# Formulation and evaluation of zidovudine cyclodextrin inclusion complex to enhance acid lability and palatability

By

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Magister Pharmaceuticiae

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# ABSTRACT

**Background:** Zidovudine (AZT) is a very useful drug for the management of Human Immunodeficiency Virus (HIV) infection. Its optimal use is limited by its bitter taste, sparing solubility (20.1 mg/ml) and acid lability. Cyclodextrins (CD) are a class of compounds which can be used to form inclusion complexes with drugs such as AZT to improve it is taste, solubility and palatability.

**Purpose:** This study complexed hydroxypropyl-beta-cyclodextrin (HPβCD) with AZT. The formulated inclusion complex was evaluated for suitability as a dosage form and as a tool for improving AZT's palatability, solubility and acid liability.

**Method:** AZT was complexed with HPβCD was using the lyophilisation method. The binding constant for the formulation was determined by the phase solubility method, and complex formation between AZT and HPβCD evaluated using proton nuclear magnetic resonance ($^1$H NMR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and hot stage microscopy (HSM). Tablets of the inclusion complex were formulated by direct compression, using the least possible amount of excipients, and the dosage form evaluated for hardness, friability, durability, disintegration time and dissolution.

**Results:** The binding constant of the formulation was 3.919, and the degree of incorporation was 4.0 mg AZT/g of CD per complex. $^1$H NMR showed significant chemical shifts between the inclusion complex and AZT. DSC and TGA analyses showed significant differences in the curves for the pure AZT and HPβCD. Values for tablet hardness, friability, durability and disintegration time were 236 ± 20 N, 0.7 %, 1.02 % and 10.25 minutes, respectively. The solubility of the formulation was 148.08 mg/ml, and its dissolution profile was different from that of the branded formulation.

**Conclusions:** AZT-HPβCD inclusion complex, with a 7.4-fold increase in AZT solubility, was successfully prepared using the lyophilisation method. The binding constant and friability of the formulation were within acceptable limits. Although the hardness value is high, the tablet still disintegrated within acceptable specified times. This study has significant implications for anti-retroviral complex formulations.

# DECLARATION

I declare that, *Formulation and evaluatin of zidovudine cyclodextrin inclusion complex to enhance acid lability and palatability*, is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name: **Meftah Abdulhafied Al-Derbali**

Signed: ______________________
Date: 22/12/2015

# DEDICATION

To those who gave me full support and guidance; my father, mother and family…
whom I call my small world.
To my dear friends; who shared me my dreams.
To my supporting teachers, through all my school life.
I say:
All that it takes to fulfil your dreams and aspiration:
A true prayer from the beloved one's …
A true support from a dear friend …
Guidance from a dedicated teacher …
A little bit of luck, hard work, and Grace from Allah…
To you…
…………… I dedicate the fruit of my effort.

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# ABBREVIATIONS

| | |
|---|---|
| $^{1}$H-NMR: | Proton Nuclear Magnetic Resonance |
| AIDS: | Acquired Immune Deficiency Syndrome |
| APIs: | Active Pharmaceutical Ingredients |
| ARV: | Anti-Retroviral |
| AZT: | Zidovudine |
| CD: | Cyclodextrin |
| CDC: | Center for Disease Control and Prevention |
| DMβCD: | Dimethyl-β-cyclodextrin |
| DSC: | Differential scanning calorimetry |
| FDA: | Food and Drug Administration |
| FTIR: | Fourier-transform infra-red |
| G$_2$βCD: | Maltosyl-β-cyclodextrin |
| GIT: | Gastrointestinal tract |
| HIV: | Human Immunodeficiency Virus |
| HPβCD: | Hydroxyl propyl-β-cyclodextin |
| HSM: | Hot stage microscopy |
| ICH: | International Conference on Harmonization |
| LOD: | Limit of detection |
| LOQ: | Limit of quantitation |
| MCC: | Microcrystalline cellulose |
| NNRTI: | Non-Nucleoside Reverse Transcriptase Inhibitor |
| NRTI: | Nucleoside Reverse Transcriptase Inhibitors |
| NtRTI: | Nucleotide Reverse Transcriptase Inhibitor |
| RT: | Reverse Transcriptase |
| SEM: | Scanning electron microscopy |
| TGA: | Thermogravimetric analysis |
| USP: | United States Pharmacopoeia |

# OUTPUT

Part of this thesis was delivered as an oral presentation:

Formulation and evaluation of zidovudine cyclodextrin inclusion complex to enhance zidovudine solubility, Al-Derbali M, Samsodien H., Mbamalu O., 36th Academy of Pharmaceutical Sciences of South Africa (APPSA) /South African Association of Pharmacists in Industry (SAAPI) Conference, Cedar Woods of Sandton Conference Centre, Woodmead, South Africa, 17 – 19 September 2015.

# CHAPTER 1

## 1 Introduction

### 1.1 Background

Globally, the increase in the endemic Human Immunodeficiency Virus type 1 (HIV-1) infection has resulted in concerted research efforts with huge funding resources dedicated to the disease. The ultimate aim is to eradicate this condition of the immune system caused by the virus, which if left untreated eventually leads to Acquired Immune Deficiency Syndrome (AIDS) and death. Since the first reported case of HIV, about 78 million people have been infected with the virus and over 39 million people have died from AIDS-related illnesses.[1] Infection with the virus that causes AIDS has grown from epidemic to pandemic status; an estimated average of 35 million people worldwide were living with HIV in 2012. Statistics also showed that of these 35 million, about 25 million people who were living with HIV were from Sub-Saharan Africa in 2012, though this percentage decreased from previous cases.[2] South Africa is known to have the highest number of people living with HIV globally.[3] In light of this, research still continues on ways to improve treatment and outcome measures.

According to the Center for Disease Control and Prevention (CDC), a person is considered to suffer from AIDS once their CD4+ T cell count goes below 200 cells per cubic millilitre of blood. Normal CD4 counts in a healthy person are between 500 and 1500 cells per cubic millilitre of blood. There is as yet no cure for HIV, and treatment options aim to keep the virus at bay with the use of anti-retroviral drugs (ARVs). The nucleoside reverse transcriptase inhibitors (NRTI) are one class of drugs used to manage HIV infection. The enzyme, reverse transcriptase (RT), is very important in the HIV-virus life cycle as it is known to assist in the viral replication cycle, hence is considered as a major target in the management of HIV-1. Several drugs have been produced to target RT and can be classified into three categories: nucleoside analogues (NRTI), nucleotide analogues (NtRTI), and non-nucleoside analogues (NNRTI).[4]

The first drug to be approved by the Food and Drug Administration (FDA) for the management of HIV infection is known as zidovudine. It has the chemical name, 3-azido-3-

deoxythymidine (azidothymidine, AZT) (Figure 1-1) and is classified as a nucleoside reverse transcriptase inhibitor (NRTI).[5]

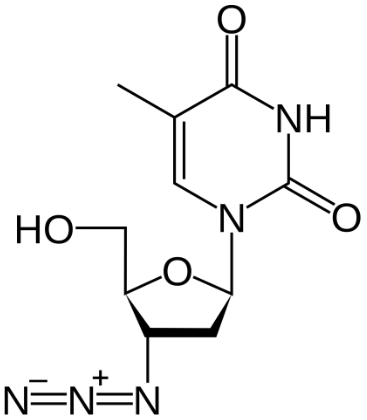

**Figure 1-1: Chemical structure of zidovudine (AZT), salt form**

In appearance, AZT is a white to off-white crystal. It has a molar mass of 267.24 amu and its molecular formula is $C_{10}H_{13}N_5O_4$. AZT's water solubility is reported as 20.1 mg/ml.[6,7] The absorption of AZT from the gastrointestinal tract is rapid as it reaches complete peak plasma concentration within 30 minutes.[8] A number of limitations however militate against the use of AZT. These limitations include dose-dependent haematological toxicity, high first-pass metabolism, poor bioavailability, low solubility and a very short biological half life.[9] A cross-sectional study conducted by Heald *et al*., (1998) of a university's infectious diseases clinic listed AZT among the drugs with undesirable taste and/or smell, qualities which may compromise druf adherence.[10] It therefore becomes imperative to mask the taste of the drug in order to promote patient adherence to drug regimens. AZT is also easily degraded in acidic media, but has been reported to be stable in alkaline media.[11] As advances in treatment and management options for HIV continue to be researched, the composition of the gastric region needs to be considered in the formulation of AZT products to ensure that its properties are not compromised due to degradation in the stomach.

The present study, therefore seeks to address the challenges of bitter taste, low solubility and gastric instability associated with AZT. These challenges will be addressed by complexation of AZT with a suitable cyclodextrin host.

## 1.2 **Cyclodextrin**

In the pharmaceutical industry, the use of cyclodextrins (CDs) continues to generate scientific interest due to their unique properties and structure. The structure is made up mainly of cyclic oligosaccharides along with a hydrophilic external surface and a lipophilic central cavity. The structural configuration of these compounds are (α-1,4)-linked α-D-glucopyranose units with a lipophilic central cavity (Figure 1-2). The different types of naturally occurring CDs are those with six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units.[12,13]

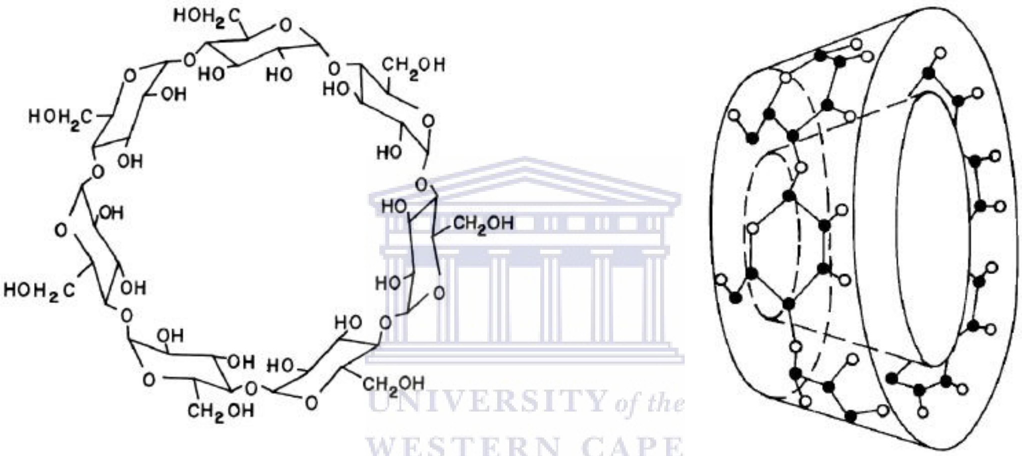

**Figure 1-2: The chemical structure and conical shape of the β-CD molecule**

## 1.3 **Aim**

The aim of the research project is to formulate AZT-CD inclusion complex as a tool to enhance AZT palatability, solubility and stability in acidic medium.

## 1.4 **Objectives**

1. To assess available CD molecules by literature retrieval with a view to selecting the most appropriate CD for complex formation.

2. To prepare AZT-CD complex using the most appropriate CD selected.

3. To conduct physical, chemical and thermal analytical tests on the AZT-CD complex using the following techniques:

    a. *Hot stage microscopy,*
    b. *Differential scanning calorimetry,*
    c. *Thermogravimetric analysis,*
    d. *Proton Nuclear Magnetic Resonace ($^1$H NMR) analysis, and*
    e. *Fourier transform infra-red (FTIR) spectroscopy*

4. To formulate tablets with the AZT-CD complex by the direct compression method, introducing excipients in a step-wise manner, if necessary, as per the formula of a branded AZT tablet excipient composition.

5. To compare the final tablet formulation of the AZT-CD complex to a commercially available AZT brand and AZT alone based on selected USP quality control tests.

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# CHAPTER 2

## 2 Literature Review

### 2.1 Introduction

Supramolecular chemistry involves intermolecular interactions in which covalent bonds are not established between the interacting species. These interacting species can exist as molecules, ions or radicals. Generally, the majority of the interactions occur in the form of host-guest relationships (Szejtli, 1998).[1] Among the hosts utilized for intermolecular interactions are cyclodextrins (CDs) which are considered very important hosts due to the following reasons[1]:

1. They are semi-natural products which can be produced from renewable natural materials such as starch *via* relatively simple enzymic conversion.
2. They are produced in huge quantities annually and through environmentally friendly technologies.
3. They are economical with potential commercial and industrial viability. Their ability to form complexes lends them to applications in modifying drug properties. Due to their unique "molecular encapsulation" property, they have been widely utilized in many industrial products, technologies, and analytical methods.
4. Their inherent secondary characteristic toxic effect can be eliminated by selecting the appropriate CD type or derivative or mode of application.
5. Very important is the fact that they can be consumed by humans as ingredients of drugs, foods or cosmetics.

Although CDs have been known for over a century, they are somewhat considered a new group of pharmaceutical excipients. Historically, CD chemistry became prominent in the early part of the twentieth century and the first patent on CDs and their complexes was registered in 1953. Their high cost of production however made them unappealing. As a result, only small amounts of CDs could be produced before 1970. With recent advances in biotechnology, significant improvement in CD production has resulted, thereby lowering their production costs. This development has also led to the availability of highly purified

CDs as well as other CD derivatives, which are well suited as pharmaceutical excipients. CDs and their derivatives are largely used to increase the aqueous solubility, stability, and bioavailability of drugs. In addition, they can also be used to convert liquid drugs into microcrystalline powders, prevent drug-drug or drug-additive interactions, reduce gastrointestinal or ocular irritation, and reduce or eliminate unpleasant taste and odour.[2]

## 2.2 Brief theory on CDs

The arrangement of component molecules and their formation in CD compounds is responsible for the CD shape. The coned shape is thought to be due to the arrangement of component glucopyranose units in chair form (Figure 2-1). The narrower and wider edges of the cone contain the primary and secondary hydroxyl groups, respectively.[3] This arrangement results in CD molecules with hydrophilic outer surface. The inner cavity however has less affinity for aqueous molecules, and its hydrophobic nature can be likened to that of an aqueous ethanol solution.[4] Several types of CDs are in existence; the most common ones which are also the naturally occurring ones are α-CD, β-CD and γ-CD consisiting of 6, 7 and 8 glucopyranose units, respectively.[5]

It is important to state that even though the natural CDs and their complexes are hydrophilic; their aqueous solubility is rather limited especially with respect to the β-CD. This has been linked to the relatively strong binding of the CD molecules in the crystal state (i.e., relatively high crystal lattice energy).[2,6]

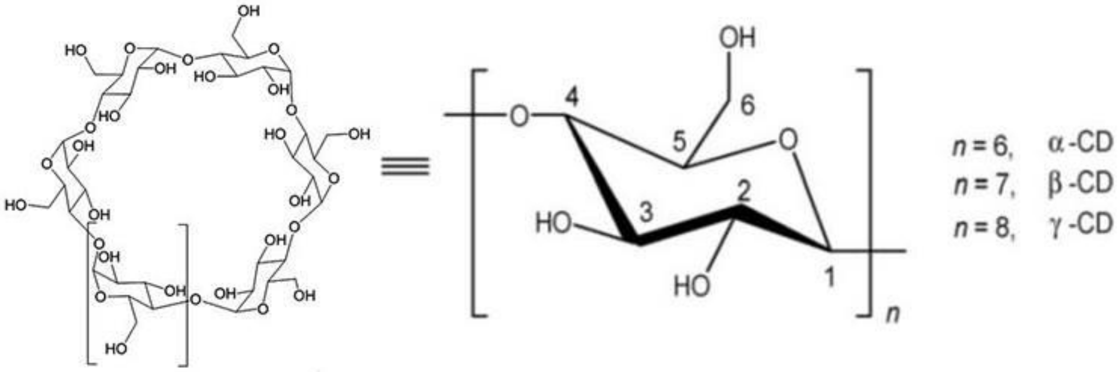

**Figure 2-1:** **The chair formation chemical structure of CDs**[7]

CD molecules are characterized by large molecular weight, hydrogen donors and acceptors and exhibit different physicochemical properties which are unique to each of the naturally occurring CDs, i.e. α, β and γ-CDs. The α- and β-CDs are not easily hydrolysed in human salivary and pancreatic amylase while γ-CDs undergo excellent hydrolysis by these enzymes.[8] The γ-CD (not the derivative) undergoes visible agglomeration especially in aqueous solutions, while β-CD exhibits nephrotoxic properties. These properties limit the suitability of β and γ-CD for parenteral formulations; allowing only the use of α-CD and hydrophilic derivatives of β- and γ-CDs for parenteral formulations. Generally, hydrophilic CDs are considered nontoxic at low to moderate oral doses.[6,9] CD monographs for the α-CD, β- and γ-CD have been incorporated into several pharmacopoeias such as US, European and Japanese pharmacopoeias.[10]

## 2.3 Synthesis of CDs

CD are important pharmaceutical materials. They have been commercially produced on an industrial scale due to the availability of the main raw material; the carbohydrate polymer, starch.[11] CDs are synthesised by exposing starch to enzymatic reactions. The enzyme, cyclomaltodextrin glucanotransferase is naturally excreted by *Bacillus macerans* and reacts with starch to produce a mixture of six-, seven- and eight-membered rings which correspond to α-CD, β-CD and γ-CD, respectively. The structural features of these parent CDs vary significantly due to the composition of the rings.[12,13]

## 2.4 Properties of CDs

CDs molecules exhibit identical physicochemical and biological properties. These include ease of hydrolysis of the α-acetal linkages of the terminal glucose units, a process enabled by the presence of glycosidic bonds as in their analogue water-soluble linear dextrins. However, when compared to linear dextrins, the cyclic structural configuration of CDs confers high resistance especially for non-enzymatic hydrolysis.[14] The solid state properties of CDs confer high stability as with other carbohydrates such as sucrose or starch. They also exhibit extended shelf life at room temperature without any observable degradation of their properties.[15] In aqueous solutions, CDs are chemically stable, especially under neutral and basic conditions.[16] The main physicochemical properties of the naturally-occurring CDs are given in Table 2-1.[17]

**Table 2-1: Selected CD properties of α-CD, β-CD and γ-CD**

| Property | α-Cyclodextrin | β-Cyclodextrin | γ-Cyclodextrin |
|---|---|---|---|
| Number of glucopyranose units | 6 | 7 | 8 |
| Molecular weight (g/mol) | 972 | 1135 | 1297 |
| Solubility in water at 25°C (%, w/v) | 14.5 | 1.85 | 23.2 |
| Outer diameter (Å) | 14.6 | 15.4 | 17.5 |
| Cavity diameter (Å) | 4.7 – 5.3 | 6.0 – 6.5 | 7.5 – 8.3 |
| Height of torus (Å) | 7.9 | 7.9 | 7.9 |
| Cavity volume ($Å^3$) | 174 | 262 | 427 |

## 2.5 Composition of CDs

The three major types of CDs are α-CD, β-CD and γ-CD. Each constitutes different structural configurations of six, seven and eight α-(1,4)-linked glycosyl units, respectively. A comparative analysis reports that β-CD is the most common form of CD found in more than 30 currently known CD-containing pharmaceutical formulations.[13] The preference for β-CD has been attributed to its low price due to ease of production.[18,19] However, β-CD is not without some limitations such as its relatively poor solubility in aqueous solution.[20] Its linear counterpart is freely soluble in water. The poor solubility of β-CD has been attributed to its structure; the molecular dimensions are optimal for the formation of a ring of intramolecular hydrogen bonds that counteract the hydration of β-CD and hence reduce its solubility.[1] As a result, the low aqueous solubility of β-CD has made it unsuitable for parenteral administration.[21] In order to address the solubility limitation of β-CD, multiple hydroxyl groups on both rims of the β-CD molecule have been substituted with 2-hydroxypropyl and sulfobutylether.[22] The theory behind this approach is that the random attachment of organic moieties causes the breakage of intramolecular hydrogen bond attachment which in turn reduces the crystallinity of β-CD and ultimately results in notably improved aqueous solubility.[20] As attestation to the popularity of CD substitutions, more than a third of all CD-containing medicines contain substituted CDs. In addition, some derivatives such as 2-hydroxypropyl (HPβCD and HPγCD) and sulfobutylether (SBEβCD) are known to possess improved toxicological profiles in comparison to their parent CDs. In view of this and with the high tolerance in the human body, new frontiers have opened in the development of injectable CD formulations with improved efficiency.[13,23]

## 2.6 CD derivatives and their characteristics

CDs are known to have numerous derivatives; each of these derivatives possesses unique characteristics often used to improve the limitations associated with their parent CDs. As such, various CD derivatives have been synthesized to improve the use of the parent CD. In this category, HPβCD is one of the most commonly used β-CD derivatives due to its high water-solubility, parenteral safety and complexation ability.[24,25] The derivatives are mostly hydroxyl while others include the 2-hydroxypropyl derivatives of both β- and γ-CD, the sulfobutylether derivative of β-CD, and the branched (glucosyl- and maltosyl-) β-CDs.[26] Substitution of any of the hydroxyl groups by hydrophobic moieties such as methoxy functions, however reportedly result in a dramatic increase in their aqueous solubility. A study by Frömming and Szejtli (1994) showed that the degree of methylation increases the solubility of β-CD (in cold water) after about two-thirds of all hydroxyl groups in the CDs have been methylated. However, the threshold of solubility decreased after further methylation.[14,27] Completely substituted derivatives of CDs therefore generally have lower aqueous solubility compared to the partly substituted derivatives. This is because the number of possible isomers decreases as the CD molecule becomes close to fully substituted.[14] The ability of the CD derivatives to form water-soluble complexes is also largely dependent on the degree of substitution. This implies that the solubility of the CD molecule depends on the accessibility of the guest molecule to the CD cavity.[28] Generally, the degree of substitution is optimized with regard to the solubilizing abilities of the CDs. For instance, the degree of substitution of the pharmaceutical grades of HPβCD is about 0.65 (i.e., on average, 0.65 hydroxypropyl-moieties are on each glucose unit) and about 1.8 for randomly methylated β-CD (i.e., 1.8 methoxy-moieties are on each glucose unit). These methods of chemical modifications are now being applied to obtain water-soluble CD derivatives.[29] In addition, several semisynthetic water-soluble cellulose derivatives such as carboxymethylcellulose and hydroxypropyl methylcellulose have been synthesized and used in large quantities in a variety of industrial products. Similar chemical modification were applied to obtain water-soluble CD derivatives.[14]

In tableting settings, HPβCD in the solid state readily lends itself to plastic deformation, which is beneficial to successful tablet formulation.[25]

Another important characteristic of CDs is hydrolysis. Hydrolysis of β-CD derivatives occurs at approximately the same rate and ring opening is known to be the dominant degradation pathway. The α-CD is approximately 1.5-times more stable and γ-CD is approximately 1.5-times less stable than β-CD. In pure aqueous solutions, the half-life ($t_{½}$) for ring-opening of β-CD was determined as approximately 15 hours at 70°C at pH of 1.1.[30] The formation of inclusion complexes significantly enhances the chemical stability of CDs.[31,32] As for non-enzymatic degradation of CDs in aqueous solution, the degradation follows specific acid-catalyzed hydrolysis of the α-acetal linkages to form glucose, maltose and non-cyclic oligosaccharides. The newly degraded CDs are known to contain as many glucose units as the original CDs.[30]

β-CD molecules also form intramolecular hydrogen bonds and this reduces their ability to form hydrogen bonds with surrounding water molecules. For the alkyl derivatives of CDs, their solubility enhancement is due to chemical manipulation *via* transformation of the crystalline α-, β- and γ-CD into amorphous mixtures of isomeric derivatives.[33] For instance, HPβCD can be obtained by treating a base-solubilized β-CD with propylene oxide. This results in an isomeric system with over 60% (w/v) solubility compared to the original solubility of β-CD at 1.85% (w/v). The number of isomers so-generated based on random substitution was also reportedly very large. In addition, the introduction of 2-hydroxypropyl introduces an optical center and the total number of isomers, i.e. geometrical and optical, is greater compared to what was obtainable before introduction of the functional group. Statistically, there are approximately 130 000 possible heptakis (2-O-(hydroxypropyl))-β-CD derivatives.[2] CD complex formation is achieved due to the release of enthalpy-rich water from the CD cavity. This is because the CD cavity is lipophilic in nature and non-covalent bond is formed during CD complex formation.

Another important characteristic of CDs is the reactivity of the hydroxyl groups on CDs. The reactivity of the three hydroxyl groups on component glucose units of CDs results in glucose units with slightly different complexing abilities compared to the parent CDs. The hydroxyl substitution is usually not random and has been found to depend on the basicity of the aqueous reaction media. The above stated reason (hydroxyl substitution) could explain the variations in the complexing abilities of identical CD derivatives from different suppliers and in some cases, of different batches from the same supplier.[34,35]

The different forms of CD derivatives formulated have resulted in a variety from which selection can be made for pre-formulation studies. However, one of the limitations associated with these derivatives is the toxicological evaluations for each derivative which are very costly; hence, only very few of these derivatives are available as pharmaceutical grade excipients (Table 2-2).'[40] In addition, availability of new CD derivatives will likely increase very slowly because the new CD derivatives may not offer better advantages over the ones currently used.

**Table 2-2: Physicochemical properties of some CDs that can be found in marketed pharmaceutical products.** [14]

| Cyclodextrin [a] | Subst [b] | MW (Da) | Solubility in water [c] (mg/mL) |
|---|---|---|---|
| **α-CD** | - | 972 | 145 |
| **β-CD** | - | 1135 | 18.5 |
| **HPβCD** | 0.65 | 1400 | >600 |
| **RMβCD** | 1.8 | 1312 | >500 |
| **SBEβCD** | 0.9 | 2163 | >500 |
| **γ-CD** | - | 1297 | 232 |
| **HPγCD** | 0.6 | 1576 | >500 |

**KEY:**

**(a)** α-CD: α-cyclodextrin; β-CD:β-cyclodextrin, HPβCD: 2-hydroxypropyl-β-cyclodextrin; RMβCD: randomly methylated-β-cyclodextrin; SBEβCD: sulfobutylether-β-cyclodextrin; γ-CD: γ-cyclodextrin; HPγCD: 2-hydroxypropyl-γ-cyclodextrin.

**(b)** Substitution: average number of substituents per glucopyranose unit.

**(c)** Solubility in pure water at about 25 °C.

## 2.7 **Applications and uses of CD**

The entire application of CDs in drug formulation involves complexation. The majority of the complexes are used as crystalline substances, especially for solid dosage drug delivery and flavour enhancement. They are also used as aqueous solutions in the case of liquid-based delivery systems. Similarly, CDs have been used to modify the physical and tabletting properties of microcrystalline cellulose. Other applications of CDs include the cross-linking of polymers in which such cross-linked polymers have been reported to perform as disintegrating agents, with results comparable to that of other disintegrants such as Ac-Di-Sol.[41,42]

Another recently proposed application of CDs is their use as fillers or as carrier materials in tablet manufacture. For instance, Shangraw *et al*. (1992) reported the use of β-CD as a direct compression filler binder and demonstrated that the compactibility of β-CD out performs all other standard direct compression fillers commonly used with the exception of microcrystalline cellulose (MCC).[43,44] The inherent advantages outlined above have made β-CD the preferred choice among the pharmaceutical excipients for co-processing with MCC. As another example, the use of MCC in a dried-mixed state has been used to facilitate tableting of β-CD. β-CD was blended with chloramphenicol in a 1:1 ratio.[45] It was reported that due to the addition of MCC to a physical or kneaded product of β-CD and chloramphenicol blend, the crushing strength of the tablets increased as the amount of MCC increased. The disintegration time of the said tablets also increased. The study also showed that increasing the crushing strength of the tablets as well as shortening the disintegration time were mutually exclusive irrespective of the state of β-CD (i.e., physical mixture or a kneaded product of β-CD with chloramphenicol) mixed with MCC.[41]

The influence of the processing method on the functionality of MCC incorporated with β-CD was also been examined. A study by Staniforth *et al.* (1988)[46] showed that MCC slurry co-dried with β-CD improved β-CD functionality as a direct-compressible excipient.[41,46] The unique properties of β-CD has resulted in its use in oral drug delivery. CD molecules are thus considered promising materials, especially as direct compression excipients due to their favourable compactibility, dilution potentials and low lubricant sensitivity. The dimethyl β-

CD (DMβCD) has also been reported to show excellent properties, however its use is still limited by toxicity issues.

In view of further applications, Amselem *et al*. (1995)[47] reported that DMβCD which is a derivative of β-CD increases AZT concentrations in the rat brain compared to the HPβCD system. In their study, there was a significant increase in concentrations of the AZT-$Q^+$ in the rat brain within 1 hour of DMβCD and HPβCD administration. Methylated CDs have been reported to improve rectal delivery and their mechanism of permeation enhancement may be associated with cellular perturbation.[47–49] Similarly, the methylated CDs possess considerable surface activity and exert detergent-like effects compared to their hydroxylated counterparts.[50] These properties of DMβCD have been responsible for their irritation to mucous membranes and haemolytic activity. Even though such effects may be more obvious in the static model examined, these effects can be considered to be of less concern for (oral dose) administration. However, irritation is often highly associated with improved drug uptake, especially for permeation enhancers. As a result, there is need for further attempts to modify HPβCD which has been shown to be parenterally and orally safe as well as non-irritating to mucous membranes.[51,52]

To improve on drug release property, CDs have been identified as appropriate drug modifiers which can be employed to meet specific characteristics. These characteristics include increasing drug bioavailability and drug dissolution as well as improving on drug stability or release times especially during gastrointestinal (GI) transit. The modification of drug release property can be achieved by modification of drug release site or time profile, decreasing local tissue irritation, and masking of objectionable taste.[39]

One of the considerations for drug optimization, especially in pharmacotherapy, is the control of drug release property in accordance with the therapeutic purpose and pharmacological properties of active substances. Therefore, the drug release rate or time types are crucial for oral preparations. This is because an appropriate drug release property from the dosage form is important in order to realize or improve on their therapeutic efficacy. The available studies have shown that there are various CD derivatives that have been used to modify drug release property, especially in oral preparations. There are three (3) main drug release types, i.e. immediate, prolonged and modified release types and the hydrophilicity and hydrophobicity of CDs determine these release types. In addition, the delayed release type formulation can be achieved by the use of 6-O-(carboxymethyl)-O-ethyl-β-CD. A typical example of a modified drug release property was recently reported for nifedipine with the developement of a double-layer tablet using HPβCD.[53,54]

A necessary part to formulation studies which may involve CDs is the production of the final dosage form. For oral dosage preparations, these dosage forms may be solutions, suspensions, capsules or tablets. A number of methods can be employed in tablet manufacture, some of which are discussed below.

## 2.8 **Methods of tablet production**

Tablet production can be accomplished by a number of methods such as direct compression, and wet/dry granulation. Among the various methods of tablet production, direct compression is regarded as a highly economical method because active pharmaceutical ingredients (APIs) and excipients can be processed without further processing as involved in granulation. One of the inherent advantages of direct compression is the fact that it is not necessary to produce granules which requires the employment of several manufacturing steps with attendant validation. In addition, this method does not require the use of a solvent. Even more important is the fact that tablets prepared by direct compression, disintegrate into primary particles and not into granulated aggregates, resulting in faster drug release compared to tablets prepared by granulation.[55]

The direct compression method is specifically used for APIs with high efficiency. APIs with high efficiency can be described as APIs which are present in a strong minority in the dosage form. In such cases, the flowability and compressibility properties of the formulation depend more on the composition and characteristics of the final product's excipients and less on that of the API. This underlies the importance of appropriate excipient or combination of excipients selection as a cornerstone of every pre-formulation study.[55]

CDs have been reported to possess good compressibility characteristics.[56] This implies that they may enhance direct compression of formulations. In cases where such direct compression is not obtained, there may be need to incorporate binders among the excipients of API for which combination with a CD is desired. A very important consideration in this case would be the selection of binder with suitable compressibility and stability characteristics.

## 2.9 Studies on drug stabilization by CDs

CDs can be used to stabilize compounds; a limiting factor is the finite space of their cavities which makes occupation by two different guests at the same time unrealistic. This however prevents interaction and reaction with other molecules. Although the ends of the cavities are open, some steric hindrance is provided to prevent the approach of other molecules at the exposed portion of the molecule. The association between the cavity or hydroxyl groups surrounding the cavity and the guest molecule can also stabilize the guest in a state of being in a less reactive form.

For instance, penicillin G is stabilized in an aqueous solution of chloroacetate buffer with HPβCD.[57] In studying the rate of degradation, it was found to be nine times slower for the complex in comparison to the uncomplexed drug. The activation energy for the degradation reaction was also similar for both the complexed and free drug. However, there was a reduction in entropy of activation which suggests that steric hindrance made the approach of the catalytic proton more difficult.

Another classical example is the study of thymopentin which is a peptide. It blocks the stimulation of smooth muscle contractions induced by (+)- anatoxin-a, which is produced by blue green algae. Thymopentin is known to be unstable in aqueous solution, hence cannot be stored in a ready to use form. However, when complexed in aqueous solution with HPβCD, thymopentin activity was retained over 14 months of storage at 25°C whereas in the absence of the CD, all of the activity was lost within 1 week.[58]

Another example is that of erythropoietin, a glycoprotein hormone known to induce an increase in red cell mass. The activity of erythropoietin increased by 50 % (i.e. from 50 % - 100 %) following complexation with HPβCD and this activity was maintained for 10 days. After 20 days, the complexed erythropoietin still retained 62 % of its activity compared to

only 24 % in the absence of CD.[37] The bioavailability of the erythropoietin in the said study was also the same for the complex and for the uncomplexed hormone.[59]
Yet another study was conducted on the photodegradation of nicardipine, a calcium channel blocker used for the management of hypertension. Nicardipine is known to be sensitive to light and decomposes on exposure to light. In the said study, nicardipine was complexed with CDs and exposed to ultraviolet (UV) irradiation. The rate of photodegradation following exposure to UV-irradiation was reduced by a factor of 10, 8, 6.5 and 5 when complexed with methylated β-CD, HPβCD, α-CD and γ-CD, respectively.[60]

Unsaturated fats such as fish and vegetable oils contain unsaturated fatty acids which are easily oxidized, resulting in unpleasant taste and odour. The use of CDs as complexing agents has been reported to protect these oils from oxidation.[61] Complexation serves to convert the oils into solids containing at least 18 % of oil in the complex.

Peroxy acids are generally used as bleaching agents and disinfectants for domestic, industrial, and medical equipment. However, their storage and thermal stability have been a source of major concern in the use of these materials. The use of CDs to complex peroxy acids has been shown to improve their storage and thermal stability.[61] The odour is also reduced for the complexed peroxy acids which are reportedly stable without the use of diluents. In addition, tablets can be made for use and thereby increase the solubility of peroxy acids which is achieved by complexation.

Explosive compounds have also been stabilized with CDs.[62] In a study performed on hair dye preparations using CDs as complexing agents, the dye was evaluated after production, and again after six months of storage following complexation with CDs.  The quality of the hair was measured by flexibility and ease of combing after application of the manufactured dye. The formulation made with complexes of CD performed similar to that without the CD immediately after preparation. On assessment after six months of storage, the hair dye complexed with the CD was rated superior (assessed by hair flexibility and ease of combing after dye application) to that which was not complexed with CD. This underscores the suitability of CD compounds as stabilizing agents.[61]

## 2.10 CD substrate binding constant

An important parameter of inclusion complexes is the affinity that the guest molecule has for the CD cavity. If the formulation of an inclusion complex is deemed necessary, it is important that the affinity or binding constant (K) of an API for CD be determined early on in development. Numerous methods can be used to determine the binding constant between CD (or derivatives) and substrate. This is often determined using the phase-solubility method with spectroscopic detection.[63] The phase-solubility process is performed by preparing an aqueous solution (buffered or unbuffered) with an excess quantity of a low solubility drug, and combining this with a highly concentrated solution of CD. An equilibrium is eventually established between solid drug ($D_{solid}$) and dissolved (complexed or free) drug.

$$D_{solid} = D_{free} + D_{complexed}$$

**Equation 2-1:**

Consequently, when a complex is formed between free drug and CD, the concentration of the free drug is replenished by the dissolution of solid drug. After attaining an appropriate equilibration time, the solutions are filtered or centrifuged and UV spectroscopic analysis is performed to determine free drug concentration. The equilibration time is ascertained on a case-by-case basis,[64,65] and this can range from minutes to days. In order to understand and determine the binding constant, the phase solubility diagram is prepared by plotting the total drug concentration on the y-axis and the total CD concentration on the x-axis. The binding constant can be extrapolated from the plot and determined for a 1:1 complex by:

$$K = \frac{slope}{S_0(1 - slope)}$$

**Equation 2-2**

where $S_0$ is the intrinsic solubility of the substrate.

There is a large dependence of K on the value as determined for $S_0$. This depends on the solubility of the API which can be close to the lower limit of detection. If the plotted phase-solubility diagram is non-linear and shows positive deviation, it is known as type $A_P$ (Figure 2-1). This implies that the ratio of CD to drug is greater than 1. Alternatively, a negative deviation from linearity is known as type $A_N$. Although the source of this deviation has not been properly articulated in literature, Higuchi and Connors (1965) states that it may due to solvent effects or self-association of CD at high concentrations.[63] $A_L$ represents direct proportionality relationship between drug solubility and CD concentration. It is also possible to combine a drug and CD with or without any improvement in solubility and at a solubility limit where precipitation of the inclusion complex is expected to occur. These systems have been labelled as type $B_S$ and $B_i$, respectively (Figure 2-2).[6,63,66]

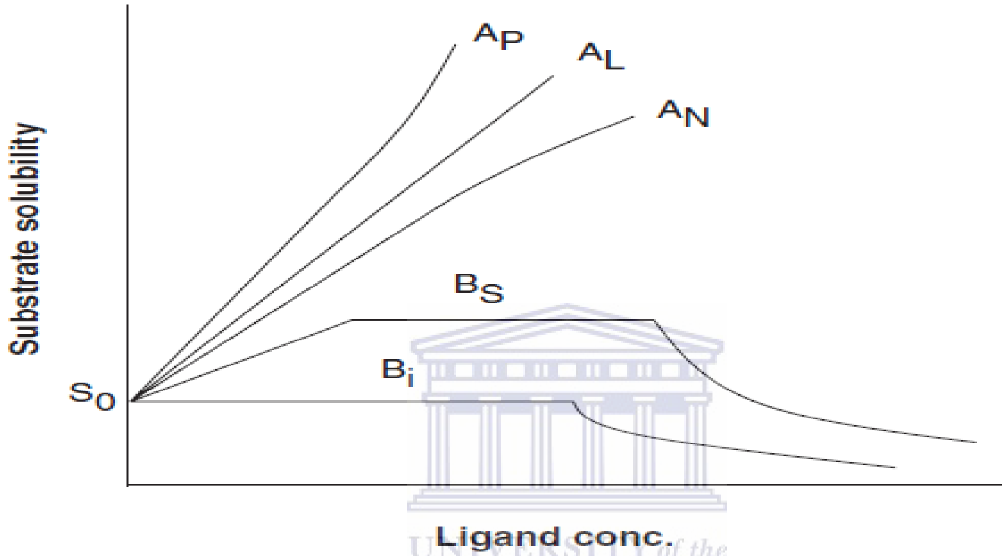

**Figure 2-2: Phase-solubility profiles and classification of complexes according to Higuchi and Connors.[63]**

## 2.11 Masking of Guest Effects

The process of masking by CDs often occurs via the inclusion of the guest in the CD molecule. As a result, the CD is kept isolated in order to prevent unwanted contact with surfaces of the body and to avoid side-effects such as irritation or an off flavour. The release of the guest from the complex is slow, resulting in an amount of free guest which is lower than if the free guest alone is used. Consequently, the amount of guest to elicit a physiological response is reduced and this results in masking or decrease in intensity of the unwanted effect of the guest. For instance, pilocarpine was administered as a prodrug, *O,O*¢-dipropionyl (1,4-xylenylene) bispilocarpine acid diester with HPβCD.[67] The severity of its accompanying irritation was decreased as the amount of CD was increased. At a concentration of 15 % CD, the irritation was reduced to the same level as the commercial formulation and the occular delivery was substantially improved.

As another example, a complex of diclofenac tablet was compared to a commercial tablet preparation, Voltaren®[68]. Lysis of the red blood cells was used to evaluate cellular lysis. In total, 50 % of the red blood cells were lysed by 10.77 mM pure diclofenac, 61.88 mM HPβCD, and 40.67 mM HPβCD diclofenac complex. It was observed that the amount of complex required to lyse the red blood cells of the complex was higher in magnitude compared to diclofenac alone. The Voltaren® lysed 22.7 % of the cells at 0.1 % concentration while under similar condition (i.e. concentration), pure diclofenac or the complex did not lyse the cells. This implies that the cell-lysing effect of diclofenac was masked in the complexed diclofenac formulation. A surfactant is used in the Voltaren® which is not needed when using the CD. The cell lysis obtained with the Voltaren® and not with the complexed formulation is likely due to the presence and absence of the surfactant in the respective formulations. It is well known that surface active agents enhance compromise of cell membranes, causing leakage. This could have promoted entrance of compounds with cell-lysing ability, resulting in the effects obtained. At a pH of 7.4, the complex had a lower permeability but a higher permeability at pH 6.0-6.5. The lag time was also reduced. At pH of 7.4, the lag time for the complex was 63 minutes while the lag time for Voltaren® was 75 minutes. At lower pH values, the lag time for the complex was 31 minutes at pH 6.5 and 35 minutes at pH 6.0. The results revealed the suitability of the HPβCD in optimizing the ophthalmic application of the drug for improved transcorneal permeability and *in vivo* tolerance based upon the haemolysis studies.

The off taste from can coatings has also been shown to be reduced or eliminated by using CDs to coat the can. Aldehydes and ketones containing 6-18 carbons atoms have been implicated in the production of tastes similar to that of stale beer in cans. Consequently, the cans were coated with and without CD and extracted with methylene chloride. The ability of CDs to keep undesirable flavour constituents out of the contents of the coated can was reported after the aldehydes were extracted from the coatings which did not contain CDs, demonstrating the ability of CDs to contain the undesirable flavour.[61]

Furthermore, a study by Marzona *et al* (1996) also demonstrated that the irritating or toxic effects of insecticides can be reduced or eliminated by complexing the insecticide with CDs.[69] This is because azinphos-methyl forms an odourless complex with β-CD. In this study, there were no systemic toxic effects observed when the insecticide was administered dermally at a dose of 4000 mg/kg in the complex while the free insecticide showed toxicity when administered dermally at 17.84 mg/kg. In addition, complexation resulted in an increase in solubility by up to about 4 orders of magnitude and the insecticidal activity was comparable to that of the commercial formulation.

## 2.12 **Formulating with CD and CD inclusion complexes**

In considering the inherent properties of CDs and their unique characteristics, the use of appropriate excipients (i.e. CDs and CD complexing material) is paramount in decision-making processes, and is applicable in the pharmaceutical industry especially in drug formulation. This is important due to the properties of material and ultimately the choice of formulation procedure. However, in the case of less desirable material properties, there may be need for granulation or re-formulation. Other factors to consider include limitations of tablet size, the effect of excipients on formation of the inclusion complex, and storage of the final product.[66]

### 2.12.1 Material Assessment

The assessment of the material properties, especially the mechanical properties must be considered for solid dosage form manufacturing and this includes other properties such as flow, density, dynamic hardness, elastic modulus and tensile strength. Of importance also are properties such as brittle fracture, bonding, and visco-elastic behaviours which are important for tabletting performance.[70,71]

For instance, Tasic *et al*. (1997)[72] utilized the scanning electron micrograph (SEM) to determine the influence of β-CD on the compression behaviour of paracetamol. This is because paracetamol is known to undergo compression by brittle fracture. In the study, four different formulations namely; Avicel PH 101 (5 %), Aerosil 200 (1 %), Ac-Di-Sol (1 %), magnesium stearate (1 %), and 460 mg of paracetamol were prepared as an inclusion complex in a physical mixture of paracetamol and β-CD or inclusion complex of paracetamol and β-CD (1:1 ratio, w/w). The inclusion complex was prepared by kneading and spray drying methods and flat-faced tablets with a diameter of 12 mm and an average weight of approximately 522 mg were produced. The tests on the tablets that contained a physical mixture of β-CD with paracetamol showed a brittle fracture compression mechanism similar to a β-CD "free" formulation. Furthermore, the paracetamol-β-CD formulations that were formed by kneading or solid spray dried dispersion methods were reported to experience plastic deformation which is consistent with the compression behaviour of CDs and correlates to the work of Shangraw *et al*. (1992).[43]

From a pharmaceutical perspective, a drug with the β-CD solid inclusion complex is considered to be most convenient for oral administration. In addition, β-CD represents the first choice in tablet formulations as filler-binder due to several reasons such as inclusion compound formation, economical and commercial availability.[72–74]

## 2.13 **Analytical techniques to characterize drug–CD complexes in the solid state**

The characterization of drug-CD solid systems and the assessment of inclusion complex formulations in the solid state requires the use of different analytical techniques. The use of these techniques can be complementary for the validation and evaluation of product formation. This is to investigate the physical, chemical, mechanical and thermal properties of the raw materials and final products.[75]

## 2.14 **Thermal analysis techniques**

Thermal analytical methods such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and hot stage microscopy (HSM) are essential for providing fundamental thermal profile data for the solid-state characterization. In particular, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) represent very diffused and widespread analytical approaches to the solid-state characterization of multi-component systems such as inclusion compounds by virtue of their reliability, simplicity and relative rapidity.[76,77]

### 2.14.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a very powerful analytical tool which may be utilized in the solid-state characterization of substances. This is because of its ability to provide detailed information on the physical, thermal and energetic properties of materials.[12,78] In particular, DSC is the most largely used thermal method for the investigation of solid-state interactions between drugs and CDs. The comparison of the thermal curves of single components, their physical mixture and the presumed inclusion compound should provide insight into the solid state modifications. It can also provide interactions between the components especially for the procedure used such as in the complex preparation and in some cases the actual inclusion complex formation. As for CDs, the complete disappearance of the crystalline drug melting peak in the DSC curve of the presumed complex is often assumed as conclusive evidence of the insertion of the drug molecule inside the CD cavity.[75]

### 2.14.2 Thermal gravimetric analysis (TGA)

TGA analysis of CD inclusion complexes is frequently used as a complementary technique to DSC analysis as it is used to support and assist in the interpretation of DSC results. TGA allows the determination of changes (weight loss) in the sample as a function of temperature gradient. The comparison of the TGA curves of pure components, their physical mixture and the interacted mixture should allow for evidenced changes in the weight loss profile of the putative complex. Ultimately, this is indicative of interactions between the components or formation of a true inclusion complex.[75]

### 2.14.3 Hot stage microscopy (HSM)

Hot stage microscopy (HSM) is an analytical technique useful for performing solid-state physical characterization of materials as a function of temperature. HSM is often used as a complementary thermal technique to DSC analysis in order to corroborate the results and to explain the nature of the thermal effects observed in the DSC curves.[75]

## 2.15 Fourier-transform infra-red (FTIR) spectroscopy

The structural functional groups present in a compound can be determined using a technique such as FTIR. It is widely used in the study of drug-CD solid complexes to identify which vibrational modes of the drug and the CD are being disturbed during the inclusion process, as well as the possible interactions between these molecules in the solid state. The changes in the characteristic bands of the guest molecule such as disappearance, broadening, variations in peak intensity or shifts in their wave number can be indicative of complex formation. These occurrences could be the result of preferential and selective restriction of the stretching vibrations of the guest molecule as a result of their inclusion into the CD cavity. It may also suggest a weakening of the inter-atomic bonds as a consequence of an altered environment around these bonds upon complexation.[75]

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# CHAPTER 3

## 3 Methodology

### 3.1 Experimental materials and methods

In this chapter the materials and methods used in this study will be presented. These include methods for selection of the appropriate cyclodextrin (CD) using information from different literature sources, analytical tests for pure zidovudine (AZT) and the selected CD and preparation of the solid inclusion AZT-CD complex, phase solubility study, tablet formulation and quality control tests of the tablet.

### 3.2 Materials

The active pharmaceutical ingredient (API), AZT, was a donation from *Aspen Pharmaceuticals* (batch no: ZD3911013, Exp date: 01/AUG /2016). The complexing agent, 2-hydroxypropyl-β-cyclodextrin, was supplied by *Sigma-Aldrich (Germany)*, microcrystalline cellulose (MCC) was supplied by *Sigma-Aldrich (Ireland)*. Reference AZT tablet, 300mg, was obtained from a local supplier in South Africa. Concentrated hydrochloric acid was obtained from (*Sigma-Aldrich*) and all water was from a Millipore distillation system (*Milford, MA, USA)*. The NMR tubes were imported from Sigma-Aldrich *(WILMAD®, USA)*.

#### 3.2.1 Selection of the most appropriate CD for inclusion studies

The shortcomings of AZT such as poor palatability, acid lability, short half-life and first pass metabolism effects are related to the oral administration route. In order to select the most appropriate CD for AZT-CD complexation, the parameters advocated by Lipinski and other scientists were considered.[1-3] These parameters assess the theoretical likelihood of an orally administered compound’s bioavailability and include molecular weight, number of hydrogen bond donors and acceptors, freely rotating bonds and polar surface area. The degree of incorporation as well as the cytotoxicity of each CD was obtained from literature sources.[4-6]

### 3.2.2 Analytical tests for pure AZT and HPβCD

AZT and HPβCD were analysed using differential scanning calorimetry (DSC) and hot stage microscopy (HSM) to assess their purity. The values obtained were compared to that in the certificate of analysis for each compound provided by the suppliers.

### 3.3 Preparation of AZT-HPβCD solid inclusion complex

The method used for the inclusion complexation of AZT with HPβCD has been described by Silion *et al*.(2011).[7] Briefly, AZT was added to a solution of HPβCD in a 1:1 ratio, and the mixture stirred for 24 hours. The suspension formed was filtered, the filtrate frozen in liquid nitrogen and lyophilyzed for 24 hours using Lobaconco freeze dry system (Free zone 6) at –45°C ± 1°C with 0.370 mBar vacuum.

### 3.3.1 Preparation of physical mixture of AZT and HPβCD

Physical mixtures of drug and CD were obtained by gentle grinding of AZT and HPβCD in a1:1 molar ratio.

### 3.3.2 AZT-HPβCD inclusion complex identification

AZT-HPβCD inclusion complex was identified using various analytical techniques, the results of which were compared to those of the individual compounds, AZT and HPβCD. Firstly, the melting point, presence of solvent and morphology of each compound was determined using hot stage microscopy (HSM) and differential scanning calorimetry (DSC). Thereafter, the thermal profiles were investigated using thermal gravimetric analysis (TGA) in order to determine the degree of each compound's degradation and weight loss. Scanning electron microscopy (SEM) was employed to prove evidence of any morphological changes which can be related to interactions between the components as well as to reveal the existence of a single component in the preparations obtained. Fourier-transform infra-red (FTIR) spectroscopy identified the vibrational modes of the drug and CD which were being disturbed during the inclusion process thereby suggesting interactions between these molecules in the solid state. Finally, proton nuclear magnetic resonance ($^1$H-NMR) technique confirmed the replacement of water molecules by AZT in the cyclodextrin cavity, hence confirming complexation.

### 3.3.3 Analytical techniques

#### *3.3.3.1 Hot stage microscopy (HSM)*

Hot stage microscopy (HSM) is an analytical technique used in performing solid-state physical characterization of materials as a function of temperature. This technique is often used as a complimentary thermal technique to DSC in order to corroborate DSC results and to help elucidate the nature of the thermal effects observed in the DSC curves. Samples were treated under the microspcope with silicon oil, which is important for solvent detection in the sample. A Linkam TH MS600 temperature control stage connected to a T95 Linkpad System Controller was used. The heater for the products was connected to an Olympus US30 colour video camera and this was used to capture pictures for the visual observation of degradation and for characterization. Images were recorded and saved by Stream Essential® software.

#### *3.3.3.2 Differential scanning calorimetry (DSC)*

Differential scanning calorimetry (DSC) is an analytical tool useful in the solid-state characterization of substances. This is because of its ability to provide detailed information about the physical and energetic properties of substances. DSC is the most largely used thermal method to investigate solid-state interactions between drugs and CDs. The comparison of the thermal curves of single components, their physical mixture and the presumed inclusion compound should provide insight into the solid-state modifications and interactions between the components.[8] Thermal analysis of the sample was performed using a Perkin Elmer differential scanning calorimeter DSC7 connected to a Perkin Elmer thermal analysis controller TAC7/DX and a Perkin Elmer gas station. The sample was scanned at a rate of 10°C/min under nitrogen gas at a flow rate of 20 ml/min. Each weighed sample with a mass gradient of 1.5-2.9 mg was crumpled in an aluminium pan and a sealed empty pan was used as reference. The calibration of the DSC instrument was performed using indium which has a melting point (mp) of 165°C. Samples were heated over the range 30-285 °C, and data were collected and analyzed using Pyris™ software.

### *3.3.3.3 Thermogravimetric analysis (TGA)*

TGA analysis of CD inclusion complexes is frequently used as a complimentary technique to DSC analysis and other thermal analytical techniques such as HSM. In TGA analysis, changes in the sample weight are monitored with respect to the temperature gradient. As a result, comparison of the TGA curves of pure components as well as their physical mixture and the interacted mixture should show changes in the weight of the putative complex. The resulting changes in weight could be indicative of interactions between the components and formation of a true inclusion complex.[9] TGA analyses of samples was performed using a Perkin Elmer thermogravimetric analyser, TGA 4000, connected to the Polyscience digital temperature controller under nitrogen gas flowing at a rate of 20 ml/minute. The calibration of the instrument was performed using indium (mp = 156.6°C) and aluminium (mp = 660.3°C). The sample was weighed in a porcelain crucible and analysed over the range 30-300°C. Data was collected and analysed using Pyris™ software.

### *3.3.3.4 Scanning electron microscopy (SEM)*

SEM is an analytical technique that provides high resolution imaging of the surface of particles. It is useful for the examination of sample morphology. In this type of microscope, a beam of electrons generated by a thermal emission source is focused on the sample. The interaction between the generated electron and the atoms of the material produces various signals which are released, thus providing information regarding sample morphology and composition. A small quantity of powder was placed on carbon adhesive tape; the latter applied onto an aluminium stub and then dried completely in a fume hood. Thereafter, the dried powder was coated with gold palladium using an Emitech™ K550X (England) sputter coater and viewed on an Auriga™ F50 HR-Scanning Electron Microscope with working distances of 6.6, 6.7 and 6.8 mm and an accelerating voltage of 5 kV as the instrument operating parameters. The micrograph images of the particles were obtained within a range of (5-50) k x and demonstrated the shape of the powder.

### *3.3.3.5 Proton Nuclear Magnetic Resonance ($^1$H NMR) analysis*

A small amount of the sample (8-10 mg) was weighed and dissolved in sufficient amount of deuterated water ($D_2O$). The sample solution was transferred into NMR tubes. Nuclear magnetic resonance (NMR) analyses were conducted and spectra recorded by a Bruker 400 MHz Avance III HD Nanobay spectrometer equipped with a 5 mm broadband observe (BBO) probe. The spectrum was recorded on TopSpin™ 3.2, with chemical shifts in ppm. Complex formation was confirmed by NMR shift in peaks which could be observed for both the CD and the guest. The resulting shifts in peaks for CD were attributed to a simultaneous change in the environment around the hydrogen atoms in relation to cavity changes in association with the guest. Similarly, shifts can be observed for peaks corresponding to atoms of the guest which penetrate into the cyclodextrin cavity.[10]

### *3.3.3.6 Fourier-transform infra-red (FT-IR) spectroscopy*

A Perkin Elmer 100 Fourier transform infra-red (FTIR) spectrum was used to confirm AZT-CD inclusion complex formation. All spectra were collected by Spectrum® software version 6.3.5. The IR spectrum of the new compound was compared to those of the parent compounds since each molecule has a specific infra-red absorption. Changes in the characteristic bands of the guest molecule such as disappearance, broadening, variations in peak intensity or shifts in wave number can be indicative of complex formation.

### *3.3.3.7 UV spectrophotometry*

UV spectra were recorded on a Cintra® 2.2 GBC Scientific Equipment Pty. Ltd. The UV/visible spectra were collected over a wavelength range of 190-450 nm at a scanning rate of 1200 nm/min. Samples were placed in 2 mm quartz cuvettes and the concentrations suitably adjusted such that all absorbance readings were measurable between zero and one absorbance unit. Calibration curve for AZT was constructed using readings collected at the lambda maximum value of the appropriate drug and the absorbance of solutions then determined.

### 3.3.4 Palatability assessment and solubility determination of the inclusion complex formed

The palatability of the formulated AZT-HPβCD inclusion complex was assessed by its taste, which was compared to the taste of the reference AZT powder. The solubility of the formed complex was determined by successive addition of increasing quantities of complex until saturation was established. The solubility of AZT in the inclusion complex was calculated from the concentration of the saturated solution.

### 3.3.5 Phase solubility study

The phase solubility measurements were performed according to Higuchi and Connors method.[11] An excess amount of drug was added to 1.5 mL of aqueous CD solution (concentration range from 4–64 mg/ml) in 10 mL poly top vials and agitated at 25 ± 0.5°C to ensure complete dissolution. At equilibrium after 24 hours, aliquots were withdrawn, filtered using a 0.45 μm pore size filter and spectrophotometrically assayed for drug content at 266 nm. The apparent binding constants of the AZT-CD complexes were calculated from the slope and intercept of the straight lines from the phase solubility diagrams according to the equation:

$$K = \frac{slope}{S_0(1 - slope)}$$

**Equation 3-1**

### 3.3.6 Formulation of AZT-HPβCD inclusion complex tablet

#### *3.3.6.1 Direct Compression Process*

In the direct compression process, the tablet press was used in such a way that a given mass of AZT-CD complex powder was measured and then compressed. This was in contrast to normal operations where the fill volume of the die cavity is used to predict the amount of powder to be compressed. An easier understanding of the above is as follows: the researchers took a given mass of powder and compressed the powder mass, whereas in an industry setting, the amount of the powder used per tablet is dependent on variables such as die cavity volume and overfill modifications. A delicate aspect which required concentration was the actual compression of the tablets. Calibration of the tablet press was quite a challenging and wearisome process. There were no adjustment indicators for the punch and die and for the hardness of the tablet. The optimal settings were obtained by trial and error after compression and analysis of individual tablet’s hardness value and disintegration times.

#### *3.3.6.2 Evaluation of tablets*

The AZT-CD complex tablet formed was subjected to palatability assessment and a number of quality control tests such as resistance to crush, friability, durability, disintegration and dissolution, evaluated as per USP specifications where available. The palatability, resistance to crush and durability are non-official parameters while friability, disintegration and dissolution are official parameters according to the United States Pharmacopoeia (USP). The non-official parameters, i.e., resistance to crush and durability, do not have specification limits whereas the official parameters are known to have specification limits.[12]

#### *3.3.6.3 Resistance to Crush*

The apparatus used to measure the hardness of the tablet was a Pharma Test PTB 301 with a pair of jaws facing each other. The operational procedure was such that one jaw remained motionless while the other jaw moved toward the tablet (i.e., force was applied to the tablet). Ten tablets were used for this test. The maximum, minimum and mean forces were expressed in Newtons.

#### *3.3.6.4 Friability*

An Eureka TAD Gmbh® friabilator was used to assess friability in order to ensure that quality of the product was not compromised as at when it reaches the end user. Twenty (20) tablets were weighed and their cumulative mass recorded as W1X. The tablets were placed in the friabilitor and underwent a hundred revolutions (25 revolutions per minute [rpm] for 4 minutes). The brushed tablets from the 20 tablets were weighed and their mass recorded as W2X. Percentage mass lost was calculated thus:

$$\left(\frac{W1X - W2X}{W1X}\right) x\ 100$$

**Equation 3-2**

A percentage mass loss not greater than 1.0 % is considered acceptable for most products

#### *3.3.6.5 Durability*

The Erueka TAD Gmbh® friabilator was used to determine the percentage mass lost in the durability test. This is similar to the friability test; however, in this case, the plexidrum is rotated for 15 minutes and loss is calculated in terms of percentage. In measuring durability, a mass loss of ≤ 2 % is within the acceptable limit.

#### *3.3.6.6 Disintegration*

The Veego® tablet disintegration test apparatus was used to assess AZT-CD tablet disintegration. Two disintegration media (water and 0.1 N HCl) maintained at 37°C (i.e., body temperature) were used. A total of 6 tablets were used for this test. If one or two tablets from the 6 tablets failed to disintegrate completely within 30 minutes, then the test should be repeated with another 12 tablets. If less than 16 tablets from the total number of tablets used for the test, disintegrate, then the batch has failed the test according to USP (2008) specifications.

## 3.4 **Dissolution of AZT formulations**

### 3.4.1 Dissolution test method

Dissolution tests of the AZT powder, AZT-CD complex tablet, and branded AZT tablet were assessed using the USP paddle method in the SOTAX T7 smart automated dissolution testing unit (*7100-1, CH-4123 ALLschunil*) in 0.1 N hydrochloric acid (pH 1.2). Each vessel was filled with 900 ml of the dissolution medium maintained at 37 ± 0.5°C. A single dosage unit (one AZT-CD complex tablet, one branded AZT tablet or 50 mg AZT powder) was placed in each dissolution vessel, and vessel contents stirred at 75 rpm.

At predetermined time intervals (5, 10, 15, 20, 30, 45, and 60 minutes), 4.5 ml of sample was withdrawn from each vessel through a 0.45 micron nylon membrane and replaced with an equal volume of the same pre-warmed medium in order to maintain constant volume and temperature.

### 3.4.2 Analysis of dissolution samples

The withdrawn samples were analysed using the Agilent 1200 series HPLC system equipped with an in-line degasser, quaternary pump, automated loading sampler, thermostatted column compartment and photodiode array detector. No separation was conducted and therefore a column was not used. The mobile phase consisted of acetonitrile and water (2:98 v/v), each with 0.1 % formic acid, and was filtered through a 0.45 µm filter and degassed with helium prior to use. The mobile phase was in isocratic mode with flow rate maintained at 1.0 ml /minute. Injection volume was 20 µl. The eluent was monitored over a number of wavelengths, with the specific wavelength of interest as 266 nm. Data acquisition and processing was by the OpenLAB™ CDS Chemstation Edition software.

Data was plotted as percentage dissolved ± standard deviation *versus* sample dissolution time. Dissolution profiles of the reference and test AZT formulations were compared using the similarity factor, f2.[14]

The ultraviolet (UV) spectroscopic method used for analysis of dissolution samples was validated for linearity and precision according to guidelines set by the International Conference on Harmonization (ICH, 1995). Stability of the working standards was evaluated by exposure of reference samples at low, medium and high concentrations to heat, light, and consecutive freeze-thaw cycles.

The limit of detection (LOD) and the limit of quantitation (LOQ) are defined at signal-to-baseline ratios of 3:1 and 10:1, respectively. The LOD and LOQ were calculated from the formulae:

$$\boldsymbol{LOD = 3\left(\frac{SD}{S}\right)}$$

**Equation 3-3**

$$LOQ = 10\left(\frac{SD}{S}\right)$$

**Equation 3-4**

where SD and S are the standard deviation of the response and the slope of the calibration curve, respectively.

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# CHAPTER 4

## 4 Results and discussion

In this chapter, the results obtained from the experimental study on zidovudine-cyclodextrin (AZT-CD) inclusion complex, and the results of all identification and characterization tests performed on the complex are presented. Results of quality control tests on the tablets formulated are also presented and discussed.

### 4.1 Introduction

The objective of this study was to prepare an inclusion complex between AZT and hydroxyl propyl beta cyclodextrin (HPβCD) as a means of addressing some of the limitations related to the pure AZT such as palatability, acid lability and short half life. Several stages are involved before complex formation can occur. It is thus important and appropriate that each of these stages be properly executed in order to achieve successful formulation of the CD inclusion complex. These stages included the choice of the most appropriate CD where toxicity and degree of incorporation between the drug and CD was considered. Another stage involved identification tests for the compound and CD of interest. Following preparation of the drug-CD complex, analytical tests were conducted in order to characterise the formed complex.

The following analytical techniques were employed in this study: hot stage microscopy (HSM), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy. After successful complex formation, the powdered complex was formulated into tablets. Quality control tests were conducted on the formulated AZT-CD tablet and the commercially available generic AZT tablet, and the results of these tests compared.

### 4.2 Selection of the most appropriate CD for inclusion studies

The most appropriate CD for inclusion studies was selected following careful consideration of the set of variables in Table 4-1, for available CDs. These variables are from published determinants of the bioavailability of orally formulated compounds as well as from incorporation and toxicity studies.[1–6] According to these literature sources, an orally active drug generally has no more than one violation of the following criteria:

- No more than 5 hydrogen bond donors,
- No more than 10 hydrogen bond acceptors,
- A molecular mass less than 500 Daltons,
- An octanol-water partition coefficient (log P) value less than 5.

Other variants of this rule propose for not more than 10 rotatable bonds and polar surface area equal to or less than 140 Å$^2$.

**Table 4-1: Summary of the CDs complexsed with AZT**

| **VARIBALE** | **G$_2$βCD** | **HPβCD** | **DMβCD** |
|---|---|---|---|
| **Molecular formula** | $C_{66}H_{110}O_{55}$ | $C_{63}H_{112}O_{42}$ | $C_{56}H_{98}O_{35}$ |
| **Molecular weight** | 1783.6 g/mol | 1541.5 g/mol | 1331.4 g/mol |
| **H-bond acceptors** | 55 | 42 | 35 |
| **H-bond donors** | 32 | 21 | 7 |
| **Freely rotating bonds** | 51 | 28 | 21 |
| **Polar surface area** | 859.65 Å$^2$ | 619 Å$^2$ | 400 Å$^2$ |
| **Degree of incorporation (mg/g complex)** | 1.2 | 4 | 4 |
| **Cytotoxicity** | ________________ | Well-tolerated and safe in majority of patients receiving HP-β-CD at daily oral doses of <16 g. | Shows high cytotoxicity and disrupts structures of lipid rafts |

From the table, none of the CD derivatives comply with and passed *Lipinski's* rule or its other variants.[1-3] In consideration of all the variables highlighted in Table 4-1, it was observed that dimethyl beta cyclodextrin (DMβCD) which is a methylated cyclodextrin was considered the best in terms of drug delivery and degree of incorporation for AZT, as well as in terms of the variables set by Lipinski and other authors. However, methylated cyclodextrins like DMβCD possess considerable surface activity and exert detergent effects unlike their hydroxylated counterparts.[4] These properties, i.e., surface activity and detergent effects, cause DMβCD to be haemolytic and irritating to mucous membranes thus making it quite toxic. As a result, its other favourable properties are limited by its toxicity which makes it not suitable for oral formulations. For this reason, the next best choice, HPβCD, which has been shown to be parentally and orally safe as well as non-irritating to mucous membranes was selected for formulation of the inclusion complex.

## 4.3 Characterization of pure AZT and HPβCD

The analytical techniques (previously mentioned in Section 3.3.3) were used to investigate the thermal, chemical and morphological properties of AZT and HPβCD. Results from these analytical techniques are presented in the sections below.

### 4.3.1 Hot stage microscopy (HSM) for AZT

HSM is a thermal analytical method that is used to determine the melting point of a sample.
In this process, a small amount of powder immersed in silicone oil to assess the presence of water or organic solvent as would be indicated by bubble formation, was heated at a rate of 10°C per minute. Changes associated with sample heating were observed under the microscope over the temperature range 20°C to 230°C. The result of HSM analysis for AZT is presented in Figure 4-1.

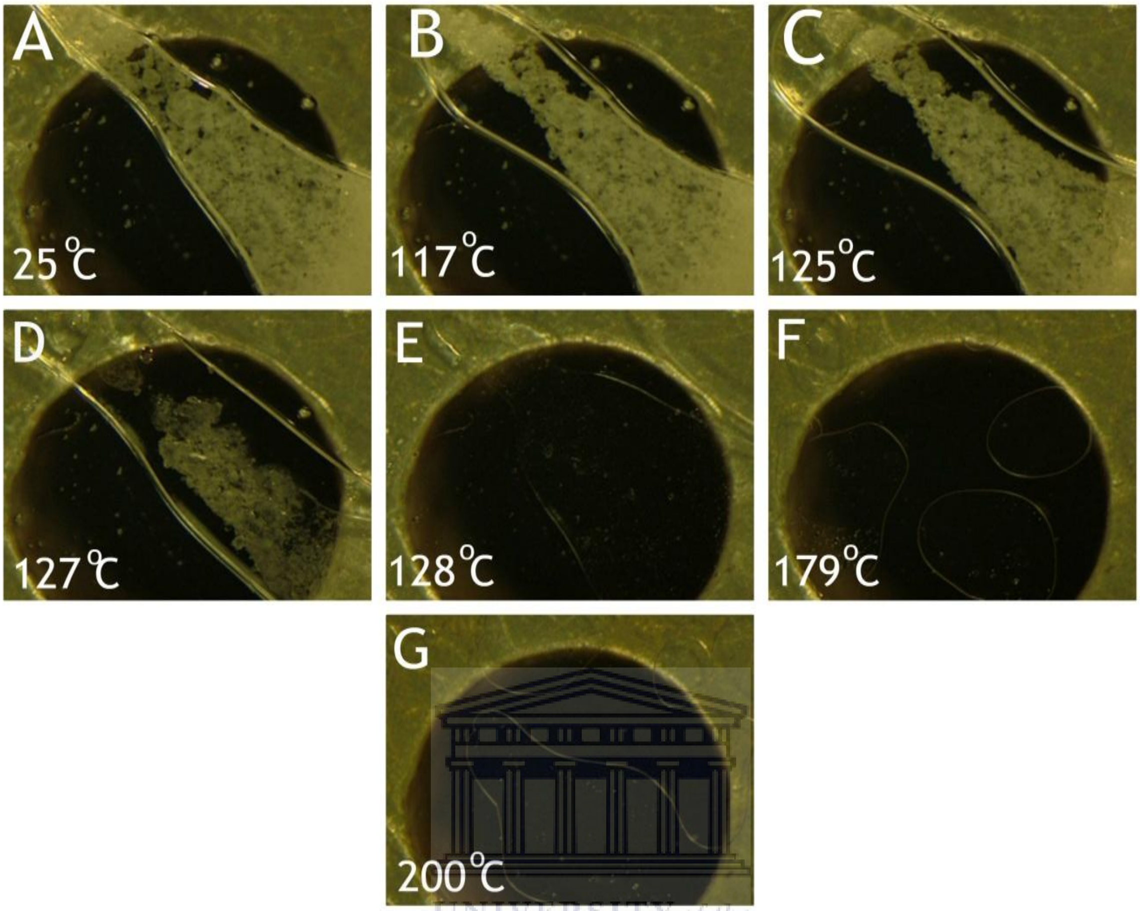

**Figure 4-1: HSM of the reference AZT**

The figure shows that melting of AZT commenced at 117°C (Figure 4-1B). The presence of bubbles was not observed which could indicate that the AZT sample did not contain moisture. In addition, Figure 4-1D revealed that at 127°C, a fragment of continuous melting of AZT was observed while complete disappearance due to melting occurred from 127°C (Figure 4-1D) up to 128°C (Figure 4-1E). The HSM results obtained for the AZT sample in Figure 4-1 agreed with the certificate of analysis which indicated that the melting point of AZT occurs between 122°C and 129°C. The commencement of AZT decomposition, indicated by discolouration, was observed from 179°C (Figure 4-1F).

### 4.3.2 Differential scanning calorimetry (DSC) for AZT

DSC is a complimentary thermal analytical method used to consolidate thermal properties of samples. The DSC analysis of AZT was performed at a constant rate of 10°C per minute under nitrogen gas purged at 20 ml per minute. Figure 4-2 shows the DSC plot for the reference AZT.

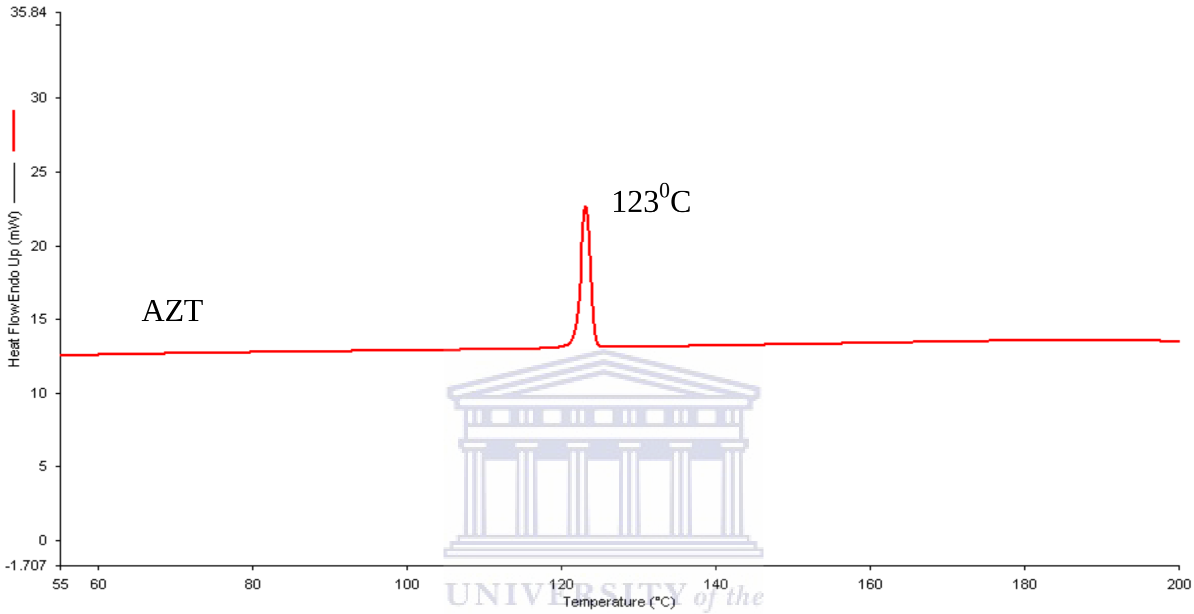

**Figure 4-2: DSC curve of the reference AZT**

The DSC endotherm profile for AZT (Figure 4-2) shows a single endotherm in the temperature range 121°C to 124°C with a peak observed at 123°C and with onset temperature identified at 121.04°C. The DSC endotherm profile for AZT did not indicate the presence of other substances or impurities which could have been observed with an additional endotherm profile; thus the AZT used in this study can be considered pure. In addition, DSC analysis supported the result observed from the HSM analysis of the reference AZT. This validation is significant because the melting point of AZT from HSM analysis can be a useful evaluation stage especially during complex formation. The two analytical tests (HSM and DSC) conducted for the reference AZT were also conducted for the HPβCD, results of which are presented in the following sections.

### 4.3.3 Hot stage microscopy (HSM) for HPβCD

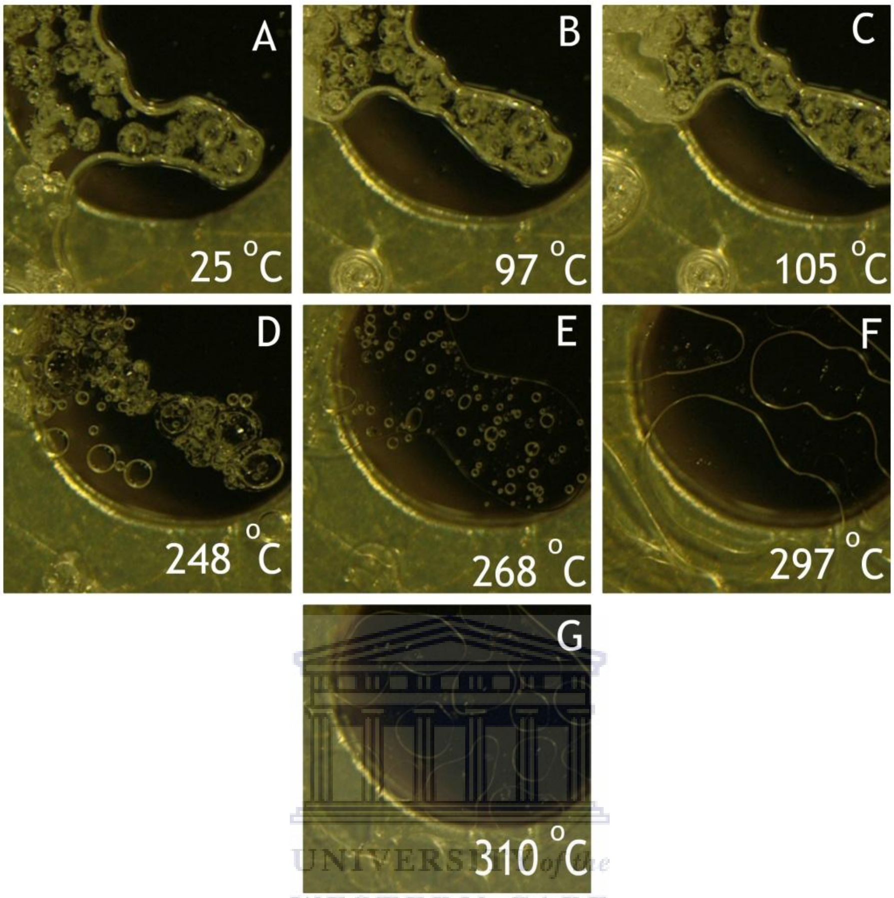

**Figure 4-3: HSM of the reference HPβCD**

In Figure 4-3, the result from HSM analysis of HPβCD is presented. The presence of bubbles was observed at 97°C as seen in Figure 4-3B; this suggests that the pure HPβCD contained trace of water (moisture). The presence of moisture in HPβCD could be attributed to the composition of HPβCD especially with the presence of the hydroxyl (-OH) groups. In addition, it was shown that melting of the reference HPβCD occurred between 248°C (Figure 4-3D) and 268°C (Figure 4-3E) although the presence of bubbles was still prevalent. Thermal decomposition of the reference HPβCD was observed to occur at approximately 300°C, indicated from the traces Figures 4-3F and 4-3G. The physical examination of the final product obtained after HSM analysis of HPβCD showed that the product had a glassy appearance which can be considered to indicate that HPβCD has been transformed from a crystalline to an amorphous product (Figure 4-3G). The melting point of the HPβCD agreed with the safety data sheet supplied with the sample thereby confirming identity of the compound. The melting point of the reference HPβCD indicated by the supplier is 267°C.

### 4.3.4 Differential scanning calorimetry (DSC) for HPβCD

The thermal analysis of HPβCD by DSC technique was used to determine the melting point and the possibility of the any trace of impurities or moisture in the HPβCD sample. DSC analysis for HPβCD was performed at a constant rate of 10°C per minute under nitrogen gas purged at 20 ml per minute. The result of this analysis for the reference HPβCD is presented in Figure 4-4.

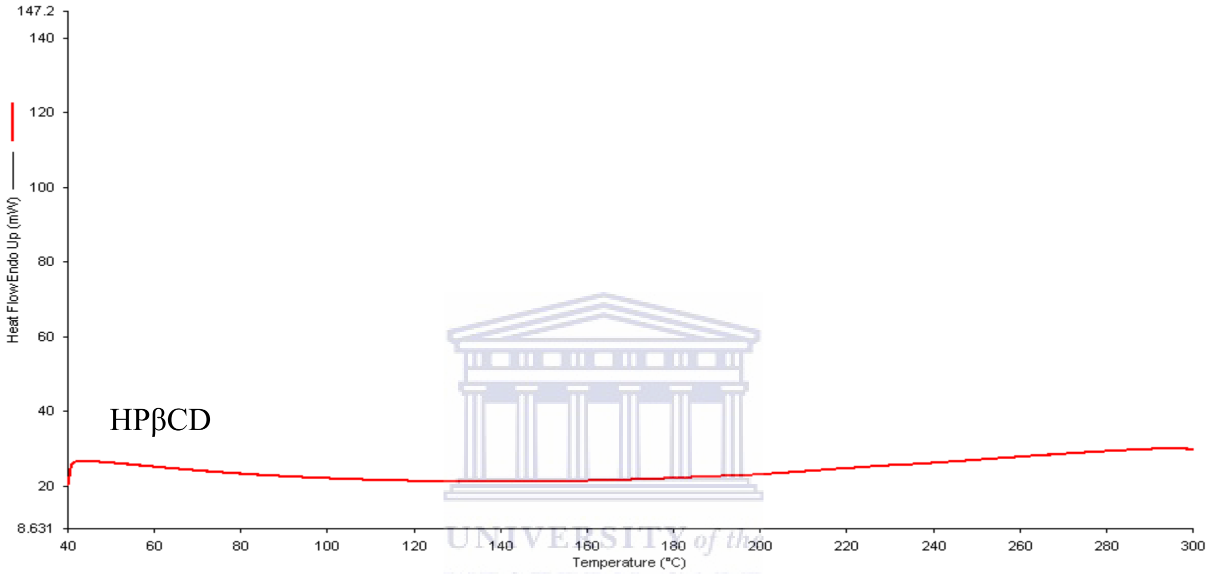

**Figure 4-4: DSC curve for HPβCD**

The DSC plot of HPβCD (Figure 4.4) revealed a broad endotherm profile of very weak inflection without any distinct peak. The broad endotherm can be attributed to water loss from HPβCD which results in the transformation of HPβCD from crystalline to amorphous form. In addition, Figure 4-4 did not show any characteristic endotherm which suggests an amorphous form of the solid. The next sections present results obtained from analytical tests on the formulated solid inclusion complex.

## 4.4 Preparation of solid AZT-HPβCD inclusion complex

The AZT-HPβCD solid inclusion complex was prepared by the freeze-drying method, briefly described.

### 4.4.1 Freeze-drying method

A weighed amount (0.262 g) of HPβCD was dissolved in 3 mL of distilled water to form a colourless solution. Thereafter, 0.050 g of AZT was added to the solution and stirred for 24 hours at room temperature. The suspension formed was filtered and the filtrate was freeze-dried to produce the complex in powdered form. The formulated AZT-HPβCD inclusion complex was white with a soft fluffy appearance.

## 4.5 Characterizations of AZT-HPβCD inclusion complex formation

### 4.5.1 Hot stage microscopy (HSM)

HSM analytical technique was used to investigate the thermal properties of the newly prepared inclusion complex especially to determine its melting point. The HSM analysis of the inclusion complex was performed by adding a single drop of silicon oil to the sample in order to identify the presence of solvent in the inclusion complex sample. The presence of any solvent will be registered by bubble formation prior to melting. Figure 4-5 shows the result of the HSM analysis.

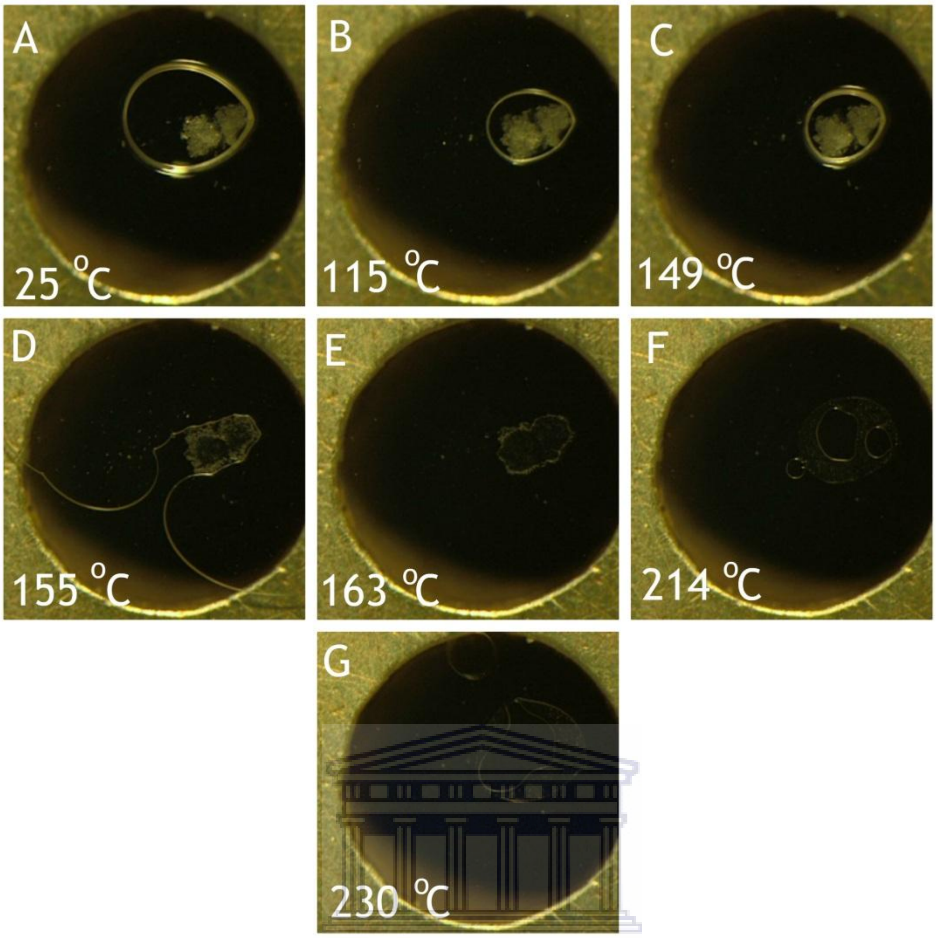

**Figure 4-5: HSM of AZT-HPβCD inclusion complex**

It was observed that the inclusion complex did not undergo any noticeable change in its physical appearance at room temperature until 149°C (Figure 4-5A to Figure 4-5C). This implied that the AZT-HPβCD inclusion complex formulation retained its original form as a white powder. Melting of the complex was observed between 155°C (Figure 4-5D) and 163°C (Figure 4-5E) while thermal decomposition was observed at 214°C with characteristic spontaneous bubble formation (Figure 4-5F). The decomposition temperature of the AZT-HPβCD inclusion complex can be considered to indicate that the sample had lost its intrinsic thermal properties. A comparison between the HSM results in Figure 4-5 and that obtained for the reference AZT (Figure 4-1) shows significant variation in the thermal behaviours of the pure AZT (Figure 4-1) and the AZT-HPβCD inclusion complex (Figure 4-5); indicating that a complex was formed between AZT and HPβCD. This is because the thermal stability of the newly formed AZT-HPβCD inclusion complex was higher than that of the reference AZT sample analysed by HSM method.

### 4.5.2 Differential scanning calorimetry (DSC)

DSC analysis of the newly formed AZT-HPβCD inclusion complex was performed to determine its thermal stability as well as to identify the presence of characteristic endotherm associated with the AZT-HPβCD inclusion complex.

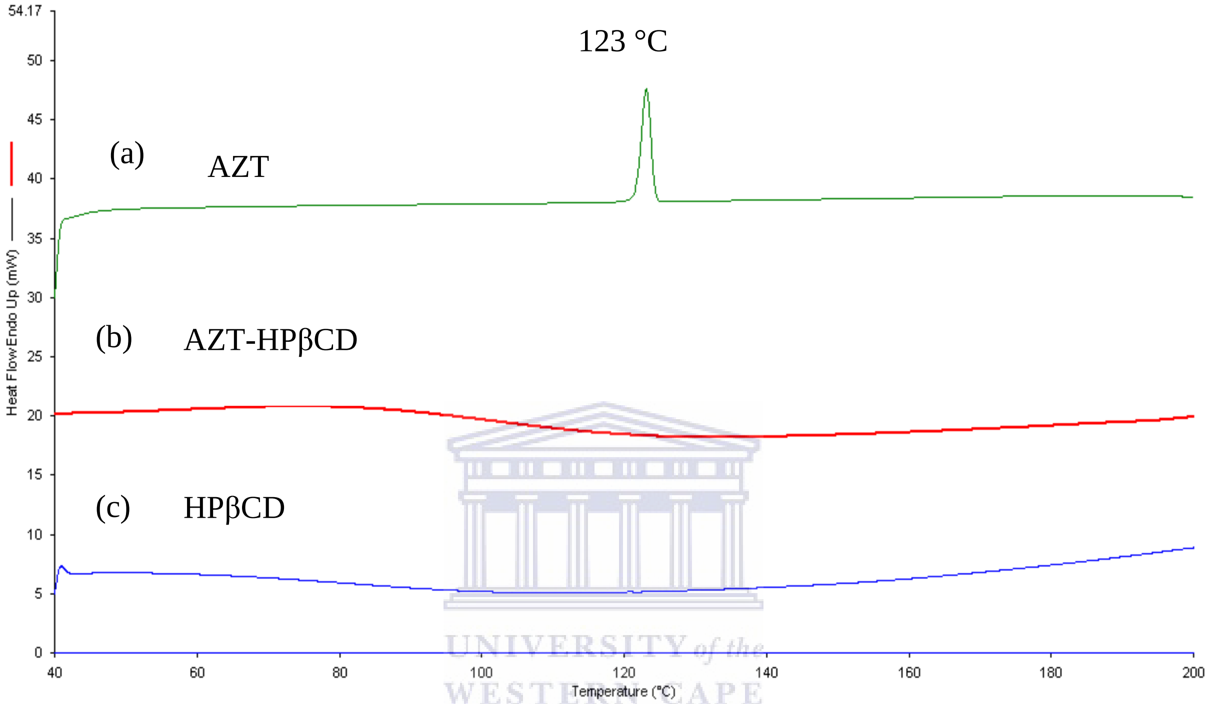

**Figure 4-6: DSC curves of AZT, AZT- HPβCD inclusion complex and HPβCD**

The DSC endotherm for AZT (Figure 4-6a) showed the presence of a single endothermic peak located at 123 °C. The DSC endotherm curves for HPβCD (Figure 4-6c) and AZT-HPβCD inclusion complex (Figure 4-6b) did not show any such characteristic peak. The DSC profile of the newly formed AZT-HPβCD inclusion complex indicated complete disappearance of the endothermic peak associated with the reference AZT which was identified in Figure 4-6a. The disappearance of the endothermic peak validated the interaction between the AZT and HPβCD, and suggesting indicative of drug inclusion complex formation.[7]

### 4.5.3 Thermogravimetric analysis (TGA)

The change in thermal stability, evolution of volatile species and degradation profile of AZT, HPβCD and AZT-HPβCD inclusion complex was performed to assess for differences in thermal properties of the newly formed inclusion complex and the starting materials, i.e., AZT and HPβCD. Figure 4-7 presents the TGA results of AZT, HPβCD and newly formed AZT-HPβCD inclusion complex.

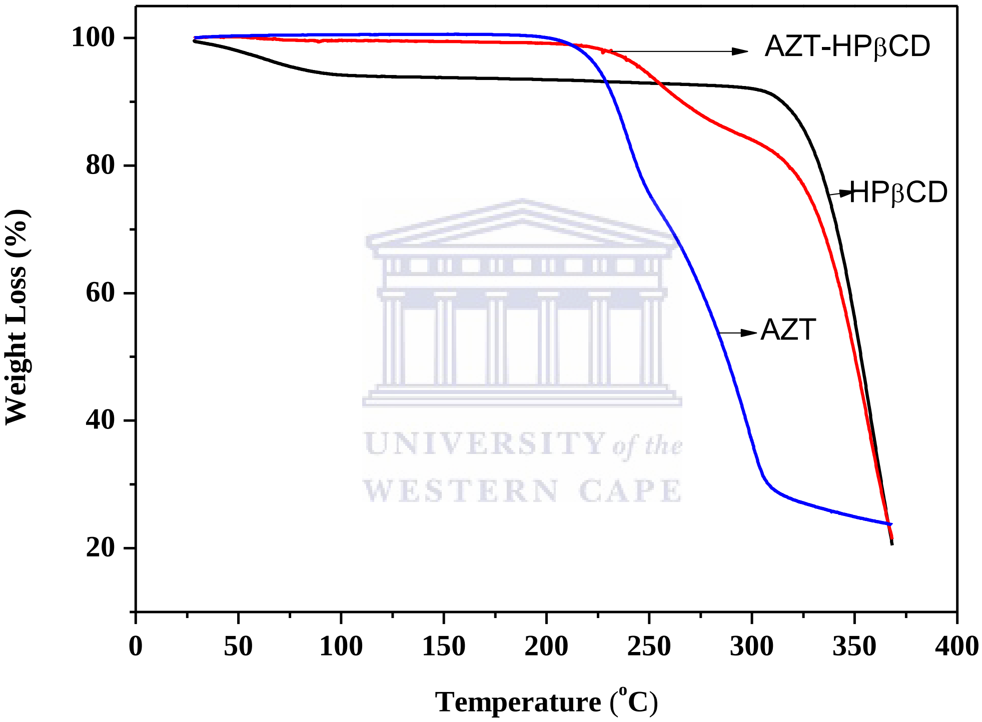

**Figure 4-7: TGA curves of the HPβCD, AZT and AZT-HPβCD inclusion complex**

The TGA analysis of AZT, HPβCD and AZT-HPβCD inclusion complex (Figure 4-7) shows the thermal responses of the individual materials to heat. It was observed that both AZT and AZT-HPβCD inclusion complex did not indicate the release of volatile species or weight loss associated with moisture. Thermal stability of AZT-HPβCD inclusion complex was also improved by approximately 50°C compared to the thermal stability profile of AZT as seen in the figure. In addition, the first mass loss of AZT was about 25 % compared to the mass loss for AZT-HPβCD inclusion complex (Figure 4-7) which was approximately 10 %. In the case of HPβCD, a mass loss of about 6 % was observed before heating to a temperature of 100°C which could be attributed to moisture or trace of water in the sample. As seen from the TGA thermogram in Figure 4.7, the reference AZT showed its initial degradation at 200°C while the HPβCD started to degrade at about 300°C. The TGA thermogram of the AZT-HPβCD inclusion complex showed two distinct degradation steps with the first step of degradation at 250°C, and the second step commencing at about 310°C. In the case of AZT, several degradation steps were observed which showed spontaneous degradation at high temperatures. Generally, with successful insertion of AZT into the HPβCD cavity, the TGA thermal analysis of the newly formulated inclusion complex should differ from and be more stable than that of the free AZT. The thermal stability of AZT-HPβCD had improved similar to what was obtained in an earlier study by Ishiguro *et al.* (1995).[8]

### 4.5.4 Fourier-transform infra-red (FT-IR) spectroscopy

Infra-red (IR) technique is used to reveal the identity of functional groups present in a sample and to identify the susceptibility of these functional groups to chemical reactions *via* reduction in band intensities or complete disappearance of characteristic bands. The infrared spectra of AZT, HPβCD and AZT-HPβCD inclusion complex are presented in Figure 4-8.

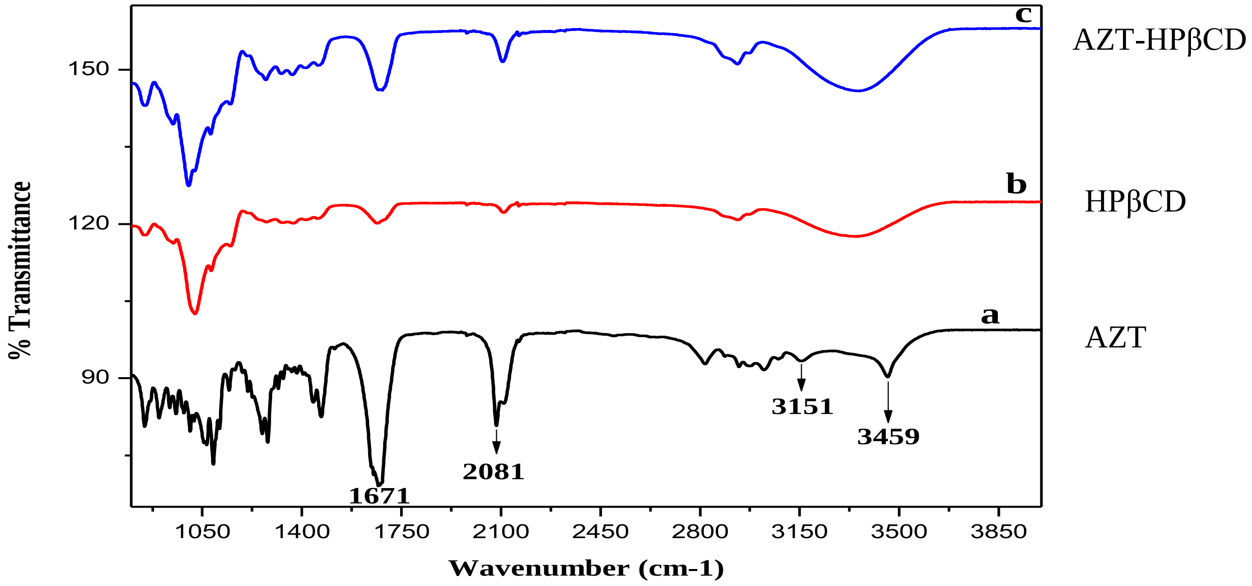

**Figure 4-8: FTIR spectrum of AZT, HPβCD and AZT-HPβCD inclusion complex**

The following characteristic peaks were identified within the AZT spectrum (Figure 4-8a) and assigned accordingly. The band located at 3459 $cm^{-1}$ represents the amide (N-H) stretch while the bands at 3151 $cm^{-1}$ has been assigned to the hydroxyl group (O-H stretch).[9] The nitrile ($N_3$) species was observed with a characteristic band at 2081 $cm^{-1}$ and the carbonyl moeties of acetyl group indicated a spectrum at 1671 $cm^{-1}$ (C=O stretch of acetyl group – CO-NH-CO-). The FTIR spectrum analysis of the inclusion complex, AZT-HPβCD (Figure 4-8c), when compared to that of the pure AZT (Figure 4-8a), shows disappearance of the AZT characteristic bands at 3151 $cm^{-1}$ and 3459 $cm^{-1}$ in the AZT-HPβCD inclusion complex (Figure 4-8c). In addition, there was reduction in intensities for the bands at 2081 $cm^{-1}$ and 1671 $cm^{-1}$ for both HPβCD (Figure 4-8b) and AZT-HPβCD (Figure 4-8c). These changes in band intensities suggest the existence of a new compound, the formed complex,[10] with different spectroscopic bands.

### 4.5.5 Scanning electron microscope (SEM)

The surface analysis of samples by the SEM technique can be used to understand the change in morphology, shape and particle size distribution of powdered samples. In Figure 4-9, results of the SEM morphological analysis for the AZT, HPβCD and the newly formed inclusion complex, AZT-HPβCD; is presented.

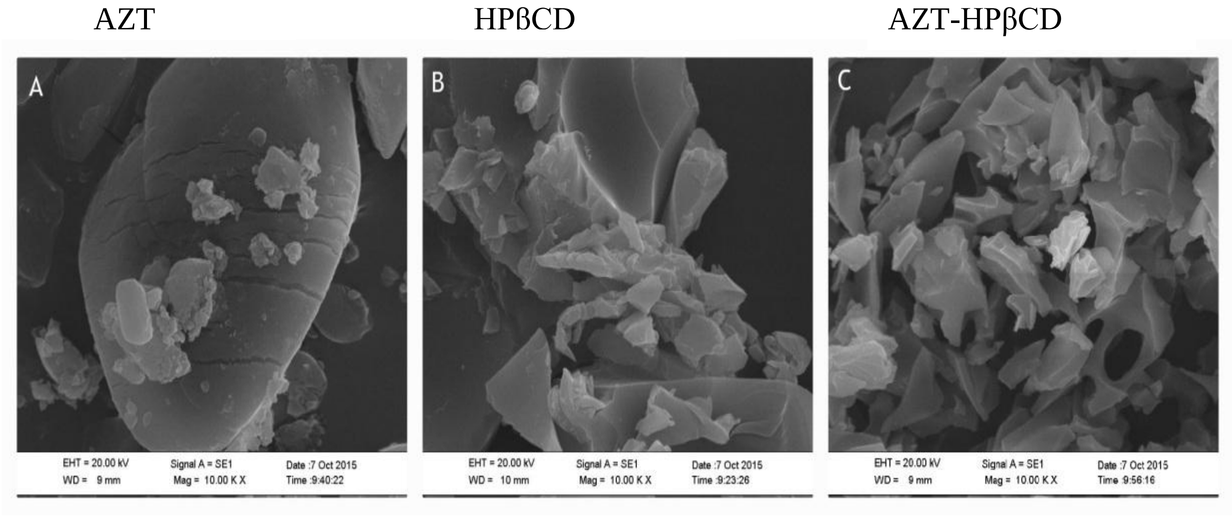

**Figure 4-9: SEM of AZT, HPβCD and AZT-HPβCD inclusion complex.**

The surface morphology of AZT, HPβCD and AZT-HPβCD as shown in Figure 4-9A, 4-9B and 4-9C showed significant variation in the morphology and particle shapes of the powdered samples. The SEM micrograph image of AZT (Figure 4-9A) shows spherically shaped particles with coarse surface and presence of distinct cracks on the surface. The SEM micrograph of HPβCD (Figure 4-9B) on the other hand revealed fragmented particles with sharp edges and rhombic shape. The surface of these particles (for HPβCD) was smooth and defect-free. After formation of the inclusion complex, i.e., AZT-HPβCD (Figure 4-9C), the SEM morphology showed unagglomerated particles with depressions on the surfaces. The distinct difference in shapes between the AZT, HPβCD and AZT-HPβCD inclusion complex showed that a complex of the inclusion components had indeed formed.

### 4.5.6 Nuclear magnatic resonance ($H^1$-NMR)

The $^1$H-NMR spectra of AZT, HPβCD and the inclusion complex are presented in Figure 4-10. Deuterated water ($D_2O$) was used as solvent; the chemical shift for the solvent is at 4.80 ppm.

The inner H3’ proton of HPβCD significantly shifted upfiled (0.07ppm), whereas the outer H1’, H2’, H4’ and methyl protons of the 2-hydroxy propyl group showed small shifts of about 0.01 ppm. Unfortunately, the inner H5’ proton of HPβCD could not be quantitatively monitored because of overlap with other protons, as has been reported in a previous study.[11] These results suggested that AZT has been included in the HPβCD cavity. Chemical shift changes were observed in most protons of AZT: 5’-H protons of the furanose ring upfield shift (0.02), 3’-H protons of furanose ring downfield shift (0.01), 4’-H protons of the furanose ring upfield shift (0.02), 1’-OH proton of furanose ring upfield shift (0.02), and 6’-proton of thymin upfield shift (0.02).

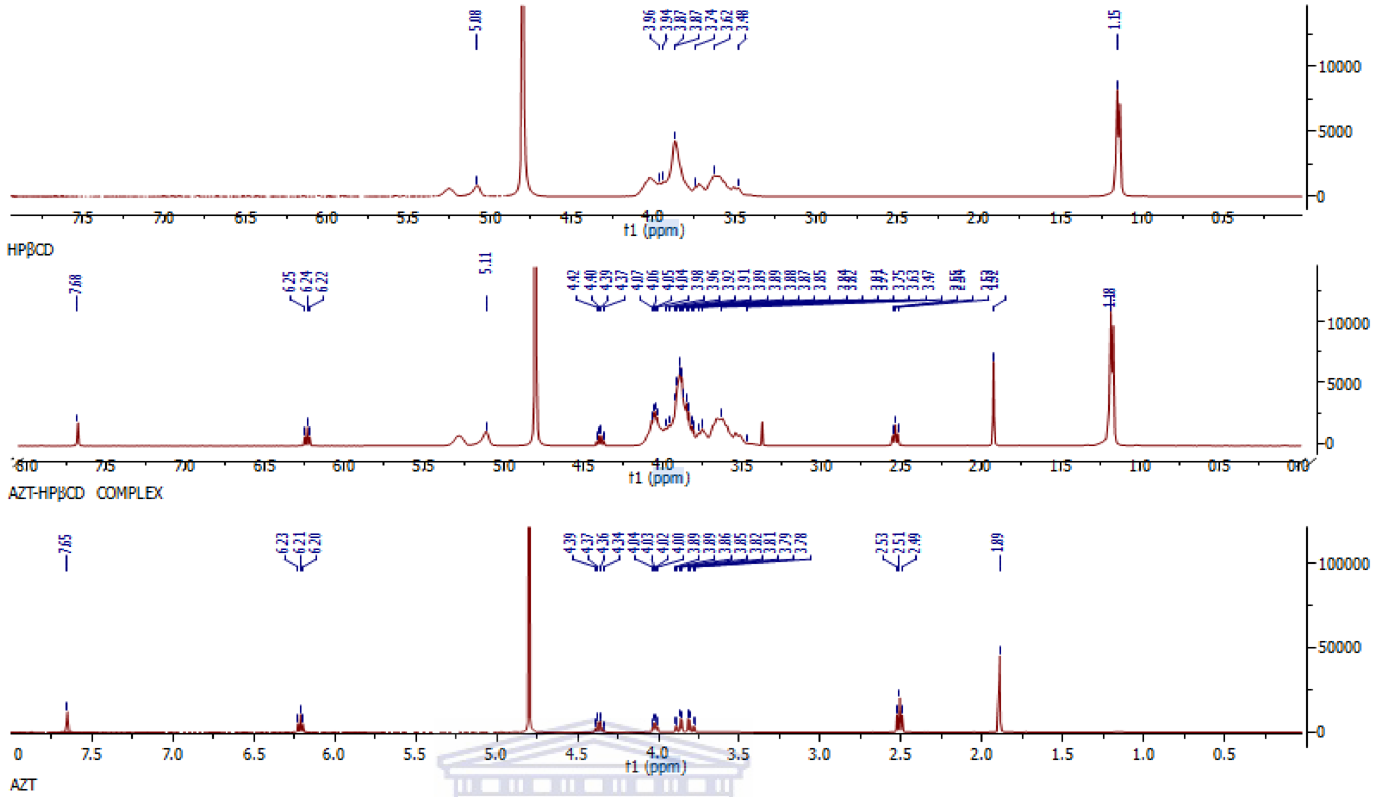

**Figure 4-10: H$^1$-NMR of the HPβCD, AZT and AZT-HPβCD inclusion complex.**

## 4.6 Physical mixture of AZT and HPβCD

To compare the complex formed with uucomplexed AZT-HPβCD preparation, physical mixtures of the host (HPβCD) and guest (AZT) compounds were prepared. The procedure for the preparation of the physical mixture was as follows. AZT and HPβCD were carefully weighed and mixed in a 1:1 molar ratio and thereafter pulverized using the mortar and pestle. The physical mixture was subjected to the same analytical tests as the newly formed AZT-HPβCD inclusion complex and subsequently characterized for differences, if any, between the complex and the physical mixture. The melting point of the physical mixture occurred within the same range as for pure AZT (Figure 4-11). This suggests that no inclusion complex was formed between AZT and HPβCD in the physical mixture, unlike the DSC result of the AZT-HPβCD inclusion-complex.

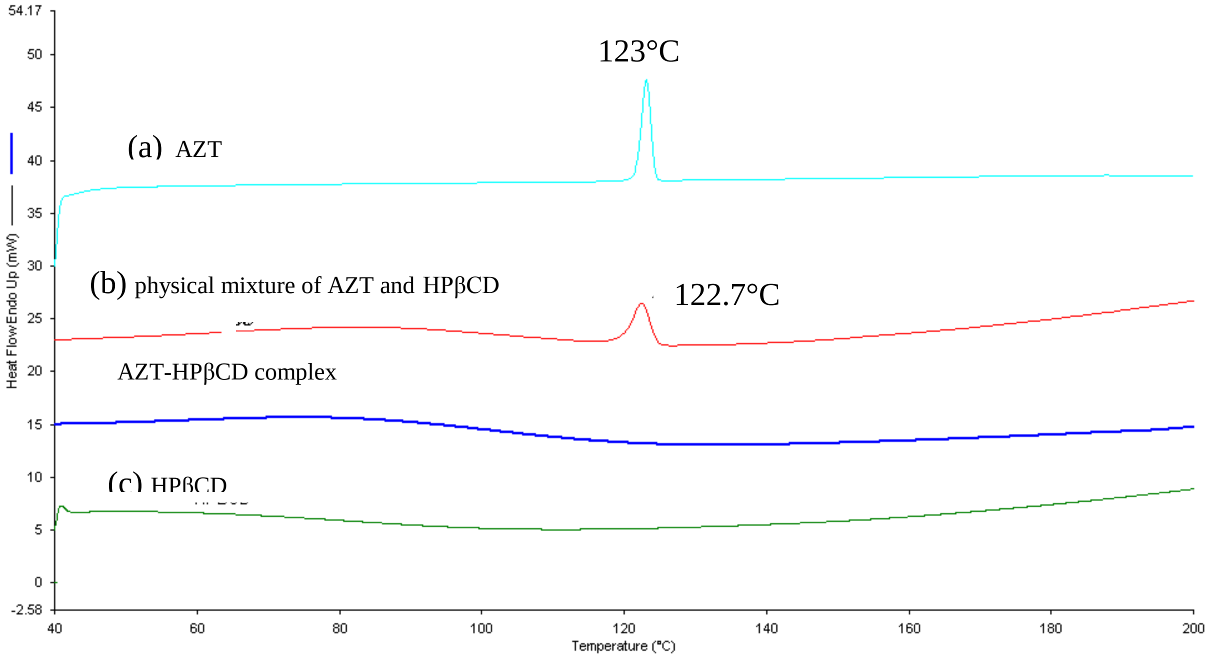

**Figure 4-11: DSC curves of the physical mixture, HPβCD and AZT**

The DSC analysis therefore shows that the physical mixture (Figure 4-11b) and AZT (Figure 4-11a) were identical as indicated by the presence of the single endotherm peak at 122.37°C and 123.00°C, respectively. In the case of HPβCD (Figure 4-11c), a broad endotherm was observed without any distinct or characteristic peak. The absence of any peak in Figure 4-11c is an identification of HPβCD.

## 4.7 **Phase solubility study**

The phase solubility relationship between AZT and HPβCD is presented in Figure 4-12.

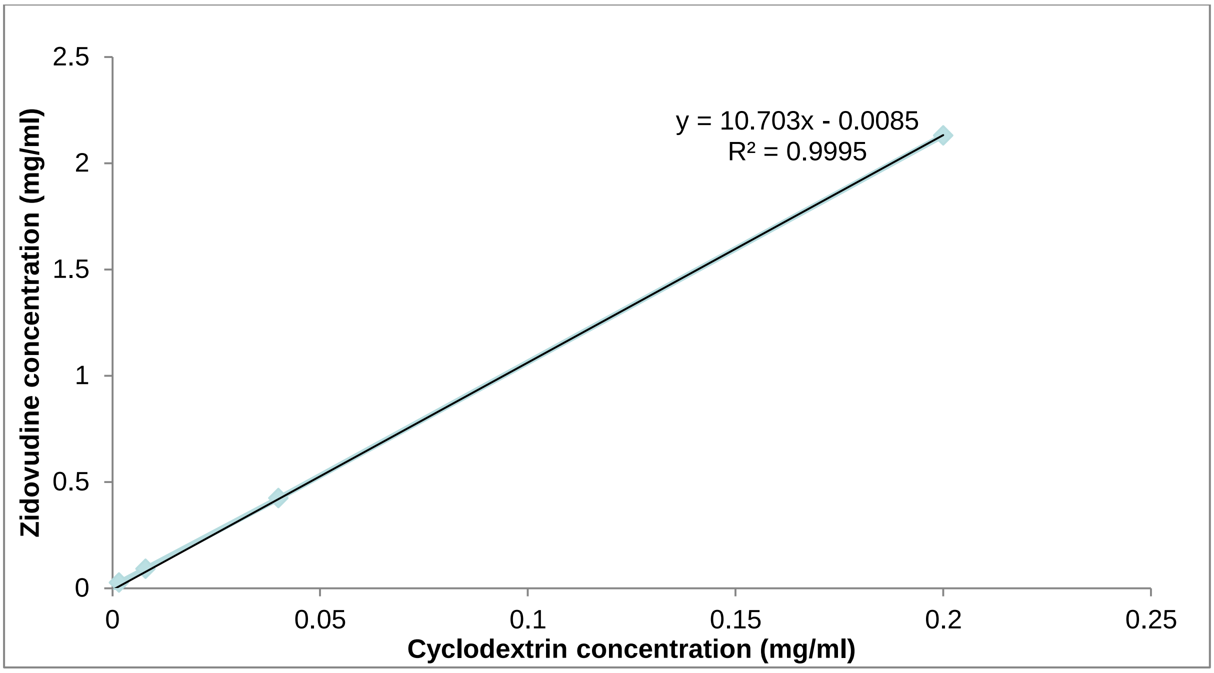

**Figure 4-12: Phase solubility relationship**

The plot of AZT concentration *versus* HPβCD concentration (Figure 4-12) was linear for the relationship between AZT and HPβCD concentrations. The phase solubility relationship between AZT and HPβCD showed a first order relationship with linear and direct proportionality and $A_L$ type of solubility. The graph in Figure 4-12 indicates that the solubility of HPβCD increases with an increase in concentration of AZT. This suggests that the AZT and HPβCD have strong chemical interaction, hence the ease of solubility.

The binding constant was calculated using Equation 2-1, previously discussed in Section 3.3.3.9:

$$K = \frac{slope}{So\ (1 - slope)}$$

The acceptable range for binding constants is from 0 to 100 000 $M^{-1}$.[12] The results obtained following calculation of the binding constant (using equation 1) for the inclusion complex gave binding constant value of 3.919 $M^{-1}$. This implies that a maximum of 4 moles of AZT could be bound to 1 mole of the HPβCD, and is considered within acceptable limits for the study purpose.

### 4.7.1 Palatability assessment and solubility determination for the inclusion complex formed

Palatability of the formulation was assessed by taste of the AZT-HPβCD formulation which was compared to the taste of the reference AZT powder. The AZT-HPβCD formulation had a slightly sugary taste, in contrast to the AZT reference powder which had a bitter taste. The taste of the AZT-HPβCD complex is probably due to the masking effect introduced by the glucose units on the HPβCD molecule. The inclusion complex was therefore deemed to have masked the bitter taste of AZT. The solubility of the formed complex was determined by successive addition of increasing quantities of the complex to a known volume of solvent until saturation was established. The solution was filtered and the UV-absorbance of the filtrate determined. The absorbance reading of the filtrate was used to determine AZT concentration (in the complex) from the AZT calibration plot. The concentration obtained was 148.08 mg/ml. This shows that AZT solubility increased from 20 mg/ml (as specified in literature) to 148 mg/ml (obtained for the formulated AZT-HPβCD inclusion complex), representing a 7.4 fold increase in solubility.

### 4.7.2 Formulation of AZT-HPβCD inclusion complex tablet

Due to the increased AZT solubility obtained with the formed complex, the amount of AZT to be used in formulation studies was reduced compared to the amount in the branded formulation. An arbitrary amount of 75 mg AZT was used for the formulation of the AZT-HPβCD inclusion complex tablet. The value of the binding constant implies that a maximum of four moles of AZT could be complexed with one mole of the HPβCD. As a result, 75 mg of AZT was complexed with 393 mg of the HPβCD to obtain a complex in a 1:1 mole ratio. The procedure for calculating the amount of AZT and HPβCD to be used in the formation of inclusion complex is described below.

#### *4.7.2.1 Calculations*

A formula was developed to establish how much of the AZT and HPβCD would be included in the inclusion complex at 1:1 molar ratio.

At 1:1 molar ratio for inclusion complex formation, the following calculation is then presented for the number of moles of AZT:

AZT: MW = 267.242 g/mol

HPβCD: MW = 1460 g/mol

Given mass (AZT) =75 mg

No. of moles (AZT) = mass ÷ MW

= 75 mg ÷ 267.242 g/mol

= 0.075 g ÷ 266.30 g/mol

= 0.000280 mol

Ratio of moles of AZT: HPβCD = 1: 1

Mass of HPβCD to be used

Mass = no. of moles X MW

= 0.000280 mol x 1460 g/mol

= 0.4097 g

=409.7 mg

### *4.7.2.2 Scaling up*

On scaling-up, identification of the inclusion complex was achieved by DSC and HSM. The AZT melting point ranged from 123-129°C. The peak indicative of AZT's melting point was not observed in the DSC plot of the AZT-HPβCD inclusion complex (Figure 4-13), suggesting a positive DSC result and therefore successful inclusion complex formation on scaling up (Table 4-2).

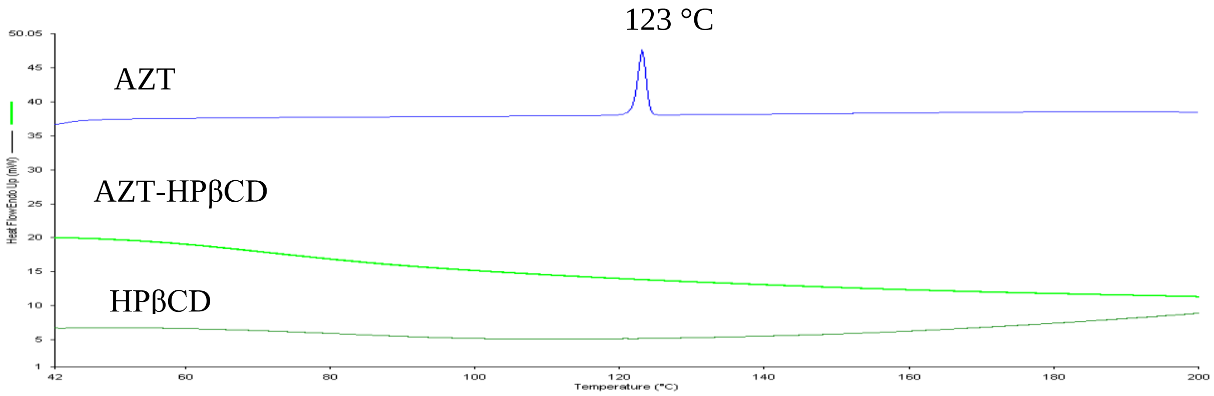

**Figure 4-13: DSC curves of the AZT-HPβCD inclusion complex and AZT, after scale-up**

**Table 4-2: Scaling up of AZT-HPβCD: Master formula**

| **Master Formula** | | | | |
|---|---|---|---|---|
| **Batch 1** | **Amount (mg)** | **Total (mg)** | **With 10% excess (mg)** | **Total (g)** |
| **AZT** | 75 mg | X 25 =1875 | 187.5 mg | 2.0625 g |
| **HPβCD** | 409.7 mg | X 25 = 10242.5 | 1024.25 mg | 11.2668 g |
| **Total** | **484.7mg (1 tablet)** | | | **13.3 g (27 tablets)** |

| **Master Formula** | | | | |
|---|---|---|---|---|
| **Batch 2** | **Amount (mg)** | **Total (mg)** | **With 10% excess (mg)** | **Total (g)** |
| **AZT** | 75 mg | X 37 = 2775 | 277.5 mg | 3.05251 g |
| **HPβCD** | 409.7 mg | X 37 = 15158.9 | 1515.89 mg | 16.67479 g |
| **Total** | **484.7mg (1 tablet)** | | | **19.8 g (41 tablets)** |

### 4.7.3 Direct compression of inclusion complex

Upon identification of successful inclusion complex formation, 10 tablets were prepared by direct compression. Initially, one tablet was used for the hardness test, one tablet for the disintegration test, four tablets for the friability test and four tablets for the durability test. While the tablets showed acceptable results for hardness (80 N) and disintegration time (10 minutes), the results for friability and durability did not meet the requirements for acceptable tablet dosage forms. This was attributed to the absence of binding agents in the formulation, and so a decision was made to formulate tablets with the lowest acceptable amount of binder

(20 % of the formulation). The tablet manufacturing process was modified to include 20 % of microcrystalline cellulose (MCC) as binding agent for the formed complex. This percentage is considered the minimum that can be used for binding purposes.

Subsequently, a set of 68 tablets were made in order to achieve optimal friability and durability results. Positive results were then obtained from the quality control tests for disintegration time, friability and durability. However, the hardness test showed a very high value (240 N) probably due to the presence of the binder in the sample.

### 4.7.4 Evaluation of the final formulation

After direct compression, the tablets underwent a number of quality control tests. These tests were resistance to crush, friability, durability, disintegration and dissolution. The final formulation was compared to the branded formulation and to the acceptable USP standards.[13]

### 4.7.5 Resistance to crush

The results obtained showed that the formulated tablets had a hardness value of 236.3 ± 20.3 N, compared to the branded AZT with hardness value of 71.0 ± 12.4 N (Table 4-3). This perhaps suggests that less of the binder could have been used for the formulation; however, the 20 % used is the lowest percentage approved by the USP. The high hardness value obtained for AZT-CD inclusion complex tablet may also be due to cyclodextrins' compressibility characteristics.

**Table 4-3: Force required to break tablets (in Newtons)**

| Tablet | Force required (in Newtons) | |
|---|---|---|
| | Branded AZT formulation | AZT-HPβCD inclusion complex |
| 1 | 80 | 234 |
| 2 | 64 | 229 |
| 3 | 84 | 207 |
| 4 | 59 | 281 |
| 5 | 73 | 244 |
| 6 | 49 | 226 |
| 7 | 92 | 231 |
| 8 | 72 | 217 |
| 9 | 68 | 249 |
| 10 | 69 | 245 |

### 4.7.6 Friability and durability

Friability and durability tests ensure the quality of the product when it reaches the end user. The friability test is used to measure weight loss when tablets are subjected to a uniform tumbling motion for a specified time. The branded formulation and the AZT-HPβCD inclusion complex had mass losses of 0.16 % and 0.7 %, respectively (Table 4-4). The friability percentage loss of a tablet must be less than or equal to 1 % in order for a tablet formulation to be considered acceptable. The branded AZT and AZT-HPβCD inclusion complex are therefore deemed to have passed the friability test since the results obtained show less than 1 % mass loss.

**Table 4-4: Friability and durability tests (percentage mass loss)**

| | Branded AZT formulation | AZT-HPβCD inclusion complex | USP specification |
|---|---|---|---|
| Friability | 0.16 % | 0.7% | 1% |
| Durability | 0.03 % | 1.02% | |

### 4.7.7 Disintegration

Disintegration measures the time taken for the tablet to break into component particles or granules. If one or two tablets from a batch of 6 tablets fail to disintegrate completely within 30 minutes, then the test should be repeated with another 12 tablets. A minimum of 16 tablets in total (from the first and second sets) should completely disintegrate in the specified time for the batch to be considered acceptable. This test was conducted in two different media (distilled water and 0.1 N HCl).

From the results shown in Table 4-5, tablets of the branded AZT and tablets formed from the inclusion complex disintegrated within 30 minutes in both media assessed, considered acceptable according to USP specification limits.

**Table 4-5: Disintegration time of tablets (branded AZT and the AZT-HPβCD inclusion complex)**

| **Disintegration medium: water** | | | |
|---|---|---|---|
| Disintegration time (sec) | AZT-HPβCD inclusion complex | Branded AZT | USP specification |
| From tablet to fine particle | 630 | 50 | < 1800 |
| **Disintegration medium: 0.1 N HCl** | | | |
| Disintegration time (sec) | AZT-HPβCD inclusion complex | Branded AZT | USP specification |
| From tablet to fine particle | 480 | 60 | < 1800 |

### 4.7.8 Dissolution study

#### *4.7.8.1 Validation of dissolution procedure*

As a category III procedure, i.e., analytical procedure for determination of performance characteristics, the dissolution method for the study was evaluated for precision by repeatability. For the dissolution validation test, powdered AZT samples were dispersed in the dissolution medium. The powder was chosen because its assessment may introduce errors, more than may be obtained with a tablet like the formed inclusion complex. Six replicate samples were collected from the dissolution apparatus at time 30 minutes. The withdrawn samples containing AZT powder in dissolution medium were used to validate dissolution method precision by repeatability, and the values expressed as relative standard deviation (% RSD) between samples.[14,15] The % RSD was 4.23 %, within the specified criterion of ≤ 5 %.[15] The result here was obtained for the powdered material, which may be prone to more errors than the tablet due to its physical state, i.e. powder *versus* tablet. This implies that the dissolution method described is suitable for the analysis of AZT tablets, with % RSD for the tablets expected to be even less than that obtained with the powder.

#### *4.7.8.2 Validation of spectroscopic procedure*

The ultraviolet (UV) spectroscopic method used for analysis of dissolution samples was validated for linearity and precision according to guidelines set by the International Conference on Harmonization (ICH, 1997).[16] In addition, samples were assessed for stability after exposure to light, heat and consecutive freezing and thawing sessions.

#### *4.7.8.3 Linearity*

A stock solution of 1 mg/ml AZT was prepared by dissolving specific amount of the reference compound in the dissolution medium. Calibration samples were prepared by diluting the stock solution with dissolution medium. The linearity of the curve for AZT was assessed in triplicate over nine concentrations in the range, 1 to 156 µg/ml. Calibration curves were constructed by linear regression of the peak area plots *versus* AZT concentration.

Calibration curves were constructed at nine progressive concentrations ranging from 1 to 156 µg/ml. The concentration range covered from 0.33 % to over 150 % of the labelled dose strength in order to accommodate for all possible concentrations in the dissolution medium. The relationship between AZT peak areas and its nominal concentrations was linear with correlation coefficient of 0.9995 (Figure 4-14).

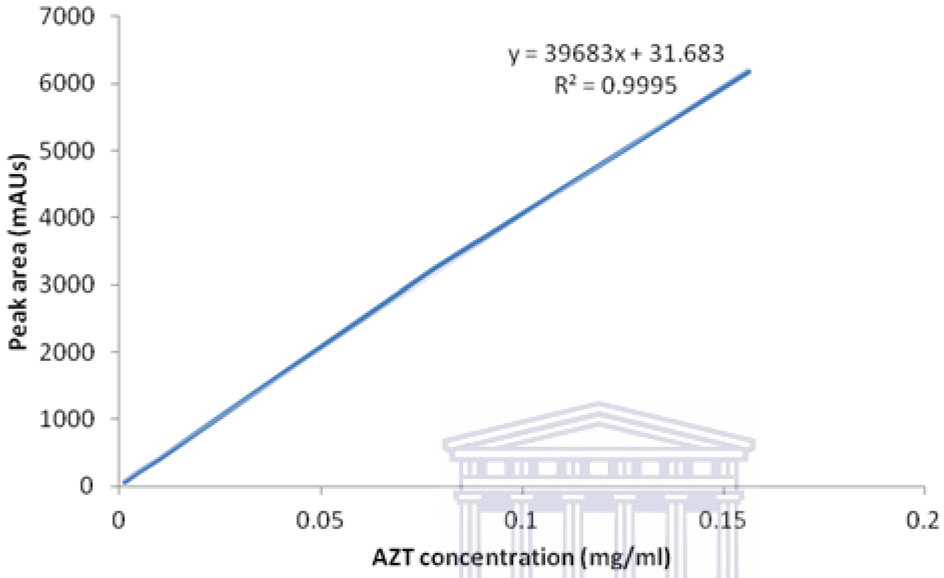

**Figure 4-14: Calibration curve of AZT**

The limit of detection (LOD) and the limit of quantitation (LOQ) are defined at signal-to-baseline ratios of 3:1 and 10:1, respectively. The LOD and LOQ were calculated from the formulae:

$$LOD = 3\left(\frac{SD}{S}\right)$$

$$LOQ = 10\left(\frac{SD}{S}\right)$$

Where SD and S are the standard deviation of the response and the slope of the calibration curve, respectively.

The LOD and LOQ values, calculated from the equations 1 and 2 above, are given as 0.043 µg/ml and 0.143 µg/ml, respectively. These limits are deemed suitable for the assay of AZT in the prepared inclusion complex and in dissolution medium.

### *4.7.8.4 Stability*

The stability assessment involved evaluation of the working standards at low, medium and high concentrations, following exposure to light, heat, and consecutive freeze-thaw cycles. Results of these evaluations are presented in Table 4-6.

**Table 4-6: Average peak areas of analyte (in mAUs) before and after stability tests**

| | **Before test** | **After test** | | | | | |
|---|---|---|---|---|---|---|---|
| **AZT conc (mg/ml)** | | **Light** | **% change** | **Heat** | **% change** | **Freeze-thaw** | **% change** |
| 0.0012 | 68.2 ± 2.8 | 23.3 ± 1.8 | -65.9 | 65.9 ± 1.7 | -3.5 | 110.1 ± 15.9 | 61.4 |
| 0.0195 | 850.6 ± 4.3 | 615.3 ± 28.4 | -27.7 | 848.2 ± 1.7 | -0.3 | 840.5 ± 3.7 | -1.2 |
| 0.1563 | 6416.0 ± 3.3 | 5986.0 ± 17.2 | -6.7 | 6230.6 ± 24.0 | -2.9 | 7861.2 ± 1473.9 | 22.5 |

Following ambient light exposure (Table 4-6), peak areas indicative of AZT at low (0.0012 mg/ml), medium (0.0195 mg/ml) and high (0.1563 mg/ml) concentrations decreased, as shown by percentage changes of -65.9 %, -27.7 % and -6.7 %, respectively. These traces indicate that the reference compound may not be stable to ambient light exposure, with such instability increasing with decreasing concentrations. Similar results were also obtained in a previous study for AZT.[17] Samples for AZT analyses should therefore ideally not be exposed to such conditions.

Heat stability was evaluated by heating AZT samples contained in closed vials. The samples were heated in a water bath maintained at 60°C for one hour. After heat exposure, AZT peak areas for the low (0.0012 mg/ml), medium (0.0195 mg/ml) and high (0.1563 mg/ml) concentrations differed by -3.5 %, -0.3 % and -2.9 %, respectively. Heat exposure therefore resulted in decreased AZT peak areas; however, such decrease was not as much as that observed following light exposure. The prepared AZT samples therefore seem more stable to heat exposure at the evaluated temperature and duration than to ambient light exposure. A slight decrease in active drug has also been noted for AZT samples refluxed in water for six hours at 60ºC.[18]

The results of five freezing (at 180°C) and thawing (at room temperature) sessions show that the reference standards were not stable to freezing and thawing sessions, as indicated by the peak areas following such sessions. Peak areas changed for reference AZT samples at low (0.0012 mg/ml), medium (0.0195 mg/ml) and high (0.1563 mg/ml) concentrations by +61.4 %, -1.2 % and +22.5 %, respectively. This implies that samples containing AZT, utilized for this study, may not be stable to freezing and thawing sessions; therefore, may not be subjected to such sessions during analyses

Intra-day and inter-day precision of the analytical method was assessed by analyses of six replicate AZT samples at the highest analytical concentration. The % RSD for inter-day analyses ranged from approximately 0.5 % to 0.7 % and for intra-day analyses from 1.1 to 1.5 %. Intra-day and inter-day precision values less than 2 % are generally acceptable for analytical procedures (ICH, 1997). This indicates that the analytical method employed for AZT analysis is suitable, with acceptable precision

### *4.7.8.5 Dissolution tests*

The dissolution study investigates drug release in any given media and can be used to categorize the release property of a drug. Figure 4-15 presents the dissolution profiles of the newly formed AZT-HPβCD inclusion complex and the branded AZT formulation.

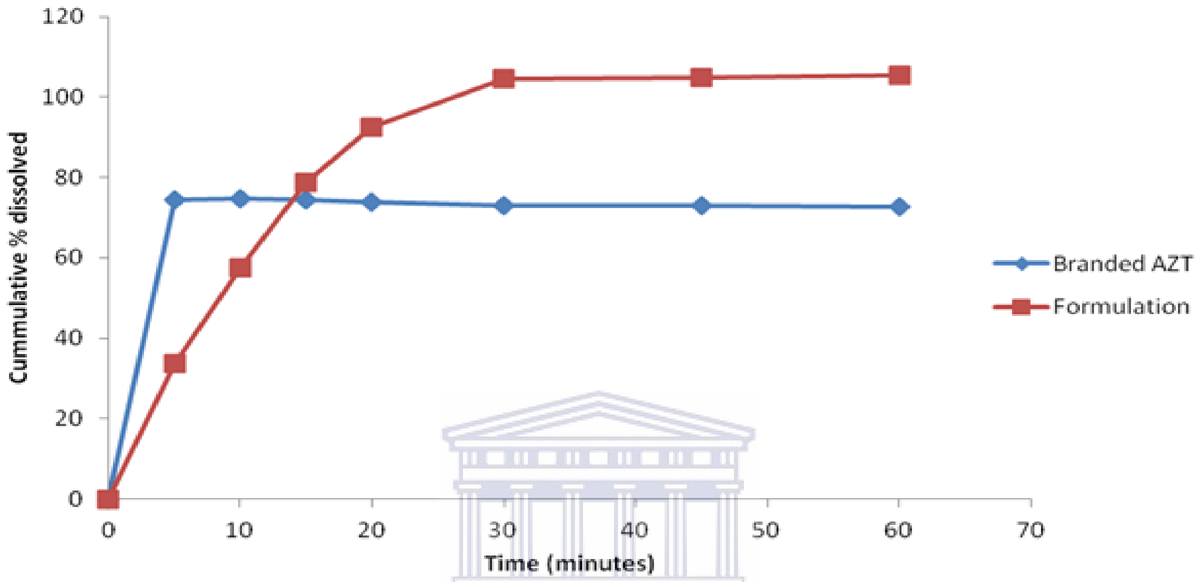

**Figure 4-15: Dissoultion profile of AZT-HPβCD inclusion complex and branded AZT**

The figure shows that the dissolution rate of the branded AZT is notably faster than that of the AZT-HPβCD inclusion complex, with the branded AZT profile attaining maximum dissolution in 5 minutes. On the other hand, the dissolution profile for the AZT-HPβCD inclusion complex shows a slower dissolution rate, with maximum concentration only attained in 30 minutes. With the short half-life of AZT (less than 3 hours), the slow dissolution rate observed with the AZT-HPβCD formulation may be an advantage since the drug will remain in the body for longer.

For the branded AZT, about 75 % of the 300 mg AZT content was released at maximum dissolution. This shows that the analyte was not fully dissolved in the dissolution medium, despite the fact that maximum dissolution was attained and maintained. In contrast, at least 100 % of the formulated AZT-HPβCD inclusion complex was released at full dissolution, though this was obtained at a longer time compared to that of the branded AZT.

Complexation of AZT with HPβCD therefore improved its solubility, which resulted in full dissolution unlike the branded AZT which did not attain full dissolution.

A comparison of both dissolution profiles using the similarity factor ($f_2$) returned an $f_2$ value of 15, implying that the dissolution profiles of the branded and formulated AZT were quite different. This perhaps may also translate to differences in their onset and duration of action; for instance, the AZT-HPβCD inclusion formulation exhibits a slower release rate than the branded formulation, a characteristic which can extend its duration in the body, perhaps leading to extended release formulation that can be administered less frequently.

The dissolution profile of the formulated complex also showed stability to the acidic dissolution medium in that the AZT-HPβCD formulation did not show a significant decrease in cumulative amount dissolved over the dissolution time period. This has significant implications for the fight against HIV.

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# CHAPTER 5

## 5 Conclusions and Recommendations

The optimal use of AZT as an anti-retroviral agent may be limited by its undesirable tase, poor solubility and acid lability. This study set out to formulate an inclusion comlex of AZT and CD as a means to address these limitations to the use of AZT.

The specific objectives of this study were:

- To select an appropriate CD for inclusion comples formation, and prepare AZT-CD complex using the selected CD
- To conduct analytical characterization tests on the AZT-CD complex formed
- To formulate tablets of the AZT-CD complex formed, and compare these tablets to a commercially available brand and to USP specifications by means of selected quality control tests.

From the results obtained, the following conclusion could be made:

- HPβCD was selected as the most appropriate CD for inclusion studies. This CD was successfully used for the preparation of AZT-HPβCD complex.

- Employment of various analytical techniques showed differences between the formulated AZT-HPβCD complex and its component compounds, alluding to successful insertion of AZT into the HPβCD molecule.

- Friability and disintegration tests, as well as hardness and durability tests showed differences between the branded AZT and the AZT-HPβCD complex; however, both formulations met acceptable USP specifications.

- Dissolution tests showed that the branded formulation showed faster release than the AZT-HPβCD formulation; however, the former did not attain full dissolution possibly due to solubility limitations. The AZT-HPβCD formulation on the other hand, exhibited a slower dissolution but attained full dissolution; possibly due to an increase in its solubility as a result of AZT complexation with HPβCD. Dissolution profiles of the two formulations showed significant differences between the two formulations.

This study provides preliminary data on AZT-HPβCD formulation, which can be advanced for production of dosage forms with desirable release characteristics. This has significant implications for the fight against HIV.