing of tablets from powdered substances depends on some important properties such as the flowability of powdered samples. The flow properties simply describes the ability of powdered samples to resist the differential movement between particles especially when the powdered sample is subjected to external stresses. Generally, the resistance of movement of the powdered sample may be caused by the cohesive forces between particles.[20] In Table 3.4 below, the parameters as well as the standards that are often considered in order to determine the angle of repose of powdered samples are presented.[20]

Table 3-4: Angle of repose[20]

Angle of repose (°)	Type of flow
25-30°	Excellent
31-35°	Good
36-40°	Fair (flow aid not needed)
41-45°	Passable
46-55°	Poor
56-65°	Very poor
Over 66°	Very, very poor

In this study, the angle of repose was investigated for CTRC and HP-*β*-CD and was found to be 28.07° and 20.79°, respectively. According to previous studies reported in the literature (Table 3.4).[20] The flowability property of both CTRC and HP-*β*-CD used in this study were found to exhibit excellent flow properties.

3.4.2 Compressibility (Carr's index) and Hausner's ratio

The Carr's index and Hausner's ratio have always been used to determine the flow properties of powders and compressibility (Table 3.5).[20]

Table 3-5: Relationship between powder flowability, % compressibility and Hausner's ratio. [20]

%Compressibility (Carr's index)	Type of flow	Hausner's ratio
1-10	Excellent	1.00
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
> 38	Very, very poor	>1.60

The Carr's index of CTRC was 37.59 which represents a very poor flowability whereas the flowability value of HP-*β*-CD was 22.41 which is considered as passable flowability. The calculated Hausner's ratio of CTRC was 1.60 which suggests that the powder exhibits very poor flow properties. The Hausner's ratio calculated for HP-*β*-CD was 1.28 which represents fair flowability of the powder. The experimental Carr's index and Hausner's ratio for CTRC and HP-*β*-CD correlate with each other but not with the angle of repose.

3.4.3 Moisture content

Moisture Content (MC) is defined as the amount of water or water vapour contained within a substance. The lower the moisture content, the lesser the excipients needed during compression. One of the main disadvantages of the presence of a high water content is that it confers a higher cohesion to the powder particles and also allows plastic deformation and capping. The MC is expressed as the percentage by mass of water in a sample of a mixture or form of matter. The MC for the CTRC used in this study was analysed and found to occur between 1.5 % and 2 % at heating temperature of 105 °C for 80 seconds. In the case of HP-*β*-CD, the MC was determined as 6.47 % for 200 seconds. The result showed that there was a relatively low percentage in moisture content of CTRC compared with HP-*β*-CD.

3.4.4 **Disintegration time**

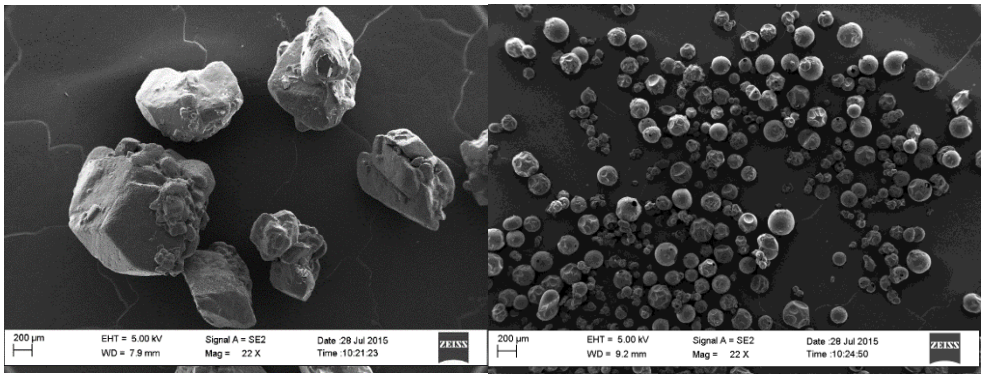
100 mg of each of CTRC and HP-*β*-CD were weighed separately and each transferred into the single punch machine to compress it into a tablet dosage form without any excipients. The disintegration time was measured as the time taken for the tablet to break into smaller particles. From the results shown, both the CTRC and HP-*β*-CD disintegrated within less than 30 minutes as stated for uncoated tablets according to the USP.[21] The disintegration time for CTRC was 110 seconds (1.83 min) while the HP-*β*-CD disintegrated within 300 seconds (5 min).

3.4.5 **Manufacturing process problems**

The problems that are associated with the use of either HP-*β*-CD or CTRC in the preparation of tablets have shown that tablets prepared from CTRC compress easily compared with the tablets prepared from HP-*β*-CD. This was attributed to the moisture content present in HP-*β*-CD which was measured to be at least 6 %. In order to confirm the presence of moisture during tablet manufacturing the compressibility force of the machine was increased but the presence of moisture could also cause powdered sample of HP-*β*-CD or CTRC to stick to the punch faces.[22]

3.4.6 **Particle size analysis**

The particle size of the drug substance is important as it can affect drug characteristics such as content uniformity in tablets and bioavailability. The particle size comparison of the CTRC and the HP-*β*-CD showed that CTRC has a particle size of 671.0 x 738.5 μm with the crystal structure being orthorhombic which was significantly large compared to HP-*β*-CD. The particle size of HP-*β*-CD was found to be 166.4 x 159.1 μm (figure 3.3) and observed as “shrinked” cylindrical spheres.



(a) (b)

Figure 3-3: SEM pictures of (a) citric acid monohydrate and (b) hydroxypropyl-β-CD

3.4.7 Resistance to crush/Hardness

The resistance to crush is the force in Newton that is applied to a tablet and required to break a tablet along its diameter by applying compression loading. The resistance to crush was investigated for CTRC and HP-*β*-CD and was found to be 35 N and 60-101 N, respectively.

3.5 Summary

According to the investigated variables that were required to determine the suitability of co-former or CD for tabletting, it was found that citric acid monohydrate was the better option to proceed to large scale laboratory preparation of the preformulation stage. CTRC was more economical than HP-*β-CD*, had good solubility with no side effects and a good safety profile. The flowability property of both the CTRC and HP-*β*-CD showed that they exhibited excellent flowability which lies within the acceptable range. As for the Carr's index analysis of CTRC, its value was 37.59 and this represents a very poor flowability whereas the flowability value of HP-*β*-CD was 22.41 which is considered as passable flowability. The MC result showed that there was a relatively low percentage in moisture content within CTRC compared with HP-*β*-CD. The disintegration analysis for CTRC show that its disintegration time was 110 seconds while the HP-*β*-CD disintegrated within 300 seconds. The manufacturing problem encountered during the process of making tablets was such that the HP-*β*-CD did not compress easily unlike the CTRC.

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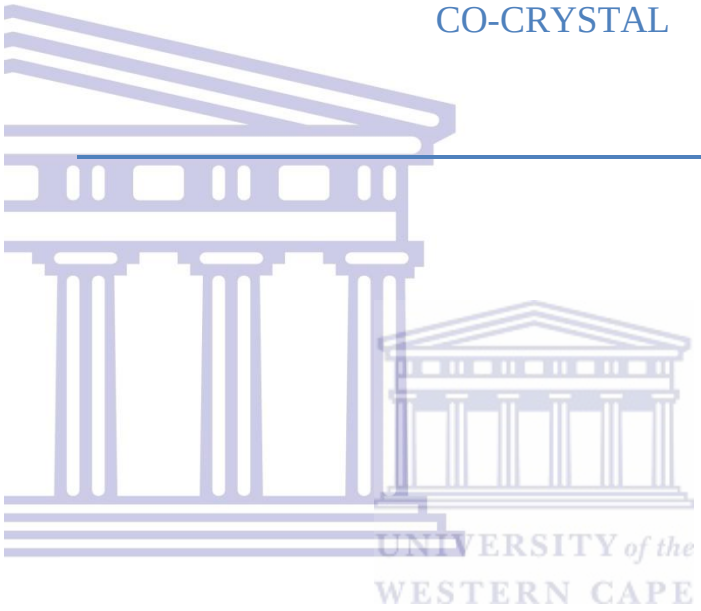
CHAPTER 4

SMALL LABORATORY SCALE PRODUCTION OF EFA/CTRC CO-CRYSTAL



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4 CHAPTER 4

4.1 Introduction

This chapter 4 will present the characterization results of the active pharmaceutical ingredient (API) i.e. efavirenz (EFA) (B030675) and co-former, citric acid monohydrate (CTRC) by hot stage microscopy (HSM) and differential scanning calorimetry (DSC). This chapter will also describe the different experiments that were performed to prepare small laboratory scale of Efavirenz/Citric acid monohydrate (EFA/CTRC) co-crystals by the solvothermal method.

4.2 Characterization of EFA (API)

4.2.1 Hot stage microscopy (HSM)

Hot stage microscopy (HSM) was used to investigate the thermal behaviour of EFA after heating the sample at a rate of 10 °C/min. A small amount of the sample powder was immersed in silicone oil to assess the presence of water or organic solvent which is often indicated by the formation of bubbles. The HSM measurement was performed from 30 °C to 250 °C. In Figure 4.1, the HSM results of EFA is presented and followed by a discussion.

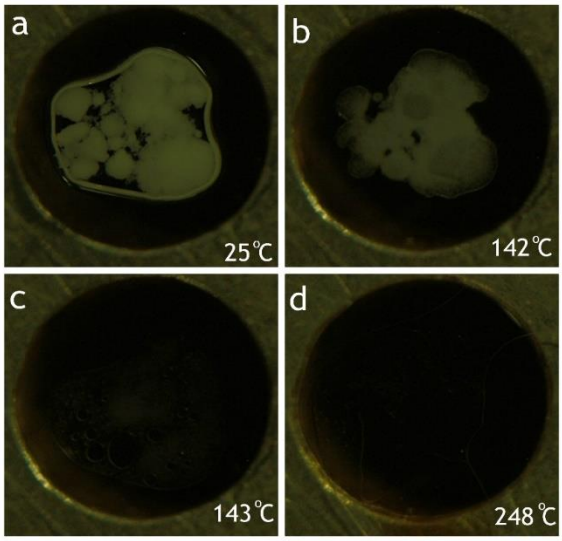


Figure 4-1: HSM analysis of EFA

The HSM analysis (Figure 4.1) of EFA showed a sequence of events in thermal behaviour. The EFA sample started to melt at 142 °C (b) and completely melted at 143 °C (c). There was no presence of bubbles between 25 °C (a) and 142 °C (b) which suggests that the EFA did not contain volatile impurities or any trace of water. However, in (d) bubbles were observed at 248 °C and the EFA decolourized of which both events is indicative of the decomposition stage of the sample.

4.2.2 **Differential scanning calorimetry (DSC)**

The differential scanning calorimetry (DSC) analytical technique can be used to establish the thermal profiles such as melting point, phase transition and decomposition of a sample. DSC analysis was performed on EFA in order to determine its thermal properties in Figure 4.2.

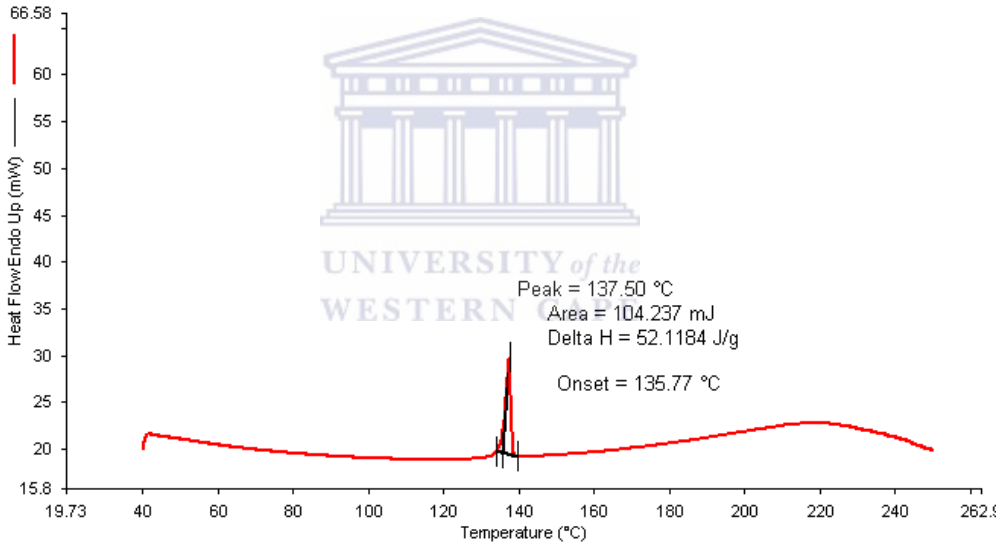


Figure 4-2: DSC analysis of EFA

The DSC result of EFA showed a single endotherm commencing from 135.77 °C to 140 °C and with a characteristic peak identified at 137.50 °C. The peak at 137.50 °C suggests the melting point of EFA. The DSC analysis of EFA as well as the HSM result showed that the melting point of EFA was within same range. In addition, an endothermic bump was observed between 220 °C and 250 °C associated with the degradation of the EFA sample concurring with the HSM analysis.

4.3 Characterization of citric acid monohydrate (CTRC)

4.3.1 Hot stage microscopy (HSM)

HSM was performed from room temperature (25 °C ±2) to 300 °C at a heating rate of 10 °C/min. In Figure 4.3 below, the HSM images of CTRC is presented.

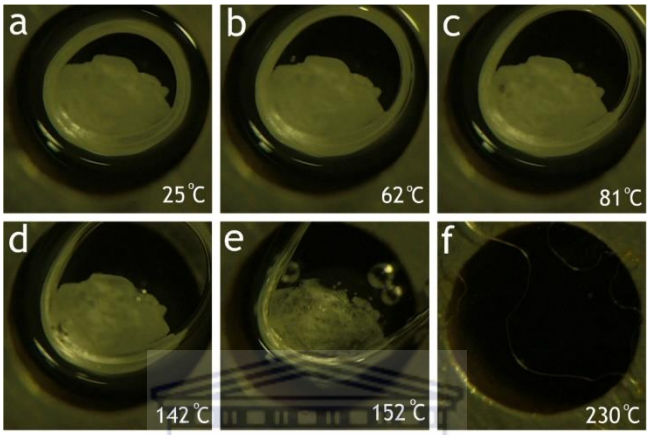


Figure 4-3: HSM images of citric acid monohydrate

The HSM result of CTRC (Figure 4.3) did not indicate any noticeable change in the physical appearance of the CTRC sample from 20 °C to 81 °C (a to c). After heating to 142 °C (d), bubbles were observed suggesting desolvation if the sample followed by the onset of melting and a complete melt was observed at 153 °C (e) due to the large sample size. The CTRC sample decomposed at 230 °C (f) with a characteristic discoloration not clearly visible on the picture talent above.

4.3.2 Differential scanning calorimetry (DSC)

DSC study for CTRC (co-former) was performed using the Perkin Elmer DSC7 instrument. The melting point, phase transition and decomposition of the samples were investigated and the DSC result is presented in Figure 4.4.

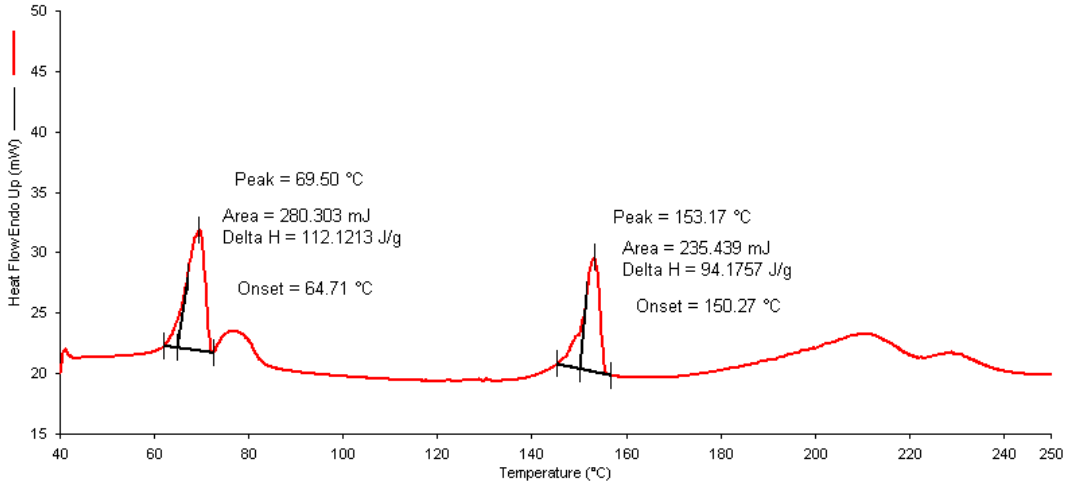


Figure 4-4: DSC analysis of CTRC (co-former)

The DSC analysis showed the presence of two endothermic peaks prior to the melt peak 153 °C. The endotherms at 69.5 °C and 78 °C was attributed to the desolvation of water from CTRC while the peak that was observed at 153.17 °C corresponds to the melting point of the CTRC sample. The melt of the sample fell well within the range of 140 to 154°C. Finally, two additional bumps were noticed between 220 °C and 250 °C and these events represent the degradation stage of the sample. The DSC analysis of CTRC agreed with the observed HSM analysis.

4.4 Preparation challenges during laboratory (small) scale production of the EFA co-crystal

Scaling up is an important industrial milestone especially for commercial viability of products that have been successfully prepared in the laboratory. This process involves the manufacture of larger batches from small-scale production. However, there are several challenges associated with a scaling-up process, hence it was considered as one of the main focal points of this study. At the laboratory scale, the EFA/CTRC co-crystal was successfully prepared by using the solvothermal method described by Chadha *et al.* (2012).[1] There were some ambiguities in the method described by Chadha *et al.*, 2012 as there was limited information on how to prepare the efavirenz co-crystal. For instance, the concentration of ethanol was not stated.

4.4.1 Differential scanning calorimetry (DSC)

According to the DSC results reported by Chadha *et al*. (2012),[1] the DSC scan of EFA/CTRC co-crystal showed a melting peak at 123.36 °C which is different from the DSC results observed for the physical mixture of the starting compound. Also, the melting peak of EFA was between 139 °C-141 °C and CTRC was at 154 °C. It was thus established that the EFA/CTRC m.p. was significantly different from the parent compounds suggesting that EFA/CTRC was a co-crystal.[1]

4.4.2 Experiment 1 (Exp 1) 50 % ethanol

In Exp1 of this study, ethanol 50% was added to 0.02 g EFA and 0.013 g CTRC in a 1:1 stoichiometric ratio. The EFA and CTRC were weighed separately and transferred to the same glass vial. The mixture (i.e. EFA and CTRC) was dissolved in 5 ml ethanol (50%) at 10 °C below the boiling point of the solvent. The mixture was continuously stirred using a magnetic stirrer until a clear solution was obtained and filtered using a 0.45 μm filter attached to a syringe. The filtered solution was transferred to a rota-vapour (V- 700/710 Buchi, Switzerland). The clear hot solution was then evaporated immediately under vacuum at a reflux temperature of 80 °C for 3 hours. A solid powder was obtained and scratched from the walls of the test tube and stored in a vial for analysis.

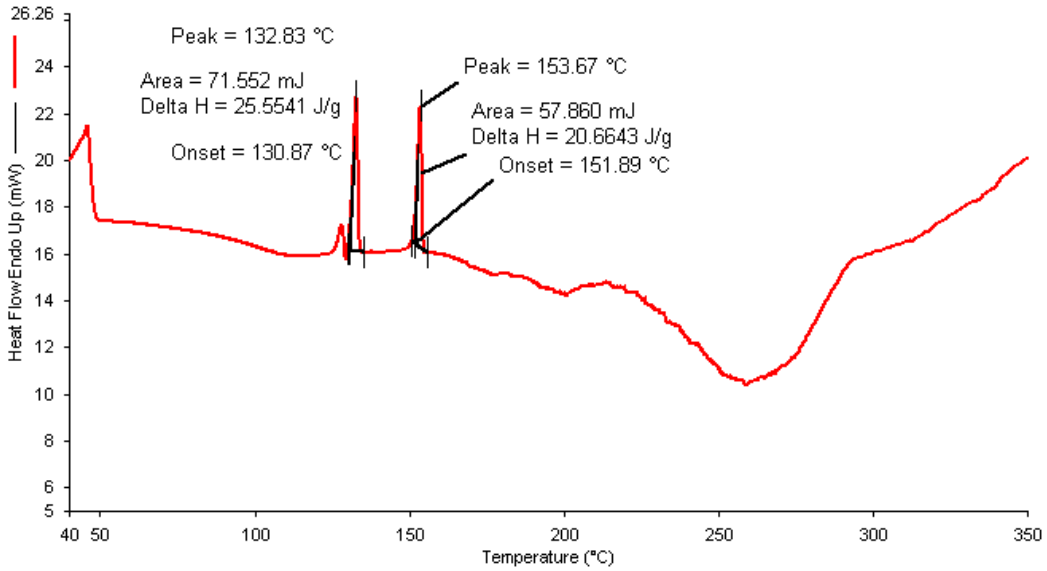


Figure 4-5: DSC analysis of EFA/CTRC (Exp 1)

The DSC analysis in figure (4.5) showed three peaks, a small peak observed at 125 °C which could indicate EFA/CTRC co-crystals while the second peak was observed at 132.83 °C and the third peak was observed at 153.67 °C assigned to CTRC. The DSC result implied that a mixture of the co-crystal, EFA and CTRC had precipitated.

4.4.3 **Experiment 2 (Exp 2) 99.9 % ethanol**

In Exp 2 (Figure 4.6), ethanol 99.9 % was used with the same quantity of the materials as in the Exp 1 and the same method as Exp 1 was applied. The mixture was stirred for 30 minutes.

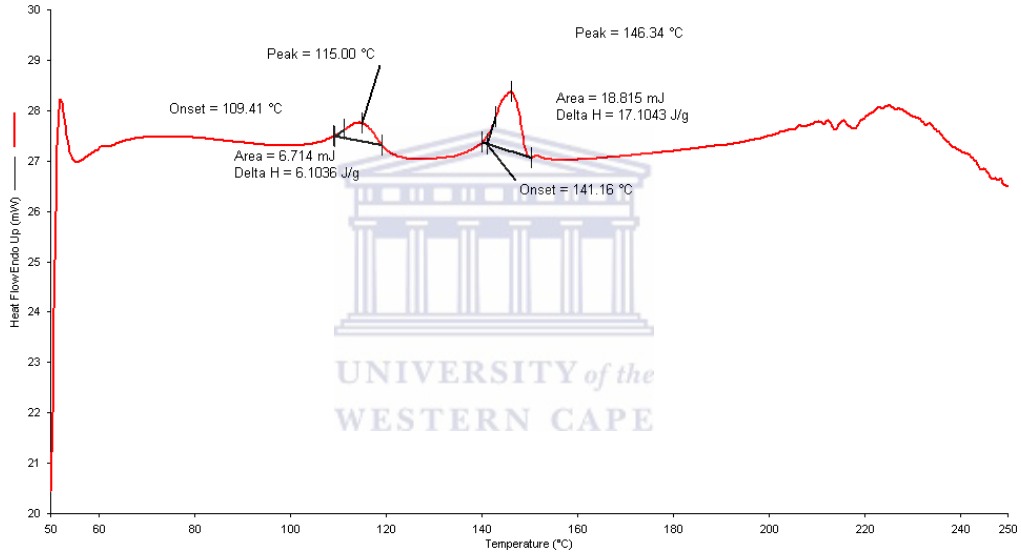


Figure 4-6: DSC analysis of Exp 2 with ethanol 99.9 %

In figure 4.6 the DSC analysis showed two peaks obtained at different positions compared with Exp 1. Although, the first broad endotherm observed from 115 °C to 119 °C could indicate the present of water and was nearly similar to the EFA/CTRC co-crystal according to the study by Chadha *et al*. at 2012,[1] the second peak was observed at 146.34 °C, which meant that EFA was still present in the sample (EFA m.p. 137-141 °C), with an onset at 141.16 °C.

After careful and systematic use of variables, the best method for the preparation of small scale EFA/CTRC co-crystal was achieved by Exp 3 below.

4.4.4 Experiment 3 (Exp 3) preparation of EFA/CTRC co-crystal

The EFA/CTRC co-crystal was prepared by using the solvothermal method (i.e. fast evaporation method) from the solvent under reduced pressure.[1] In small scale production, 0.02 g EFA and 0.013 g CTRC were individually weighed in suitable glass vials in a stoichiometric 1:1 molar ratio. Thereafter, each solid powder was separately dissolved in 2.5 ml ethanol (99.9 %) at 10 °C below the boiling point of ethanol. The dissolved mixtures were continuously stirred using a magnetic stirrer until clear solutions were obtained. The two solutions were mixed together for a period of time (10 minutes). The effect of stirring was also investigated. It was observed that stirring with different times, especially above 30 minutes, the co-crystal was not formed whereas stirring between 5-10 minutes resulted in the formation of co-crystals. The solution was filtered using a 0.45 μm filter with a syringe. The solution was transferred to a test tube in a rota-vapour (V-700/710 Buchi, Switzerland). The clear hot solution was then evaporated under the vacuum; the refluxing was set at 80 °C for 3 hours. A solid powder was obtained and scratched from the walls of the test tube and stored in a vial. The schematic procedure is shown in Figure 4.7. DSC and TGA analysis, confirmed the identity and integrity of the co-crystal.

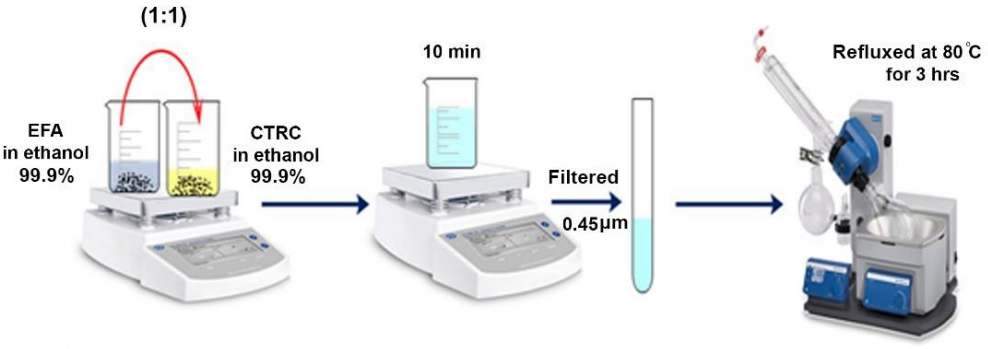


Figure 4-7: Preparation efavirenz co-crystal by solvothermal method

4.4.4.1 **Differential scanning calorimetry (DSC)**

The DSC (Figure 4.8) confirmed the formation of the EFA/CTRC co-crystals according to Chadha *et al*., 2012.[1]

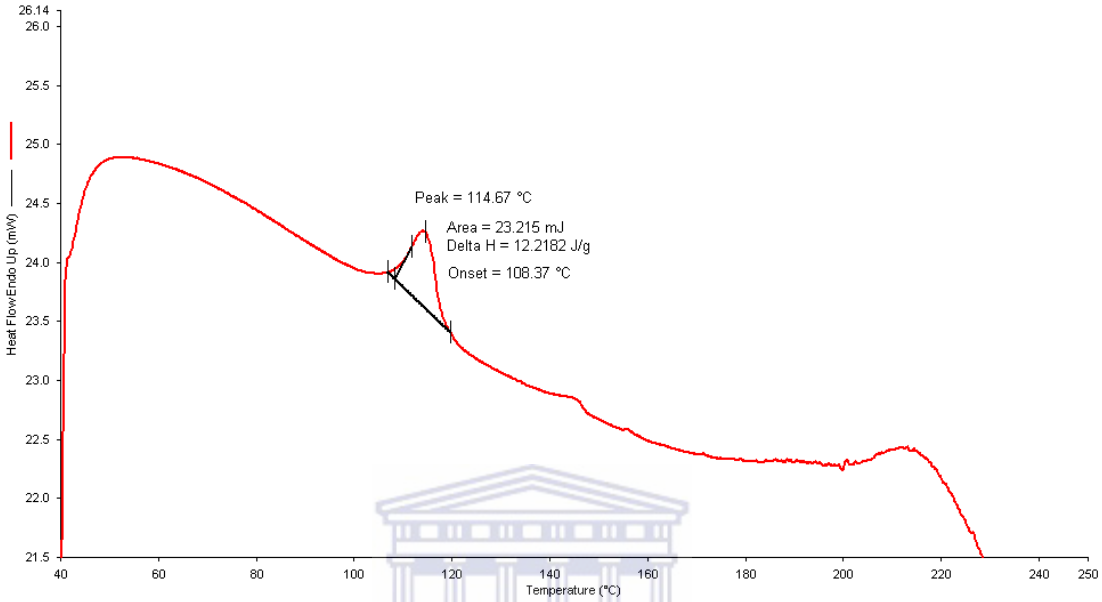


Figure 4-8: DSC analysis of Exp 3 with ethanol 99.9 %

In Exp 3 (Figure 4.8). The DSC scan of EFA/CTRC co-crystals showed a broad endotherm between 109 ˚C and 120 °C with a peak at 114 °C and a small bump was observed at 141 °C possibly suggesting that some EFA might not have complexed. The decomposition of sample was observed from 190 °C.

4.4.4.2 **Thermal Gravimetric Analyses (TGA)**

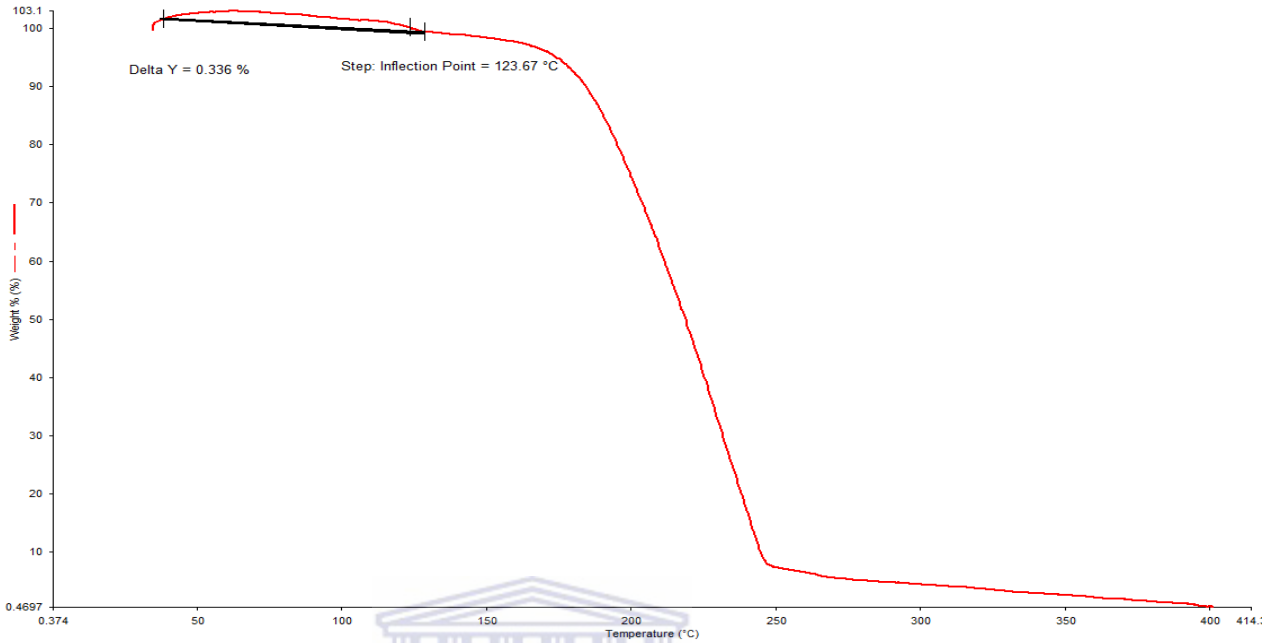


Figure 4-9: TGA analysis of Exp 3 method

In figure 4.9, the TGA result of EFA/CTRC prepared from Exp 3 indicated that the sample experienced a negligible amount of mass loss of approximately 0.336 % in the temperature range between 30 °C and 123 °C. This concurred with the DSC analysis and with Chadha *et al*[1] suggesting that the melt commenced in the range of 123 °C. The thermal studies further revealed that the EFA/CTRC co-crystal decomposition commenced at 180 °C.

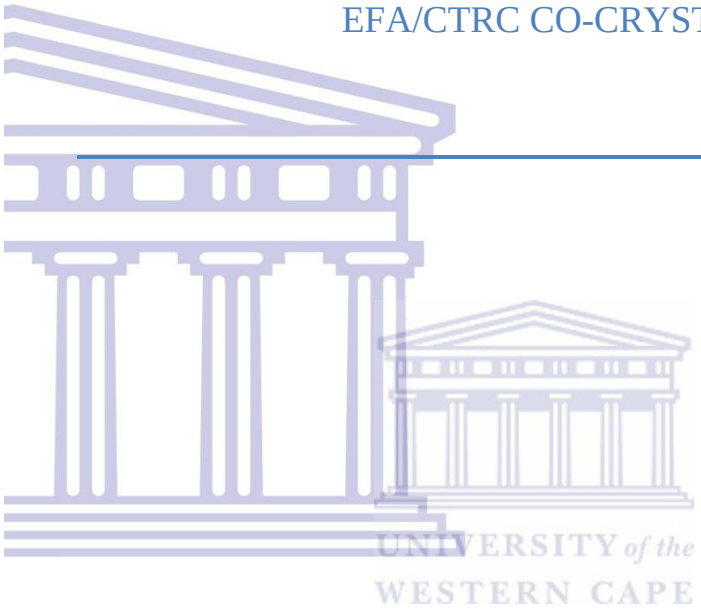
Exp 3 was considered as the best preparation method of the EFA/CTRC co-crystal obtained, the next task was to scale up this method to prepare a larger laboratory scale of the EFA co-crystal for a tablet dosage form. The experiments were performed in batches based on the Exp 3 during notably different weather seasons.

References

1. Chadha, R.; Saini, A.; Arora, P.; Chanda, S.; Jain, D. Cocrystals of efavirenz with selected coformers: preparation and characterization. *Int J Pharm Pharm Sci* **2012**, *4*, 244-250.

CHAPTER 5

LARGE LABORATORY SCALE PRODUCTION OF THE EFA/CTRC CO-CRYSTAL



School of PHARMACY

5 CHAPTER 5

5.1 Introduction

This chapter presents the various methods of preparing EFA/CTRC in a large laboratory scale; namely batch; 1, 2, 3, 4, 5 and 6. This chapter also presents the different analytical techniques used to investigate the prepared batches. These analytical techniques are: SEM, HSM, DSC and TGA. In addition, the compressibility characteristic of the powder in each batch was investigated.

5.2 Large laboratory scale production of the EFA/CTRC co-crystal based on Exp 3

The manufacture of tablets under various experimental conditions are conducted by using different batches and each batch has a unique set of variables that have to be investigated. In this chapter, table 5.1 below starts with the original set of variables (Exp 3). Table 5.1 shows the different parameters that were considered for large laboratory scale production. These parameters include EFA/CTRC ratio, mass of EFA and CTRC, amount of solvent i.e. ethanol (99.9%) used, the use of different batches of EFA, CTRC and ethanol purchased, drying time during refluxing, drying time in oven, season of preparation and brand of the rotatory vapour used.

Table 5-1: Parameters for production of efavirenz co-crystal based on Exp 3

Batch no	*Ratio*	*EFA*	*CTRC*	*Ethanol 99.9 %*	*Drying time during reflux*	*Drying time in Oven*	*Brand rot-vapour*	*Season*
1	1:1	0.230 g	0.153 g	5 ml	3 hours	0 hours		summer
2	1:1	1.152 g	0.765 g	7 ml	4 hours	0 hours		summer
3	1:1	1.152 g	0.765 g	7 ml	4 hours	8 hrs (40 °C)		winter
4	1:1	2 g	1.331 g	7 ml	8 hours	4 hrs (40-80 °C)		winter
5	1:1	2 g	1.331 g	7 ml	4 hours	0 hours	√	winter
6*	1:1	2 g	1.331 g	7 ml	4 hours	0 hours	√	winter

* Different batch numbers and different rotatory vapour used for batch 6

5.3 Batch 1

5.3.1 Preparation of EFA/CTRC

In batch 1, 0.230 g of EFA and 0.153 g of CTRC (1:1 ratio) were weighed in separate glass vials. Each of the EFA and CTRC were separately dissolved in 2.5 mL of ethanol (99.9 %), stirred with a magnetic stirrer at 10 °C below the melting point of ethanol until the EFA and CTRC were completely dissolved into clear solutions. The clear solutions of EFA and CTRC that were separately obtained were then mixed together for 10 minutes to obtain a homogenous EFA/CTRC mixture. Thereafter, the EFA/CTRC mixture was filtered using a 0.45 μm filter with a syringe. The EFA/CTRC solution was transferred into a test tube and mounted on a rota-vapour (V- 700/710 Buchi, Switzerland) to evaporate the ethanol solvent under vacuum to obtain the co-crystal. The conditions for the evaporation of the ethanol solvent was 80 °C for 3 hours. The solid (co-crystal) obtained was carefully collected from the walls of the test-tube via scratching and stored in a clean vial. The results from the analysis of the newly formed EFA/CTRC co-crystal in batch 1 is discussed below.

5.3.2 Physical appearance of batch 1 granules collected

The use of colour change observed from prepared co-crystals can be used to identify or describe the prevailing environmental factor. Also, this characteristic (i.e. colour change) can serve as an indicator to indicate the presence of solvent or degradation as well as variation in particle size distribution of EFA/CTRC co-crystals. Consequently, the instability of a drug in pharmaceutical formulations may be noticed by a change in the physical appearance such as colour, odour, taste, or texture of the formulation.[1] In Figure 5.1, the pictorial features of EFA/CTRC prepared in batch 1 experiment is presented.

Figure 5-1: Physical features of batch 1 of EFA/CTRC

The EFA/CTRC obtained from batch 1 showed a distinct white colour of a powdery, crystalline sample. Batch 1 exhibited no colour change during the process of drying of 3 hours. The absence of discoloration from batch 1 did not suggest any absorption of moisture from the environment, which could have resulted in agglomeration of the powder.

5.3.3 Scanning electron microscopy (SEM)

SEM is a microscopy technique used to probe the surface morphology of samples. This technique provides a high resolution image of the surface of a samples showing distinct features associated with the sample.

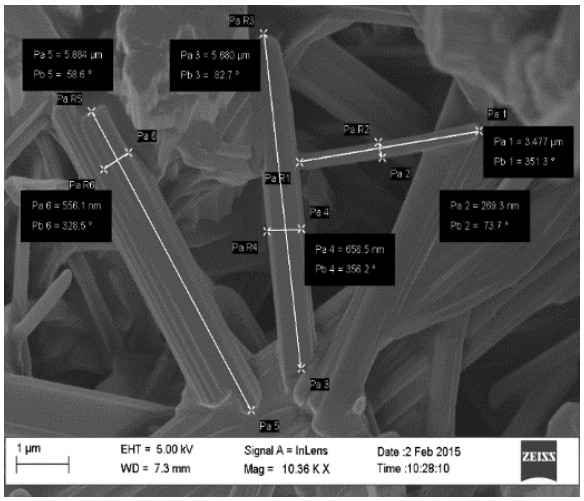


Figure 5-2: SEM analysis of batch 1 of EFA/CTRC

The SEM micrograph images of batch 1 (Figure 5.2) showed the presence of a needle-

like shaped crystals of EFA/CTRC. The needle-like shapes were not mono-dispersed but mostly agglomerated to each other. The surfaces of the needle-like crystal was smooth without distinct defects i.e. pore, holes or surface roughness. The average length and width of the needles were between 3.5 μm and 269 nm, respectively. The needle shape observed from SEM analysis in Figure 5.2 could prove to be a suitable platform to explore its dissolution rate and ease of tableting. This is because needle-shapes have a higher surface area compared to a sphere with respect to magnitude. For instance, Aulton, M. E and Taylor, K. M. (2013)[2] reported that the shape i.e. ratio of surface area to volume of a drug can alter its properties such as dissolution rate. The principle of rational formulation depends on adequate understanding of the physicochemical properties of any active pharmaceutical material. Similarly, it is essential to determine the mechanical properties at an early stage especially during the development process. This is mainly because the properties of the newly formed sample often result in changes such as particle size and morphology during development and these properties ultimately affect the compaction properties of the prepared material.

5.3.4 **Hot stage microscopic analysis (HSM)**

HSM was used to investigate the thermal behaviour of the EFA/CTRC sample obtained from batch 1. The crystal was immersed in silicone oil in order to identify the presence of "so-called" impurity such as water and other solvent that could be entrapped in the molecular structure of the newly formed crystals. The sample was heated at a constant rate of 10 °C/min and its images were collected in the temperature range from 30 °C to 300 °C. The Essential Stream® software was used to observe the change under the microscope and to analyse the data collected. In Figure 5.3, the HSM images of EFA/CTRC from batch 1 is presented.

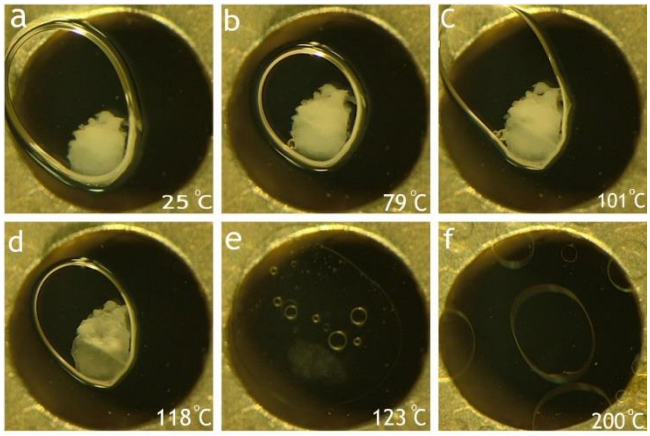


Figure 5-3: HSM analysis of batch 1 of EFA/CTRC

In Figure 5.3, batch 1 remained unchanged until 79 °C where the presence of a single bubble was observed suggesting desolvation of ethanol since its boiling point occurs at 70 °C. Bubbles were also observed at 101 °C in (c), indicating the release of moisture since the boiling point of water occurs at 100 °C. (d) and (e) showed the complete melting of the EFA/CTRC co-crystal with bubbles between 118 °C and 123 °C, respectively. The complete melt of EFA/CTRC at 123 °C concurs with reported data in the study by Chadha *et al.* (2012).[3] (f) the decomposition of EFA/CTRC was characterised by a physical discoloration from 200 °C.

5.3.5 **Differential scanning calorimetry (DSC)**

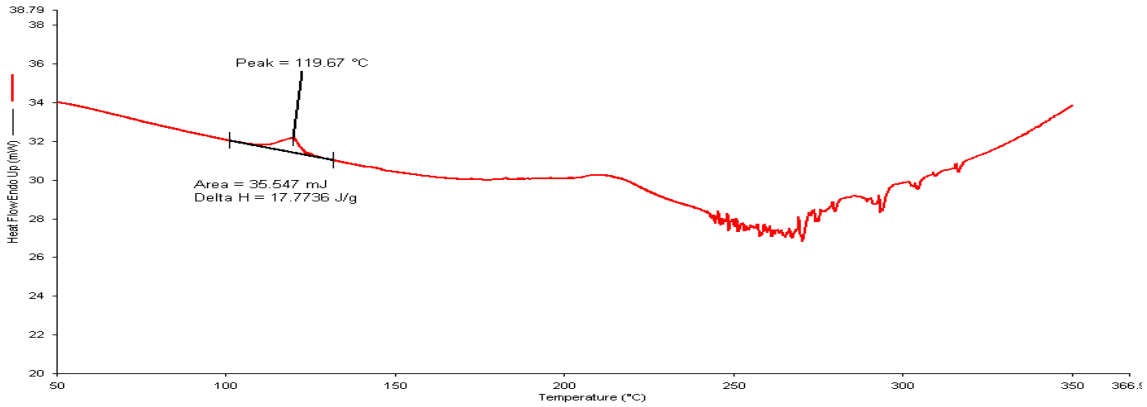


Figure 5-4: DSC analysis of batch 1 of EFA/CTRC

The DSC result of EFA/CTRC obtained from batch 1 (Figure 5.4) showed an endotherm i.e. peak between 118 -120 °C. In addition, the EFA/CTRC from batch 1 showed relative stability since the onset of decomposition was observed from 200 °C.

5.3.6 Thermal gravimetric analyses (TGA)

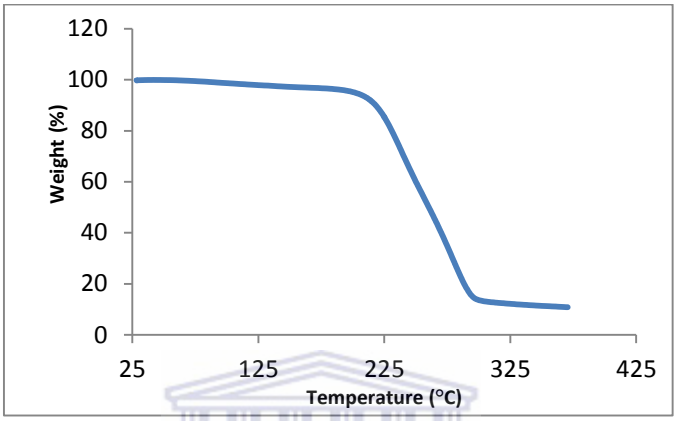


Figure 5-5: TGA analysis of batch 1 of EFA/CTRC

The TGA result of EFA/CTRC co-crystal (Figure 5.5) prepared from batch 1 suggests thermal stability from 25 °C to 125 °C. At 125 °C, the mass loss (%) experienced by EFA/CTRC is approximately 1.6 %. This mass loss is associated with moisture and/or ethanol solvent loss used during the preparation of the EFA/CTRC sample. The thermal degradation profile of EFA/CTRC obtained from batch 1 showed that the sample experienced a significant mass loss of about 85 % from 200 °C until about 300 °C. This region of degradation is preceded by the melting stage at 125 °C of the EFA/CTRC which concurs with the HSM and DSC results presented in Figures 5.3 and 5.4, respectively.

5.4 Batch 2

5.4.1 Preparation of EFA/CTRC

The preparation procedure of EFA/CTRC in batch 2 was similar to batch 1. However, after identifying the limitation during the preparation of batch 1, the amount of EFA, CTRC and ethanol (99.9 %) was increased in batch 2. The limitation in batch 1 was

that the prepared EFA/CTRC was observed to be wet which could have to been due to the presence of ethanol (99.9 %) in the prepared sample. To address this limitation, the amount of EFA, CTRC and ethanol (99.9 %) was increased to 1.15 g, 0.75 g and 7 mL respectively in batch 2 compared with 0.230 g, 0.15 g and 5 mL in batch 1. The drying in batch 2 was 4 hours. During the processes of weighing and transferring of EFA/CTRC into glass vials after drying, it was noted that there were often significant differences in the total mass before and after drying of the sample. One of the reasons for the weight loss could be as a result of the attachment of the sample to the wall of the test-tube during drying. It therefore became imperative to use a working formula to determine the precise variation in weight loss. In addition, the working formula was used to determine the numerical constituents for the preparation of five (5) tablets of an EFA co-crystal. The details of the working formula is presented below.

Before scraping 0.33 g - after scraping 0.28 g = 0.053 g

0.053 g / 0.33 g X 100 = 15 % loss

EFA 200 mg + 15 % = 230mg

No of moles of EFA = 0.230 g / 315.675 g/mol = 0.00073 mol

Mass of CTRC = 0.00073 mol X 210.14 g/mol = 0.15310659 g

1 tablet EFA = 0.230 g X 5 tablet = 1.15 g

1 tablet CTRC = 0.15310659 g X 5 tablet = 0.76553297 g

Total = 1.15 g + 0.76553297 g = 1.9155329 g

Total weight after scraping from wall of test-tube= 1.7649 g of EFA/CTRC co-crystal.

The working formula used above was to obtain the precise amount EFA and CTRC for preparation of EFA/CTRC and also to determine the appropriate stoichiometry ratio for the preparation of an EFA/CTRC co-crystal. This working formula was used as a guide in the preparation of the EFA/CTRC co-crystal as indicated in the subsequent batches.

5.4.2 Physical appearance of EFA/CTRC batch 2 granules

The characteristic physical features such as dryness, colour and other physical properties associated with EFA/CTRC can be determine by visual inspections for batch 2 (Figure 5.6).

Figure 5-6: Physical features of batch 2 of EFA/CTRC

Batch 2 was observed as white powder with finely defined particles. The sample was dry as the powdered sample did not cluster or become attached to the wall of the mortar. The white colour of batch 2 was characteristic of EFA/CTRC while the dryness of the powdered sample could be as a result of the extended time of drying which was 4 hours. In addition, the duration of drying could have facilitated the release of solvent such as ethanol (99.9 %) and moisture present in the sample.

5.4.3 Scanning electron microscopy (SEM)

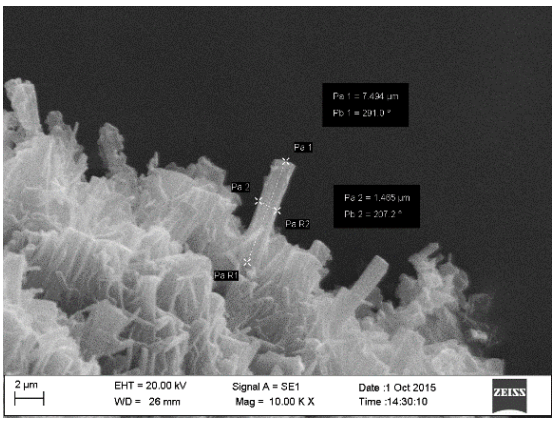


Figure 5-7: SEM analysis of batch 2 of EFA/CTRC

The surface morphology of batch 2 (figure 5.7) showed the presence of irregular and agglomerated spiked particles of different size dimensions i.e. width and height. The average size of the newly prepared sample was approximately 1.465 μm in width and 7.494 μm in length. Also, the SEM morphology of the sample in Figure 5.7 showed a highly rough surface.

5.4.4 Hot stage microscopic Analysis (HSM)

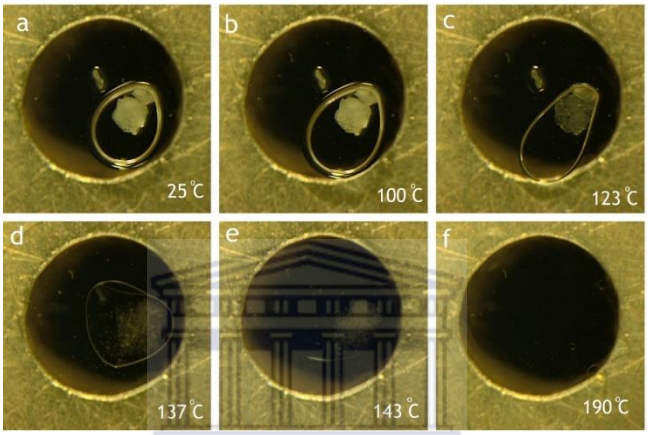


Figure 5-8: HSM analysis of batch 2 of EFA/CTRC

The powder obtained from batch 2 (Figure 5.8) did not experience any obvious change until 123 °C as observed in (c). Between 123 °C and 143 °C, (c to e) the sample melted with only a small fragment left seen in (e). In (f), sample was completely melted at 190 °C without any trace of the fragment observed.

5.4.5 Differential scanning calorimetry (DSC)

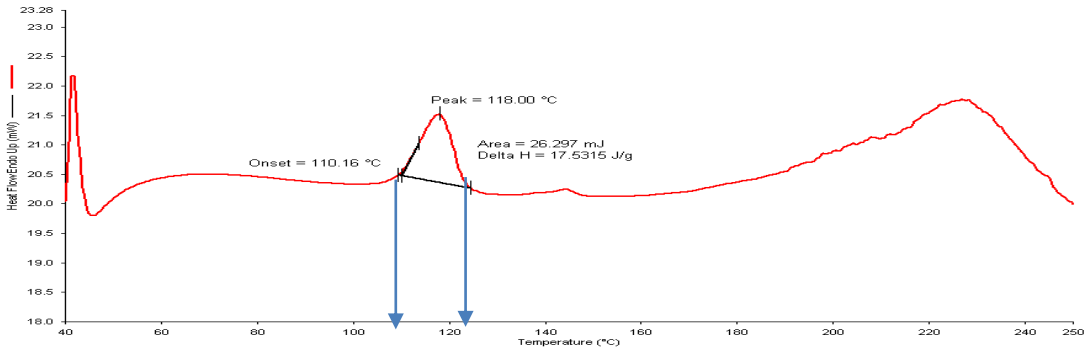


Figure 5-9: DSC analysis of batch 2 of EFA/CTRC

The DSC analysis of batch 2 (figure 5.9) showed a broad endotherm in the temperature range between 110 °C – 123 °C peaking at 118 °C. The endotherm between 110 °C and 123 °C can be related to the melting point region of batch 2 while the endotherm at 141 °C suggests the degradation of EFA. The decomposition of batch 2 was seen to occur from 180 °C.

5.4.6 Thermal gravimetric analyses (TGA)

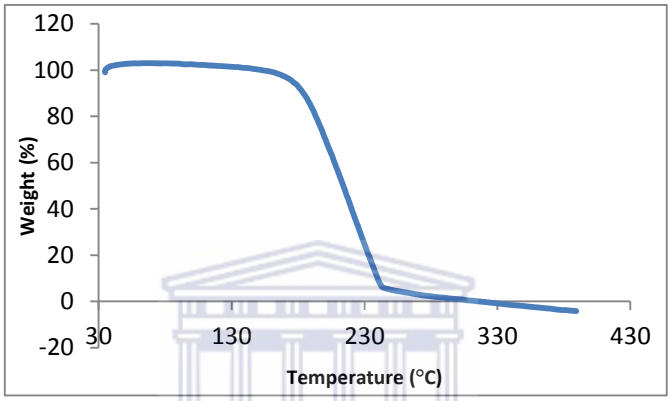


Figure 5-10: TGA analysis of batch 2 of EFA/CTRC

The thermal behaviour of batch 2 (Figure 5.10) showed a small bulge of the thermogram in the temperature range between 30°C and 125 °C. At 125 °C the sample started to melt. The mass loss experienced by batch 2 was 0.758 %. Degradation was observed from 170 °C with a single thermal profile up to 235°C. The weight loss between 170 °C and 235 °C was determined to be approximately 97 %. From the TGA analysis of batch 2, the presence of moisture or other volatile species was negligible. This was due to the drying time of 4 hours at 80 °C. The single thermal degradation profile from 170 °C observed in Figure 5.10 was within the melting point range of batch 2 as previously observed in Figure 5.8 and 5.9 for the HSM and DSC results, respectively.

5.4.7 Compressibility of batch 2

A tabletting machine is one of the most sophisticated machines used in the pharmaceutical manufacturing environment because it requires careful calibration for a tablet press which is a challenging process. In addition, the understanding of basic

principles in tablet press operation is crucial especially to achieve successful batch production. The tablet machine used did not have adjustment indicators for the punch and die, nor for the hardness of the tablet. Also, the machine settings always have to be corrected and optimized to avoid problems such as laminating, capping, weight variation and hardness variation that are associated with tabletting defects. Generally, the optimal settings for this machine were obtained by trial and error after compression, and by testing (characterizations) of the properties of individual tablets such as hardness and disintegration time.

The EFA co-crystal tablets prepared in this study were compressed using a Single Punch Press (Manesty machine LTD, Type F3, No 1 L188, UK). The tablet machine used was adjusted by several means so as to obtain an optimal size of the tablet. Firstly, the upper punch was adjusted to penetrate the die cavity at a given depth and thereafter the lower punch was adjusted in a similar manner as the upper punch. In the final stage, dies with different cavity volumes were used until the optimal size was obtained. In order to determine if there will be need for the addition of excipients, only the uniform EFA co-crystal was compressed and two tablet products were prepared (figure 5.11). The die volume equipped with an 8 mm concave punch was adjusted to produce a tablet of theoretical weight of 200 mg each. After producing the tablets, two quality control tests such as hardness and disintegration time according to the USP[3] were used to determine the quality standards of the tablets. The hardness of the tablets was investigated to see whether the tablets were able to withstand mechanical shocks of handling especially during manufacture, packaging and shopping. The hardness measurement of the two EFA co-crystal tablets were analysed after adjustments were made to the tablet machine and the best hardness achieved was 7 N. This value indicated that the tablets were weak because the acceptable hardness value should be between 40-60 N. Also, the disintegration time of these tablets was more than 30 minutes which indicates that the tablet failed because the USP[3] requirement stipulates that the disintegration time must be within 30 minutes in order for it to be acceptable. To improve the hardness of the EFA co-crystal tablet, the lowest amount of microcrystalline cellulose (MCC) i.e. 20 % MCC was added to the EFA co-crystal as binder/diluent in a direct compression process and thereafter blended together

manually for a specific period of time. The blended mixture of the EFA co-crystal and MCC was forced through a sieve No. 18 so as to obtain uniform powder and also achieve good compressibility. The outcome of this approach resulted in extremely hard tablets with hardness value of 120 N, hence the tablets failed the quality control tests. A similar problem was observed for the disintegration time of the tablets (more than 4 hours).

In Figure 5.11, the resultant compressibility test of EFA/CTRC co-crystals obtained from batch 2 experiment is presented.

Figure 5-11: Morphological features of batch 2 tablets.

The compressibility results of batch 2 showed that the prepared EFA/CTRC co-crystal resulted in the formation of tablets (Figure 5.11) with an excellent hardness value, uniformity of size, diameter and a smooth surface. The mass of the tablets was investigated and observed to be identical for all tablets with an approximate weight of 200 mg. In addition, batch 2 tablets failed its disintegration testing. This failure in disintegration suggests that batch 2 have highly compacted tablets which could make the tablet unsuitable for medicinal purpose especially in the human gastrointestinal tract.

5.5 Batch 3

5.5.1 Preparation of EFA/CTRC

Batch 3 was a replication of batch 2 experiment and all conditions was similar without any change or alteration of the parameters used in batch 2. However, in batch 3, the timing of the experiment was such that it was performed at the start of the winter season and this season is known with unique and prevailing environmental conditions such as relatively high humidity. The sample obtained from batch 3 experiment was inspected after 4 hours of drying at 80 °C and it was observed that the sample contained moisture with a slightly waxy texture. As a result of the first drying experiment of sample at 80 °C for 4 hours, the sample was further oven dried at 40 °C and for 8 h to obtained a dry powder. The choice of using a lower temperature i.e. 40 °C compared to the first drying temperature of 80 °C was to ensure that the sample did not undergo degradation or weight variation which could affect the samples physicochemical properties.

5.5.2 Physical appearance of batch 3 granules collected

Figure 5-12: Physical features of Batch 3 of EFA/CTRC

Batch 3 (Figure 5.12) showed a colour change of the co-crystal sample. The observed colour change was from white to bright pink (Figure 5.12). In addition, the texture was observed to be waxy and the particle shape was seen to be mostly spherical. Also, batch 3 was observed to contain a high moisture content before the colour and texture inspections.

5.5.3 Scanning electron microscopy (SEM)

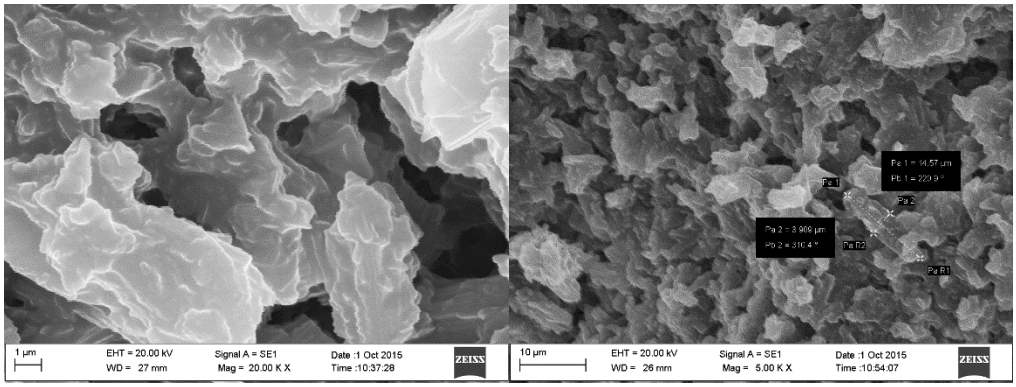


Figure 5-13: SEM analysis of batch 3 of EFA/CTRC

The SEM micrograph image of batch 3 (Figure 5.13) showed irregular surfaces of the sample with the presence of globular particulates. Unlike the previous SEM analysis for batch 1 (Figure 5.2) and batch 2 (Figure 5.7), the surface of batch 3 did not indicate the presence of needle-like or spiked shaped particulates. The particle size distribution for batch 3 was observed to be uniform and highly clustered. Also, the surface of the batch 3 did not indicate the presence of pores (holes).

5.5.4 Hot stage microscopic analysis (HSM)

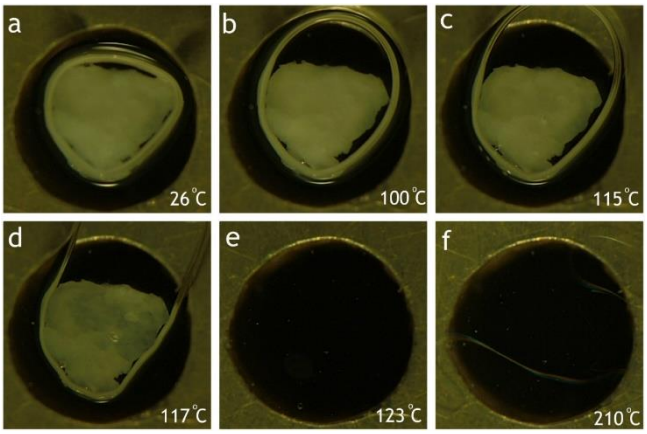


Figure 5-14: HSM analysis of batch 3 of EFA/CTRC

The melting temperature of batch 3 by HSM (Figure 5.14) showed that the melting of the batch was unchanged form (a-c) which correspond to 26 °C, 100 °C and 115 °C, respectively. At 117 °C (d) melting occurred with complete melting observed at 123

°C (e). The presence of bubbles were observed at (b) which suggests the release of moisture present in the sample. The bubbles were also observed in (c) and (d). The characteristic decomposition of batch 3 was observed from 190 °C.

5.5.5 Differential scanning calorimetry (DSC)

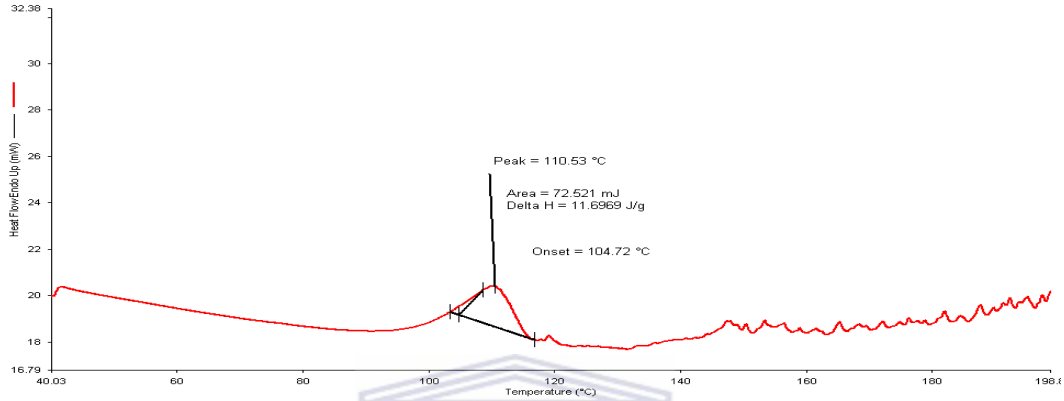


Figure 5-15: DSC analysis of batch 3 of EFA/CTRC

The DSC analysis for batch 3 (Figure 5.15) showed a broad endotherm between 98 °C and 117 °C with a characteristic peak located at 110 °C. A small but sharp endothermic bump was also located at 120 °C suggesting the presence of the co-crystal. This is because at this temperature, co-crystals have been reported in previous studies.[4] Decomposition of batch 3 started at 150 °C.

5.5.6 Thermal gravimetric analyses (TGA)

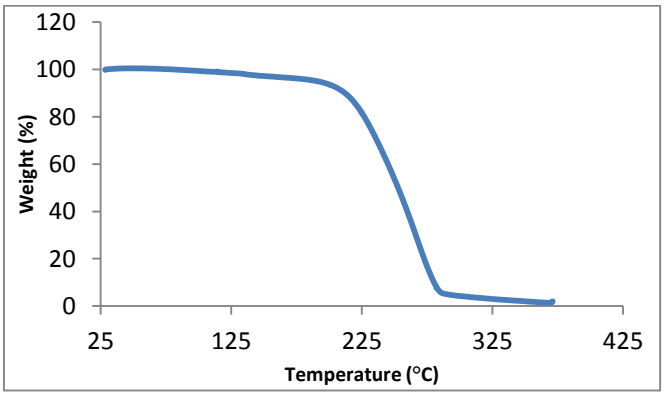


Figure 5-16: TGA analysis of batch 3 of EFA/CTRC

The TGA result of batch 3 (Figure 5.16) showed a relative stable thermal profile until 125 °C at which a mass loss of 1.178 % occurred between 125 °C and 185 °C. Also, between 185 °C and 280 °C, a single degradation thermal profile was observed which resulted in a mass loss of approximately 94 %. The initial mass loss identified at 125 °C (1.178 %) may be due to the presence of moisture while the second mass loss of 94 % experienced by the sample was the melt followed by degradation of the sample agreed with the HSM results.

5.5.7 **Compressibility of sample**

Batch 3 showed that different shapes, different mass of the co-crystal and a wet powder was formed. One of the plausible explanations for the wet powder was attributed to the change in weather as the experiment was performed during the winter season. Before compressing the powder, the oven was used to dry the sample at 40 °C for 8h and the sample did not experience any change in weight even after 8 hours of drying. Batch 3 was compressed and the hardness test was conducted which indicated a zero N value. Microcrystalline cellulose (MCC) was added as a binder in the range between 20 % and 90 % to improve the quality of the powder at different percentages, firstly at 20 %, then at 30 %. The addition of 20 % of MCC showed a hardness value between 20 N - 22 N while the addition of 30 % of MCC resulted in a hardness value of 39 N-44 N. Physical examination of the tablets showed that they were still a wet shape despite the addition of MCC percentages, hence resulting in poor disintegration.

5.6 **Batch 4**

5.6.1 **Preparation of EFA/CTRC**

In batch 4, the amount of EFA was increased from 0.230 g in batch 1 to 2 g in batch 4 while 1.33 g of CTRC was used. The total amount of ethanol (99.9 %) added was 7 mL i.e. 3.5 mL each for the EFA and CTRC and the mixture of EFA/CTRC was dried for 4 hours at 80 °C using a rotary vapour. In this batch, after 4 hours of drying by the rotary vapour, a wet sample of batch 4 was observed, the sample was returned to the rotary vapour and dried for another 4 hours, with regular monitoring of the sample at

every hour. The sample was also dried in oven under different temperature (i.e. 40 to 80 °C) for 4 hours before compression.

5.6.2 Physical appearance of batch 4 granules collected

Figure 5-17: Physical features of batch 4 of EFA/CTRC

Batch 4 (Figure 5.17) resulted in a powdered sample with highly clustered particles. The sample was observed to be wet despite the extended drying time. The colour of batch 4 changed from white to pink. The shape of the particles were spherical and waxy in texture.

5.6.3 Scanning electron microscopy (SEM)

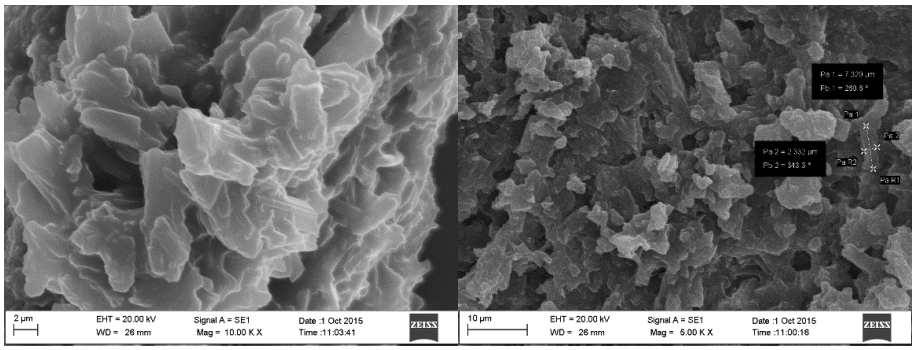


Figure 5-18: SEM analysis of batch 4 of EFA/CTRC

In Figure 5.18, the SEM micrograph image of batch 4 showed clustered particles with random sizes and different shapes. The particles were observed to be mostly non-spherical with ‘sheet-like’ shape which existed as smaller sizes, the surface of the

particles were fairly smooth. The batch 4 did not indicate needle-like shaped particles (batch 1) and there was no presence of spikes compared with batch 2. The preference in shape, particle size and distribution could be as a result of prevailing environmental effects due to high moisture present in the sample.

5.6.4 **Hot stage microscopy (HSM)**

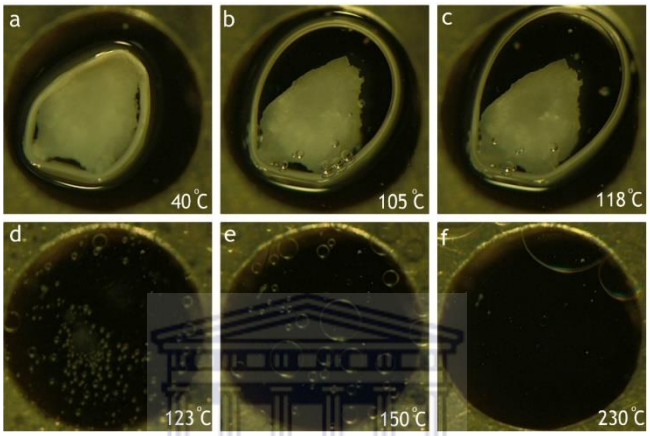


Figure 5-19: HSM analysis of batch 4 of EFA/CTRC

The HSM result (Figure 5.19) for batch 4 indicated that the sample was affected by temperature at 105 °C (b) with the presence of bubbles and the sample began to melt at 118 °C (c). The continuous heating of the sample resulted in an increase in melting with several bubbles as seen in (d) which represents 123 °C. The presence of bubbles at 105 °C was associated with the moisture while above this temperature, the release of other solvents with higher melting point or volatile compounds present in the sample was considered. The decomposition of the sample resulted in discoloration which was observed at 230 °C (f).

5.6.5 Differential scanning calorimetry (DSC)

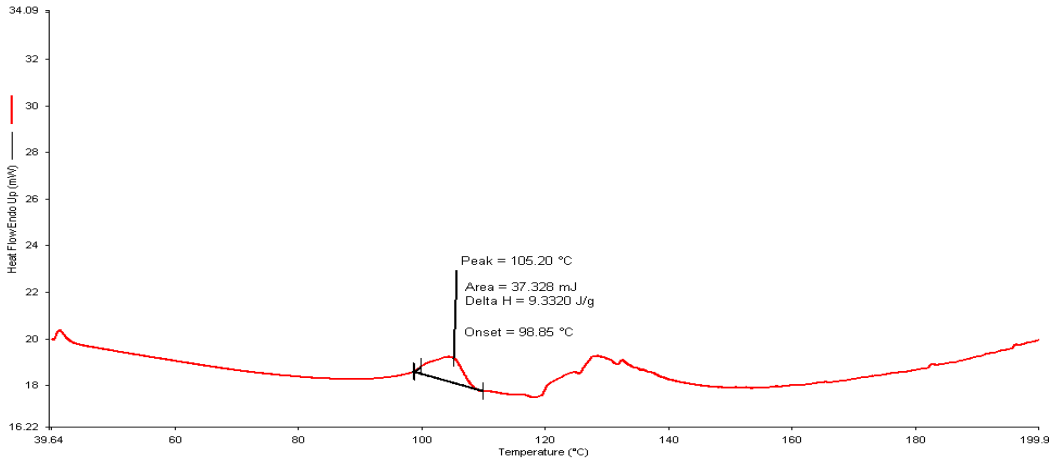


Figure 5-20: DSC analysis of batch 4 of EFA/CTRC

The DSC result of batch 4 (Figure 5.20) showed the presence of several multiple endothermic peaks with the first peak showing a broad endotherm between 98 ˚C and 110 °C along with a peak located at 105 °C (Figure 5.20). A similar endotherm was observed for batch 3. The second peak showed a triple headed endotherm in a temperature range between 120 °C -140 °C. The first endotherm located between 120 °C – 125 °C suggested the EFA/CTRC co-crystal. The third head indicated EFA, observed between 131.9 °C -140 °C. Alternatively, the sample could be experiencing early degradation, given the many peaks followed by the melted.

5.6.6 Thermal gravimetric analyses (TGA)

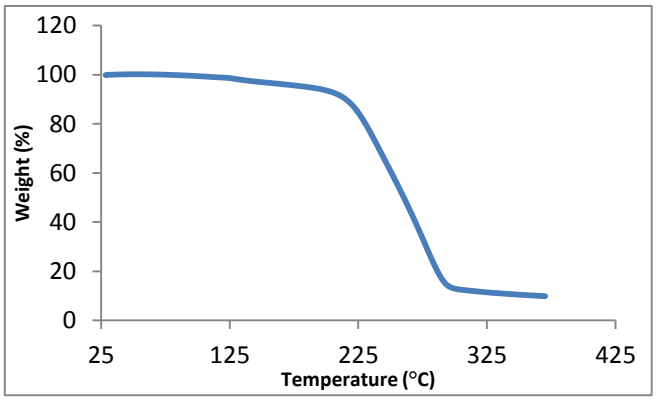


Figure 5-21: TGA analysis of batch 4 of EFA/CTRC

The TGA analysis of batch 4 (Figure 5.21) showed three (3) distinct degradation stages experienced by the sample. The first stage of the TGA result between 28 °C and 125 °C showed a mass loss of 1.176 %. In the second stage of the TGA showed a mass loss of about 6.98 % observed between 125 °C and 210 °C and this degradation temperature is associated with the onset of the melt of the batch 4. Followed by decomposition. The third stage of batch 4 was observed between 220 °C and 280 °C with a single thermal profile and mass loss of over 90 %, suggesting further degradation of the sample.

5.7 Batch 5

5.7.1 Preparation of EFA/CTRC

In batch 5, the same amounts of EFA, ethanol 99.9 % and CTRC were used as in batch 4. However, a new rotary vapour was used in this batch to prepare the co-crystal. The choice of using a new rotary vapour was to determine if the rotary vapour was functional or faulty. Interestingly, batch 5 showed different results compared to batch 4.

5.7.2 Physical appearance of granules collected

Figure 5-22: Physical features of batch 5 of EFA/CTRC

The physical appearance of batch 5 (Figure 5.22) showed similar change in colour from white to pink as previously observed in batches 3 and 4. The particles were observed to exist in clustered forms and coarse in texture. The presence of moisture in

batch 5 could be associated with the unique change in colour from white to pink which also indicates that the prepared sample has the ability to absorb moisture from the environment.

5.7.3 **Scanning electron microscopy (SEM)**

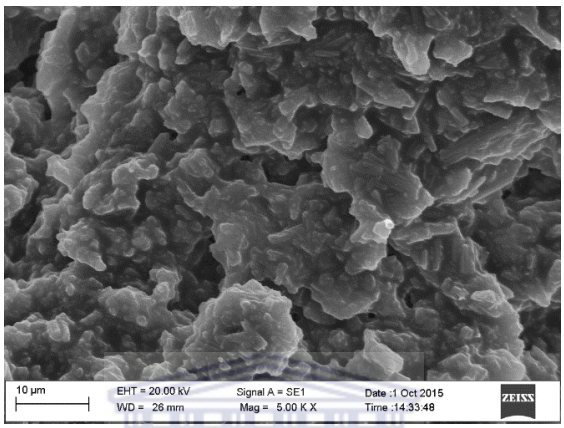


Figure 5-23: SEM analysis of batch 5 of EFA/CTRC

The SEM micrograph image of batch 5 (Figure 5.23) showed an irregular surface morphology without the distinct presence of needle-like, spiked or spherical particles. Also, the surface morphology of the batch 5 showed that the particles are highly clustered and not mono-dispersed or separated. The particle surfaces are relatively rough and their sizes are undefined.

5.7.4 Hot stage microscopic analysis (HSM)

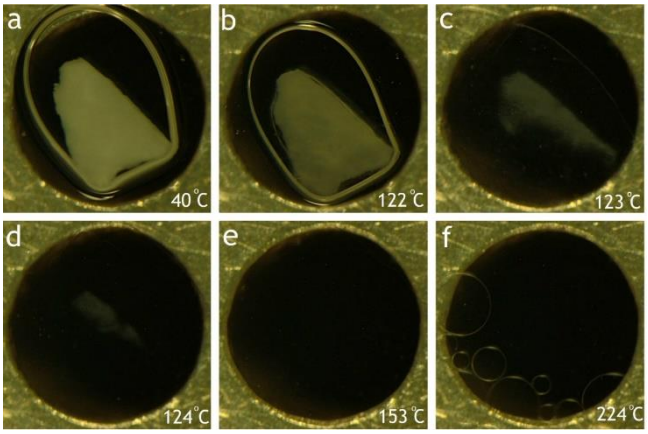


Figure 5-24: HSM analysis of batch 5 of EFA/CTRC

The HSM images of batch 5 (Figure 5.24) showed that the melting of samples started at 121 °C (b) while the final melt occurred at 125 °C (d). Although a great portion of the sample melted, a fragment of the sample was observed to be unaffected and this left-over quantity was observed up to 125 °C from the initial 123 °C. Also, the decomposition of batch 5 was observed by the presence of bubbles as well as physical discoloration which occurred from 224 °C.

5.7.5 Differential scanning calorimetry (DSC)

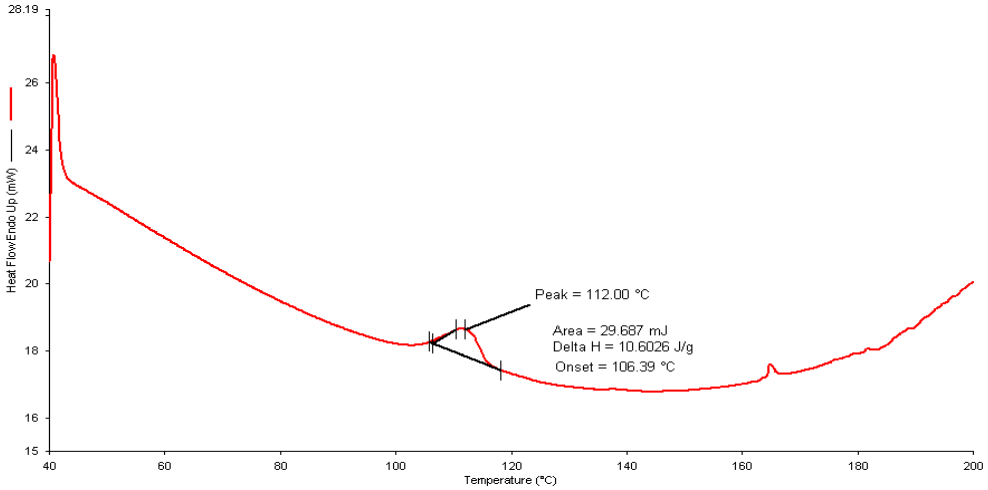


Figure 5-25: DSC analysis of batch 5 of EFA/CTRC

The DSC indicated a peak melting point of batch 5 (Figure 5.25) at 112 °C along with a broad endotherm between 100 °C and 118 °C. In addition, a small endothermic peak appeared at 165 °C. The DSC analysis did not show the presence of endotherm peaks at 141 °C and 154 °C which are characteristic of EFA and CTRC. The endothermic profile located at 112 °C is consistent with the melting temperature region of EFA/CTRC co-crystal.

5.7.6 Thermal gravimetric analyses (TGA)

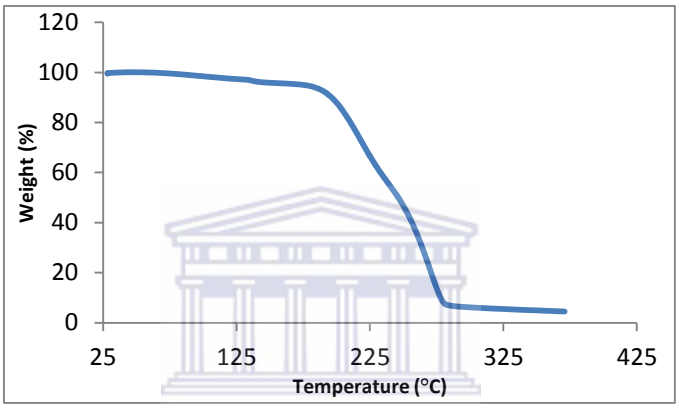


Figure 5-26: TGA analysis of batch 5 of EFA/CTRC

Thermogravimetric profile of the sample showed that the batch 5 (Figure 5.26) experienced thermal stability up to 76 °C. Between 76 °C and 125 °C a mass loss of 2.235 % appears. The mass loss in this region was attributed to the presence of water and ethanol which were used during the preparation stage of batch 5. Moreover, there is another step at 225 °C which suggested the further degradation. This mass loss in this batch was significant which was above 90 % as observed in other batches previously reported.

5.8 Batch 6

5.8.1 Preparation of EFA/CTRC

To improve the quality of the co-crystal, batch 6 involved the use of similar ingredients used in the preparation of previously reported batches i.e. 1,2,3,4 and 5. However,

batch 6 involved the use of the same brands of EFA and CTRC but with different batch numbers. Also, the new rotary vapour equipment was used. The motivation was to attempt reproducibility studies of the EFA/CTRC co-crystal obtained from previous batches. The characterization of the EFA/CTRC co-crystal sample prepared from batch 6 is presented and discussed below.

5.8.2 Physical appearance of granules collected

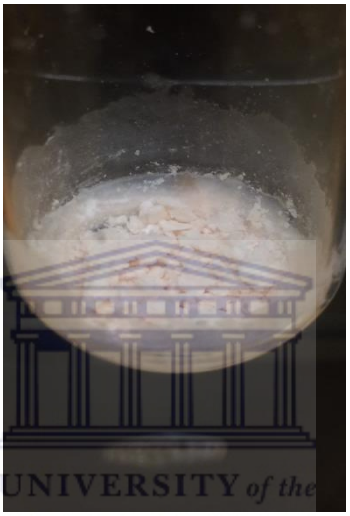


Figure 5-27: Physical features of batch 6 of EFA/CTRC

It was observed from the physical characteristics of batch 6 (Figure 5.27) that the samples surface was a highly coarse powder which changed from a white to pinkish colour. The sample was waxy in texture and wet with solvent which could have been either water or ethanol. In addition, the sample did not indicate the presence of finely defined particles but irregular cracks were observed on the surface of the sample.

5.8.3 **Scanning electron microscopy (SEM)**

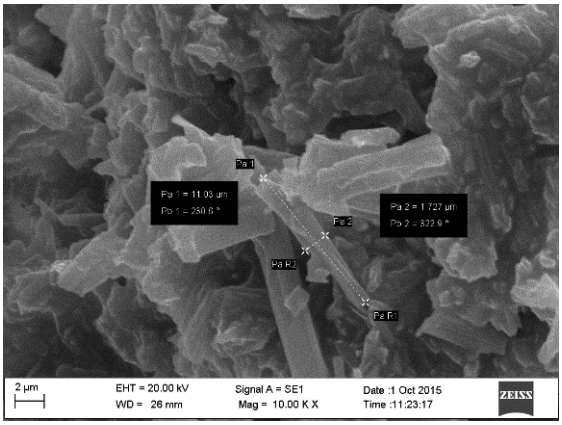


Figure 5-28: SEM analysis of batch 6 of EFA/CTRC

The SEM micrograph images of batch 6 (Figure 5.28) indicated the presence of needle-like shapes as well as clustered smaller particles. The surface of the particles as well as the needle-like shaped particles was smooth without any distinct morphological features such as pores. The particle size for the needle-like particles of batch 6 was measured and found to be 11.03 μm X 1.727 μm.

5.8.4 **Hot stage microscopic analysis (HSM)**

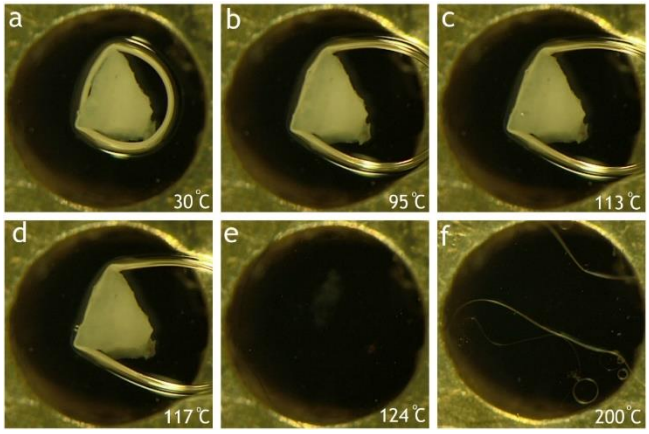


Figure 5-29: HSM analysis of batch 6 of EFA/CTRC

The HSM images in Figure 5.29 (b) shows that the heating of batch 6 did not cause any noticeable change such as melting or presence of bubbles until 110 °C. The first

indication of bubbles in the HSM analysis occurred from 117 °C as seen in (c) which could suggest the presence of moisture in the sample. The amount of EFA/CTRC decreased in size from (c) and complete melting occurred at 124 °C (e). The onset of decomposition of batch 6 was indicated by the appearance of bubbles at 190 °C accompanied with a colour change.

5.8.5 Differential scanning calorimetry (DSC)

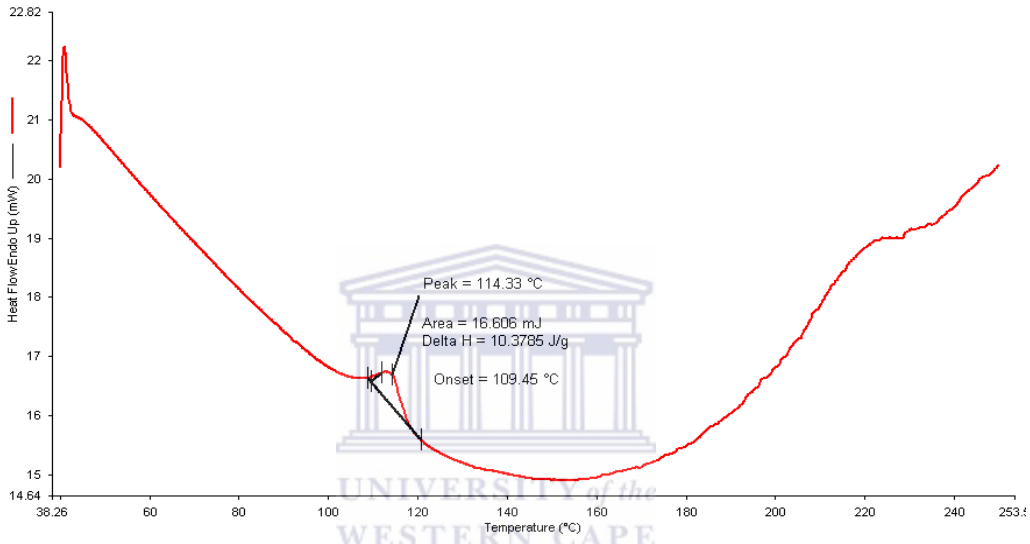


Figure 5-30: DSC analysis of batch 6 of EFA/CTRC

The DSC analysis of batch 6 (Figure 5.30) showed that the sample registered a single endotherm between 109 °C and 120 °C peaking at 114.33 °C. The DSC analysis did not reveal the presence of endotherms that are characteristic of the melting points for EFA or CTRC. However, the decomposition of the EFA/CTRC sample occurred from 180 °C. This decomposition temperature corresponds with the HSM result previously reported in Figure 5.29.

5.8.6 Thermal gravimetric analyses (TGA)

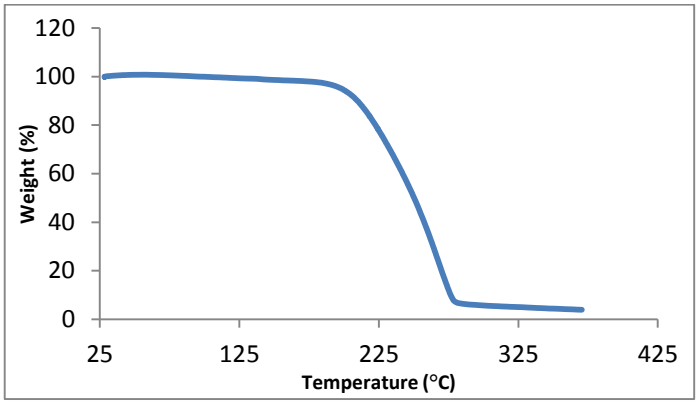


Figure 5-31: TGA analysis of batch 6 of EFA/CTRC

The TGA result of batch 6 (Figure 5.31) indicated that the sample experienced a negligible amount of mass loss of approximately 0.546 % in the temperature range between 30 °C and 123 °C. The thermal studies further revealed that batch 6 was thermally stable up to 200 °C after which decomposition of the sample occurred. The decomposition of batch 6 was observed at 190 °C with a mass loss of approximately 96 %. At this decomposition temperature, the results of HSM agreed with the TGA analysis of the sample.

Table 5-2: Summary of large laboratory scale production of EFA/CTRC

Batch	Physical appearance	SEM	HSM M.P.	DSC M.P.	TGA Mass loss (%)	Compressibility	Hardness	Disintegration
1	White	Needle-like	123 °C	119 °C	1.6	X	X	X
2	White	Spike shaped	123-143 °C	118 °C	0.75	Compressed	120 N	Failed
3	Pink	Irregular and clustered	123 °C	110 °C, 120 °C	1.8	Compressed	39 N-44 N	Failed
4	Pink	Sheet-like	118 °C	105 °C, 131.9 °C, 140 °C	1.176	X	X	X
5	Pink	Clustered	125 °C	112 °C	2.235	X	X	X
6	Pink	Needle-like highly clustered	124 °C	114. 3 °C	0.546	X	X	X

Key:

X represents analysis was not performed.

5.9 Variation in physical properties of co-crystals after preparation

5.9.1 Shape, particle size and moisture effects

The principle of rational formulation depends on adequate understanding of the physicochemical properties of any active pharmaceutical material. Similarly, it is essential to determine the mechanical properties at an early stage especially during the development process. This is mainly because the properties of the newly formed sample often result in changes such as particle size and morphology during development and these properties ultimately affect the compaction properties of the prepared material. Aside of the aforementioned properties i.e. particle size and morphology, other factors such as humidity or moisture content from the environment can affect the manufacturing process of co-crystals formulation.[5] These environmental factors will be discussed in relation to the efavirenz co-crystals prepared in this study. One of the main challenges in the laboratory preparation of efavirenz co-crystals was to ensure that all environmental conditions for moisture-sensitive materials and products were carefully controlled. This is because previous studies have shown that seasonal humidity variation may affect product quality during manufacturing or packaging processes.[6] For instance, a manufacturing environment may facilitate the ease of drug preparation such that the presence of humidity (low or high) ultimately determines the physicochemical properties of a product. As a result, all environmental conditions must be considered and reported where possible, since they must be controlled.

5.9.2 Effects of environmental conditions on the prepared efavirenz co-crystals

5.9.2.1 Colour change

The observed colour change from the prepared co-crystals can be used to identify or describe the prevailing environmental factors. This characteristic (i.e. colour change) can serve as an indicator to indicate the presence of solvent, sample degradation and variation in particle size distribution of efavirenz co-crystals.

The reason for the colour change in this study has been attributed to the different weather conditions during the preparation of these EFA/CTRC batches. For instance,

the small scale EFA/CTRC as well as EFA/CTRC obtained in batches 1 and 2 experiments were prepared in the summer season in South Africa and this season is known with low humidity and high temperature conditions, hence the presence of moisture will be relatively low. Batches 3, 4 and 5 of EFA/CTRC were prepared during the winter season and the colour changes associated with these newly formed EFA/CTRC products was due to the high humidity conditions and variation in moisture in the environment as discussed by Armin H. Gerhardt.[6] This variation in moisture seemed to have affected the EFA/CTRC sample on a batch-by-batch basis, hence the colour change that was observed for batches 3, 4, 5 and 6. Also, the SEM surface morphology and particle distribution, shape, size and texture of the EFA/CTRC was different from one batch to another. In batches 1 and 2, it was observed that the EFA/CTRC particles were in the form of needles and spikes, respectively whereas in other batches i.e. batches 3, 4, 5 and 6, these particles took the shape of an irregular and highly clustered morphology with relative smoothness. However, based on Table 5.2, the HSM, DSC and TGA analyses for all the batches showed good agreement in the thermal properties such as melting point and mass loss of the EFA/CTRC.

Selection of the most "ideal" batch from Table 5.2 for tableting formulation purposes was decidedly very difficult. Every batch resulted in the EFA/CTRC co-crystal. Reproducibility was finally proven but with slight imperfections in particle size, shape, moisture content and colour. During the life-span of a product it is imperative that the production pharmacist becomes aware of these imperfections which will not necessarily deem the product as unworthy. However, with each of these imperfections the production pharmacist has to be acutely aware of how to deal with a specific batch when the opportunity presents itself. Table 5.2 made two suggestions with two of the batches (batch 2 and 3) upon compression of the sample. Batch 2 and 3 failed the compression exercise which took the production pharmacist back to the drawing board. Every other batch will have the same implication upon formulation of the product into a dosage form. The careful selection of excipients would now be imperative to the success of each batch for its formulation into a dosage form. Thereafter, quality control

testing would be the standard of acceptance for the particular batch to ensure acceptance or recall of the batch.

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CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

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6 CHAPTER 6

Conclusions and recommendations

6.1 Summary of findings

The review of the various co-formers of efavirenz co-crystals and cyclodextrin (CD) inclusion complexes and the different methods for the improvement of their physicochemical properties was achieved. According to the investigated variables in this study, it was found that the citric acid monohydrate (CTRC) was the preferred option to proceed to large scale laboratory preformulation of tablets. The flowability property of both the CTRC and hydroxypropyl-beta-cyclodextrin (HP-β-CD) showed that they exhibited excellent flow which lies within USP acceptable range. As for the Carr's index analysis of CTRC, its value was 37.59 was very poor flow whereas the flow value of HP-β-CD was 22.41 which is considered as passable. The moisture content result showed that there was a relatively low percentage in moisture content of CTRC compared with the HP-β-CD. The disintegration analysis for CTRC show that its disintegration time was 110 seconds while the HP-β-CD disintegrated within 300 seconds. The manufacturing problem encountered during the process of making tablets was such that the HP-β-CD did not compress easily unlike the citric acid monohydrate. Hence, the small scale laboratory preparation of EFA/CTRC was successfully accomplished after several attempts of preparation by varying selected variables not mentioned in the official method of the EFA/CTRC co-crystal in literature.

The preparation of the EFA/CTRC co-crystal was successfully prepared on a larger laboratory scale in batches 1, 2 and 6 whereas in batches 3, 4 and 5, the preparation of EFA/CTRC was not successful, hence reproducibility was not guaranteed. In batch 2, tablets of the EFA/CTRC were prepared of which the hardness and disintegration tests indicated the tablets have acceptable hardness properties but did not disintegrate after several hours. The different analytical techniques used to analyse the prepared EFA/CTRC was by SEM, HSM, DSC, TGA showed general agreement in the thermal properties but significant particle morphologies was observed from the SEM analysis.

The reason for the batch-to-batch variation in the physicochemical properties of the batches was attributed to the different weather conditions during the preparation of the batches. For instance, batch 1 and 2 were prepared in the summer season in South Africa. This season has a low humidity with high temperature, hence the presence of moisture in the prepared EFA/CTRC was very low. Batches 3, 4, 5 and 6 of EFA/CTRC were prepared during the winter season and the colour changes associated with these newly formed products was due to the high humidity conditions and variation in moisture in the environment as discussed by Armin H. Gerhardt.[1] This variation in moisture seemed to have affected the EFA/CTRC samples on a batch-by-batch basis including the colour change that was observed for batches 3, 4, 5 and 6. The SEM surface morphology, particle distribution, shape, size and texture of the EFA/CTRC also differ from batch to batch. For example in batch 1 and 2, it was observed that the EFA/CTRC particles were in form of needle and spikes, respectively whereas in batches 3, 4, 5 and 6, the SEM analysis showed that the particles were mostly of irregular shapes with highly clustered morphology and a relative smooth surface. The HSM, DSC and TGA analysis for all the batches showed good agreement in the thermal properties such as melting point and mass loss of the co-crystal.

6.2 Recommendations

The following recommendations should be considered for a successful preparation of EFA/CTRC at large scale laboratory quantity.

a. The laboratory environmental conditions must be controlled especially for humidity
b. The preparation of EFA/CTRC must be considered for all seasons and the prevailing conditions for successful preparation may require intervention during alternate seasons for formulation into dosage form.

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