DESIGN AND EVALUATION OF FAST DISPERSIBLE TABLETS OF LAMIVUDINE USING SELECTED NATURAL SUPERDISINTEGRANTS

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Keywords

Fast dispersible tablets, dysphagia, natural polymers, phytochemicals, superdisintegrant, disintegration, dispersion, dissolution, adherence, wetting, swelling, multipurpose excipient, flowability, compressibility, direct compression, wet granulation, micromeritics, high performance liquid chromatography, palatability.

Abstract

Fast dispersible tablets (FDTs) are solid single-unit dosage forms that are placed in the mouth and allowed to disperse or dissolve in the saliva without the need of water. The basic approach to formulating FDTs consists of adding a superdisintegrant to a tablet formulation. These tablets offer both the advantages of conventional tablets and liquid dosage forms along with distinctive properties which include accurate dosing, ease of administration, quick onset of action, enhanced bioavailability, and increased patient adherence.

FDTs have been found to be effective in remedying therapeutic in-adherence caused by dysphagia (swallowing difficulties) particularly in paediatric and geriatric subjects. There is a strong correlation between therapeutic success and patient adherence especially with HIV/AIDS treatment regimens, consequently the dosage form should be patient friendly and devoid of unappealing characteristics. This study aimed at developing a cost effective fast dispersible tablet of lamivudine using alternative excipients and conventional techniques. Only conventional tablets and oral liquid dosage forms of lamivudine are available on the South African market.

Two natural polymers reported to have superdisintegrating properties were selected to serve as multipurpose excipients in this study. The polymers were identified, characterised and compared using thermal, spectroscopic and micromeritic analytical tools. The polymer that displayed the best characteristics in terms of micromeritic, tableting and disintegrating properties was retained and used for the optimum formulation.

The optimum formulation was composed of 150 mg of lamivudine, 23% w/w unripe banana powder and 2% w/w magnesium stearate. FDTs of lamivudine were obtained using the compression technique with and without wet granulation. The tablets were assessed as per the United States Pharmacopoeia (USP) guidelines and other evaluation procedures pertaining to

FDTs. The wet granulated tablets were found to be less friable and thus more resilient than the directly compressed tablets. *In-vitro* disintegration of the wet granulated tablets occurred within 50±3 sec in deionised water (pH 7) and 35±2 sec in a phosphate buffer solution (pH 6.8). Consequently, the innovative tablets fulfilled the core requirement of FDTs i.e. rapid disintegration.

Drug release studies were carried out by analysing dissolution aliquots of the innovative tablets using a validated High Performance Liquid Chromatography (HPLC) method, and comparing them to Aspen Lamivudine®, a conventional tablet of lamivudine presently on the South African market. Complete dissolution in deionised water (pH 7) was attained within 10 minutes and 30 minutes for the innovative tablets and Aspen Lamivudine® respectively.

Declaration

I declare that the thesis, **Design and evaluation of fast dispersible tablets of Lamivudine using selected natural superdisintegrant** is my own work, that it has not been submitted before for any degree examination at any other university and that all the sources I have used or quoted have been indicated and acknowledged by complete reference.

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Dedication

I dedicate this thesis to Yahweh, the Omnipotent and most gracious, from Whom all blessings flow. No amount of words will suffice to express my gratitude for His mercies and bounties in my life.

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List of abbreviations

AIDS Acquired Immune Deficiency Syndrome

API Active Pharmaceutical Ingredient

ARV Antiretroviral

BCS Biopharmaceutical Classification System

DSC Differential Scanning Calorimetry

FDA Food and Drug Administration

FDT Fast Dispersible Tablet

FTIR Fourier Transform Infrared

GRAS Generally Regarded As Safe

HIV Human Immunodeficiency Virus

HPLC High Performance Liquid Chromatography

HSM Hot Stage Microscopy

IHP Ispaghula Husk Powder

SEM Scanning Electron Microscopy

TGA Thermogravimetric Analysis

UBP Unripe Banana Powder

USP United States Pharmacopeia

Chapter 1

Introduction

This chapter is an overview of the research problem of this project. It gives the reader a glimpse of what this study offers. The key themes of the project are prefaced. The motivation behind this investigation, the aim and the set objectives are clearly elaborated.

1.1 Problem and background

Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) contribute tremendously to the global burden of disease with sub-Saharan Africa being the region with the highest prevalence rate in the world. Southern Africa is the most affected region and is widely regarded as the epicentre of the global HIV epidemic.[1] With an estimated 7.03 million people living with HIV/AIDS in 2016, roughly 12,7 % of the total population, South Africa is the country with the highest number of people living with HIV/AIDS in the world.[2] Since the late 1990s, HIV/AIDS has progressed from a fatal illness to a chronic manageable disease due to the development of several drugs that can halt the replication of the causative virus at various steps of its lifecycle. Some of these drugs include abacavir, emtricitabine, lamivudine, tenofovir, and zidovudine.[3]

Lamivudine is a synthetic nucleoside analogue indicated for the treatment of HIV-1 infection in combination with other antiretroviral agents.[4] Lamivudine appears on the essential medicine list of South Africa, and it is one of the first line antiretroviral drug treatments in the country.[5] A combination of zidovudine and lamivudine is often used as a post exposure prophylaxis for individuals and health care workers after accidental contact with HIV contaminated tissues or fluids.[6]

Several formulations of Lamivudine can be found on the South African pharmaceutical market in the form of tablets and syrups. Although tablets and syrups are conventional oral dosage forms and are widely accepted, they have several drawbacks which may dissuade patients from taking them based on individual factors.[7]

Self-administration, accurate dosing, compactness and ease of manufacture account for the popularity of tablets amongst other dosage forms. One important disadvantage of tablets is the difficulty some patients have in swallowing them (dysphagia). Roughly a third of the

population (mostly paediatrics and geriatrics) has swallowing difficulties.[8] Dysphagia is prevalent in geriatric patients due to damage in the head and neck anatomy as a result of ageing, as well as changes in neural and physiologic mechanisms supporting swallowing function, and in paediatric patients due to their underdeveloped muscular and nervous systems. Difficulties in swallowing solid dosage forms is also prompted by upper respiratory tract affections such as laryngitis and the unavailability of potable water (especially in developing countries).[9] The United Nations Children's Fund (UNICEF) reports that 17 countries in Africa including Somalia and Ethiopia are drought stricken with children being at a higher risk. Also, recent developments in the drought-stricken Cape Town metropole have seen water restrictions and rationing being implemented due to the probability of the town running out of potable water if stringent measures are not taken.

Although syrups and other oral liquid formulations somehow address the problem of dysphagia, several practical issues may arise from their use which include instability of the active pharmaceutical ingredient, spillage, bad taste, inaccurate dosing, bulkiness, transportation and storage.[10]

The issues enumerated above can dissuade patients from taking their medications, consequently, they may become in-adherent to the prescribed therapy. Adherence to medication is a crucial part of patient care and indispensable for attaining therapeutic goals. In the case of HIV/AIDS, inadherence to therapy can lead to drug resistance and virologic treatment failure.[11] The World Health Organisation (WHO) 2003 report on medication adherence states that "increasing the effectiveness of adherence interventions may have a far greater impact on the health of the population than any improvement in specific medical treatment"[12] In an attempt to increase patient adherence by mitigating the drawbacks of oral liquid formulations (syrups, solutions, emulsions and suspensions) and conventional tablets,

orally disintegrating dosage forms such as fast dispersible tablets have been developed and have attracted a great deal of attention.[13]

Fast dispersible tablets are solid single-unit dosage forms that are placed in the mouth, and allowed to disperse or dissolve in the saliva without the need of water and provide a quick onset of action. Other appellations for fast dispersible tablets include: quick dissolves, fast melts, fast dissolving, fast disintegrating, rapid-dissolve, or orally dissolving tablets.[14] The fast dissolving solid dosage form turns into a soft mass or liquid form to facilitate swallowing; consequently, the risk of choking is reduced considerably.[15] Fast dispersible tablets offer both the advantages of solid dosage forms and liquid dosage forms along with distinctive properties which include accurate dosing, ease of administration, quick onset of action, enhanced bioavailability, and increased patient compliance.[16] A dispersible tablet is flexible and can be prescribed across all age groups; this simplifies the task of prescribers and reduces the work load of manufacturers as one dosage form suits all.[17]

The basic approach in the development of fast dispersible tablets is the use of superdisintegrants which provide rapid tablet disintegration in the mouth.[15] Over the past few years, new pharmaceutical excipients have been introduced referred to as “superdisintegrants”. A “superdisintegrants” is an excipient, which is incorporated into an oral solid dosage form blend to speed up disintegration i.e. the breakdown of the compact mass into smaller fragments that can be easily swallowed. Superdisintegrants are relatively more effective at lower concentrations with greater disintegrating efficiency and mechanical strength.[18] Superdisintegrants are classified into two categories based on their source i.e. natural and synthetic. Natural superdisintegrating agents are preferred over their synthetic counterparts because they are comparatively cheaper, facilely available, non-toxic in nature, eco-friendly and capable of a myriad of chemical modifications.[19]

Several methods of preparing fast dispersible tablets have been described to date, including lyophilisation, moulding, and the compression of wet powders to construct highly porous structures. Although these methods are effective, they are time consuming and technically difficult, often requiring special processing equipment. Furthermore, although tablets produced by these methods disintegrate rapidly, they are usually very weak and friable. The mechanical strength of the tablets may not be sufficient to endure packaging, transportation, and patient handling. Tablets obtained by the conventional compression method are less friable but disintegrate more slowly.[20] The compression method, with or without wet granulation, is a convenient and cost-effective way to prepare tablets with sufficient structural integrity. Formulating fast dispersible tablets using conventional compression is attractive because of the low manufacturing cost and ease of technology transfer.[20]

1.2 Aim and objectives

The aim of this project was to develop a cost effective fast dispersible tablet of lamivudine using alternative excipients and conventional techniques. The motivation behind this study is the unavailability of an oral dosage form of lamivudine on the South African market that caters for the "missing middle" i.e. patients who suffer from dysphagia, bedridden patients and patients who are reluctant to take syrups or conventional tablets for one reason or the other. In order to fulfil this aim, the following objectives were set:

1. To select suitable natural polymers to be used as superdisintegrants
2. To characterise the selected natural polymers.
3. To assess the compatibility of lamivudine with the selected natural polymers by analysing 1:1 drug-excipient mixtures using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR).

4. To formulate a fast dispersible tablet of lamivudine by direct compression using the natural polymer that displays the desired micromeritic and tableting properties.
5. To determine whether the selected natural polymers can be used as multipurpose excipients (binder, disintegrant, diluent and lubricant) by assessing the various powders blends for flowability and compressibility and evaluating the resultant tablets.
6. To assess the innovative tablets according to the United Stated Pharmacopoeia (USP) prerequisites and comparing them to a lamivudine tablet brand on the South African market.
7. To design, develop and validate a high performance liquid chromatography (HPLC) method for assaying lamivudine in the innovative tablets.
8. To perform drug release studies of the innovative tablets and branded tablets using the validated HPLC method to analyse the samples.

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Chapter 2

Literature review

This chapter gives an account of published work pertaining to the research problem of this project. The following themes are explored in this section: human immunodeficiency virus (HIV), lamivudine, problems associated with conventional oral dosage forms, fast dispersible tablets, various formulation techniques of fast dispersible tablets and disintegrants.

2.1 Human immunodeficiency virus (HIV)

2.1.1 Background

The human immunodeficiency virus (HIV) is a member of the retrovirus family. It is a single-stranded ribonucleic acid virus with an icosahedral nucleocapsid and a lipid envelope. HIV-1 and HIV-2 are the two main types of HIV.[1] Although both types are known to cause acquired immune deficiency syndrome (AIDS), HIV-2 is less virulent and less transmissible than HIV-1. The HIV-2 group is confined to West Africa, while the HIV-1 group is spread across the globe and its various subtypes are responsible for the AIDS pandemic.[2] HIV is transmitted by sexual contact, by contact with infected blood or body fluids containing blood, and by infected mother to infants (intrapartum, perinatally, or peripartum through breast milk).[3]

2.1.2 Epidemiology

HIV is a major contributor to the global burden of disease, especially in sub-Saharan Africa. According to the joint United Nations programme on HIV/AIDS (UNAIDS), there were roughly 36.7 million people living with HIV/AIDS at the end of 2015. The majority of people living with HIV are in low and middle income countries. In 2015, About 66 % of new HIV infections occurred in Sub-Saharan Africa with an estimated 25.6 million people living with HIV.[4] South Africa has the highest number of people living with HIV in the world. In 2016, South Africa had an estimated overall HIV prevalence rate of 12,7% of the total population. The total number of people living with HIV in 2016 was estimated to be 7.03 million.[5]

2.1.3 Pathogenesis

The main target of HIV is the cluster of differentiation 4 (CD4) T lymphocytes; the virus enters by interacting with CD4 and the chemokine coreceptors (CCR5 or CXCR4). Other cells bearing CD4 and chemokine receptors are also infected, including resting CD4 T cells,

monocytes, macrophages, and dendritic cells.[6] A range of host proteins interact with HIV proteins or HIV deoxyribonucleic acid (DNA) to either counter or enhance virus replication in a specific cell type. The innate immune response to HIV is mediated by natural killer cells to a large extent and is also crucial for virus control.[7] The peculiarity of the HIV infection is the progressive depletion of CD4 T cells as a result of an increase in reduction and decrease in production thereof.[8]

In most patients, there are no symptoms of HIV infection, or its complications, for several years. This asymptomatic phase is characterised by ongoing viral replication, and progressive CD4 cell depletion. The average patient has a viral load set-point of 30,000 copies/ml of HIV-1 ribonucleic acid and loses 50 CD4+ T cells per year.[1] The absolute value of the CD4 count is the best-established surrogate marker to predict time to AIDS, risk of specific opportunistic infections, or death, especially once counts have fallen from the normal range of 800–1200 to ≤300.[9]

2.2 Antiretroviral therapy

Since the late 1990s, HIV has progressed from a fatal illness to a chronic manageable disease due to the development of combination antiretroviral therapy regimens capable of suppressing viral replication. There are more than 25 licensed drugs available that halt the replication of the virus at various steps of its lifecycle. Current antiretroviral therapy regimens are more suitable than the initial protease inhibitor based regimens in that they are dosed less frequently, they are less toxic, have a lower pill burden and are more effective in viral load reduction.[10]

Standard antiretroviral therapy regimens consist of two nucleoside reverse transcriptase inhibitors (emtricitabine or lamivudine together with abacavir, tenofovir, or zidovudine) with a non-nucleoside reverse transcriptase inhibitor, protease inhibitor, or integrase inhibitor.

Several effective nucleoside reverse transcriptase inhibitor-sparing regimens can be used if intolerance or resistance to nucleoside reverse transcriptase inhibitors develops.[1]

2.3 Lamivudine

Lamivudine (Figure 2.1) is a synthetic nucleoside analogue with activity against hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Lamivudine is indicated for the treatment of HIV-1 infection in combination with other antiretroviral agents.[11]

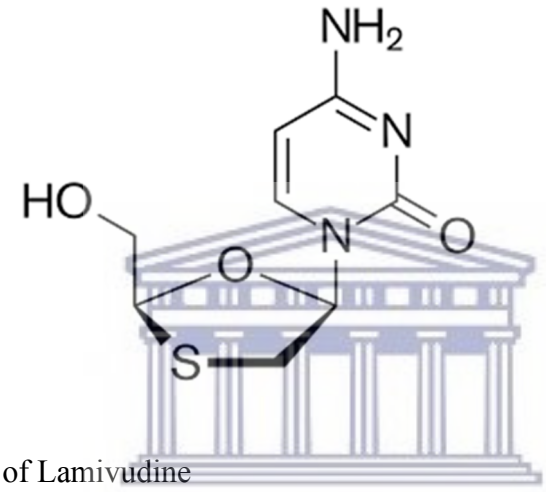


Figure 2.1: Structure of Lamivudine

With a molecular formula of $C_8H_{11}N_3O_3$, lamivudine has a molecular weight of 229.26 g/mol. The melting point occurs between 160-162 °C. At 20 °C, the white powder is soluble in water to an extent of 70,000 mg/L. The partition coefficient (LogP) of lamivudine is -9.54 at 25 °C. Lamivudine is a class I drug based on the biopharmaceutical classification system which entails that it is highly soluble and highly permeable.[12]

2.3.1 Dosing and status in South Africa

Lamivudine is a schedule 4 medication (prescription only). Therapy should be initiated by a physician experienced in the management of HIV-1 infection. Several generic formulations of lamivudine are available on the South African market in the form of conventional tablets and solutions for oral use. The recommended dosage of lamivudine in adults and adolescents over 16 years of age is 300 mg daily, administered either as 300 mg once daily or 150 mg twice daily.[13] Lamivudine appears on the essential medicine list of South Africa, and it is one of the first line antiretroviral drug treatments in the country. A combination of zidovudine and lamivudine is often used as a post exposure prophylaxis for individuals and health care workers after accidental contact with HIV contaminated tissues or fluids.[14]

2.3.2 Mechanism of action of Lamivudine

Intracellularly, lamivudine is phosphorylated to its active metabolites lamivudine triphosphate (L-TP) and lamivudine monophosphate (L-MP). In HIV, L-TP inhibits HIV-1 reverse transcriptase (RT) via deoxyribonucleic acid (DNA) chain termination after incorporation of the nucleoside analogue into viral DNA. In HBV, incorporation of L-MP into viral DNA by HBV polymerase results in DNA chain termination. L-TP is a weak inhibitor of mammalian DNA polymerases alpha and beta, and mitochondrial DNA polymerase.[11]

2.3.3 Clinical efficacy

The efficacy of lamivudine has been investigated in several randomised, prospective clinical trials combined with other antiretroviral agents. These studies have shown substantial reduction in plasma HIV RNA and a surge in CD4 cell counts when used in combination with other nucleoside analogues and either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor(PI). In recent studies by intention-to-treat analysis more than 75% of

participants achieved plasma HIV RNA less than 50 copies/ml after 48 weeks of combination antiretroviral therapy.[15]

2.3.4 Absorption and bioavailability

Lamivudine is promptly absorbed following oral administration. Bioavailability ranges between 80 and 85%. Peak plasma concentrations occur within 1 hour after administration. In healthy participants, at a therapeutic dose of 150 mg twice daily, mean steady-state C_{max} and C_{min} of lamivudine in plasma were 1.2 µg/ml and 0.09 µg/ml, respectively. The mean area under the curve (AUC) over a dosing interval of 12 hours is 4.7 µg.h/ml. At a therapeutic dose of 300 mg once daily, mean steady-state C_{max}, C_{min} and 24h AUC are 2.0 µg.h/ml, 0.04 µg/ml and 8.8 µg.h/ml, respectively.[12]

2.3.5 Distribution

The estimated volume of distribution is 1.3 l/kg. The experimental half-life ranges between 5 to 7 hours. The mean systemic clearance of lamivudine is roughly 0.32 l/h/kg, with predominantly renal clearance (greater than 70 %) via the organic cationic transport system. Protein binding is minimal.[11]

2.3.6 Metabolism and elimination

The active moiety intracellular L-TP has a prolonged half-life in the cell (16 to 19 hours) compared to the plasma half-life. Lamivudine is mainly cleared unchanged by renal excretion.[12]

2.3.7 Pregnancy and lactation

Lamivudine is assigned FDA Pregnancy category C status, i.e. risk cannot be ruled out. No increased risk of birth defects has been reported for Lamivudine.

Lamivudine is excreted into the breast milk of lactating mothers. Due to the potential for HIV transmission and adverse effects caused by lamivudine in nursing infants, HIV- infected mothers should be discouraged from breastfeeding.[11]

2.3.8 Adverse effects

At the onset of treatment; nausea, vomiting, headaches, abdominal pain, fatigue and diarrhoea may occur. These reactions are usually trivial and short-lived.

The following adverse effects have been reported in controlled clinical trials and case series during treatment of HIV-1 infection with lamivudine.[15]

Table 2.1: Adverse effects of Lamivudine

System	Adverse Effects	Occurrence
Blood and Lymphatic	Neutropenia	Uncommon
Nervous	Insomnia and headache	Common
Gastrointestinal	Nausea and diarrhoea	Common
Skin and subcutaneous tissue	Hair loss	Common
General	Fatigue	Common

2.4 Fast dispersible tablets

2.4.1 Background

Tablets and capsules are considered as conventional dosage forms since they are widely accepted i.e. between 50-60% of all dosage forms. Self-administration, accurate dosing, compactness and ease of manufacture account for the popularity of tablets amongst other dosage forms. One important disadvantage of solid dosage forms is the difficulty in swallowing (dysphagia) or chewing in some patients especially paediatric and geriatric patients.[16] Dysphagia is prevalent in geriatric patients due to hand tremors, fear of choking, and in paediatric patients due to their underdeveloped muscular and nervous systems. Difficulties in swallowing solid dosage forms is also prompted by the unavailability of potable water, ailments such as diarrhoea, allergic conditions, common cold and other upper respiratory tract infections.[17] Roughly a third of the population (mostly paediatrics and geriatrics) has swallowing difficulties, resulting in poor compliance to solid dosage form drug therapies which leads to poor therapeutic outcomes. For these reasons, fast dispersible tablets (FDTs) have attracted a great deal of attention.[18]

Fast dispersible tablets are solid single-unit dosage forms that are placed in the mouth and allowed to disperse or dissolve in the saliva without the need of water and provide a quick onset of action.[19]

It has been established that rapid dissolution yields a faster drug absorption (applicable to the unionized form of drug) thus a quick onset of action. Some drugs are absorbed in the oral cavity, pharynx and oesophagus as the saliva transits into the stomach. Thus, the bioavailability of a drug formulated as a fast dispersible tablet is considerably higher than a conventional tablet. The disintegration time of fast dispersible tablets is generally considered to be less than

one minute. The fast dissolving solid dosage form turns into a soft mass or liquid form to facilitate swallowing; consequently, the risk of choking is reduced considerably.[20]

2.4.2 The need for developing fast dispersible tablets

Dysphagia

Tablets and capsules are the most popular and preferred dosage forms for oral drug administration due to their convenience, ease of administration and economical aspects. [21] However, some individuals have difficulties in swallowing solid dosage forms, thus they may resort to modifying the dosage form to facilitate swallowing. When individuals find it difficult to swallow their medications, they may desist from taking their medication, incorporate them to food substances that may reduce the efficacy of the drug, and/or modify the dosage form. Solid dosage form modification may involve splitting, crushing, chewing tablets, or opening capsules.[22] The potential problems associated with modification of dosage forms are well reported in literature, e.g. undesirable effects, increased toxicity, decreased efficacy, alongside legal implications that arise as result of altering a particular dosage form.[23]

Paediatrics

The absence of paediatric friendly dosage forms limits the use of certain medications that are potentially beneficial to children. Despite the fact this has been a long-standing issue, little attention has been paid to resolve it until recently [14]. Optimisation of oral drug delivery is one of the biggest setbacks in paediatric pharmacology. Although children above six years of age can be trained to swallow solid dosage forms, most find it cumbrous until they reach adolescence. Even though modifying solid oral dosage forms to meet the needs of children has been a common practice in paediatric healthcare, it is undoubtedly not an ideal alternative.[24] Crushing tablets to mix them with food or water may decrease the rate or extent of drug

absorption. Despite the fact that breaking tablets is a widespread practice and is acceptable for some medications, it can however introduce significant dose inconsistency. In the case of drugs with a narrow therapeutic index, this inconsistency may be sufficient to produce significant changes in clinical response.[25] When it is deemed necessary to cut a tablet, caregivers should receive adequate counselling on how to effectively carry out this task, including the appropriate use of a pill cutter. Caregivers involved in dose preparation should be knowledgeable about the disposal of unused medications and the danger of continuous exposure to carcinogenic and teratogenic drugs.[26]

A way of minimising the issues enumerated above is to have a pharmacist prepare an extemporaneous oral solution or suspension. However, a relatively simple change in the process, such as switching to a sugar-free suspending agent, incorporating a flavourant, or generic substitution, may affect the stability of the final product or the absorption properties of the drug. Although preparing extemporaneous formulations is common practice in developed countries, it may be limited in some parts of the world lacking basic resources such as potable water.[27] Oral liquid formulations provide a more reliable, ready-to-use preparation for paediatrics but bioequivalence with solid oral dosage forms is not guaranteed. The drawbacks associated with the currently available formulations call for the need to develop products that are easily administered and capable of providing desirable and steadfast serum drug concentrations. Over the past few decades, several alternatives to conventional dosage forms have been introduced in an attempt to resolve these issues. Orally disintegrating tablets as well as prolonged released tablets and films are suitable for children unable to swallow tablets and capsules.[28]

Geriatrics

Changes in swallowing function as a result of age predispose geriatric patients at risk for dysphagia for two main reasons. Firstly, healthy ageing gradually damages head and neck anatomy, as well as neural and physiologic mechanisms supporting swallowing function. Such mechanisms of naturally diminishing functional reserve contribute to swallowing alterations in healthy older adults which are known as presbyphagia. Secondly, diseases associated with aging including neurological (e.g. Parkinson's, Alzheimer's disease and stroke) and non-neurological diseases (e.g. gastro-oesophageal reflux disease) may trigger swallowing disorders. Lastly, geriatric patients suffering from multiple chronic diseases are often treated with complex polypharmacy regimens, and some medications may trigger the onset of swallowing disorders by several different mechanisms. The clinical repercussions of dysphagia are complex and potentially precarious. Swallowing disorders could lead to poor therapeutic adherence and menial therapeutic outcomes.[29, 30]

Adherence

Therapeutic adherence is generally considered as a major factor in achieving desired therapeutic goals. In the case of HIV and AIDS, poor therapeutic adherence has the potential to adversely impact therapeutic outcomes on multiple levels. Inadherence to antiretroviral therapy (ART) can result to less effective viral suppression, which can adversely affect the health of the patient, and trigger resistance to a drug or a group of drugs. This can affect the cost of treatment as well as therapeutic outcomes. [31]

When patients are unable to take their medications due to swallowing difficulties, therapeutic adherence is considerably reduced. There is a correlation between adherence to therapy and good health, thus in-adherence can adversely affect health, with significant costs for the patient, the government or medical schemes. According to the health belief model, patients carry out

their own cost-benefit analysis for each treatment they are offered, weighing up the expected benefits (usually symptomatic relief) against the severity of their symptoms and the perceived risks of treatment (side effects, dependence, time and effort involved, and stigma) according to their lay beliefs and the information at their disposal.[32] One of the roles of healthcare professionals is to encourage people to feel capable of taking their medications. The concept of concordance stipulates that healthcare professionals must understand patients' experiences and behaviours towards their diseases and treatment in order to help them to adhere to the prescribed therapy. [33]

Manufacturing and marketing factors

It is customary practice for manufacturers to optimise and design a new dosage form for a specific drug when the drug approaches the end of its patent life. A new dosage form allows a manufacturer to extend market uniqueness, product differentiation, value-added product line extension, and extend patent protection, while offering patients a more suitable dosage form. This leads to a surge in income, whilst targeting undertreated and underserved patients. [34]

Effectiveness factor

Dispersion of the dosage form in the mouth leads to pre-gastric absorption of the dissolved drug (Figure 2.2). Buccal, pharyngeal and gastric regions are potential absorption sites for several drugs. The main advantage of pre-gastric absorption is the avoidance of first pass hepatic metabolism which increases bioavailability. Additionally, safety profiles may be improved for drugs that produce substantial amounts of toxic metabolites as a result of first-pass liver metabolism and gastric metabolism and for drugs that have a considerable fraction of absorption in the oral cavity and pre-gastric segments of the gastrointestinal tract (GIT).[35]

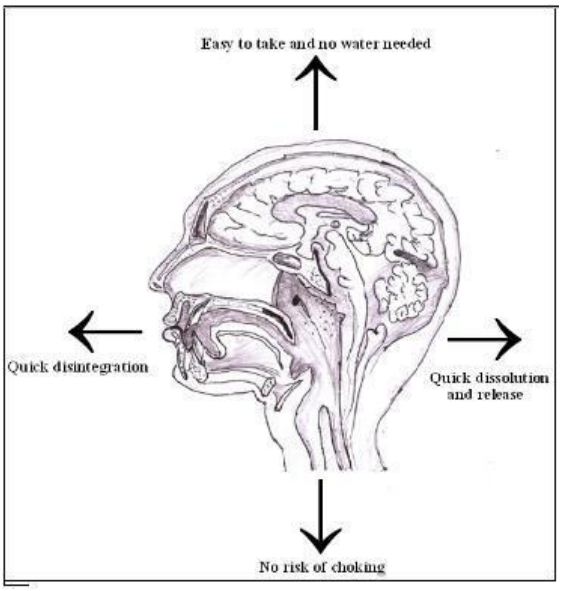


Figure 2.2: Effectiveness of fast dispersible tablets[35]

2.5 Development of fast dispersible tablets

The basic approach in the development of fast dispersible tablets is the use of superdisintegrants which provide rapid tablet disintegration in the buccal cavity. [20].

2.5.1 What are Superdisintegrants?

Over the past few years, new pharmaceutical excipients have been introduced referred to as "Superdisintegrants". A "Superdisintegrant" is an excipient, which is incorporated into an oral solid dosage form blend to speed up disintegration i.e. the breakdown of the compact mass into smaller fragments that can be easily swallowed. This process is sought for immediate release products where rapid release of the product is desired. Superdisintegrants are relatively more effective at lower concentrations with greater disintegrating efficiency and mechanical strength. Superdisintegrants play a significant role in the dissolution and disintegration of tablets. It is imperative to choose an optimal superdisintegrant concentration so as to ensure rapid disintegration and high dissolution rates of tablets.[36]

The rapid disintegrating effect of superdisintegrants is as a result of water absorption and swelling. Swelling of superdisintegrants leads to an increase in the wetted surface of the carrier, this enhances the wettability and dispersibility of the system, thus promoting disintegration and dissolution. The optimal concentration of the superdisintegrant can be selected according to the critical concentration of the disintegrant. Below this concentration, the rate of tablet disintegration is directly proportional to the concentration of the superdisintegrant, whereas above this concentration the disintegration time remains almost constant or even increases.[37]

2.6 Various techniques for preparing fast dispersible tablets

Disintegrant addition

This method involves the incorporation of an optimum concentration of a superdisintegrant into the formulation blend to achieve rapid disintegration or dissolution. The yield is similar to conventional tablets with a higher percentage of disintegrants, lower hardness and higher friability.[38]

Freeze drying or lyophilisation

The drug is dissolved or dispersed in an aqueous solution of a carrier. The mixture is poured into the wells of preformed blister packs. Liquid nitrogen is applied to the blisters packs in order to freeze the drug solution. The frozen blister packs are kept in refrigerated cabinets to continue the freeze-drying. The resultant tablets are highly porous, have high specific surface area, dissolve rapidly and show enhanced absorption and bioavailability.[39]

Moulding

A water-soluble ingredient with a hydro-alcoholic solvent is used and is moulded into tablets under a pressure lower than that used in conventional tablet compression. The moulded tablets

are relatively less compact compared to compressed tablet, and their high porosity enhances disintegration and dissolution. Therefore, drug absorption is increased.[40]

Sublimation

Inert solid ingredients that volatilise rapidly like camphor, urea, ammonium bicarbonate, ammonium carbonate, and hexamethylenetetramine are incorporated into the formulation blend and the mixture is compressed into tablets. The volatile excipients are then removed via sublimation, which produces a porous structure.[41]

Spray-Drying

Hydrolysed and non-hydrolysed gelatines are used as supporting agents, mannitol as a diluent, sodium croscarmellose or sodium starch glycolate as disintegrating agents and an acidic excipient (e.g. citric acid) and or alkali excipient (e.g. Sodium bicarbonate) to promote disintegration or dissolution. This disintegration time of the tablet is roughly 20 seconds when immersed in an aqueous medium.[42]

Mass-Extrusion

This technique involves softening the active blend using a solvent mixture of water soluble polyethylene glycol and methanol. The softened mass is expelled through the extruder or syringe to get a cylindrical shape of the product into even segments using a heated blade to form tablets. The dried product can be used to coat granules of bitter tasting drugs, thereby making them more palatable. [43]

Direct compression

Conventional tableting equipment, facilely available excipients and a limited number of processing steps are involved in direct compression. It is the most cost effective tablet manufacturing technique.[44]

Cotton candy process

Polysaccharide matrices are formed by the concurrent action of flash melting and spinning. This candy floss matrix is then milled and blended with active ingredients and excipients after re-crystallization and then compressed to fast dispersible tablets. The tablets can accommodate high doses of active ingredient and display enhanced mechanical strength.[45]

Compaction

a) Melt granulation

This is done by incorporating a hydrophilic waxy binder (super polystate) PEG-6-stearate. Super polystate not only acts as binder and increase physical resilience of tablet, but also enhances tablet disintegration. The tablet melts in the mouth and solubilises rapidly leaving no residue.[46]

b) Phase-transition process

This technique involves compressing a powder containing two sugar alcohols with high and low melting points, and subsequently heating the powder at a temperature between their melting points. The tablet hardness is increased after the heating process due to increase of inter particle bond induced by phase transition of lower melting point sugar alcohol. Increased compactibility and thus sufficient hardness gained by the formulation. [47]

Nanonisation

The drug size is reduced to nano-size by milling the drug using a proprietary wet-milling technique. The nano-crystals of the drug are stabilized against agglomeration by surface adsorption on selected stabilisers which are then incorporated into fast dispersible tablets. This technique is used for poorly water soluble drugs. It leads to an increase in bioavailability and reduction in dose, cost effective manufacturing process, conventional packaging due to exceptional durability and wide a range of doses (up to 200 mg of drug per unit).[40]

Fast dissolving films

A non-aqueous solution is prepared containing a water-soluble film forming polymer (pullulan, carboxymethylcellulose, hydroxypropyl methylcellulose, hydroxyl ethyl cellulose, hydroxyl propylcellulose, polyvinyl pyrrolidone, polyvinyl alcohol or sodium alginate), drug and other taste masking ingredients are used to form a film after the solvent has evaporated. In the case of a bitter drug, resin adsorbate or coated micro particles of the drug can be incorporated into the film. The dissolution of the film is within 5 seconds, the drug is delivered instantly with a pleasant after taste. [48]

2.7 Requirements for fast dispersible tablets

The following are the desired criteria for fast dispersible tablets.[49, 50]

- ✓ The tablets should disintegrate or dissolve in the mouth within few seconds, and not require water to be swallowed. This requirement highlights the effectiveness of a FDT since the tablet is conveniently administered, the risk of choking is drastically reduced, and the active pharmaceutical ingredient (API) is made available for absorption promptly.

- ✓ The tablets should allow for high drug loading. This speaks particularly to medications that require a relatively high amount of the API in a single dosage unit such as antibiotics (e.g. amoxicillin 500mg), oral anti-diabetic drugs (e.g. metformin 500 mg) and antiretroviral drugs (e.g. lamivudine 150 mg and efavirenz 600 mg)
- ✓ The active ingredient should be compatible with the excipients used in the formulation. This is very crucial since drug-excipient incompatibilities can adversely affect the integrity and the therapeutic activity of the API.
- ✓ Should be palatable. Since FDTs come into close proximity with the tongue for a substantial amount of time, it is thus imperative for the tablets to leave a pleasant taste in the mouth. Adequate taste masking techniques should be employed for bitter and unpleasantly tasting drugs.
- ✓ Little or no residues should remain in the mouth after oral administration. This suggests that the FDT should be formulated with excipients which disintegrate into fine non-gritty particles and leave a pleasant mouth-feel. Also, the tablet size and the amount of excipients used should be kept minimal.
- ✓ Should be resilient enough to withstand the manufacturing process and post manufacturing handling. FDTs are usually more porous and less resilient than conventional swallow tablets so as to ensure rapid disintegration. Consequently, a suitable tablet hardness which confers adequate resilience to the tablet whilst allowing for instantaneous disintegration should be sought.
- ✓ Should not be affected by environmental conditions such as temperature and humidity. FDTs are usually prone to moisture due to their relatively high porosity and the hygroscopicity of the excipients used. This can be quite problematic in tropical regions such as Central and West Africa which are characterised by high levels of humidity. Consequently, adequate moisture proof packaging should be designed for FDTs.

- ✓ Should be manufactured at a low cost using conventional manufacturing techniques. This entails the use of cheap facilely available excipients and simple manufacturing processes.

2.8 Desired characteristics of fast dispersible tablets

Since the administration of FDTs differs from that of conventional tablets, FDTs should possess several unique characteristics as enumerated below.

Fast disintegration

FDTs should disintegrate in the mouth without the need for water. The saliva of the patient provides the disintegration medium. The disintegrated dosage form should form a soft paste or suspension providing a pleasant mouth feel and ease of swallowing. Generally, a tablet should disintegrate within a minute in order to fulfil the criterion for fast disintegration, but instantaneous disintegration is highly sought.[51]

Taste of active ingredients

Since FDTs dissolve or disintegrate in the patient's mouth, the drug is in close proximity with the tongue. There should be little or no residues in the mouth after swallowing. A pleasant taste is one of the key factors for patient acceptance. Taste masking should be employed for bitter and poor tasting drugs. Taste masking excipients should be kept as low as possible in order not to increase the tablet mass. The taste-masking technology employed should be compatible with the FDT formulation [42].

Drug properties

The tablet properties should not be significantly affected by the drug properties. Several drug properties can negatively impact the performance of FDTs. For example, the hygroscopicity, bulk density, compressibility, solubility, particle size, and crystal morphology of a drug can

considerably influence the characteristics of the resultant tablet, such as tablet strength and disintegration. The FDT technology employed should be flexible enough to accommodate distinctive properties of individual drugs. [52]

Tablet strength and porosity

The porosity of FDTs is usually maximised to ensure fast tablet disintegration and dissolution. The key factors are rapid water absorption or wetting, and break down of agglomerated particles into smaller particles for fast dissolution. For quick disintegration to take place, the excipients should have a high wettability and the tablet matrix should be highly porous. Since tablet strength is directly proportional to compression pressure, and porosity is inversely proportional to compression pressure, it is important to find the porosity that allows fast water absorption while maintaining high mechanical strength. Moreover, low compression pressure yields soft and friable FDTs, thus unsuitable for packaging in conventional blisters or containers. A technique to enhance tablet mechanical strength without decreasing tablet porosity or requiring special packaging to handle fragile tablets should be employed.[51]

Moisture sensitivity

FDTs should have low sensitivity to humidity. Tackling the issue of moisture sensitivity can be very challenging since most excipients employed in the formulation of FDTs are highly water soluble to ensure quick dissolution and a good mouth feel. Highly water soluble excipients are susceptible to moisture; some are deliquescent at high humidity. An adequate packaging or other strategy should be employed to shield FDTs from various environmental conditions. [51]

2.9 Advantages of fast dispersible tablets

FDTs offer both the advantages of solid and liquid oral dosage forms along with distinctive properties which include:

Accurate dosing

As unit solid dosage forms, they provide dosing accuracy, easy manufacturing and transportability, good physical and chemical stability and an ideal alternative for paediatric and geriatric patients.[49]

Enhanced bioavailability

Bioavailability of drugs is enhanced due to quick dissolution and absorption from the buccal cavity, pharynx and oesophagus.[52]

Quick onset of action

FDTS provide rapid onset of therapeutic action as tablet are instantly disintegrated alongside quick dissolution and absorption in the mouth.[53]

Patient compliance

Little or no water is needed to swallow FDTs. Hence, it is convenient for patients who are traveling and do not have immediate access to water.[51]

Ease of administration

Since FDTs are easily swallowed, they are an ideal alternative for patients who cannot swallow, such as paediatrics, geriatrics, stroke victims, bedridden patients and psychiatric patients. [51]

Obstruction free

The risk of suffocation in airways due to physical obstruction when swallowed is considerably reduced, thus providing enhanced safety and compliance.[49]

Enhanced palatability

Good mouth-feel, especially for paediatric patients as taste masking techniques can be employed to attenuate the unwanted taste of drugs.[49]

Simple packaging

Conventional packaging such as blister packs can be used for FDTs.[51]

Business avenue

Provide new business opportunities in the form of product differentiation, line extension, uniqueness and life cycle management.[51]

Cost effectiveness

FDTs are manufactured at low cost since conventional formulation processes and material are employed.[52]

2.10 Challenges in formulating fast dispersible tablets

Mechanical strength and disintegration time

FDTs are formulated to disintegrate within a minute. While doing so, maintaining a good mechanical strength is a prime challenge. Many FDTs are fragile and they are prone to damage during packing, transport or handling by patients. It is evident that increasing the mechanical strength of the tablet will delay the disintegration time. So, a good compromise between these two parameters is always essential.[54]

Taste masking

Many drugs have a bitter taste. A FDT constituting of a bitter or poor tasting drug will adversely impact patient compliance and acceptance for the dosage form. So, effective taste masking of bitter drugs must be done so that the taste of the drug is not felt in the mouth.[55]

Hygroscopicity

Many orally disintegrating dosage forms cannot uphold physical integrity under normal conditions of temperature and humidity as they are hygroscopic. Hence, they need protection from humidity which demands specialised product packaging.[56]

Mouth-feel

FDTs should not break down into large fragment in the mouth. The disintegrated particles should be as small as possible. Little or no residues should be left in the mouth after swallowing. The incorporation of cooling agents and flavours enhances the mouth-feel.[57]

Amount of drug

The choice of technology employed in the formulation of FDT is motivated by the amount of drug to be used per single dosage form.[57]

Cost

The technology used for FDTs should be acceptable in terms of the cost of the final product. Certain FDTs that require special technologies and specific packaging increase the cost of production remarkably.[54]

Tablet size

The diameter of the easiest tablet to swallow has been reported to be 7-8 mm, while tablets that are easily handled have diameters greater than 8 mm. Consequently, it is difficult to attain a tablet size that can be easily handled and easily swallowed.[54]

2.11 Limitations of fast dispersible tablets

Due to the high porosity of the tablet matrix, FDTs usually possess unsatisfactory mechanical strength. Consequently, careful handling is vital during manufacturing, transportation and administration.

The tablets may leave an unpleasant taste and or grittiness in the mouth if not formulated properly.

Drugs with larger doses are usually difficult to formulate as FDTs e.g. Rifampicin (600 mg) and Ethambutol (1000 mg).[55, 58]

2.12 Direct compression of fast dispersible tablets

Fast absorption of water into the tablets and disintegration of aggregated particles into individual components for instantaneous dissolution are the most important characteristics of FDTs. Attaining a highly porous tablet is the most common strategy to ensure fast disintegration, which will ensure fast water absorption into the matrix. For this to be possible, the excipients used should have high ‘‘wettability’’ to enhance water penetration into the tablet matrix. However, the porosity of a tablet is inversely proportional to the compression pressure, which in turn is directly proportional to the mechanical strength of the tablet. Therefore, it is important to find the optimum porosity that allows both fast water absorption and high mechanical strength. Furthermore, low compression pressure causes FDTs to become too fragile for packaging in conventional blisters or bottles. A formulation strategy is necessary to

enhance tablet mechanical strength without compromising porosity or necessitating special packaging.[59]

Several methods of preparing FDTs have been described to date, including lyophilisation, moulding, and the compression of wet powders to construct highly porous structures. Although these methods are effective, they are time consuming and technically difficult, often requiring special processing equipment. Furthermore, although tablets produced by these methods disintegrate rapidly, they are usually very weak and friable. The mechanical strength of the tablets may not be sufficient to endure packaging, transportation, and patient handling. Tablets obtained by the conventional compression method are less friable but disintegrate more slowly.[56]

The compression method, with or without wet granulation, is a convenient and cost-effective way to prepare tablets with sufficient structural integrity. Formulating FDTs using conventional compression is attractive because of the low manufacturing cost and ease of technology transfer. However, tablet presses were originally designed to make conventional tablets with high mechanical strength. When making conventional tablets, maintaining high tablet porosity is not a primary concern, and high compression force is applied to achieve high tablet strength.[56]

2.13 Excipients used in the direct compression of fast dispersible tablets

A direct compression FDT powder blend usually comprises of a disintegrant, diluent, lubricant, flow aid, flavourant, sweetener, and occasionally a colourant. Most FDTs on the market use mannitol as a diluent. Directly compressible grades of mannitol display sought properties, such as; sweetness, mouth feel, solubility, and rapid dispersion as a result of wicking. Hence, mannitol has gained popularity for FDTs over other diluents typically used in direct compression such as lactose.[60]

FDT formulations usually contain high levels of superdisintegrants to ensure rapid tablet disintegration. Depending on the level and properties of the active pharmaceutical ingredient and the desired release profile, the concentration of the superdisintegrant used can be between 10–20 % of the weight of a single tablet. Consequently, the choice of superdisintegrant is critical when formulating a FDT.[60]

As with most direct-compression formulations, additional excipients such as flow aids and lubricants are likely to be included. Since the tablet is meant to disintegrate in the buccal cavity, flavourants and sweeteners are often incorporated to mask unpleasant tastes of actives. A colourant can be added to enhance the aesthetic appeal of the tablet and to aid identification.[60]

2.13.1 Requirements for excipients used in fast dispersible tablets

Since FDTs are designed to disintegrate in the mouth, the dosage form should have a pleasant taste with the formation of a smooth paste or suspension after disintegration so that patients should have a good mouth feel. Excipients with good water solubility will enhance disintegration and dissolution. Pharmaceutical grade saccharides such as xylitol, lactose, glucose, mannitol, and sucrose are often used to formulate FDTs. Mannitol is one of the most common excipients used for this dosage form because of its water solubility and non-hygroscopic nature. It also produces a unique cooling sensation in the mouth and has a pleasant taste when chewed or dissolved. Sugars can be used as diluents, binders, and or enhancing agents hence they are not categorized with respect to a single specific function. Consequently, sugars are often incorporated into FDT formulations to serve several purposes.[61]

The particle size of the excipients in a FDT must be considered. The smaller the particle size, the better the patients' compliance as larger particles leave a ''gritty'' feel in the mouth. Smaller particles ensure a smooth tablet surface, which also enhances aesthetic appeal. Nonetheless, smaller particles may impart poor material properties such as poor flowability, high

segregation, moisture sensitivity, and or low porosity of the tablet matrix. It is therefore important for the formulator to strike a balance with the properties of the powders to achieve processability, stability, optimal therapeutic adherence and efficacy.[60]

2.14 Disintegrants

A disintegrant is an excipient added to a tablet formulation to enhance the fragmentation of the tablet matrix when in contact with water. Fast disintegration of the tablet matrix in the mouth eases swallowing and increases the surface area of the tablet particles, thereby increasing the rate of absorption of the drug to achieve the desired therapeutic outcomes. Every marketed tablet has a certain level of disintegrant and it is important to investigate which and how much disintegrant is necessary for a given tablet formulation. Disintegration is triggered when a small amount of water or saliva comes into contact with the solid dosage form (wetting) and penetrates the tablet matrix by capillary action. Therefore, the material properties of pharmaceutical excipients and also the matrix structure including pore size and distribution need to be considered for successful formulation development. Since most disintegrants swell to some extent, swelling pressure is generally considered the main factor for tablet disintegration.[39]

Rapid swelling without an increase in viscosity (gel formation) is one of the most sought properties of disintegrants. A high viscosity will slow disintegration by hindering the penetration of water into the tablet matrix. There are several disintegrants on the market which can be considered in the formulation of FDTs. Some of which include crospovidone (cross-linked PVP), croscarmellose (cross-linked cellulose), sodium starch glycolate (cross-linked starch), and low-substituted hydroxypropyl cellulose.[62,63]

Most disintegrants are water insoluble materials that swell on contact with water, therefore excessive disintegrant addition can lead to grittiness following tablet disintegration. The

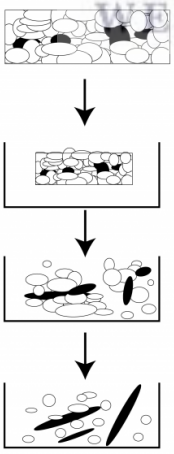
appropriate disintegrant concentration should be carefully investigated for a given FDT formulation. The processing technique employed, and the purpose of the formulation influence the choice of the disintegrant.[62,64]

2.14.1 Mechanism of action of disintegrants

Tablets break into individual particles by one or more of the mechanisms listed below

Porosity and capillary action (wicking)

Capillary action is always the first step in tablet disintegration. A suitable aqueous medium into which a tablet is placed penetrates into the tablet and replaces the air adsorbed on the particles there by weakening intermolecular bonds and breaking the tablet into smaller particles (Figure 2.3). The hydrophilicity of the drug and tableting conditions affect the rate and extent at which the tablet absorbs water. For these types of disintegrants, maintenance of a porous structure and low interfacial tension towards aqueous fluid is vital which helps in disintegration by creating a hydrophilic network around the drug particles.[65, 66]



The disintegrant pulls water into the tablet matrix via the pores which results in the weakening of interparticulate bonding

Figure 2.3: Illustration of tablet disintegration due to wicking

Swelling

Swelling is the general mechanism of tablet swelling. Tablets with high porosity show poor disintegration due to lack of adequate swelling force (Figure 2.4). On the other hand, sufficient swelling force is exerted in tablets with low porosity. It is important to note that if the packing fraction is very high, fluid is unable to penetrate the tablet and disintegration is slowed down.[64]

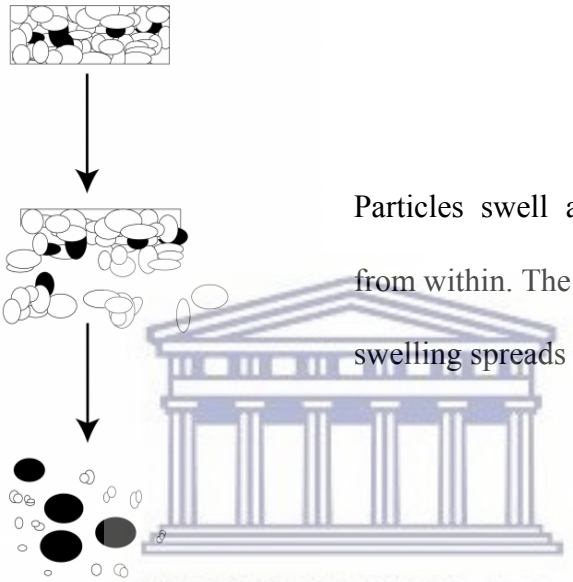
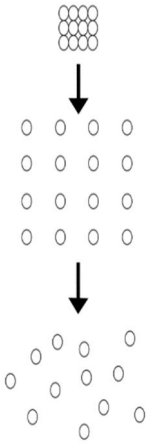


Figure 2.4: Illustration of tablet disintegration due to swelling

Due to disintegrating particles/particle repulsive forces

Another mechanism of disintegration which attempts to explain the swelling of tablets made with non swellable disintegrants. Guyot-Hermann has proposed a particle repulsion theory based on the observation that non-swelling particle also cause disintegration of tablets. The electric repulsive forces between particles constitute the mechanism of disintegration and water is required for it (Figure 2.5). Researchers found that repulsion is secondary to wicking.[67]

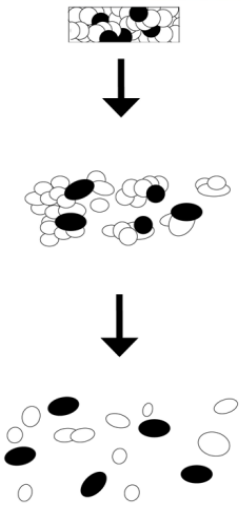


Water is drawn into the pores and particles repel each other as a result of electrical forces

Figure 2.5: Illustration of tablet disintegration due to repulsive forces

Due to deformation: elastic recovery

As a result of stored potential energy, most materials which undergo plastic deformation during compression try to return to their original shape as soon as possible. Return to the initial shape is not possible in the tablet matrix. As soon as water penetrates into the tablet matrix and the forces which hold the particles together are reduced, those particles gain the ability to return to their original shape (Figure 2.6)[68]



Particles swell to precompression size and break up the tablet matrix

Figure 2.6: Illustration of tablet disintegration due to deformation

Due to the release of gases

Carbon dioxide is released within tablets on contact with water due to interaction between bicarbonate and carbonate with citric acid or tartaric acid. The generation of pressure within the tablet matrix accounts for tablet disintegration. These disintegrants are mainly used when the need to formulate a very rapidly disintegrating or dissolving tablet arises, as these disintegrants are highly sensitive to slight changes in humidity level and temperature, strict control of environment is required during manufacturing of the tablets. The effervescent blend is either added immediately prior to compression or can be added in two separate fractions of formulation.[69]

By enzymatic action

In this case, enzymes present in the body act as disintegrants. The cohesive effect of the binder is destroyed by enzymes thus enhancing disintegration. As a result of swelling, pressure exerted in the radial direction causes the tablet to burst, or the speeded uptake of water leading to a considerable increase in the volume of granules to promote disintegration.[70]

Because of heat of wetting (air expansion)

When disintegrants with exothermic properties are wetted, localized stress is generated due to capillary air expansion which helps in disintegrating the tablet. This explanation however is limited to only a few types of disintegrants and cannot describe the action of most modern disintegrating agents.[69]

2.15 Superdisintegrants

The term superdisintegrant refers to substances which achieve disintegration faster than the substances conventionally used. A solid oral dosage form disaggregates into smaller particles that dissolve more rapidly than in the absence of such disintegrants. Superdisintegrants are

usually used at low level in solid oral dosage forms, typically between 1-10% of the total weight of a given dosage form.[63] The disintegration of dosage forms depends on various physical factors of superdisintegrants which are enumerated below.[64]

- ✓ Percentage of superdisintegrant in the formulation. The activity of superdisintegrants increases with increasing superdisintegrant percentage below their critical concentration. Above this concentration, no further increase or a decrease in their disintegrating activity can be observed.
- ✓ Proportion of superdisintegrants used. The extent to which the material properties of the superdisintegrant impart the properties of the tablet is dependent on the portion of the tablet comprised of the superdisintegrant.
- ✓ Compatibility with other excipients. The occurrence of physical or chemical incompatibilities between the superdisintegrant and the other excipients can adversely influence the integrity and the stability of the tablet.
- ✓ Presence of surfactants. Surfactants are added to enhance the disintegration of tablets containing hydrophobic constituents. The absence of surfactants in a tablet containing hydrophobic constituents can negatively impact the disintegrating activity of the superdisintegrant.
- ✓ Hardness of the tablets. The rate of disintegration is inversely proportional to tablet hardness due to the decrease in the number and size of pores on the tablet surface which enhance the penetration of water into the tablet matrix. Since the swelling of superdisintegrants is dependent on the wettability of the tablet, increase in tablet hardness can thus lead to a decrease in super disintegrating activity.
- ✓ Nature of drug substances. The presence of poorly soluble and hydrophobic constituents in a tablet can impede the activity of a superdisintegrant by countering

interactions between water and the tablet. The reverse is true for hydrophilic and highly soluble substances.

2.15.1 Selecting a superdisintegrant

Although the superdisintegrant mainly enhances the rate of disintegration, when used at high levels it can also affect mouth feel, tablet hardness, and friability. Thus, several factors must be considered when selecting a superdisintegrant.[60]

Disintegration

The disintegrant must quickly wick saliva into the tablet to generate the volume expansion and hydrostatic pressures necessary to provide rapid disintegration in the mouth.[60]

Compactability

In order to avoid the need to use specialise packaging, it necessary to formulate tablets with adequate hardness at a given compression force to produce resilient tablets while enhancing production speed. Consequently, a more compactable disintegrant will yield stronger and less-friable tablets.[60]

Mouth-feel

In order to ensure patient compliance, FDTs must be palatable. Large particles can result in a gritty feeling in the mouth. Consequently, smaller particles are preferred. If the tablet forms a gel-like consistency on contact with water, it produces a gummy texture that many patients find distasteful.[60]

Flow

As far as direct compression is concerned, content uniformity and good flow are vital in attaining drug content uniformity. In typical tablet formulations, superdisintegrants are used at 2–10 % of the formulation blend. In cases where the superdisintegrant concentration is considerably higher, the flow properties of the superdisintegrant become more important because of the greater impact on the flow characteristics of the total blend.[71,72]

2.15.2 Types of superdisintegrants

Superdisintegrants can be classified into two categories on the basis of their source:

1 Natural Superdisintegrants

2 Synthetic Superdisintegrants

Natural superdisintegrants

Presently, there is a number of plant based pharmaceutical excipients and various researchers have explored the utility of some of these plant-based materials as pharmaceutical superdisintegrants. These superdisintegrating agents are natural in origin and alternative over synthetic substances because they are comparatively cheaper, easily available, non-irritating and non-toxic in nature. Therefore, natural gums and mucilage have been extensively used in the field of novel drug delivery for their easy availability, cost effectiveness, eco-friendliness, emollient, and non-irritant nature and non-toxicity, capable of a myriad of chemical modifications, potentially degradable, and compatible due to their natural origin.[73, 74]

Advantages of natural superdisintegrants

The advantages of natural superdisintegrants include:

Biodegradability

They are biodegradable compared to their synthetic counterparts since they are naturally sourced and are produced by living organisms.[75]

Biocompatibility and non-toxicity

They are mainly plant materials made up of reiterating sugar polysaccharides. As such, they are compatible with the human body and non-toxic.[75]

Low cost

Since they are naturally sourced, the cost of production is considerably less compared to synthetic superdisintegrants. Many developing countries are dependent on agriculture, and there are substantial financial investments on agriculture.[50]

Environmental-friendly processing

There are many types of natural compounds obtained from different plant sources which are widely utilised in the pharmaceutical industry and collected in immensely large quantities due to the simple production processes involved.[50]

Local availability (especially in developing countries)

In some developing countries, the government promotes the cultivation of plants to be used as pharmaceutical excipients. It thus provides the facilities for large scale production, like gum and mucilage because of their wide applications in industries.[75]

Patient tolerance as well as public acceptance

The lower incidence of adverse effects and allergenicity associated with the use of natural superdisintegrants compared to their synthetic counterparts has been reported.[75]

Examples of natural polymers used as superdisintegrants

All these polymers are approved by the US Food and drug administration (FDA). The FDA recognizes these polymers as GRAS (Generally Recognized as Safe)

Gellan gum

Approved as a food additive in the European community under the number E 418, with ADI (acceptable daily intake) confirming its status as a safe food additive. The Gellan gum food grade fully meets the standards and the purity criteria issued in different regions of the world or internationally, such as the Food Chemicals Codex, the US Pharmacopoeia, and the European Directives.[76]

Chitin and Chitosan

Chitin (β-(1→4)-N-acetyl-D-glucosamine) is a natural polysaccharide obtained from the exoskeleton of crustaceans such as shrimps and crabs, and cell walls of fungi. It possesses an amino group covalently linked to an acetyl group as compared to a free amino group in chitosan. Chitosan is produced commercially by the deacetylation of chitin. Chitosan is the best known natural polysaccharide used for its myriad of applications in the pharmaceutical industry.[77]

Gum Karaya

Gum karaya is a vegetable gum produced as an exudate by trees of the genus *Sterculia*. Chemically, gum karaya is an acid polysaccharide composed of the sugars galactose, rhamnose, and galacturonic acid. The high viscosity nature of the gum limits its uses as binder and disintegrant in the development of conventional dosage form. Gum karaya has been investigated for its potential as a tablet disintegrant. Different results showed that modified gum karaya produces rapid disintegration of tablets.[78]

Agar and treated agar

The dried gelatinous substance obtained from *Gelidium amansii* (*Gelidanceae*) and several other species of red algae like *Gracilaria* (*Gracilariaceae*) and *Pterocladia* (*Gelidaceae*). Agar is yellowish-grey or white to proximately colourless, odourless with mucilaginous taste and is available in the form of digests, sheet flakes, or coarse powder. Agar consists of two polysaccharides, agarose and agar pectin. Agarose is responsible for gel vigour and agar pectin is responsible for the viscosity of agar solutions. The high gel vigour of agar makes it a potential disintegrant candidate.[79]

Fenugreek seed mucilage

Trigonella foenum-graceum commonly known as fenugreek is a herbaceous plant of the leguminous family. Fenugreek seeds contain a high percentage of mucilage. Although it does not dissolve in water, mucilage forms a viscous tacky mass when exposed to fluids. Like other mucilage-containing substances, fenugreek seeds swell up and become slick when they are exposed to fluids.[79]

Soy polysaccharide

Soy polysaccharide does not contain any starch or sugar. A study evaluated soy polysaccharide (a group of high molecular weight polysaccharides obtained from soy beans) as a disintegrant in tablets made by direct compression using lactose and dicalcium phosphate dihydrate as fillers. Cross-linked sodium carboxymethyl cellulose and corn starch were utilized as control disintegrants. Soy polysaccharide performed well as a disintegrant in direct compression formulations with results similar to cross-linked carboxymethyl cellulose.[76]

Mango peel pectin

Mango peel which constitutes 20–25% of the mango processing waste was found to be a good source for the extraction of high quality pectin, suitable for the preparation of film, and acceptable jelly. Pectin is an involute hetero-polysaccharide which is a hydrophilic colloid. A study found that mango peel pectin is a prospective superdisintegrant candidate. Although not as more efficient than synthetic superdisintegrants, its good solubility and higher swelling index makes it suitable to be utilised in the formulation of FDTs.[80, 81]

Examples of synthetic superdisintegrants

Croscarmellose Sodium

It is modified cellulose and is a cross linked polymer of carboxymethylcellulose. The disintegration rate of croscarmellose sodium is higher than that of sodium starch glycolate and the mechanism of disintegration also differs. Cross linking makes it hydrophilic, insoluble, highly absorbent, resulting in exceptional swelling properties and its unique fibrous nature gives it excellent water wicking abilities. It is used in oral pharmaceutical formulations as a superdisintegrant for granules, tablets and capsules. Concentrations of croscarmellose sodium range between 1-5% w/w of the formulation blend.[82, 83]

Sodium starch glycolate

It is a cross linked polymer of carboxymethyl starch. Although sodium starch glycolate can be synthesised from a wide range of native starches, potato starch is commonly used as it yields the product with the best disintegrating properties. The introduction of large hydrophilic carboxymethyl groups aims at disrupting the hydrogen bonding within the polymer structure. This allows water to penetrate into the molecule thereby making the polymer cold water soluble. The aim of the cross-linking is to reduce both the water-soluble fraction of the polymer and the viscosity of dispersion in water. The natural pre-dried starches swell in water to the extent of 10-20 per cent and the modified starches increase in volume by 200-300 per cent in water. Tablets formulated by using these superdisintegrants usually disintegrate within two minutes.[82, 84]

Cross-linked polypyrrolidine

Crospovidones are synthetic, insoluble, cross-linked homopolymers of N-vinyl-2- pyrrolidone. When inspected under a scanning electron microscope, crospovidone particles appear as granular and are highly porous. Due to its high crosslink density, crospovidone swells rapidly in water without gelling. The unique particle morphology of Crospovidones makes them highly compressible. Crospovidone is used as superdisintegrant at low concentration levels (2- 5%) in direct compression and in wet and dry granulation processes.[82, 85]

2.16 Banana

2.16.1 Taxonomical classification

Kingdom	*Plantae*
Phylum	*Magnoliophyta*
Class	*Liliopsida*
Order	*Zingiberales*
Family	*Musaceae*
Genus	*Musa*
Species	*Musa paradisiaca, Musa sapientum*
Common name	Banana

2.16.2 Description

Musa paradisiaca is a herbaceous plant (up to 9 m long) with a robust tree-like pseudostem and a crown of large elongated oval deep-green leaves (up to 365 cm in length and 61 cm in width). Each plant produces a single inflorescence 15-20 cm long, concave, dark red in colour and somewhat fleshy. Fruits are oblong, fleshy, 5-7cm of length in the wild form and longer in the cultivated varieties. *Musa sapientum* is a tree-like perennial herb that grows 5 - 9 m in height with a tuberous rhizome (Figure 2.7). The inflorescence is big with a reddish-brown bract. The fruit pulp is fleshy, juicy and contains numerous seeds. From its native south-western Pacific home, the banana plant spread to India around 600 BC and later on it spread all over the tropical regions. It is possibly the world's oldest cultivated crop and the most common tropical fruit. [86]

Figure 2.7: Banana tree with young fruits and inflorescence

2.16.3 Cultivation and distribution

About 300 varieties of bananas are grown of which a vast majority are spread throughout tropical and subtropical countries.[86]

2.16.4 Traditional uses

The fruit of *M. paradisiaca* and *M. sapientum* is traditionally used in diarrhoea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uraemia, nephritis, gout, hypertension, and cardiac disease.[87].

2.16.5 Pharmacological activities

Reduced risk of high blood pressure

Bananas are reported to be one of the best sources of potassium, an essential mineral for maintaining normal blood pressure and heart function. A medium-sized banana provides 350 mg of potassium. A number of studies have demonstrated the effectiveness of potassium rich foods in reducing blood pressure. The FDA has endorsed claims from the banana industry of

its ability to reduce the risk of hypertension and stroke. The FDA asserts that consuming foods containing high amounts of potassium and low amounts of sodium considerably reduces the risk of high blood pressure and stroke. Additionally, potassium plays an important role in maintaining fluid and electrolyte balance in biological cells. Researchers have reported that some phytochemicals found in banana act similarly to some antihypertensive drugs. A group of researchers studied six popular banana varieties and found that they all possess ACE (angiotensin converting enzyme) inhibitory properties, though unripe bananas displayed a weaker inhibition than the ripe ones. A 10% drop in blood pressure was observed in subjects who consumed bananas daily for two weeks.[88]

Reduced risk of stroke

Scientists suggest that individuals who have a low potassium intake are prone to strokes. A study of 5,600 people aged over 65 found that those with the lowest potassium intake were 50% more likely to suffer a stroke. Foods rich in potassium like bananas may lower the risk of stroke, however more substantial evidence is required to confirm whether increasing potassium in the diet can prevent strokes.[88]

Restore normal bowel activity

Bananas can help restore normal bowel function and help alleviate diarrhoea and constipation because of its high content of non-digestible fibres (including alpha glucans, hemicellulose, and cellulose). The high content of pectin in bananas which is water absorbent confers them a bulk producing ability. Bananas are an excellent source of fructooligosaccharide, a prebiotic which enhances the growth of probiotics (beneficial bacteria) in the colon. These beneficial bacteria produce vitamins and digestive enzymes that improve our ability to absorb nutrients, plus compounds that protect the body against unfriendly microorganisms. In a study, 57 babies (5-12 months) with persistent diarrhoea of at least 14 days duration were administered a week's

treatment with a rice-based diet containing either green banana, apple pectin or the rice diet alone. Treatment with both green banana and apple pectin resulted in a 50% reduction in stool weights, indicating that the babies were absorbing significantly more nutrients.[89]

Protection from ulcers and heartburn remedy

Bananas are well known for their antacid properties that confers protection against gastric ulcers and ulcer damage. Leucocyanidin, a flavonoid found in banana, has been found to considerably increase the thickness of the gastric mucous membrane. Bananas are effective in alleviating heartburn due to their potential to neutralise acid. In an animal study, a simple mixture of banana and milk significantly suppressed acid secretion.[90]

Protection against neurodegenerative diseases (Alzheimer's disease)

Researchers at Cornell University investigated the effects of orange, banana, and apple extracts on neuron cells and found that the phenolic phytochemicals of the fruits prevented neurotoxicity on the cells. Apples were found to contain the highest content of protective antioxidants, followed by bananas then oranges. These results suggest that introducing fresh oranges, bananas, and apples in our diet along with other fruits may protect neuron cells against oxidative stress-induced neurotoxicity and may play an important role in reducing the risk of neurodegenerative disorders such as Alzheimer's disease.[88]

Cholesterol lowering effect

It has been demonstrated through animal studies that bananas have the potential to lower cholesterol. The dietary fibre component of banana is responsible for its cholesterol-lowering effect. Ripening doesn't alter the amount of dietary fibre in the pulp.[91]

Kidney health

Bananas have been shown to enhance kidney function. This is as a result of the high potassium content of banana. A normal intake of potassium suppresses calcium excretion in the urine and minimizes the risk of kidney stones. The results of a Swedish population based prospective study (13.4 years) of 61,000 women aged 40-76, show that women eating more than 75 servings of fruits and vegetables per month (which translates into 2.5 per day) cut their risk of kidney cancer by 40%. Among the fruits, bananas were especially protective. Women eating bananas four to six times a week halved their risk of developing the disease compared to those who did not eat this fruit.[88]

Immunity booster

Bananas contain 25 % of the recommended daily allowance (RDA) for vitamin B6, necessary for the synthesis of red blood cells and antibodies as well as aiding in the metabolism of fat. Additionally, vitamin B6 serves as an immunity booster. Consequently, bananas strengthen the body defence against infectious diseases. An average sized banana contains about 15% of the RDA for vitamin C, one of the strongest antioxidants.[88]

2.16.6 Adverse effects

The presence of tryptophan in banana combined with the high carbohydrate content is believed to affect the consumer's state of awareness influencing brain activity and triggering sleepiness. Consuming bananas and alcohol simultaneously may aggravate migraines. Thus, it is not advisable to consume bananas with alcohol. Few people have been reported to be allergic to bananas.[88]

2.16.7 Nutritional value of banana

Bananas are an excellent source of potassium. Potassium can be found in a variety of fruits, vegetables, and even meats, however, a single banana provides 23% of the potassium required daily amount. Potassium is beneficial to the muscles as it helps maintain their proper functioning and prevents muscle spasms.[88]

Bananas are an excellent source of vitamins, including:

1. A - helps in maintaining healthy bones, teeth, soft tissue, among others

2. B6 - enhances the body's immune system, promotes heart health, brain health, among others

3. C - aids in healing, growth of tissue, ligaments, among others

4. D – enhances Calcium absorption

2.16.8 Phytochemical profile

Carbohydrates have been isolated from M. *sapientum*. Catecholamines such as norepinephrine, serotonin, dopamine, tryptophan, indole compounds, and pectin have been found in the pulp. Several flavonoids and related compounds (Leucocyanidin, quercetin and its 3-Ogalactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside) were isolated from the unripe pulp of plantain. Serotonin, nor-epinephrine, tryptophan, indole compounds, tannin, starch, iron, crystallisable and non-crystallisable sugars, vitamin C, B-vitamins, albuminoids, fats, mineral salts have been found in the fruit pulp of M. *paradisiaca* and M. *sapientum*. Acyl steryl glycosides such as sitoindoside-I, sitoindoside-II, sitoindoside-III, sitoindoside-IV and steryl glycosides such as sitosterol gentiobioside, sitosterol myo-inosityl- β-D-glucoside have been isolated from fruits of M. *paradisiaca*. Cellulose, hemicelluloses, arginine, aspartic acid, glutamic acid, leucine,

valine, phenylalanine and threonine have been isolated from the pulp and peel of M. *paradisiaca* [92].

2.16.9 Properties of unripe banana powder

Banana is rich in carbohydrate with starch being the principal component of unripe banana accounting for about 70 to 80% dry weight basis. Its high starch content accounts for its use as a tablet disintegrant.[93] The starch undergoes important changes during ripening and the average starch content drops in the pre-climacteric (prior to starch breakdown) period to less than 1% at the end of the climacteric period, when sugars, mainly sucrose, accumulate to more than 10% of the fresh weight of the fruit. Banana starch has been reported to be resistant to α-amylase and glucoamylase hydrolysis and has been shown to possess health benefits similar to dietary fibre. It has been shown to prevent colorectal cancer, lower the risk of heart disease, and influence metabolic and inflammatory bowel diseases such as diabetes and diverticulitis [94].

Recent studies have reported the binding properties of banana starch obtained from the unripe fruit of *Musa sapientum* in comparison with official corn starch Banana starch was found to compare favourably with corn starch as the binding agent in tablet formulations although the tablets produced had lower crushing strength and faster disintegration time, suggesting its potential usefulness when fast tablet disintegration is desired. However, no work has been done to evaluate the physicochemical and material properties of banana starch to determine their functional properties and their usefulness. Moreover, the disintegrant properties of the starch have remained largely uninvestigated.[95]

2.16.10 Unripe banana powder as a superdisintegrant

Singh MC et al prepared and evaluated powders of the unripe fruit of various varieties of *Musa pariadisiaca* for angle of repose, bulk density, tapped density, Carr's index, Hausner ratio, swelling capacity, hydration capacity and moisture content. Angle of repose ranged from 28.2º to 32.5º, which indicates good flow property. Bulk density ranged from 0.38 to 0.45 g /cm2. Tapped density ranged from 0.5 to 0.68 g /cm^2. Carr's index ranged from 24 to 33.82% which indicates poor compressibility of the powder. Hausner ratio revealed good flowability. The moisture content for all the powders was within 0.53 to 0.72% w/w.[96]

Hamsanandini J *et al* formulated orodispersible liquisolid compacts of Meloxicam using unripe banana powder as a superdisintegrant. The *in-vitro* dispersion time was found to be in the range of 36.66±1.52 - 96.66±1.52 seconds. The mean disintegration time for all evaluated tablets was found to be 2 minutes, which is aligned with pharmacopoeial requirements. The disintegration time was found to be in the range 35.00±1.00 - 88.00±2.00 seconds. The disintegrating effect of unripe banana powder was investigated by varying the concentration of the polymer. From the results, it was deduced that the disintegrating effect of unripe banana powder is inversely proportional to the amount used in the formulation.[97]

Arun Raj R undertook a comparative evaluation of potato starch and unripe banana powder as disintegrants in a tablet formulation of Aceclofenac. It was observed that the percentage release of tablets containing banana powder and potato starch was higher as compared to tablets formulated with microcrystalline cellulose.[98]

Sathali AH and Suganya M designed fast dispersible tablets of Amiodarone HCL using a variety of synthetic and natural superdisintegrants. Five formulations were prepared using 2%, 3%, 4%, 6%, 8% w/w of unripe banana powder as a superdisintegrant. The cumulative percentage of drug release showed 95.9%, 96.9%, 98.07%, 98.24%, 99.07% respectively at

12.8 seconds. The formulation containing 8% unripe banana powder showed maximum drug release (99.07%) at 12.8 seconds [99].

Saudagar R.B developed and evaluated mouth dissolving tablets of Lisinopril using varying concentrations of unripe banana powder as a superdisintegrant. It was observed that the tablets formulated with unripe banana powder as sole disintegrant showed nearly identical and improved results in terms of wetting time, disintegrating time *in-vitro* dispersion time, water absorption ratio and percentage drug release as compared to the tablets formulated with synthetic superdisintegrants. Studies revealed that unripe banana powder can be used on its own as a disintegrant in the formulation of mouth dissolving tablets of some drug candidates such as Lisinopril. It was thus deduced that preferring superdisintegrants of natural origin over their synthetic counterparts should be motivated by their cost effectiveness and nutritive potential.[100]

Figure 2.8: Unripe banana powder

2.17 Ispaghula

2.17.1 Taxonomical classification of ispaghula

Kingdom	*Plantae*
Phylum	*Magnoliophyta*
Class	*Magnolipsida*
Order	*Lamiales*
Family	*Plantaginaceae*
Genus	*Plantago*
Species	*Ovata*
Common name	Psyllium, ispaghula, isabgol

2.17.2 Description

The name Isabgol is derived from two Persian words '*Isap*' and '*ghol*' meaning horse ear, denoting the characteristic shape of the seed. The Plantago genus comprises of around 200 species of herbs and sub-shrubs which are mainly cultivated in temperate regions and few tropical regions.[101] About ten species are recorded in India, of which *Plantago ovata* is important for its seeds. Family Plantaginaceae is a stemless woolly annual herb which attains a height of 30-45 cm. Leaves are narrowly linear or filiform appearing whorled due to the short terete stem. The spikes are 1.2-4 cm long and about 0.5 cm broad, cylindrical to ovoid in shape and bear between 45-70 flowers. Flowers are bisexual, tetramerous, anemophilous and protogynous and as such favouring outcrossing. The fruits are ellipsoid capsules, about 8 mm long, obtuse, membranous, glabrous, upper half coming off as a blunt conical lid and seeds are ovoid-oblong, boat-shaped, smooth, rosy-white being concave on one side and convex on the other. The concave side of the seed is covered with a thin white membrane produced by fusing of the outer layer of the ovule together with the inner epidermis, forming the seed coat.[102].

The seed epidermis is made of polyhedral cells whose walls are thickened by a secondary deposit which is the source of mucilage. On mechanical milling, the coating of the seed provides the husk, a membranous covering of the seed, white to light pink in colour, translucent and odourless (Figure 2.9). The husk absorbs moisture and forms a tasteless mucilaginous substance which constitutes the drug.[103]

Figure 2.9: *Plantago ovata*. Left: plant. Middle: seeds, Right: husk

2.17.3 Distribution and cultivation

Plantago ovata is commonly distributed in the Mediterranean coastal region, Sinai, Isthmus desert, east of the Nile, North Africa. India, Iran, Pakistan, countries of the Arabian Peninsula and West Asia, extending up to Sutlej and Sind in West Pakistan. *P.ovata* is also distributed throughout Arabia on sandy and silty soil, often in shady and damp locations. The crop was introduced to India during the 16th century. It is cultivated as a cash crop in various parts of India and on a limited scale in some areas of west Pakistan.[104]

2.17.4 Traditional uses

In traditional medicine, dried seeds and husk are regarded as emollient, demulcent and safe laxative mainly beneficial in chronic constipation, chronic diarrhoea and dysentery.[105] Seed husk of blonde psyllium has been used in European and Asian herbal medicine for chronic

constipation since the 16th century.[101] It does not irritate the intestine and is specific in its use when the mucous membrane is disturbed by inflammatory affections. Seeds are considered as cooling and diuretic and recommended for use in febrile conditions and in the affections of the kidney, bladder and urethra. A decoction of seeds is prescribed in cough and cold and a dressing of crushed seeds are useful for rheumatic and glandular swelling.[106] The mucilage of the dried seed is used externally as an emollient. The seed coat of the dried seed is taken orally as a bulk laxative. An Infusion of the dried seed is taken orally for urinary tract inflammations.[107]

2.17.5 Phytoconstituents

Psyllium husk contains a high quantity of hemicellulose, composed of a xylan backbone linked with arabinose, rhamnose, and galacturonic acid units (arabinoxylans). Phytochemical analysis of *Plantago* species showed their high potential to produce a myriad of secondary bioactive metabolites, i.e. sterols, phenols, alkaloids, iridoids, phenols and cumatines that are useful in treating human diseases. The seed consists of 35% soluble and 65% insoluble polysaccharides (lignin, hemicellulose and cellulose). Psyllium is classified as a mucilaginous fibre due to its powerful ability to form a gel in water. This ability comes from its role as the endosperm of the *P. ovata seed*, where it functions to retain water in order to prevent the seed from drying out [108].

Psyllium seeds contain over 30% of hydrocolloidal polysaccharide (mucilage) in the outer seed coat, fixed oils, tannin, aucubin glycoside (iridoid), sugars, sterols and protein. This mucilage is colloidal in nature and its composition varies with the conditions of preparation. It is mainly composed of xylose, arabinose and galacturonic acid with rhamnose and galactose. Two polysaccharide fractions have been separated from the mucilage. One of them is soluble in cold water and on hydrolysis yields D-xylose (46%), an aldobiouronic acid (40%), L-arabinose (7%) and insoluble residue (2%); the other fraction is soluble in hot water forming a

highly viscous solution which sets to a gel on cooling and yields on hydrolysis D-xylose (80%), L-arabinose (14%), aldobiouronic acid (0.3%) and traces of D-galactose.[109]

2.17.6 Pharmacological activity

Irritable Bowel Syndrome (IBS)

Plantago ovata is widely used for the treatment of inflammatory bowel disease (IBD). *P. ovata* seeds ameliorated the development of colonic inflammation in transgenic rats as evidenced by an improvement of intestinal cytoarchitecture, significant decrease in some of the pro-inflammatory mediators and higher production of short-chain fatty acid. [110]

Antihypertensive effect

The alcoholic extract of *P.ovata* seeds exhibits cholinergic properties. It has been found to reduce blood pressure in anaesthetized cats and dogs, inhibit the isolated and perfused hearts of rabbits and frogs and stimulate intestinal movement in rabbits, rats and guinea-pigs. The activity of the extract on smooth muscle is inhibited by atropine.[102]

Cholesterol-lowering effect

The seed oil has the ability to reduce cholesterol level of serum in rabbit. The use of linoleic acid-rich oil obtained from embryo has been suggested as a dietary hypocholesterolemic agent in place of corn oil. Feeding of the embryo oil as dietary supplement for lowering serum cholesterol level gave promising results in experimental animal.[103]

Hypoglycaemic effect

The aqueous extract of *P. ovata* seeds reduced hyperglycaemia in type 1 and 2 diabetes in rats. These properties show that this extract can be used in the treatment of diabetes.[111]

2.17.7 Ispaghula as a superdisintegrant

The seed husk of Plantago ovata comprises of a high amount of a xylan known as arabinoxylan, which is made up of 74.8% xylose and 23.2% arabinose. Arabinoxylan with a molar mass of 364,470 g/mol shows high swelling capacity in water and thus accounts for the tremendous disintegrating effect of *Plantago ovata* husk and mucilage. This polysaccharide based hydrogel is biocompatible and non-toxic; hence it would be a good candidate for the design of novel drug delivery systems. The hydrogels of arabinoxylan contain ferulic acid which demonstrates antioxidant and anticancer properties. Xylans are the most common hemicelluloses found in plants such as cereals, herbs and grasses. The isolation techniques, structures and properties of naturally occurring xylans have been studied over the past few years. Despite their properties and myriads of applications, xylans are not yet available in industrially required quantities for large scale processing. [112]

Prakash Goudanavar et al designed and evaluated fast disintegrating tablets of Granisetron HCL with natural and synthetic polymers. The formulation which contained 5% w/w of *Plantago ovata* as superdisintegrant showed better drug release as compared to the other formulations.[113]

Patil et al formulated and evaluated fast dissolving tablets of Candesartan cilexetil using *Plantago ovata* and croscarmellose sodium as superdisintegrants. The swelling index of *Plantago ovata* mucilage was found to be more than that of its synthetic counterpart croscarmellose sodium. *Plantago ovata* formulations showed faster drug release compared to croscarmellose formulations. This is due to the relatively higher swelling index of *Plantago ovata* mucilage.[114]

Shirsand SB et al designed fast disintegrating tablets of Prochlorperazine maleate by the direct compression technique using varying concentrations of *Plantago ovata* mucilage and

crospovidone as super-disintegrants along with microcrystalline cellulose. Mannitol was used as a filler to enhance mouth feel. The percentage loss in mass when assessing the friability of the tablets was found to be below 1%, this is an indication of good mechanical resilience. Hardness of the tablets was found to be around 2.63 kg/cm. Water absorption ratio and wetting time, which are imperative criteria for judging the ability of disintegrants to swell in the presence of a minute amount of water were found to be in the range of 50-86% and 11-47 s, respectively. Overall, the formulations containing 8% w/w of Plantago ovata mucilage and 60% w/w of microcrystalline cellulose showed an *in vitro* dispersion time ranging from 8 to 10 s.[115]

In an investigation, fast disintegrating tablets of amlodipine besylate were designed by the direct compression method using varying concentrations of *Plantago ovata* mucilage as a superdisintegrant. The formulated tablets were evaluated for weight variation, friability, hardness, disintegration time, dissolution, and drug content. The optimised formulation showed *in vitro* disintegration time of 11.69 seconds with rapid *in vitro* dissolution within 16 minutes.[116]

2.18 Formulation and evaluation of fast dispersible tablets

The formulation of fast dispersible tablets using the direct compression technique is very much similar to that of conventional tablets. However, supplementary processes might be required when botanically sourced superdisintegrants are employed. Prescribed evaluation procedures used for conventional tablets can be used for fast dispersible tablets alongside additional tests that lay emphasis on tablet dispersion.

2.18.1 Preformulation studies

This phase consists of characterising the excipients and the active pharmaceutical ingredient (API). The extent to which the ingredients are characterised, and the choice of characterisation techniques are influenced by the nature of the project, the nature of the ingredients and the amount of literature available. The following steps are usually followed.

Characterisation of the active pharmaceutical ingredient

The physicochemical properties of the API are identified or determined; these generally include molecular weight and size, density, solubility (in various solvents and different pH media), melting point, boiling point, pH and LogP. Thermal analytical tools such as hot stage microscopy (HSM), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are widely used and are highly accurate in determining the melting point, boiling point and purity of compounds.[117-119] Key functional groups of the API can be identified with spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR)[120]. Another step consists of determining the micromeritic properties of the API powder using conventional assessment techniques. This step is imperative because it guides the selection of key tableting variables such as the type and amount of excipients to be added and the compression force to

be employed. The powder properties commonly assessed include, bulk density, tapped density, angle of repose, compressibility index and Hausner ratio.[121]

Characterisation of the excipients

The excipients are assessed and characterised in the same manner as the API. In the case of botanically sourced excipients, a number of additional processes are required. Usually, extraction and purification steps are needed prior to characterising these excipients. Characterisation tests pertaining to botanically sourced excipients generally include, organoleptic analysis, microbiological load, moisture content, ash value and phytochemical analysis.[122] Spectroscopic analytical techniques such as FTIR, vibrational spectroscopy, nuclear magnetic resonance spectroscopy (NMR), as well as chromatographic methods have proven to be effective in analysing the constituents of botanically sourced excipients.

Drug-excipients compatibility

This step consists of identifying physical or chemical incompatibilities that may arise from combining the API with the excipients. These incompatibilities can adversely affect the stability of the tablet and the therapeutic activity of the API. Thermal analytical tools (HSM, DSC) and spectroscopic techniques (FTIR) can be used to identify and predict these incongruities.[123]

2.18.2 Powder blending

The API is mixed with the excipients in the desired proportions and the micromeritic properties of the powder blends are determined. The micromeritic properties of the powder blends hint at the need for adding compression aids or altering the proportion of some excipients. Table 2.2 provides examples of commonly used excipients and the role they play in fast dispersible tablets.

Table 2.2: Excipients commonly used in fast dispersible tablet formulations[124]

Excipients	Function	Examples
Superdisintegrant	Increases the rate of disintegration and hence enhances dissolution. Some superdisintegrants are multipurpose excipients	Microcrystalline cellulose, crospovidone, sodium starch glycolate, pregelatinised starch
Flavouring agent	Increases palatability thereby increasing patient acceptability and therapeutic adherence	Vanilla, peppermint, strawberry, anise oil
Sweeteners or sugar-based excipients	Exhibit high aqueous solubility and can be used as fillers. Enhance taste and mouth-feel	Aspartame, dextrose, mannitol, fructose
Surfactant	Reduces surface tension and hence enhances tablet solubilisation	Sodiumdoecylsulphate, sodiumlaurylsulphate
Binder	Confers resilience and maintains the integrity of the tablet prior to administration	Polyvinylpyrrolidone (PVP), Polyvinylalcohol (PVA)
Colourants	Enhances the aesthetic appeal of the tablet	Amaranth, red iron oxide
Lubricant	Reduces friction and wear between the die and punches of the tablet press	Magnesium stearate, liquid paraffin, talc, magnesium lauryl sulphate
Filler	Increases bulk of tablet and may serve other functions	Mannitol, xylitol, sorbitol, pregelatinised starch

2.18.3 Compression

This entails applying sufficient pressure to the powder blend in order to obtain tablets with satisfactory compactness and hardness. The most intricate part of this phase is to find a suitable compression force that confers sufficient hardness to the tablets whilst allowing the tablets to disintegrate instantly given that the rate of disintegration is inversely proportional to the compression force employed. Fast dispersible tablets are usually compressed using relatively lower compression forces so as to obtain adequately porous tablets.[54] Tablet resistance to crush and disintegration time are two key parameters that are used to assess the suitability of the compression force used.

2.18.4 Evaluation and quality control

Fast dispersible tablets are evaluated using the same tests and procedures prescribed for conventional tablets alongside key tests that lay emphasis on tablet dispersion and palatability. Parameters used to evaluate fast dispersible tablets include, hardness, weight uniformity, tensile strength, friability, durability, taste, porosity, *in-vitro* dispersion time, wetting time, water absorption ratio, *in-vitro* disintegration time and dissolution. Disintegration and dissolution are crucial parameters for fast dispersible tablets since they demarcate them from conventional swallow tablets. Stability testing is also necessary in order to assess the need for optimising the product, estimate a suitable shelf-life for the product, identify potential incompatibilities between the ingredients and recommend suitable packaging and storage conditions.[125]

2.18.5 Packaging and storage

The choice of packaging used for fast dispersible tablets is influenced by their level of resistance to mechanical stress. Highly porous and less rigid fast dispersible tablets require specialised packaging in order to prevent mechanical damage; this includes packing tablets with peelable backing foil, or dome-shaped blisters which prevent the tablets from moving vertically. Fast dispersible tablets with sufficient mechanical strength are generally packed in bottles or push-through blisters. Fast dispersible tablets should be stored in a dry place in moisture impervious packaging since they are highly porous and contain moisture sensitive excipients.[126]

2.19 High performance liquid chromatography (HPLC) methods for lamivudine assay

Several techniques have been used for the assay of lamivudine of which chromatography is the technique of choice due to its high sensitivity. Chromatography is a technique used to separate and purify compounds based on their polarity. The high performance liquid chromatography (HPLC) system consists of four main components which are enumerated below.

- ✓ A column referred to as the stationary phase which is composed of substances like silica functionalised with non-polar long carbon chains.
- ✓ The analyte i.e. the drug being assayed
- ✓ Injectors to inject samples of the analyte into the column.
- ✓ The mobile phase. The mobile phase is a solution which moves through the column and serves as a carrier for the samples. It is generally composed of water and an organic solvent such as acetonitrile, a phosphate buffer or methanol in a given ratio.

Compounds are identified, purified or separated by HPLC based on the time required for individual components to be eluted from the column. The amount of time required to elute a

component from the column subsequent to injection is referred to as the retention time (Rf) which is determined by its polarity.[127] As the concentration of the solvent in the column increases, the concentration of water decreases. This enhances the non-polarity of the mobile phase. Non-polar compounds in the sample adhere strongly to the carbon whilst the polar compounds adhere weakly.

Four key articles were selected to compile a suitable HPLC method for this study. There were remarkable similarities amongst the reviewed assessment techniques with regards to sample preparation, mobile phase and stock solutions. Literature reports that mobile phases consisting of acetonitrile or 0.015 M potassium dihydrogen ortho-phosphate with methanol have been used for the HPLC assay of lamivudine. The mobile phases reviewed had a two phase system with a polar and a non-polar solvent e.g. methanol (non-polar) and water (polar) in a 50:50 or 30:70 ratio. Orthophosphoric acid in water has also been used to assay lamivudine.[127-129]

The reviewed data showed that mixtures of methanol with pH values adjusted with orthophosphoric acid yielded adequate and clearly defined peaks. Data accuracy was tested by means of recovery studies by adding a known amount of the assayed drug to a drug solution of a known concentration (e.g. 80%, 100% and 120%) prior to HPLC analysis. Each sample was tested in triplicate and the recovery values obtained were high suggesting that the results obtained were accurate. Precision was tested by using six similar standard solutions and analysing them using HPLC. This was all completed and repeated on the same day. The relative standard deviation was determined, and the results were within an acceptable range.[127-129]

The reviewed HPLC methods (Table 2.3) were found to be speedy, precise and accurate, thus a good point of reference for the development and design of a suitable HPLC method for the assay of lamivudine tablets formulated using natural polymers

Table 2.3: Summary of reviewed literature of HPLC methods for lamivudine assay[129-132]

Variables assessed	Method 1	Method 2	Method 3	Method 4
Mobile phase	Acetonitrile, methanol and water	Potassium dihydrogen ortho-phosphate, acetonitrile	Methanol, ortho-phosphoric acid, acetonitrile	Water, ortho-phosphoric acid, methanol
Mobile phase ratio	30:45:25	45 to 55	30:70 or 70:30	50:50
Use of internal standard solution	None	Yes (Carbamazepine)	Yes	Yes (Efavirenz, Lamivudine, Zidovudine)
Was sample pulverised? / Solvent used to dissolve powder?	Yes / methanol	Yes / methanol	Yes / methanol	Yes / methanol
Formulation	Combination dose tablet	Combination dose tablet	Combination dose tablet	Combination dose tablet
Column	Phenomenex Luna C18 column	HiQ Sil C18V column	Inertsil ODS-3 c18 column	Xterra C18 (150 mm × 4.6 mm, 5µ) column
Method validation	Accuracy, precision, linearity, UV detection	Recovery studies and relative standard deviation achieved. Accuracy, precision and linearity studies	Accuracy, precision, linearity, recovery studies, relative standard deviation	Accuracy, precision, linearity, recovery studies, relative standard deviation

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Chapter 3

Materials and methods

In this chapter, the materials and methods used for the formulation of fast dispersible tablets of lamivudine are discussed. A clear description of analytical techniques and procedures is provided. This chapter gives a breakdown of the project in a chronological order i.e. sourcing of materials, preformulation studies, formulation and quality assessment of the product.

3.1 Materials

Lamivudine

Pure lamivudine powder was donated by Aspen Pharmacare (batch number: LV1400913; expiry date: August 2018). Lamivudine was used as the active pharmaceutical ingredient of the innovative tablets.

Banana

Unripe bananas of the genus *Musaceae* were purchased from a local retailer (Food lover's market). The bananas were used to prepare unripe banana powder which was used as a superdisintegrant.

Ispaghula husk

Ispaghula husk was purchased from a local retail pharmacy (Clicks pharmacy). The husk was milled into a powder and used as a superdisintegrant.

Magnesium stearate

Magnesium stearate is the magnesium salt of stearic acid. The light white powder was added to the formulation blends in order to enhance the flowability of the powders and to prevent adhesion to the punches and die. The magnesium stearate used in this study was of analytical grade.

Talc

A clay mineral composed of hydrated magnesium silicate. Talc was used as a dusting powder to reduce friction on the punches and die during powder compression. The talc used in this study was of analytical grade.

Comparator

Aspen Lamivudine® 150 mg tablet (Batch number: A851174; Expiry date: June 2018) was used as a standard for comparison.

Phosphate buffer solution

A 6.8 phosphate buffer solution (PBS) was used for *in-vitro* dispersion and disintegration studies. The PBS was prepared as follows:

Sodium chloride: 8 g

Potassium chloride: 0.2 g

Disodium hydrogen phosphate: 1.44 g

Potassium dihydrogen phosphate: 0.24 g

Deionised water: 1000 ml

Hydrochloric acid (0.1 M): *qs*

The salts were accurately measured and ground in a mortar using a pestle. The salts were transferred to a graduated beaker containing 800 ml of UV sterilised deionised water. The solution was stirred until the salts were fully dissolved. 0.1 M HCL was added to the solution dropwise whilst stirring and measuring the pH. When the desired pH was attained, the solution was adjusted to 1000 ml using deionised water.

All other reagents used in this study were obtained from the chemical company Sigma-Aldrich. They were all analytical grade reagents.

3.2 Preparation of the natural polymers

Unripe banana powder

Unripe bananas of the genus *Musaceae* were peeled and the edible part of the fruit was sliced. The sliced pulp was washed with distilled water and 0.2 % w/w of methyl paraben was added as a preservative. The pulp was ground using a domestic blender and dried in a convection oven at 45 ºC for 24 hours. The dry pulp was pulverised in a mortar, sieved using sieve No.80 (177µm), and stored in an airtight container with a desiccant (silica gel packets).[1]

Ispaghula husk powder

Ispaghula husk was milled using a domestic dry blender. The milled husk was sieved using sieve No.80, and the powder was stored in an airtight container with silica gel.

3.3 Extraction of relevant constituents from the natural polymers

The relevant constituents responsible for the disintegrating effect of the natural polymers were extracted and characterised. In the case of unripe banana powder, its high starch content accounts for its disintegrating properties.[2] The hemicellulose arabinoxylan is responsible for the disintegrating effect of ispaghula husk powder.[3]

Extraction of starch from unripe banana powder

Dehydrated banana powder (20 g) was suspended in 100 ml of distilled water and agitated for 12 hours at room temperature using a magnetic stirrer. The mixture was filtered using mesh 200. The residue was washed with distilled water, and the filtrate was centrifuged at 1000 x g for 30 minutes at room temperature. The supernatant was discarded, and the wet mass was dried in a convection oven at 40 ºC for 12 hours. The dried mass was pulverised in a mortar, sieved using sieve No.80, and stored in an airtight container with a desiccant.[4]

Extraction of arabinoxylan from ispaghula husk powder

Ispaghula husk powder (7 g) was soaked in distilled water in the ratio 1:50 (husk to water). The suspension was allowed to macerate for 24 hours at room temperature. The pH of the suspension was increased to 13 by adding concentrated NaOH dropwise to solubilise arabinoxylan. The insoluble fibrous matter was removed by sieving the alkali extract through mesh 200 (74µm). Acetic acid was added to coagulate the mucilage at pH 3. The translucent gelatinous mass obtained was washed several times with distilled water to remove excess acetic acid.[3] The gel was frozen at -80ºC and freeze dried for 5 days. The white powder obtained was pulverised in a mortar, sieved through sieve No.80, and stored in an airtight container with a desiccant.

3.4 Characterisation of the natural polymers

3.4.1 Organoleptic evaluation

Sensory appraisal of the natural polymers was conducted in order to determine their macroscopic properties and overall aesthetics. An assessment of taste, colour, odour and texture was recorded.

3.4.2 Phytochemical analysis [5, 6]

This assessment was conducted to gain an insight of the chemical composition of the natural polymers from a qualitative perspective. Phytochemicals can have potentially beneficial or detrimental therapeutically significant effects. Samples of unripe banana powder and ispaghula husk powder were evaluated for the presence of carbohydrates, saponins, phenols, proteins, tannins, lipids, flavonoids and steroids.

Iodine starch test: 1 g of powder was dissolved in 15 ml of distilled water. The solution was boiled until a mucilage was obtained. Few drops of 2N iodine solution were added to 2 ml of the mucilage. A dark blue colouration was an indication of the presence of starch.

Foam test (Test for saponins): 1 g of powder was dissolved in 10 ml of distilled water in a test tube. The test tube was sealed and agitated vigorously. The appearance of a permanent froth was a positive test for saponins.

Phenols: an aqueous extract of the polymer was treated with $FeCl_3$. A dark colouration was an indication of the presence of phenols.

Biuret test (test for proteins): a small amount of powder was added to 4% NaOH and few drops of 1% $CuSo_4$ were added. The appearance of a violet or pink colour was an indication of the presence of proteins.

Tannins: An aqueous extract of the polymer was treated with $FeCl_3$. A dark colouration was an indication of the presence of tannins.

Lipids: 10 ml of 96% ethanol were added to 1 g of powder in a test tube and agitated. The solution was diluted with distilled water and the appearance of a cloudy emulsion was an indication of the presence of lipids.

Flavonoids: four pieces of magnesium ribbons were added to the ethanolic extract of the powder sample. Few drops of concentrated hydrochloric acid were added to the extract and agitated. The appearance of a reddish colouration was an indication of the presence of flavonoids

Steroids (Liberman-Burchard test): 1g of powder sample was dissolved in dry chloroform and several drops of acetic anhydride were added followed by 2 drops of concentrated sulphuric

acid. A pink, purple or dark colouration was an indication of the presence of steroids such as cholesterol.

Monosaccharides (Benedict's test): 1 g of powder was dissolved in 10 ml distilled water and placed in a test tube. Few drops of Benedict's reagent were added, and the test tube was placed in a hot water bath for 10 minutes. A reddish, yellowish or greenish colouration indicated the presence of reducing sugars.

3.4.3 Microbiological limit test

This assessment was carried out to quantitatively estimate the number of microbial colony forming units (CFU) present in the natural polymers. Botanically sourced powders offer a propitious environment for microorganisms to thrive due to the presence of essential nutrients and moisture. The bioburden of a naturally sourced powder provides an insight on the safety, integrity, and stability thereof.

The microbiological load of the natural polymers was determined by using the spread plate method. 1 g of powder was dispersed in 9 ml of distilled water and three serial dilutions were made. Nutrient agar was dissolved in distilled water, sterilised at 121ºC using an autoclave, and allowed to set in petri dishes. The petri dishes were then inoculated with 1 ml of the test sample. To determine fungal activity, the dishes were incubated at 25 ºC for 72 hours. To assess bacterial growth, the dishes were incubated at 37 ºC for 24 hours. [7]

3.4.4 Swelling Capacity

It is important to assess the propensity and the extent at which natural polymers used as superdisintegrants swell when in contact with water as this behaviour hints at their disintegrating properties.

The swelling capacity of the natural polymers was estimated by a modification of the method of Bowen and Vadino.[8] The tapped volume occupied by 1 g of the powder Vx was recorded. The powder was then dispersed in a graduated cylinder containing 85 ml of distilled water, and the volume was made up to 100 ml with additional water. The suspension was allowed to stand for 24 hours, after which the volume of the sediment Vy was estimated. The swelling capacity was determined as follows:

Swelling capacity: Vy/Vx

The mean of three determinations was calculated.

3.4.5 Determination of pH

This analysis was carried out in order to estimate the acidity or basicity of aqueous dispersions of the natural polymers. The pH of a 1% aqueous dispersion was determined using an electronic pH metre.

3.5 Thermal analysis

Thermal analytical tools have the ability to characterise the physical structure and properties of materials. The following thermal analytical techniques were used to investigate the thermal behaviour of lamivudine and the natural polymers.

3.5.1 Loss on drying

This analysis was done to estimate the amount of moisture present in the natural polymers. In view of the fact that water is essential for microbial growth, the stability and shelf life of a naturally sourced powder is substantially affected by its moisture content.

The moisture content of the natural polymers was estimated using a halogen moisture analyser. 1 g of powder was heated to a maximum temperature of 105 ºC for 5 minutes and the percentage loss in mass was recorded. The analysis was carried out in triplicate.

3.5.2 Differential scanning calorimetry (DSC)

Differential scanning calorimetry is a thermal tool that measures the difference in the amount of heat required to increase the temperature of a sample and reference as a function of temperature. The sample and reference are kept roughly at the same temperature throughout the analysis, and the heat flow in and out of the sample is measured by a calorimeter. A differential calorimeter measures the heat of a sample relative to a reference. The temperature programme for a DSC analysis is commonly designed in such a way that the sample holder temperature increases linearly as a function of time. The analysis only requires a few milligrams of the sample. The availability, speed and simplicity of DSC make it one of the most used thermal analysis techniques. [9]

DSC thermograms were obtained using a Perkin Elmer DSC 7 instrument under nitrogen purge. A ±3 mg powder sample was placed in an aluminium pan, sealed, pierced to provide a vent hole and heated at a rate of 10 °C/min within the temperature range of 35 to 600 °C.

3.5.3 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is a thermal analysis method in which changes in physical and chemical properties of materials are measured as a function of increasing temperature with constant heating rate, or as a function of time with constant temperature and/or constant mass loss. TGA can provide information about physical phenomena, such as second-order phase transitions, including absorption, adsorption, desorption, sublimation, and vaporisation.

Similarly, TGA can be used to elucidate chemical phenomena including chemisorption, decomposition, and solid-gas reactions (e.g., oxidation or reduction).[10]

TGA thermograms were obtained using a Perkin Elmer Pyris TGA. ±3 mg of powder sample was heated in a crucible under constant nitrogen purge within the temperature range of 30 ºC to 600 ºC, at the heating rate of 10 ºC min^{-1}.

3.5.4 Hot stage microscopy (HSM)

Hot stage microscopy (HSM) is a technique that couples microscopy and thermal analysis to elucidate the thermal behaviour of materials as a function of temperature and time. Besides providing information about particle size and particle morphology, HSM allows one to visualise melting and other transitions when heating a sample. It offers a unique means of visually following thermal changes. Amongst others, HSM can be used to assess: crystal growth, melting, sublimation, evaporation and interactions between different compounds.[11]

Observations were made during heating using a HSM Mettler Toledo model connected to an Olympus BX-50 Microscope. Approximately 0.1 mg of sample was placed on a glass slide with coverglass using silicone oil as a medium. Silicone oil was used because of its resistance to decomposition at high temperatures and its inertness. The sample was heated within the temperature range of 20 to 400 ºC at a rate of 10 ºC min^{-1}.

3.6 Spectroscopy

The extent at which materials emit or absorb light and other radiation depending on the wavelength of the radiation can be used to apprehend the functional configuration of a given material.

3.6.1 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a spectroscopic technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. The raw data is converted into the actual spectrum using a Fourier transform.[12]

Infrared spectra were obtained using a PerkinElmer Spectrum 400 FT-IR in the range 650-4000 cm^{-1}.

3.7 Particle morphology and size analysis

The knowledge and control of the size of particles is of great importance in tablet formulation. This enables one to have an insight of the fundamental and derived properties of a given powder. The size of a particle can be related to the chemical, physical and therapeutic properties of a powder.

3.7.1 Scanning electron microscopy (SEM)

The scanning electron microscope uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20 X to approximately 30,000 X with a spatial resolution of 50 to 100 nm).[13]

Micrographs were obtained using a SEM Leo 1450. Particle diameter was estimated using the software imageJ® by measuring the longest axis on a particle, assuming that the particles are spherical.

3.8 Determination of micromeritic properties

When formulating a tablet, it is essential to assess the fundamental and derived properties of the powders since these properties considerably influence dose uniformity, dissolution, physical stability, drug release, and absorption.

Lamivudine, the natural polymers, and the various powder blends were assessed using the United States Pharmacopoeia (USP) recommended methods to determine their propensity to flow and compressibility. The experiments were done in triplicate and the mean was determined.

3.8.1 Angle of repose

The angle of repose is the constant, three-dimensional angle (relative to the horizontal base) assumed by a cone-like pile of material. Angle of repose is a characteristic related to interparticulate friction or resistance to movement between particles. It is calculated using the formula below.

tan (α) = height/0.5 base.

30 g of a powder sample was sieved using sieve No. 80 in order to break agglomerates. The powder was allowed to flow through a clamped funnel 4 cm above a steady base. The height and diameter of the heap were measured. [7]

3.8.2 Bulk density

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume, including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles within the powder bed. The bulk density is expressed in grams per ml (g/ml). It may also be expressed in grams per cubic centimetre (g/cm3).

30 g of a powder sample was sieved using sieve No. 80 in order to break any agglomerates. The volume of the powder was measured using a 100 ml graduated measuring cylinder readable at 1 ml.[7]

3.8.3 Tapped density

The tapped density of a powder is an increased bulk density attained after mechanically tapping a container carrying the powder sample. Tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel. After observing the initial powder volume or weight, the measuring cylinder or vessel is mechanically tapped, and volume or weight readings are taken until little further volume or weight change is observed.

The same powder used to determine bulk density was used to determine tapped density. The cylinder was fastened to a settling apparatus and subjected to 250 taps per minute. The decrements in volume were recorded until no further reduction in volume was observed.[7]

3.8.4 Compressibility index and Hausner's ratio

These parameters measure the susceptibility of a powder to be compressed. As such, they are measures of the powder's ability to settle, and they permit an assessment of the relative importance of interparticulate interactions. In a flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing

materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the compressibility index and the Hausner Ratio.[7]

3.9 Drug-excipient compatibility studies

This study was carried out to ensure that the therapeutically active drug lamivudine does not undergo changes when combined with the natural polymers.

Lamivudine and individual natural polymers were mixed in a 1:1 ratio. The blended drug samples were stored at room temperature in poly-top vials for a week. FTIR and DSC were used to analyse the mixtures for any possible interaction or incompatibility.

3.10 Formulation of fast dispersible tablets

3.10.1 Powder blending

The individual powders were sieved using sieve No.80 in order to break any agglomerates present. Only two excipients were used in this project so as to make the innovative formulation as cost effective as possible, and to assess the multipurpose properties of the natural polymers i.e. filling, binding, and disintegrating. Several blends of lamivudine with varying concentrations of individual natural polymers and magnesium stearate were mixed using a tumbler mixer for 30 minutes. The following formulation blends were prepared (Table 3.1).

Table 3.1: Different formulation blends prepared

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
L (mg)	150	150	150	150	150	150	150	150	150	150	150	150
B (mg)	3.06	3.06	8.01	17.04	27.11	46	-	-	-	-	-	-
I (mg)	-	-	-	-	-	-	3.06	3.06	8.01	17.04	27.11	46
M (mg)	-	3.125	3.22	3.41	3.61	4	-	3.125	3.22	3.41	3.61	4
S %	2	2	5	10	15	23	2	2	5	10	15	23

* L(lamivudine), B(Banana powder), I(Ispaghula husk powder), M(Magnesium stearate), S(superdisintegrant)

The amount of lamivudine used in the different formulations was constant in order to compare the innovative tablets with the branded tablets Aspen Lamivudine® 150 mg.

This formulation approach was prompted by available literature on the use of natural polymers as superdisintegrants in tablet formulations. Naturally sourced superdisintegrants are generally used in the range 2-10 % w/w of the total tablet weight.[14] The following formula was designed to determine the theoretical tablet weight and the amount of excipients to be added .

$$\mathbf{K + (x+y)W = W}$$

K: mass of Lamivudine

x: magnesium stearate percentage

y: superdisintegrant percentage

W: tablet weight

Ten tablets were compressed for each formulation. The formulation that displayed the best attributes in terms of friability, hardness and dispersion time was selected as the optimum formulation. Formulation F6 yielded the best tablets. A batch of 60 tablets were made from this formulation for further assessment and comparison with Aspen Lamivudine® as per the United States Pharmacopoeia recommendations.

3.10.2 Tableting

Tablets were compressed using the direct compression method. Direct compression was chosen because of its simplicity and cost effectiveness. A manually operated single punch tablet press was used. Different compression forces were applied (30, 35, 40, 45, 50 kN). 40 kN was found to be ideal in terms of conferring sufficient hardness to the tablets whilst allowing for instantaneous disintegration. Concave punches of 8 mm in diameter were used. The punches

and die were dusted with talc prior to compression. A 0.01 g sensitive electronic scale was used to weigh the required amount of powder for each individual tablet.

3.10.3 Wet granulation

Granulation is the process by which primary powder particles are made to adhere to form larger, multiparticle entities called granules. Wet granulation employs the use of a binder solution to improve the properties of the formulation blend in a such way that the granules possess optimal properties for tableting. The resulting tablets have desired quality attributes namely homogeneity, weight uniformity, hardness and disintegration. [15]

Wet granulation was carried out in an attempt to improve the compactability of the formulation blend and reduce the friability of the final product. Granulation was done manually using a 5% starch paste as a binder solution. Lamivudine was mixed with unripe banana powder, few drops of the starch paste were added to the powder blend and stirred to form agglomerates. The granules were dried in an oven at 50 ºC until constant mass was attained. The required amount of magnesium stearate was added to the dried granules and mixed.

3.11 Evaluation of tablets

The innovative fast dispersible tablets were evaluated as per the USP recommendations and compared to Aspen Lamivudine®.

General appearance

An organoleptic evaluation of the tablets was effectuated to determine their overall elegance. The following aspects were recorded: shape, colour, odour, surface texture, physical flaws and consistency.

Tablet diameter

Ten tablets were randomly selected from the batch. The diameter of each tablet was determined using a vernier caliper. The mean of the values obtained was determined.

Tablet thickness

The average thickness of the tablets was determined by measuring the thickness of ten randomly selected tablets using a vernier caliper. The mean of the values obtained was determined

Weight uniformity

To determine the weight consistency of the tablets and the deviation from the theoretical weight, twenty randomly selected tablets were weighed individually using a 0.001 g sensitive scale. The mean weight and standard deviation were determined.

Friability

The extent at which the outer layer of a tablet crumbles when subjected to mechanical stress was determined using an Erweka friability tester. Twenty tablets were randomly selected from the batch and dusted. The tablets were weighed and placed into the drum. The drum was rotated 100 times and the tablets removed. The tablets were dusted and accurately reweighed. The friability percentage was calculated as follows:

$$\mathbf{F: 100\ X\ (W_i\text{-}W_f)/W_i}$$

Where :

W_i: Initial weight of tablets

W_f: Final weight of tablets

Hardness

The hardness of a tablet is defined as the force applied across the diameter of the tablet in order to break it. The resilience of a tablet to mechanical stress imparted during handling and storage depends on its hardness. The hardness of the tablets was determined using a Schleuniger hardness tester. Ten randomly selected tablets were assessed, and the average force in newtons required to fracture tablets was calculated.

Wetting time

The wetting time of a tablet is related to the contact angle of constituent particles with water. The smaller the contact angle, the more favourable particle-water interactions are, and the faster tablet wetting occurs. It is paramount to assess the wetting time of a fast dispersible tablet to have an insight of its disintegrating properties. Instantaneous wetting brings about prompt tablet disintegration. To determine wetting time, a tablet was placed on a double folded piece of tissue paper and kept in a small petri dish (10 cm diameter) containing 10 ml of distilled water.[16] The time required for complete wetting was measured using a stop watch. The experiment was carried out in triplicate and the mean was determined.

Water absorption ratio

The extend at which the tablets absorb water prior to disintegrating was determined by placing a tablet on a double folded piece of tissue paper in a 10 cm petri dish containing 10 ml of distilled water. When complete wetting was attained, the wet tablet was weighed.[16] The experiment was carried out in triplicate and the mean was determined. The formula used is shown below.

$$R = 100 (W_a - W_b) / W_b$$

W_b: Weight of the tablet prior to wetting

W_a: Weight of the wet tablet

In-vitro dispersion

The time required for the tablets to disperse in distilled water and a 6.8 phosphate buffer solution (PBS) at room temperature was determined A petri dish (10 cm diameter) was filled with 10 ml of distilled water or 6.8 PBS. The tablet was carefully placed in the centre of the petri dish and the time for complete disintegration into fine particles was recorded.[17]

Disintegration

Disintegration is the physical process by which a tablet breaks down into small fragments. This process is monitored visually and pertains to the physical integrity of the tablet alone.

Disintegration times were measured *in vitro* using the standard USP disintegration method for uncoated tablets. The test was carried on six randomly selected tablets using an Electrolab tablet disintegration tester. Deionised water and a freshly prepared 6.8 PBS at 37±0.5 ºC were used as the disintegration media, and the time in seconds taken for complete disintegration of the tablets with no palpable mass remaining in the apparatus was recorded.[7]

3.12 Drug release studies

The rate and extent at which the active pharmaceutical ingredient was released from the different tablet formulations were determined using the USP dissolution test and a validated HPLC method.

3.12.1 Dissolution

Dissolution is the process by which the active pharmaceutical ingredient is released from the dosage form and dissolves into the liquid assay medium. Dissolution is monitored via chemical analysis and provides the approximate time required for full solubilisation of the drug under the test conditions

In vitro dissolution was carried out using the USP paddle method in a Vankel V 700 (220 V) dissolution apparatus with temperature control provided by the Vankel VK 650 A Heater/Circulator Benchsaver® series. Vessels containing 900 ml of freshly prepared and de-aerated deionised water (pH 7) was used as a dissolution medium. The setup was maintained at 37±0.5 ºC, and a stirring speed of 50 rpm was applied. A single tablet was placed in each vessel at time 0 minutes to commence the test.

At predetermined time intervals (10, 20, 30, 40, 50, and 60 minutes), 5 ml aliquots were withdrawn from each vessel and immediately replaced with the same volume of pre-warmed medium to maintain constant volume and temperature.[7] The aliquots were filtered using 0.45 µm nylon filters and stored in poly-top glass vials.

3.12.2 High performance liquid chromatography (HPLC) analysis

Dissolution aliquots were filtered using 0.2 µm filters, transferred to amber HPLC vials and sealed. Samples were analysed using water and methanol in a 70:30 ratio and lamivudine concentrations were determined at 275 nm. The drug release profile of the different tablet

formulations was determined by plotting the cumulative percentage of drug released in the medium against time.

HPLC conditions

A Kinetex C18 (150 mm × 4.6 mm, 5µ) column was attached to a Perkin Elmer Flexar™ HPLC. This HPLC consists of a solvent manager, LC pump and a PDA UHPLC detector. A cleaning programme was run to remove any residue present in the Kinetex C18 column.

Mobile phase

The mobile phase consisted of 70 volumes of water and 30 volumes of methanol in isocratic mode (70:30 ratio).

3.12.3 Preparation of a standard stock solution

A 1 mg/ml standard stock solution of lamivudine was made by accurately weighing 1.07 mg of lamivudine in a 1 ml eppendorf tube containing deionised water (pH 7). The solution was vortexed for two minutes, filtered using a 0.2 µm filter and transferred to an amber HPLC vial and sealed.

The volume required for each standard concentration was calculated as follows:

$$C_1V_1 = C_2V_2$$

Where:

C1 = 1070 µg/ml C2 = x

V1 = ? V2 = 0.5 ml

Table 3.2 below presents the different concentrations prepared using the standard stock solution

Table 3.2: Standard concentrations of lamivudine

Concentration (µg/ml)	Volume (µl)	Medium (µl)	Total Volume (µl)
1. 100	47	453	500
2. 150	70	430	500
3. 200	94	406	500
4. 250	117	383	500
5. 300	140	360	500
6. 350	164	336	500

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Chapter 4

Results and Discussion

This chapter presents the findings and outcomes of the preformulation, formulation and quality control phase of this project. The results are presented using tables, figures and graphs. The outcome of each analysis is discussed with reference to literature and inferences are made.

4.1 Identification and characterisation of the natural polymers

This section presents the results and inferences of the different tests and analyses carried out on the natural polymers and their respective extracts for the purpose of identification and characterisation.

4.1.1 Relevant constituents extracted from the natural polymers

Banana starch

Unripe banana has a high content of carbohydrates with starch being the main constituent accounting for about 60 to 80% on a dry weight basis.[1] The percentage yield of starch obtained from unripe bananas of the genus *Musa* by centrifugation was found to be 77% of the pulverised dried pulp.

Starch is facilely available and is one of the most popular pharmaceutical tablet excipients due to its inertness, low cost, and use as binder, disintegrant, filler and glidant.[2] Besides its therapeutic and nutritional value, the high starch content of unripe banana powder (UBP) suggests its potential usefulness as a multipurpose tablet excipient.[3]

Arabinoxylan

Arabinoxylan is a gel-forming polysaccharide which comprises of about 23% arabinose and 75% xylose on molar basis.[4] The hydrogel with a molar mass of 364,47 g/mol has a high swelling capacity in water, hence it can be used as a tablet disintegrant.[5] The high content of the hydrophilic hemicellulose arabinoxylan in ispaghula husk powder (IHP) motivated its use as a disintegrant in this study.

3.22 g w/w of arabinoxylan was obtained from 7 g of the whole husk of ispaghula upon freeze-drying the mucilaginous precipitate obtained from the alkali extract. The 46% yield was rated

as good in view of the fact that the hemicellulose arabinoxylan accounts for 45-60% of the weight of ispaghula husk.[6]

The extraction yield of the constituents responsible for the disintegrating effect of the natural polymers is presented in Table 4.1 below.

Table 4.1: Percentage yield of starch and arabinoxylan

No	Polymer	Amount used	Extract	Mass	Percentage yield
1	UBP	20 g	Starch	15.4 g	77%
2	IHP	7 g	Arabinoxylan	3.22 g	46%

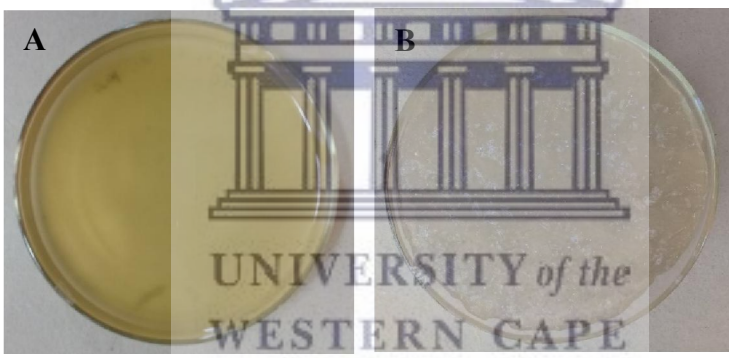


Figure 4.1: a) alkali extract of arabinoxylan b) arabinoxylan gel coagulated with acetic acid

The photographs were taken immediately after extraction at room temperature (25 ºC). Figure 4.1-a shows the extent at which arabinoxylan is solubilised in an NaOH at pH 13. Figure 4.1-b shows the gelatinous nature of arabinoxylan.

4.1.2 Organoleptic evaluation of the natural polymers

Table 4.2 below presents the organoleptic properties of the natural polymers and their respective extracts (starch and arabinoxylan). A pale-yellow powder was obtained from the dried pulp of unripe bananas of the genus *Musa*. This powder was smooth to the touch with a

faint-sweet and floury smell. Starch, a white smooth powder with no distinctive smell was obtained by centrifuging a suspension of UBP.

IHP is a fleecy pale-brown powder, soft and gritty to the touch with a distinctive herby smell. No adulterants or foreign matter were found in the powder. Arabinoxylan is a white smooth powder with no distinctive smell. However, a faint acetic acid smell could be perceived from the sample suggesting the presence of residual acetic acid or sodium acetate. Acetic acid was used to coagulate arabinoxylan from its alkali extract. Washing of the extracted gelatinous mass with water after extraction might have not been sufficient to remove residual acetic acid and sodium acetate. Overall, UBP and IHP had desirable organoleptic properties, hence they had no potential to adversely impact the aesthetic appeal of the final product.

Table 4.2: Organoleptic evaluation of the natural polymers and their respective extracts

No	Property	IHP	UBP	Arabinoxylan	Banana starch
1	Colour	Pale brown	Pale yellow	White	White
2	Odour	Herby	Floury	Acetic acid smell	No distinct smell
3	Taste	Herby taste	Faint sweet	No distinct taste	No distinct taste
4	Texture	Soft and gritty	Soft and smooth	Soft and smooth	Soft and smooth

Figure 4.2: Photographs showing the natural polymers. A) Unripe banana powder B) Ispaghula husk powder. Photographs taken at room temperature (25 ºC), immediately after preparing the powders.

4.1.3 Phytochemical analysis

The qualitative profile of phytoconstituents identified in the natural polymers is presented in Table 4.3 below. (+) indicates the presence of a phytoconstituent while (-) indicates the absence thereof. Starch, saponins, phenols, proteins, tannins, lipids, flavonoids and reducing sugars were identified in UBP. The phytochemical profile of UBP obtained in this study is in concordance with literature.[7] On the other hand, only saponins were identified in IHP (Figure 4.3 A-H).

The phytochemical profile of IHP obtained in this study is contradictory to what literature reports. Several studies have shown that the outer coat of *Plantago ovata* seeds contains sugars, sterols, fixed oils, tannins and protein in addition to the mucilaginous polysaccharide arabinoxylan.[8] Hence, the results obtained can be classified as "false negative''. The difficulty to qualitatively identify the phytochemicals enumerated above could have been due to the formation of a thick hydrogel when *Plantago ovata* husk powder came in contact with water. This gel could have served as an impermeable barrier preventing reagents to come into contact with phytochemicals embedded in its matrix.

Starch

Starch is the main component of UBP which accounts to about 60-80 % w/w of the dry pulp. The starch content of UBP drastically declines with ripening to less than 1% with sugars accumulating to more than 10% of the fresh fruit pulp.[9] Chemically, starch is a polysaccharide which is composed of glucose molecules joined together with α-d-(1-4) and/or α-d-(1-6) linkages. Amylose and amylopectin are the two main structural components of starch. Amylose is a linear slightly branched polymer which constitutes 15-20 % of starch, while amylopectin is an extensively branched molecule and is the major component of starch.[10]

The blue-black colouration observed when UBP was treated with iodine is as a result of the formation of an intermolecular charge-transfer complex between starch and the triiode anion. This colorimetric method of identifying starch is the basis of iodometry.[11]

Saponins

A permanent froth was observed when samples of UBP and IHP were agitated with water, this was an indication of the presence of saponins in the analytes. Saponins are a class of plant secondary metabolites which form a permanent froth in aqueous media. They include compounds that are glycosylated steroids, triterpenoids and steroid alkaloids. Extensive research on saponins have shown them to possess antifungal, antiviral, antibacterial, anticarcinogenic, hypocholesterolemic and hypoglycaemic properties.[12]

Phenols, Tannins

A purplish colouration was observed when an aqueous extract of UBP was treated with ferric chloride. This colour change was an indication of the presence of phenols in the sample as coloured complex was formed between Fe^{3+} and the phenolic compounds present such as tannins. Phenolic compounds are the largest group of phytochemicals and they possess a wide

array of pharmacological activities some of which include antioxidant, anti-inflammatory and anti-tumorigenic.[13]

Proteins

A purplish colouration was observed when an alkali suspension of UBP was treated with $CuSo_4$. This colour change is attributable to the sequential reduction of Cu^{3+} to Cu^{1+}, when the latter binds with nitrogen ions in peptides.[14] Proteins constitute about 3-5% w/w of unripe banana pulp. The therapeutic and nutritional benefits of proteins and their building units amino acids are well known and have been extensively researched and documented.[15]

Lipids

The formation of a cloudy precipitate when water was added to an ethanolic suspension of UBP indicated the presence of lipids in the sample. Fats account for less than 2% w/w of UBP.[15] Lipids play several roles in the normal functioning of the body some of which include hormone production, cell membrane production and energy storage.[16]

Flavonoids

Flavonoids are ubiquitous polyphenolic compounds that occur as glucoside, aglycones and methylated derivatives. The reddish colouration observed when magnesium and hydrochloric acid were added to the ethanolic extract of UBP indicated the presence of flavonoids in the sample. Flavonoids are powerful antioxidants and have been reported to possess a myriad of pharmacological properties including antitumor, anti-inflammatory as well as antimicrobial properties.[17]

Monosaccharides

UBP tested positive for monosaccharides. A green colouration was observed upon adding few drops of Benedict's reagent to a suspension of the analyte which indicated the presence of reducing sugars such as glucose. Benedict's solution is an aqueous alkaline solution of copper sulphate and sodium citrate. When reducing sugars are heated in the presence of an alkali, they are converted to enediols. Enediols are powerful reducing agents which convert Cu^{2+} to Cu^{+1} , followed by a red precipitate of Cu_2O .[18] The intensity of the precipitate is dependent on the concentration of reducing sugars in the sample. A brick red precipitate indicates a high concentration of reducing sugars while a green colouration indicates traces of reducing sugars. Glucose, fructose and sucrose are the most prevalent sugars found in unripe banana powder with glucose being the major contributor. On average, UBP contains between 1-2% w/w of sugars depending on the ripening stage of the fruit and the species; this percentage usually increases to 15-20% in ripe bananas.[19]

Steroids

UBP tested negative for steroids such as cholesterol as no colour change was observed when the reagents were added to the analytes. This result is in concordance with literature as several studies have found banana to be a cholesterol free fruit.[7]

Table 4.3: Phytochemical profile of the natural polymers

No	Phytochemical	Banana powder	Ispaghula husk powder
1	Starch	+++	-
2	Saponins	+	+
3	Phenols	+	-
4	Proteins	+	-
5	Tannins	+	-
6	Lipids	+	-
7	Flavonoids	+	-
8	Steroids	-	-
9	Monosaccharides	+	-

Figure 4.3 below presents the different colour changes visualised during the phytochemical analysis of unripe banana powder and ispaghula husk powder.

A) Starch: iodine solution(left), blue black colouration observed in unripe banana powder (middle), no colour changed observed in ispaghula husk powder (right)

B) Saponins: permanent froth observed in both unripe banana powder (left) and ispaghula husk powder (right)

C) Phenols/Tannins: ferric chloride (right), purplish specks observed in unripe banana powder(middle), no colour change observed in ispaghula husk powder (right).

D) Proteins: copper sulphate solution (left), purplish colouration observed in unripe banana powder (middle), no colour change observed in ispaghula husk powder (right).

E) Lipids: cloudy suspension observed in unripe banana powder (left), ispaghula husk powder (right)

F) Flavonoids: reddish colouration observed unripe banana powder (left), no colour change observed in ispaghula husk powder (right)

G) Steroids: no colour change observed for both powders.

H) Monosaccharides: Benedict's solution (right), copper colouration in ispaghula husk powder (middle), greenish colouration observed in unripe banana powder (left).

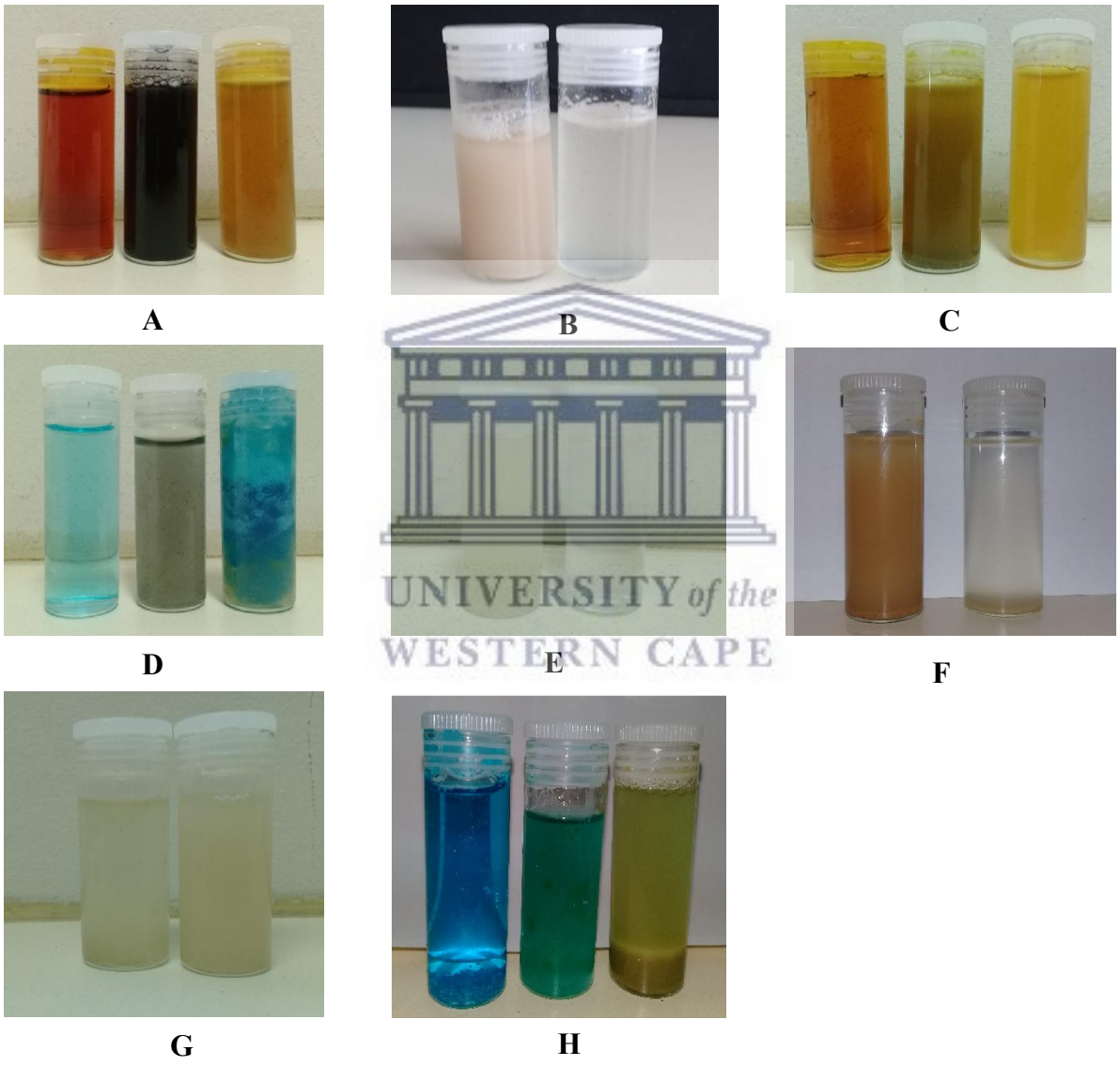


Figure 4.3 A-H: Phytochemical tests

4.1.4 Microbiological limit test

Table 4.4 below presents the average viable count of microorganisms expressed as colony forming units (CFU) in tested samples of UBP and IHP.

Table 4.4: Average CFU/g of microorganisms in incubated samples of the natural polymers

Microorganism	Unripe banana powder	Ispaghula husk powder
Bacteria	4×10^4 CFU g^{-1}	13×10^4 CFU g^{-1}
Fungi	No visible growth	No visible growth

The results presented above were calculated as follows

Calculations

Concentration of stock solution: 0.1g/ml

Volume of inoculum: 1 ml

Dilution factor of inoculum: 10^{-3}

Average colony forming units (CFU) per plate: Banana (4 CFU), Ispaghula (13 CFU)

CFU g^{-1}: (Average plate count) x 10^3 / 0.1g

Banana: 4 CFU x 10^3 /0.1g = 40000 or 4 x 10^4 CFU g^{-1}

Ispaghula: 13 CFU x 10^3/0.1 g = 130000 or 13 x 10^4 CFU g^{-1}

Fungal growth was not observed in any of the tested samples; this suggests that the natural polymers do not offer conducive conditions for the growth of yeasts and moulds. Microbial

growth was observed in the assessed sample of UBP although a preservative was added during the preparation thereof. IHP displayed a relatively higher bioburden compared to UBP.

The USP total aerobic microbial count (TAMC) acceptance criteria for non-sterile non-aqueous pharmaceutical products intended for oral administration is 10^3 CFU/g. The natural polymers did not meet this pharmacopoeial requirement. However, there is no set pharmacopoeial limit for the bioburden of raw herbal pharmaceutical products.[20]

Considering the fact that raw herbal materials are intrinsically contaminated, deviations from the maximum limit of 10^3 CFU/g are often allowed provided that the microorganisms present are potentially non-pathogenic. The presence of microbes in a pharmaceutical excipient can be quite problematic due to health implications and the adverse impact they have on the shelf-life and effectiveness of the product. Decontamination and sterilisation techniques can be employed to reduce the bioburden of naturally sourced pharmaceutical excipients on the basis of a favourable risk/benefit assessment. Some of these techniques include: dry heat, moist heat, irradiation, acid/base treatment and preservative addition.[21]

4.1.5 Swelling capacity

Table 4.5 presents the swelling index of the natural polymers used as superdisintegrants in this study. The results obtained showed that UBP has the ability to swell to more than twice its initial volume while IHP had a remarkable 2100 percent volume increase (21 times more). IHP formed an extensively gelatinised structure in water while UBP formed a suspension with a sediment of coarse particles whilst the fine particles were suspended within the supernatant (Figure 4.4).

The most common mechanism of tablet disintegration is by disintegrant swelling. The dimensional enlargement of disintegrant particles results in an omni-directional force exerted on surrounding particles. This force disrupts the cohesive forces that bind particles together resulting in the breakdown of the tablet matrix.[22] Although a high swelling index is a sought property for tablet disintegration, not all polymers with high swelling capacities are good tablet disintegrants because other factors like tablet porosity and gel formation affect the process of disintegration. A polymer that becomes gelatinised after swelling will most likely form a gel plug which impedes disintegration. The formation of a gel plug is proportional to the amount of disintegrant in the tablet, thus such disintegrants are only effective at low concentrations.[23] Although IHP displayed an high swelling capacity, a sought property for instantaneous tablet disintegration, the polymer may not be used at high concentrations due to the probable formation of a gel plug. Conversely, minimal or no gel formation in UBP suggests that the polymer can be utilised as a disintegrant over a wider range of concentrations and thus can serve as a multipurpose excipient at higher concentrations.

Table 4.5: Swelling index of the natural polymers

No	Polymer	Initial volume	Final volume	Swelling index
1	Unripe banana powder	0.53 ml	1.32 ml	2.50
2	Ispaghula husk powder	0.62 ml	13.27 ml	21.4

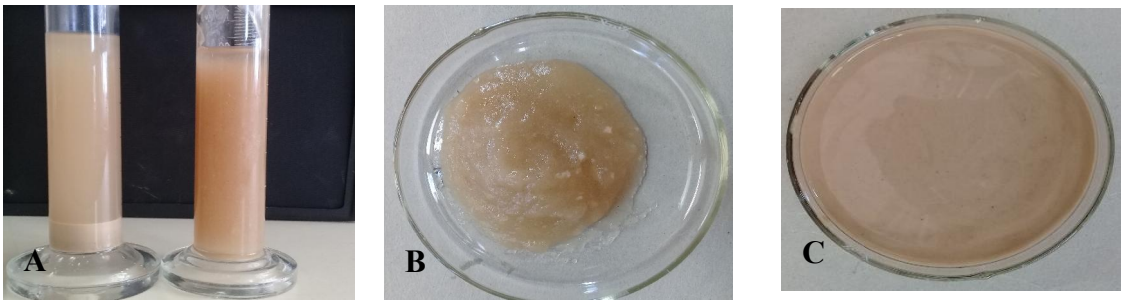


Figure 4.4: Swollen natural polymers. A) Unripe banana powder (left), ispaghula husk powder (right). B) Gelatinised ispaghula husk powder. C) Unripe banana powder paste. Photographs taken at room temperature (25 ºC) during the swelling index assessment of the polymers.

4.1.6 pH

The pH of aqueous dispersions of the natural polymers are presented in table 4.6 below. Both polymers had pH values below 7, which fall within the acidic region of the pH scale. UBP was found to be slightly more acidic than IHP.

The relevance of pH testing in this study was to predict the interaction of the natural polymers with salivary secretions. Saliva is a hypotonic fluid which is composed of 99% water and a myriad of functional constituents such as electrolytes, proteins, mucins, immunoglobulins, and nitrogenous compounds. Saliva is slightly acidic with pH oscillating between 6 and 7.[24] The pH values of the natural polymers suggest that they are susceptible to form weak acidic solutions in an aqueous medium. Generally, acidic substances are known to elicit a sour taste in the mouth thereby causing unpalatability. A correlation exists between sourness and acidity i.e. sourness increases with decreasing pH. Also, a decrease in the pH of saliva (≤ 5.5) as result of the intake of acidic substances creates a conducive environment for enamel demineralisation.[25] However, considering the pH modulating effect of saliva and its buffering capacity attributable to the presence of urea, phosphates and bicarbonates, it is unlikely that the presence of the natural polymers in the oral cavity will elicit a sour taste.[26] Also, the amount

of polymer used in the innovative fast dispersible tablet may not be significant enough to decrease the pH of the oral cavity and cause an undesirable mouth feel.

Table 4.6: pH of the natural polymers

No	Polymer	pH value
1	Unripe banana powder	5.2
2	Ispaghula husk powder	5.9

4.1.7 Loss on drying

Table 4.7 presents the moisture content of the natural polymers obtained by desiccation. The relevance of this analysis was to evaluate the stability of the natural polymers based on their respective water content, as there is a correlation that exists between the stability of botanically sourced powders and moisture content. Also, the presence of moisture in a powder can impact the flowability and compressibility thereof. The propensity of a powder to flow decreases with increasing moisture due to an increase in particle cohesiveness as a result of the formation of liquid bridges between individual particles.[27] Adequate moisture in a powder can be beneficial in reducing powder adhesion to dies and punches owing to the lubricating properties of water.[28] Interparticulate binding force increases as moisture content increases until a saturation point is reached. However, the effect of moisture on the micromeritic properties of a powder is highly dependent on the intrinsic nature thereof.[29]

IHP met pharmacopoeial specifications for moisture content as the USP monograph stipulates that the water content of an analysed sample of ispaghula husk should not exceed 12% w/w.[20] UBP was found to have a higher moisture content than IHP. Unfortunately, no monograph or official specifications for the quality assessment of UBP are available.

Desiccation by oven drying is the most common and most feasible method for water analysis. This method is based on the loss in mass after drying a sample. This moisture analysis technique is however criticisable because other volatile compounds beside water can contribute to the loss in mass of the sample. Also, the water content value does not hint at the nature of moisture in the sample i.e. whether it is free, bound, inherent or surface moisture.[30] The presence of moisture in a naturally sourced powder enhances degradation through microbial growth and water dependent biochemical reactions. Consequently, extending the shelf life of a powder entails reducing the availability of water for degradation reactions.[31] A high water content in a powder is however not absolutely proportional to microbiological activity since microorganisms might have restricted access to bound moisture.[30] Besides moisture content, water activity i.e. the ratio of the vapour pressure of water in a powder to the water vapour of pure water at the same temperature also influences microbial growth. The lower limit water activity for all microorganisms is $0.60a_w$. At low moisture levels fungal growth is inhibited, but moisture contents around 14% or above offer conducive conditions for fungal growth.[31]

In a study to investigate the effect of moisture and packaging on the shelf life of wheat flour, the flour sample having 9% moisture content was shown to be adequate with regards to the shelf life of wheat flour. Also, storage conditions and the nature of the packaging material were found to influence the moisture content of the samples and their microbiological load.[32] It can thus be inferred that the moisture content of the natural polymers powders used in this study is not potentially detrimental to their integrity and stability.

Table 4.7: Percentage loss on drying of the natural polymers

No	Polymer	Percentage loss on drying
1	Unripe banana powder	8.6%
2	Ispaghula husk powder	7.3%

4.1.8 Thermal analysis

Hot stage microscopy(HSM)

Unripe banana powder

Figure 4.5-A shows hot stage microscopy images of UBP. No physical change was observed between room temperature and 99 ºC. At 100 ºC, temperature at which the boiling point of water occurs, solvent extrusion and bubbling was observed. This indicated the presence of moisture in the sample. The physical aspect of the powder remained unchanged between 100 ºC and 190 ºC. At 200 ºC, a colour change from pale yellow to light brown was observed. The discolouration steadily intensified from light brown to dark brown due to increased thermal degradation of the powder. At 300 ºC, UBP was in an advanced degradation stage suggesting that most of its constituents had undergone decomposition.

Banana starch

In view of the fact that UBP has a myriad of components, it was fitting to analyse its major constituent in order to get a detailed insight of the thermal behaviour of the polymer. Figure 4.5-B shows hot stage microscopy imageries of starch extracted from UBP. Bubbling was observed at 100 ºC indicating the presence of moisture in the sample. As observed with UBP, discolouration commenced around 200 ºC and intensified with increased thermal degradation of the sample. No noticeable thermal events other than those observed in UBP were visualised

in this analysis suggesting that the thermal behaviour of UBP is almost exclusively determined by starch.

Ispaghula husk powder

Hot stage microscopy images of IHP are shown in figure 4.5-C below. No noticeable change was observed between room temperature and 230 ºC. Bubbling due to evolving moisture was not evident as with UBP. Discolouration commenced around 200 ºC and intensified with increased thermal degradation of the powder. At 300 ºC, ispaghula husk appeared to be highly calcined. This suggests that virtually all the constituents of IHP are decomposed around this temperature.

Arabinoxylan

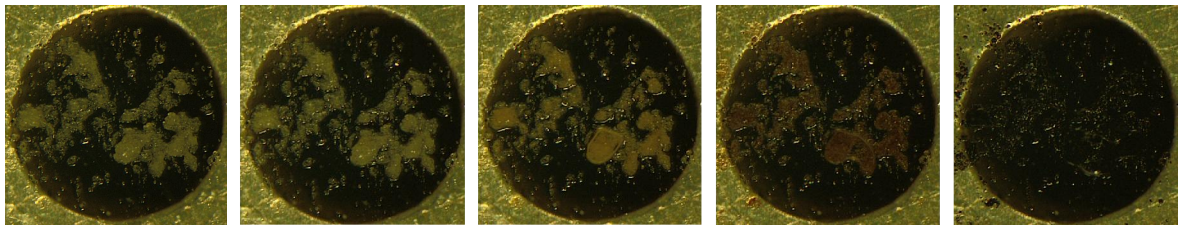
The thermal behaviour of IHP was further investigated by analysing its major constituent arabinoxylan. No noticeable change was visualised between room temperature and 90 ºC. Solvent extrusion and bubbling were observed at 100 ºC as shown in figure 4.5-D indicating the presence of moisture in the analyte. Partial melting of the powder was observed at 150 ºC. Above 150 ºC, the amount of liquid extruding from the powder increased. At 250 ºC, the colour of the unmelted portion of the sample changed from white to dark brown suggesting the onset of degradative reactions.

Arabinoxylan is a copolymer which is composed of two monosaccharide sugars of the aldopentose kind namely arabinose and xylose.[5] Arabinose and xylose melt around 164 ºC and 150 ºC respectively.[33, 34] The partial melting of the powder around 150 ºC suggests that the monosaccharide xylose had melted. The increase in the amount of liquid extruding from the sample around 180 ºC shows that both sugars had transitioned from solid to liquid. The sugar residues where most probably cleaved from the hemicellulose chain during alkali extraction and/or during coagulation with acetic acid. Unmelted crystalline solids lingering in

the analyte above 180 ºC hint at the presence of impurities in the sample. These impurities are most likely sodium acetate crystals formed during the extraction of arabinoxylan from IHP. The hemicellulose arabinoxylan was extracted from IHP using sodium hydroxide and coagulated with acetic acid. The formation of sodium acetate as the yield of the reaction between sodium hydroxide and acetic acid is depicted in the equation below.

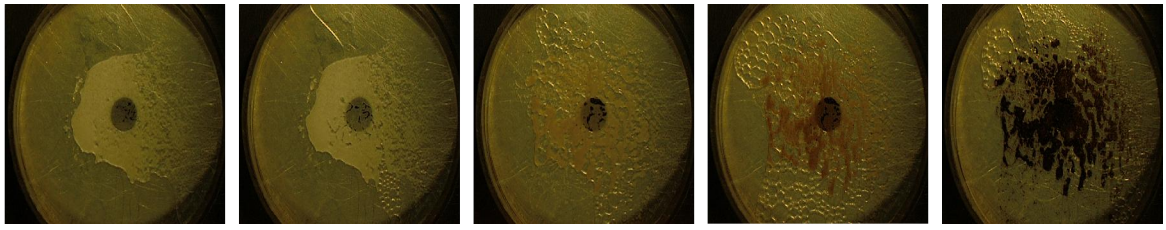
$$CH_3COOH_{(aq)} + NaOH_{(aq)} \longrightarrow H_2O_{(l)} + NaCH_3COO_{(aq)}$$

Sodium acetate is a colourless deliquescent salt which melts around 324 ºC.[35] The presence of sodium acetate in arabinoxylan shows that the extraction technique used does not yield a product of high purity. The dark brown discolouration visualised at 250 ºC can be attributed to the caramelisation of arabinose and xylose and decomposing hemicellulose.



21ºC 100 ºC 200 ºC 250 ºC 300 ºC

Figure 4.5-A: HSM images of unripe banana powder

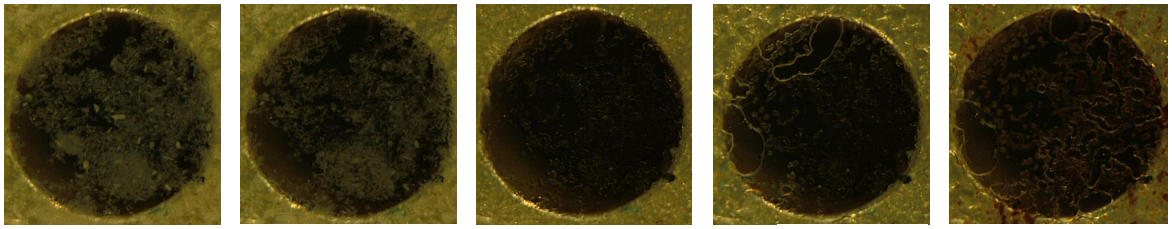


21 ºC 100 ºC 200 ºC 250 ºC 300 ºC

Figure 4.5-B: HSM images of banana starch

21 ºC 100 ºC 200 ºC 250 ºC 300 ºC

Figure 4.5-C: HSM images of ispaghula husk powder



21 ºC 100 ºC 150 ºC 180 ºC 250 ºC

Figure 4.5-D: HSM images of arabinoxylan

Differential scanning calorimetry(DSC)

Unripe banana powder

The DSC curve of UBP (figure 4.6-A) reveals a shallow endothermic peak at 340 ºC. This can be attributed to the decomposition of UBP as observed using HSM.

Banana starch

The DSC thermogram of starch extracted from UBP (figure 4.6-B) reveals a shallow endothermic peak at 305 ºC. This temperature falls within the range of temperatures at which the decomposition of starch occurs.[36] The observations made are in concordance with the HSM imageries of banana starch discussed earlier.

Ispaghula husk powder

The DSC curve of IHP (figure 4.6-C) reveals a sharp exothermic peak at 310 ºC. This temperature falls within the range of temperatures at which the hemicellulose arabinoxylan decomposes.[37] The observations made are in concordance with the HSM imageries of IHP.

Arabinoxylan

The DSC thermogram of arabinoxylan extracted from IHP (figure 4.6-D) reveals a shallow endothermic peak around 160 ºC. This temperature is within the melting range of the constituent sugars of arabinoxylan which are arabinose and xylose.[33, 34] This enthalpy change confirmed the presence of the aforementioned sugars in the analysed sample. Another shallow endothermic peak can be observed around 320 ºC. This might be as the result of the melting of residual sodium acetate crystals found in the sample and/or the decomposition of arabinoxylan. The observations made are in concordance with the HSM imageries of arabinoxylan discussed earlier.

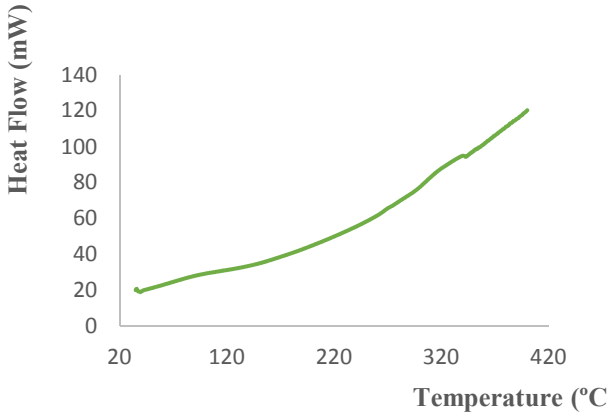


Figure 4.6-A: DSC curve of unripe banana powder

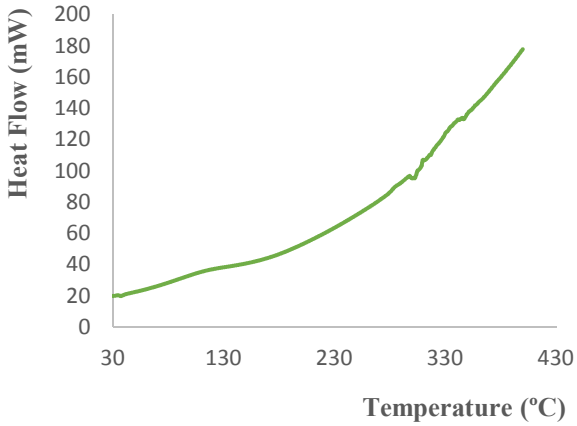


Figure 4.6-B: DSC curve of banana starch

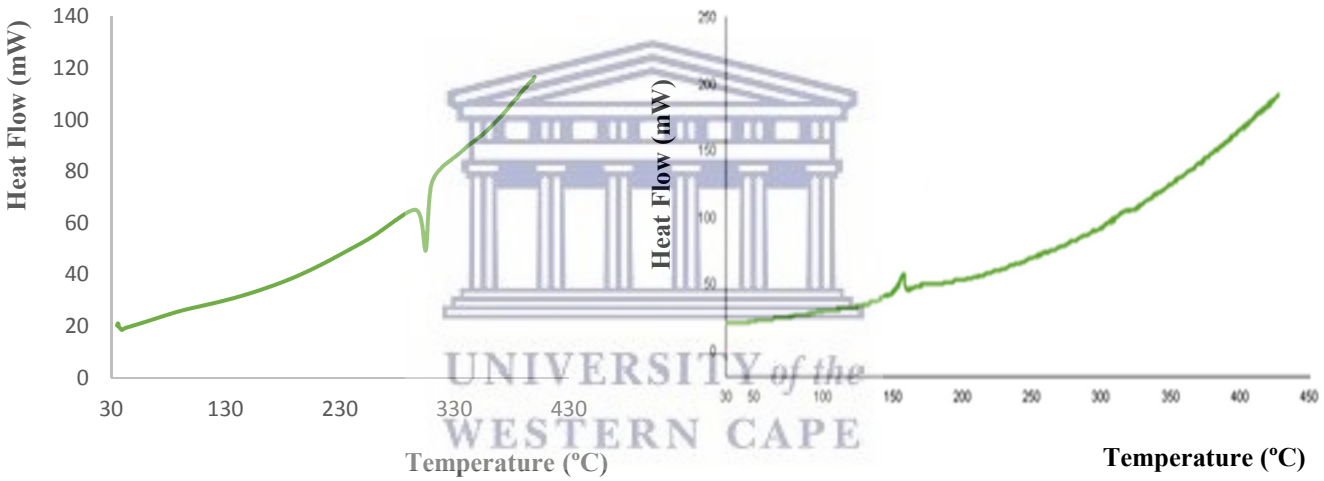


Figure 4.6-C: DSC curve of ispaghula husk powder

Figure 4.6-D: DSC curve of arabinoxylan

Thermogravimetric analysis

Unripe banana powder

The TGA thermogram of UBP (figure 4.7-A) reveals a 9.80% (n=3) loss in mass between 20 and 150 ºC. This mass loss can be attributed to the evolution of moisture and highly volatile constituents as observed with the halogen moisture analyser. A 50% loss in mass can be observed between 300 and 350 ºC. This degradation step is attributable to the decomposition of starch within the powder. Above 350 ºC, the steady mass loss is as a result of the further pyrolysis of banana powder into carbonaceous and inorganic residues. These observations are in concordance with the HSM and DSC analysis results of UBP.

Banana starch

The TGA curve of banana starch (figure 4.7-B) is almost identical to that of UBP. This suggests that the thermal properties of UBP are determined by its major constituent starch. A weight loss of 9.8% (n=3) can be observed at 150 ºC due to dehydration of the sample. A degradation point around 350 ºC can be attributed to the pyrolytic decomposition of starch. These observations reaffirm the results of the HSM and DSC analysis of banana starch discussed earlier.

Ispaghula husk powder

The TGA thermogram of IHP (figure 4.7-C) reveals a 9.7%(n=3) loss in mass at 150 ºC attributed to the loss of moisture and highly volatile components. No degradation point can be observed around the melting range of the constituent sugars of IHP i.e. 150-160 ºC. This suggests that the sugars are highly confined within the fibrous network of ispaghula husk. The degradation point observed around 320 ºC can be attributed to the decomposition of the hemicellulose arabinoxylan and cellulose. Further pyrolysis of the sample into carbonaceous

and inorganic residues is responsible for the steady mass loss observed above 320 ºC. These observations are in concordance with the HSM and DSC analysis results.

Arabinoxylan

The TGA curve of arabinoxylan (figure 4.7-D) reveals a mass loss of 3%(n=3) at 110 ºC due to dehydration of the sample. The subsequent thermal event observed at 160 ºC can be attributed to the melting of the constituent sugars of arabinoxylan i.e. arabinose and xylose. The degradation point around 325 ºC coincides with the melting point of sodium acetate, thus indicating the presence of the salt in the sample. Also, the decomposition of arabinoxylan which occurs around this temperature can be attributed to this thermal event. These observations reaffirm the results of the HSM and DSC analysis of arabinoxylan.

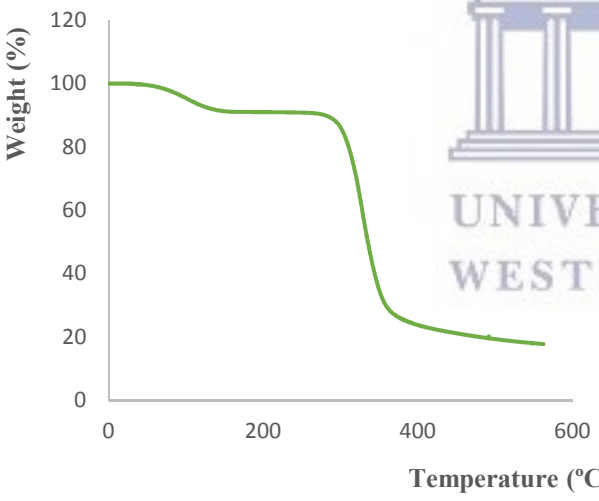


Figure 4.7-A: TGA curve of unripe banana powder

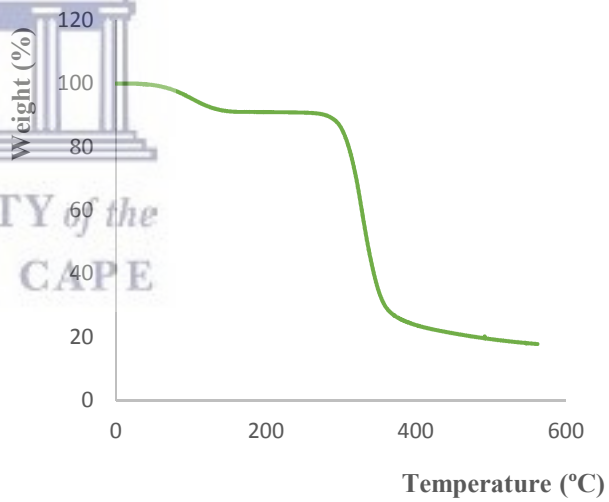


Figure 4.7-B: TGA curve of banana starch

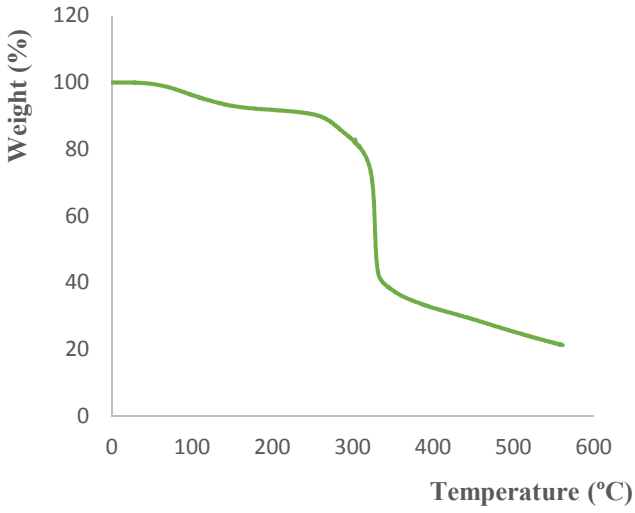


Figure 4.7-C: TGA curve of ispaghula husk powder

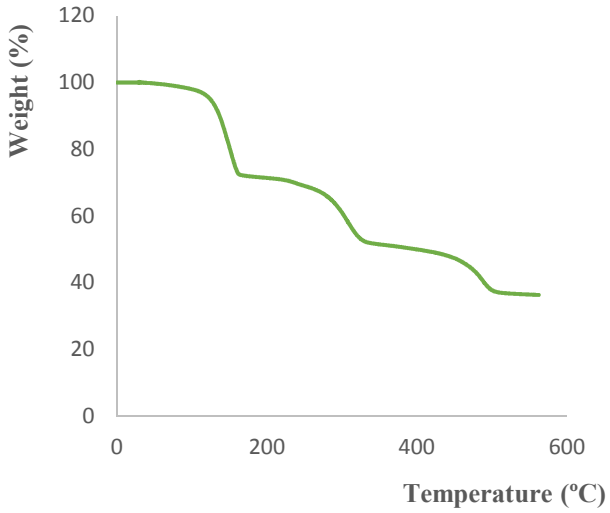


Figure 4.7-D: TGA curve of arabinoxylan

4.1.9 Spectroscopy

Fourier transform infrared spectroscopy(FTIR)

FTIR spectra of the natural polymers and their respective extracts are shown in figure 4.8. Table 4.8 provides a list of functional groups found in cellulose and hemicellulose and their corresponding wavelengths. Figure 4.9-A and B depicts the chemical structure of the natural polymers with annotated key functional groups.

Unripe banana powder

The FTIR spectrum of UBP (figure 4.8) reveals a broad peak at 3278.37 cm^{-1} attributed to the vibration of the hydroxyl groups (O-H). The peak observed at 2925.49 cm^{-1} is as a result of the C-H stretching of the constituents of starch, which are amylose and amylopectin. The peak at 1630.66 cm^{-1} reveals the presence of water molecules tightly bound within the powder. The band at 1076.17 cm^{-1} is attributable to a complex mode involving the CH_2OH side chain of amylose. Stretching modes in amorphous starch present in banana powder are responsible for the peak at 1000 cm^{-1}.

Banana starch

The FTIR spectrum of banana starch (figure 4.8) is almost identical to that of UBP with no major shift in the peaks. This suggests that starch is the main constituent of UBP. The peaks observed on the UBP spectrum at 3278.37 cm^{-1}, 2925.49 cm^{-1}, 1630.66 cm^{-1}, 1076.17 cm^{-1}, and 1000 cm^{-1}, can be observed on the banana starch spectrum at 3293.50 cm^{-1}, 2930.34 cm^{-1}, 1638.23 cm^{-1}, 1076.69 cm^{-1}, and 1000 cm^{-1} respectively. Signals present at 927.89 cm^{-1}, 859.88 cm^{-1}, and 762.33 cm^{-1} are due to β-glycosidic linkages between glucose residues in amylose and amylopectin chains.

Ispaghula husk powder

The FTIR spectrum of IHP (figure 4.8) reveals the very complex nature of the analyte since only few characteristic peaks are present on the spectrum. The band at 3298.91 cm^{-1} is attributed to the stretching of hydroxyl groups (-OH). Vibration of the C-H group is represented by a peak at 2924.41 cm^{-1}. The signal observed at 1635.02 cm^{-1} is as a result of absorbed water since ispaghula husk has a high water affinity. The peak at 1410.97 cm^{-1} can be attributed to the C-H, O-H, or C-O bending vibration of the constituents of ispaghula husk.

Arabinoxylan

The FTIR spectrum of arabinoxylan (figure 4.8) reveals more characteristic peaks pertaining to hemicelluloses as compared to the spectrum of IHP. The bands present at 3412.30 cm^{-1}, 3278.07 cm^{-1}, 3164.57 cm^{-1} can be assigned to the stretching of the hydroxyl groups (-OH). The signal present at 1636.97 cm^{-1} is attributable to the bending mode of bound water molecules. Skeletal vibration of C-C and C-O functional groups is responsible for the peaks present at 1400 cm^{-1} and 1339.01 cm^{-1}. The peak present at 1019.57 cm^{-1} is due to the stretching and bending vibration of the C-C, C-O, and C-O-C functional groups found in hemicellulose.

Dominant β-glycosidic linkages between monosaccharides units in hemicellulose i.e. xylose and arabinose, are responsible for the peaks present at 928.10 cm-1 and 796.41 cm^{-1}.

Table 4.8: Characteristic of cellulose and hemicellulose in the FTIR fingerprint region[38]

Wavelength (cm^{-1})	Cellulose assignment	Hemicellulose assignment
Anomeric region (950-700 cm-1) A small sharp band	Dominant β-glycosidic linkages between glucose residues in cellulose	Dominant β-glycosidic linkages between the monosaccharide units in hemicellulose
1200-1000	C-C, C-O-C and C-O stretching and bending vibration	C-C, C-O-C and C-O stretching and bending vibration
1400-1300	CH_2 wagging (crystallised cellulose I), C-OH in plane bending (amorphous cellulose), CH bending	C-C and C-O skeletal vibration
1378	C-H ester band due to partial acetylation of hydroxyl groups	C-H ester band due to partial acetylation of hydroxyl groups
1425	CH_2 bending (crystallised I and amorphous cellulose)	C-H and C-O vibration in hemicellulose
1640	Bending mode of absorbed water	Bending mode of absorbed water
1735		C=O stretching of unconjugated ketone aldehydes and carboxyl groups
2900 – 2800		C-H stretching and CH bond deformation of CH_2-CH_3 groups
3000-2800	Stretching of asymmetric and symmetric methyl and methylene cellulose groups	
3600-3400	Stretching of O-H group	

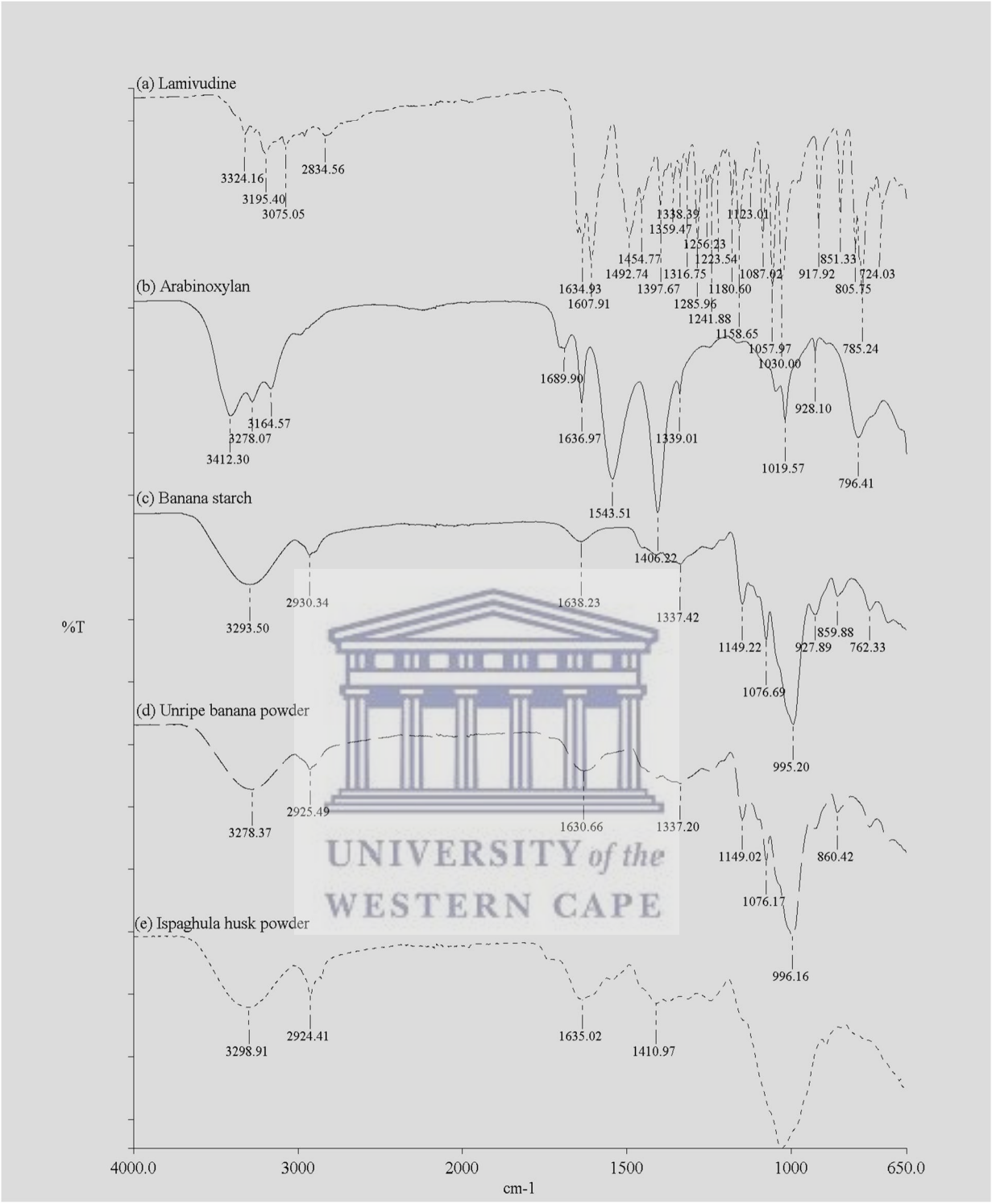
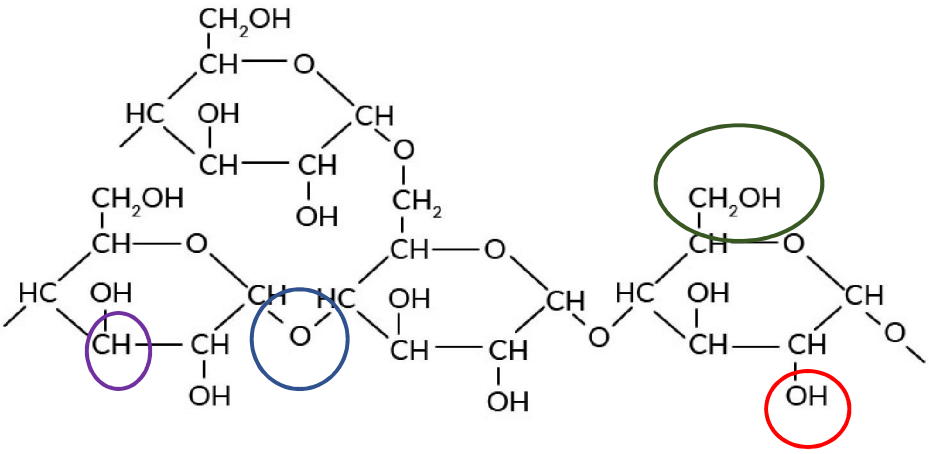
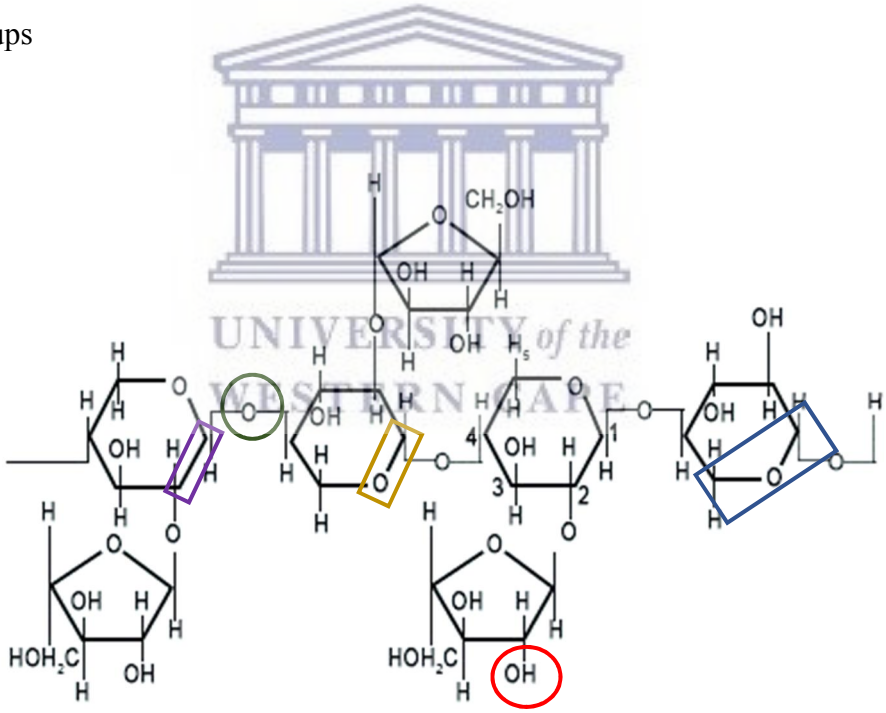


Figure 4.8: FTIR spectra of the parent powders



Purple: C-H group, Blue: β-glycosidic linkage, Green: CH_2OH side chain, Red: hydroxyl group

Figure 4.9-A: chemical structure of the starch polymer amylopectin with annotated key functional groups



Purple: C-C group, Green: β-glycosidic bond, Gold: C-O group, Red: hydroxyl group, Blue: C-O-C group

Figure 4.9-B: chemical structure of arabinoxylan with annotated key functional groups

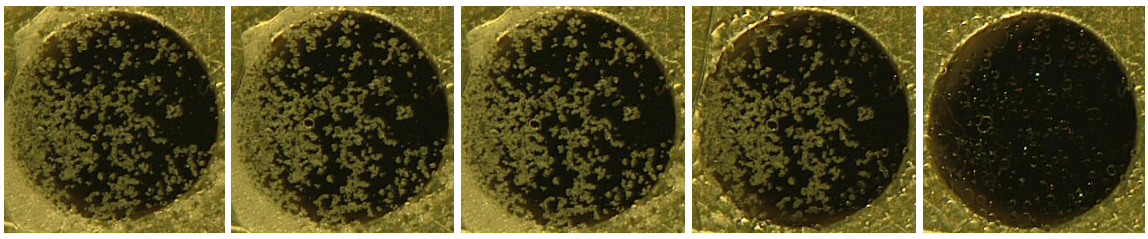
4.2 Identification and characterisation of the active ingredient

The active ingredient lamivudine was identified and characterised by means of thermal and spectroscopic tools. The melting point of lamivudine was determined using HSM and DSC, while key functional groups of lamivudine were identified using FTIR spectroscopy as discussed below.

4.2.1 Thermal analysis

Hot stage microscopy

HSM imageries of lamivudine are shown in figure 4.10. No noticeable change was observed in the physical appearance of lamivudine between 21 ºC and 170 ºC. No solvent extrusion or evolution of gases were observed; this suggests the absence of moisture in the sample. Melting of lamivudine commenced at 170 ºC, and at 180 ºC, complete phase transition of the drug from solid to liquid had occurred. It can thus be inferred that the drug powder used in this study was of high purity as no other phase transition besides the melting of lamivudine was observed. Also, the melting point of lamivudine was found to be around 177 ºC as stipulated by literature.[39]



21 ºC 100 ºC 160 ºC 170 ºC 180 ºC

Figure 4.10: HSM images of lamivudine

Differential scanning calorimetry

The DSC thermogram of lamivudine (figure 4.11) reveals a sharp endothermic peak at 177 ºC. This temperature falls within the melting range of lamivudine stipulated by literature, thus confirming the purity of the drug sample used in this study.[39] The observations made are in concordance with the results obtained in the HSM analysis.

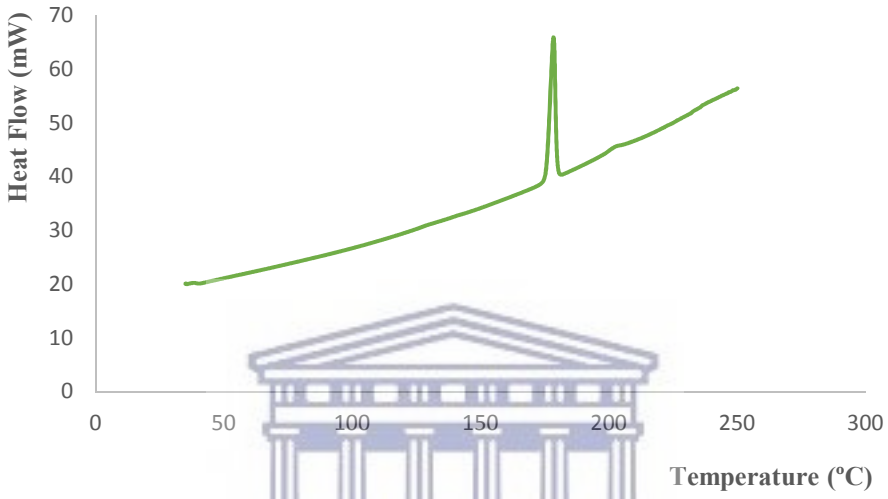


Figure 4.11: DSC thermogram of lamivudine

4.2.2 Spectroscopy

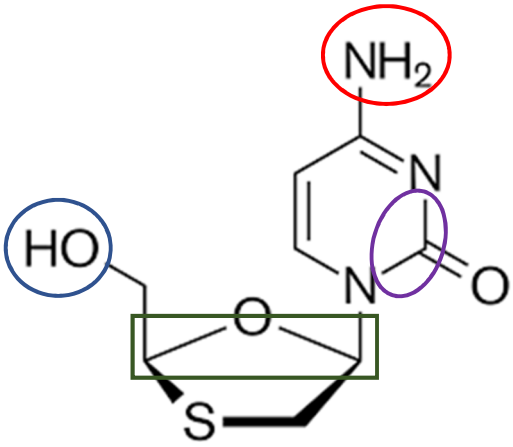
Fourier transform infrared spectroscopy

The FTIR spectrum of pure lamivudine (figure 4.8) reveals a characteristic peak at 1634.93 cm^{-1} which can be attributed to the carbonyl group present in the cystidine nucleus. The amino and hydroxyl groups present in lamivudine are responsible for the characteristic peaks observed at 3324.16 cm^{-1} and 3195.40 cm^{-1}. Peaks present at 1285.96 cm^{-1} and 1158.65 cm^{-1} are attributable to the asymmetrical and symmetrical stretching of the C-O-C functional group found in the oxathiolane ring of lamivudine.

Table 4.9 provides a list of functional groups which can be found in lamivudine and their corresponding wavelengths. Figure 4.12 depicts the chemical structure of lamivudine with annotated key functional groups

Table 4.9: Functional groups and their corresponding wavelengths[40]

Wavelength (cm^{-1})	Functional groups	Compounds
3600–3000	OH stretching	Acid, methanol
2860–2970	C–Hn stretching	Alkyl, aliphatic
1700–1730		Aromatic
1510–1560	C=O stretching	Ketone and carbonyl
1632	C=C	Benzene stretching ring
1613, 1450	C=C stretching	Aromatic skeletal mode
1470–1430	O–CH_3	Methoxyl–O–CH_3
1440–1400	OH bending	Acid
1402	CH bending	
1232	C–O–C stretching	Aryl-alkyl ether linkage
1215	C–O stretching	Phenol
1170, 1082	C–O–C stretching vibration	Pyranose ring skeletal
1108	OH association	C–OH
1060	C–O stretching and C–O deformation	C–OH (ethanol)
700–900	C–H	Aromatic hydrogen
700–400	C–C stretching	



Blue: hydroxyl group, Green: C-O-C group in oxathiolane ring, Purple: carbonyl group in cystidine nucleus, Red: amino group

Figure 4.12: chemical structure of lamivudine with annotated key functional groups

4.3 Particle size and morphology analysis

The particle size and shape of the parent powders were assessed using scanning electron microscopy (SEM). The data obtained from this assessment was used to predict key powder behaviours as they relate to tabletting properties of the powders.

4.3.1 Particle morphology analysis

Unripe banana powder

SEM micrographs of UBP (figure 4.13-A) below reveal a heterogenous particle population. Particles are oval shaped, roughly spherical with a smooth surface morphology. Cohesiveness is evident amongst the particles as specks of agglomerates can be observed.

Ispaghula husk powder

Figure 4.13-B below shows SEM micrographs of IHP. Particles have an irregular rectangular morphology with rough dented surfaces. IHP shows a relatively less varied particle population as compared to UBP. Cohesive interactions between particles are restricted and agglomeration is not evident.

Lamivudine

SEM micrographs of lamivudine (figure 4.13-C) reveal a heterogenous particle population. The photomicrographs show irregular shaped crystalline structures with defined edges and smooth surfaces. Interparticulate cohesive interactions and agglomeration are evident.

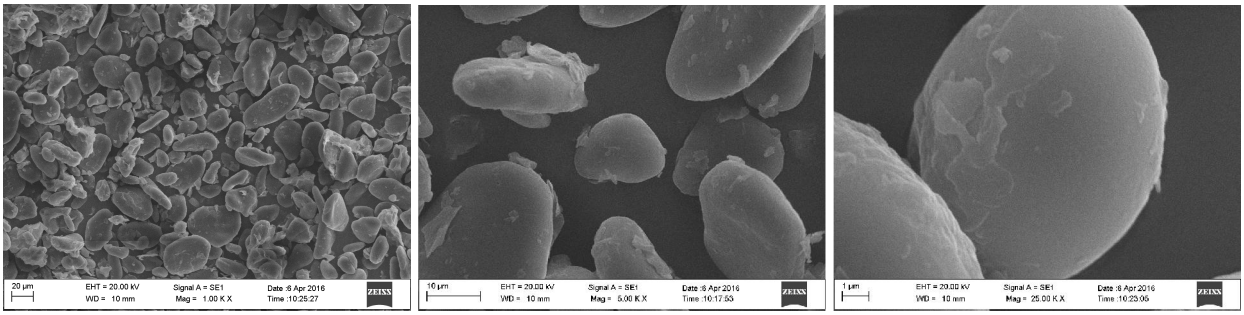


Figure 4.13-A: SEM images of unripe banana powder

Figure 4.13-B: SEM images of ispaghula powder

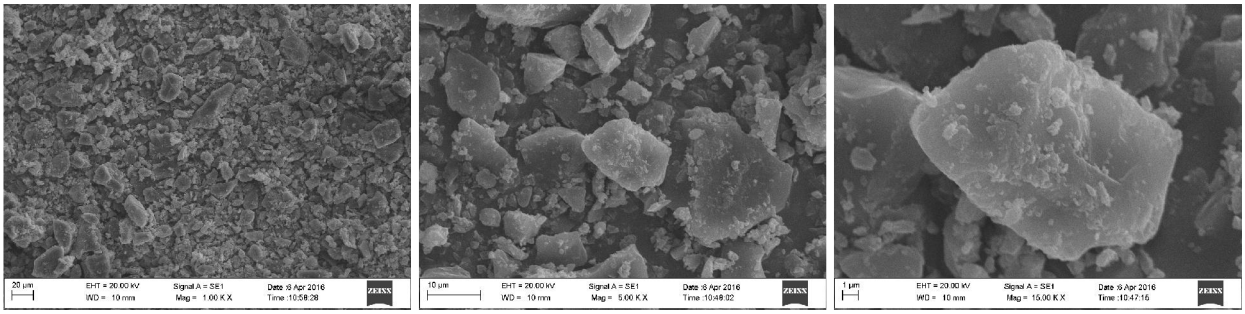


Figure 4.13-C: SEM images of lamivudine

4.3.2 Particle size analysis

Particle size was determined by measuring the diameter of individual particles i.e. the longest axis length, with the assumption that each particle is a sphere. This analysis was carried out to assess the correlation between the characteristics of individual particles and powder properties. The data obtained was computed into statistical parameters as depicted below (table 4.10 and table 4.11) and inferences were made. A cumulative frequency particle size distribution cure of the parent powders is shown in figure 4.14.

Table 4.10: Particle size ranges of the parent powders and their respective frequency percentage

	Frequency percentage (%). Sample size (n)= 200		
Range (µm)	**Lamivudine**	**Unripe banana powder**	**Ispaghula husk powder**
$0 < x \leq 5$	82.4	12.9	3
$5< x \leq 10$	11.6	20.8	5.5
$10 < x \leq 15$	4	17.5	-
$15< x \leq 20$	2	24	11
$20 < x \leq 40$	-	22	14
$40 < x \leq 60$	-	2.8	22
$60 < x \leq 80$	-	-	28
$80 < x \leq 100$	-	-	11
$100 < x \leq 120$	-	-	-
$120 < x \leq 140$	-	-	-
$140 < x < 160$	-	-	5.5

Table 4.11: Particle size distribution of the parent powders with statistical parameters

No	Parameter	Lamivudine	Banana	Ispaghula
1	Mode	4 µm	20 µm	80 µm
2	Range	0.5≤ x ≤20 µm	1≤ x ≤50 µm	5≤ x ≤150 µm
3	Mean particle size	3.9 µm	19 µm	60 µm
4	Median	2 µm	14 µm	52 µm

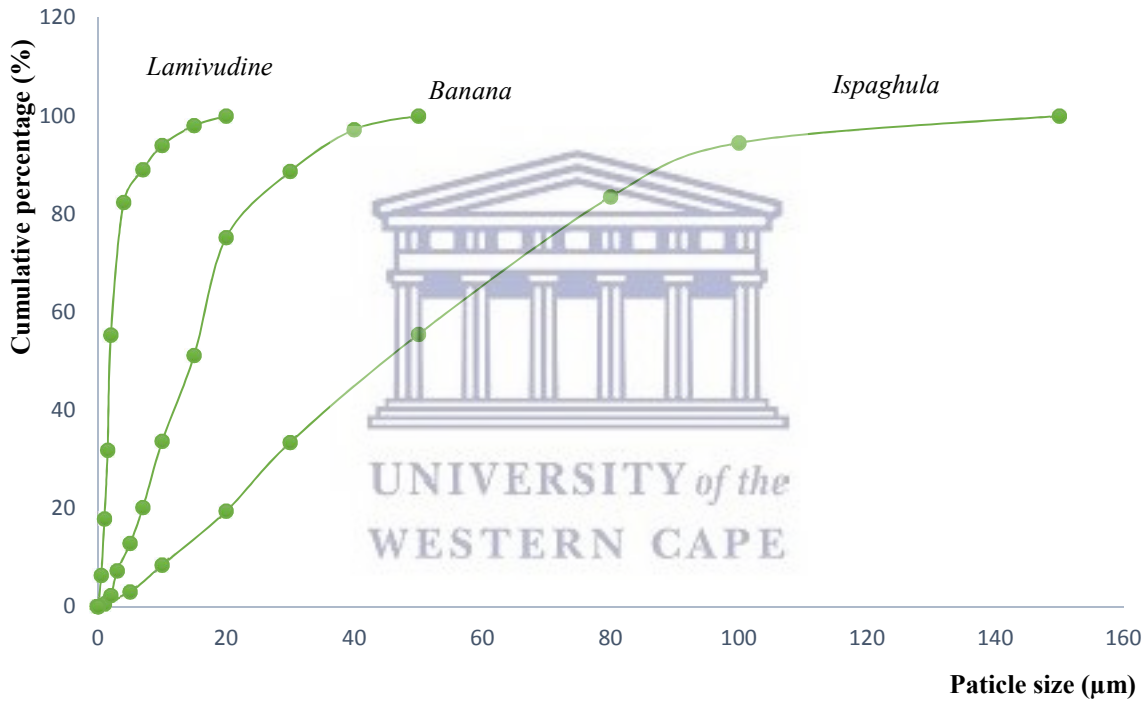


Figure 4.14: Particle size distribution of the individual parent powders vs cumulative percentage

Unripe banana powder

The particle size distribution plot of UBP (Figure 4.15) reveals a non-symmetric and positively skewed distribution i.e. the measures of central tendency which are mean, median and mode do not equate to the same value. The average particle size and the most common particle size

recorded were found to be 19 µm and 20 µm, respectively. UBP also had varied particle size distribution with particle diameter ranging between a minimum of 1 µm to a maximum of 50 µm.

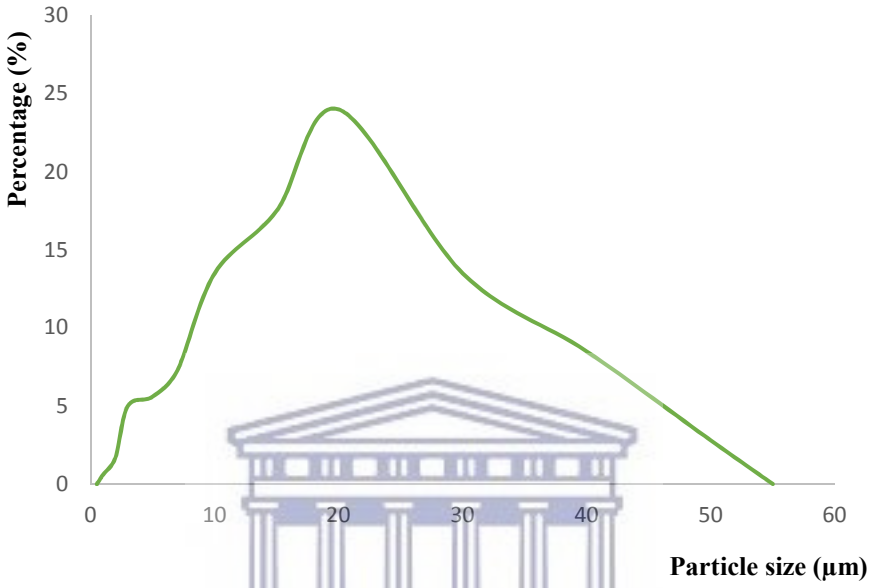


Figure 4.15: Particle size distribution of unripe banana powder

Ispaghula husk powder

The particle size distribution graph of IHP (figure 4.16) reveals a positively skewed asymmetric curve. Particle diameter ranged between 5 to 150 µm, the mean particle size and modal value were found to be 60 and 80 µm, respectively. Amongst the three powders analysed, IHP recorded the largest particle size and had a wider particle size distribution range, albeit having the least particle diameter variation.

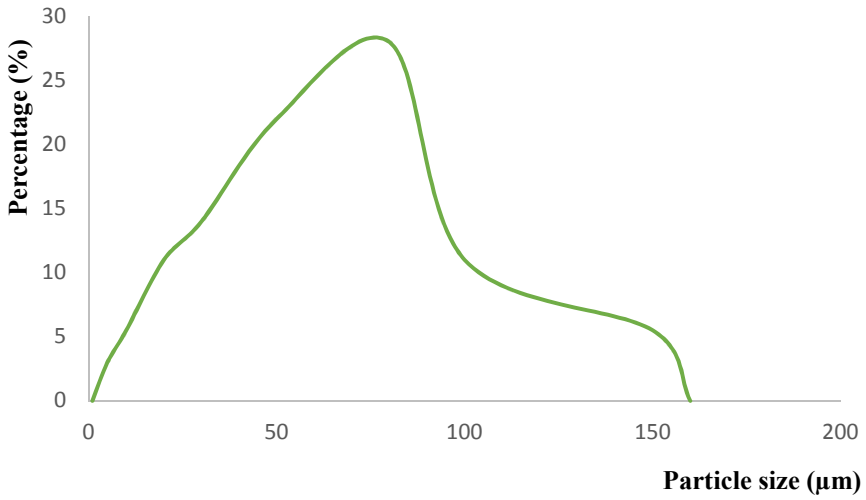


Figure 4.16: Particle size distribution of ispaghula husk powder

Lamivudine

The particle size distribution plot of the active pharmaceutical ingredient is represented in figure 4.17 below. The graph reveals an asymmetrical curve heavily skewed to the right. The average particle diameter and the most recurrent particle diameter recorded were 3.9 and 4 µm respectively. Particle size ranged from a minimum of 0.5 µm to a maximum of 20 µm. Lamivudine exhibited a relatively narrow particle size range compared to the natural polymers but had a significantly varied particle size population.

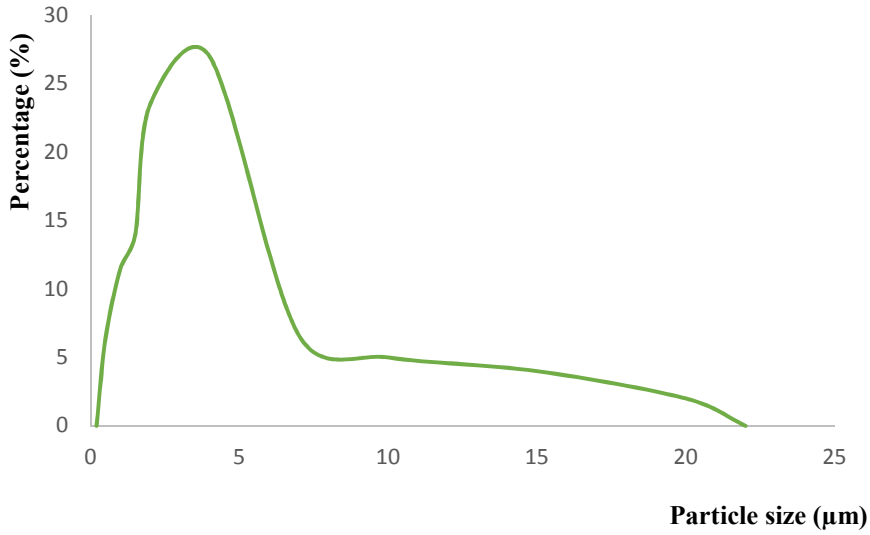


Figure 4.17: Particle size distribution of Lamivudine

4.3.3 Relevance of particle size and morphology in tablet formulation

The knowledge of particle size and morphology is crucial in tablet preformulation studies as it enables the formulator to predict key powder behaviours which include flowability, and compressibility. These powder attributes influence the integrity and the characteristics of the resultant product considerably.

Particle size greatly influences powder flowability which in turn affects tablet content uniformity. It has been well established that flow-propensity increases with increasing particle size owing to gravity. Also, fine particles are less inclined to flow due to substantial adhesion forces between the particles which restrict powder motion.[41] Powder compactness increases with decreasing particle size due to an increase in interparticulate cohesion. The smaller the particle size, the smaller the interstices between the particles.[42] This was visualised on the SEM micrographs of the powders (figures 4.13-A,B,C); IHP with a relatively large mean particle diameter exhibited poor particle cohesiveness as compared to UBP and lamivudine which had relatively smaller particles and displayed significant particulate cohesiveness.

Additionally, the overall micromeritic properties of the powder is greatly influenced by its uniformity i.e. its particle size distribution.[43]

A wide particle size distribution is beneficial in formulating a resilient tablet because the fine particles fill the voids between the larger particles resulting in a more compact network.[44] Table 4.12 below describes the characteristics of various particle size ranges and the impact on powder flow.

Table 4.12: Characteristics of different particle size ranges. Adapted from Axelsson.[45]

Size range (µm)	Component	Bulk	Characteristic
3000-30000	Grain/lump	Fragmented solid	Free flowing
100-1000	Granule	Granular solid	Flows easily with cohesive effect if contains high percentage of fines
10-100	Particle	Granular powder	May display cohesiveness
1-10	Particle	Superfine powder	Highly cohesive; difficult to handle
<1	Particle	Ultrafine powder	Extremely difficult to handle

Findings pertaining to the effect of particle morphology on powder properties are equivocal, and it is bewildering to appraise the role particle morphology plays on powder micromeritics without alluding to particle size. The influence of particle morphology on powder attributes varies greatly with particle size and distribution of the sample, as such general statements about this effect cannot be made. Coarse particles (larger than 0.5mm) which are smooth and spherical generally have a higher propensity to flow compared to rough, sharp-edged non-spherical particles. Markedly rough particles are believed to flow better than smooth particles due to restricted interactions between the former. Also, particles with rough surfaces can

enhance the compactness of a powder since the presence of dents on the particles enable particles to interlock thereby enhancing interparticulate adhesion.[46]

4.4 Micromeritic properties of the parent powders

The ability of the powders to settle and the influence of interparticulate interactions on the overall properties of the powders were investigated using the recommended USP techniques. The results obtained are presented in table 4.13 below.

Table 4.13: Micromeritic properties of the parent powders

No	Property	Banana powder	Ispaghula husk powder	Lamivudine
1	Angle of repose (º)	34.3	31.2	38.6
2	Bulk density (g/cm3)	0.53	0.62	0.47
3	Tapped density (g/cm3)	0.81	0.77	0.76
4	Compressibility index (%)	34.0	19.4	36.0
5	Hausner ratio	1.53	1.24	1.62

Table 4.14 below depicts the relationship between the micromeritic properties of a powder and its propensity to flow.

Table 4.14: The relationship between angle of repose, compressibility index, Hausner ratio and flowability. Adapted from Carr.[47]

Flow character	Compressibility index (%)	Hausner ratio	Angle of repose (°)
Excellent	≤10	1.00-1.11	25-30
Good	11-15	1.12-1.18	31-35
Fair	16-20	1.19-1.25	36-40
Passable	21-25	1.26-1.34	41-45
Poor	26-31	1.35-1.45	46-55
Very poor	32-37	1.46-1.59	56-65
Extremely poor	>38	>1.60	>60

4.4.1 Angle of repose

This property is related to interparticulate friction or motion resistance between particles. Despite having several drawbacks (e.g. varying results), this technique continues to be used in the pharmaceutical industry as a valuable tool for assessing powder flowability and predicting manufacturing setbacks.[20] As anticipated, IHP displayed the smallest angle of repose while lamivudine powder recorded the largest angle. Based on their respective settling geometries, the flowability of IHP and UBP was rated as good while that of lamivudine powder was rated as fair. These results suggest that the angle of repose is inversely proportional to particle size. The greater the particle size, the smaller the angle of repose. Nevertheless, other factors such as particle size distribution, particle morphology, particle surface morphology, interparticulate cohesiveness and the presence of solvents also influence the geometry of a settling powder.[48]

4.4.2 Compressibility index

This is one of the most accepted and straightforward techniques of assessing the propensity of a powder to flow. This technique has been proposed as an indirect measure of bulk density, particle size and morphology, moisture content, surface area and interparticulate cohesiveness of powders as these factors can influence the observed compressibility index.[20] IHP displayed the lowest compressibility index amongst the three powders. The percentage compressibility index of IHP corresponds to a fair flow character while the values obtained for UBP and lamivudine correspond to a very poor flow character. These results suggest that the interparticulate interactions which restrict powder flow are less pronounced in IHP compared to dehydrated banana powder and lamivudine. Since flowability is an indication of the compressibility of a powder, it can be deduced that IHP has a relatively high propensity to compression as compared to UBP and lamivudine.

4.4.3 Hausner ratio

Although not an absolute property of a material, Hausner's ratio provides an indication of the flowability of a powder and hence its compressibility. This value depicts the relationship between the tapped density of a powder and its bulk density. In a free-flowing powder where interparticulate interactions are relatively less pronounced, the difference between tapped density and bulk density is minimal. The reverse is true for powders with considerable interparticulate interactions, a significant difference between tapped density and bulk density can be observed.[20] The difference between the tapped and bulk densities of IHP was relatively minimal compared to UBP and lamivudine. The Hausner's ratio of IHP was rated as fair in terms of flow behaviour while the values obtained for UBP and lamivudine were rated as very poor and extremely poor, respectively.

Overall, IHP displayed satisfactory micromeritic properties suggesting that it is a suitable candidate for direct compression. The results obtained for UBP and lamivudine were not satisfactory and therefore they may not be good candidates for direct compression. However, literature reports that powders with very unsatisfactory micromeritic properties can be successfully formulated into tablets by direct compression using suitable flow aids and binders, or by employing granulation.[49] The poor micromeritic properties of UBP and lamivudine indicates the presence of strong interparticulate cohesive forces which restrict powder flow and hence decreases their propensity to be compressed. The trend observed in this study indicates that flowability increases with increasing particle size. IHP with the largest particle sizes displayed the best flow characteristics while lamivudine with the smallest particle sizes displayed the worst flow characteristics. The values obtained from the three different flow assessment techniques used were in concordance with each other suggesting that this study provides a detailed assessment of the fundamental and derived properties of the powders.

4.5 Drug-excipient compatibility studies

4.5.1 Differential scanning calorimetry (DSC)

DSC is highly preferred over other conventional techniques for the detection of incompatibilities between drugs and excipients in the preformulation phase since it requires a small amount of sample, and the analysis can be carried out in a short period of time.[50] Also, DSC enables the formulator to predict potential issues that may arise with the use of a particular excipient (e.g. reducing the potency and shelf-life of the drug by forming complexes with the active pharmaceutical ingredient), thus, the excipient can be rejected or substituted at an early stage of product formulation.[51]

It is presumed that the thermal properties of the blend (enthalpy change, melting point, etc.) is the sum of the thermal properties of the individual components should the components be compatible with each other.[50] An absence or a significant shift in the melting point of a component, appearance of new peaks and/or variation in enthalpies are indications of incompatibilities. Despite the fact that DSC is a highly beneficial and reliable technique, confusion may arise, as such, careful interpretation of the results and/or the use of non-thermal techniques for comparison is highly recommended.[51]

The DSC thermograms of individual components were compared to thermograms obtained from 1:1 physical mixtures of the active pharmaceutical ingredient and the natural polymers.

The lamivudine + UBP DSC thermogram (figure 4.16-A) reveals an endothermic peak at 177 ºC, which coincides with the melting point of lamivudine. Consequently, it can be inferred that lamivudine remained unchanged within the mixture. Also, no new peaks can be observed on the curve suggesting that no incompatibility arose. However, no peak can be observed around 300 ºC, the temperature at which UBP is expected to decompose. The absence of a peak around this temperature might be due to the complex nature of UBP.

An endothermic peak can be seen at the melting point of lamivudine (177 ºC) on the lamivudine + IHP DSC curve (figure 4.16-A). A very shallow endothermic peak can be observed around 320 ºC, attributable to the decomposition of hemicellulose. No new peaks or shift in enthalpy can be observed on the thermogram. Consequently, it can be deduced that no incompatibilities arose from the mixture of lamivudine and IHP.

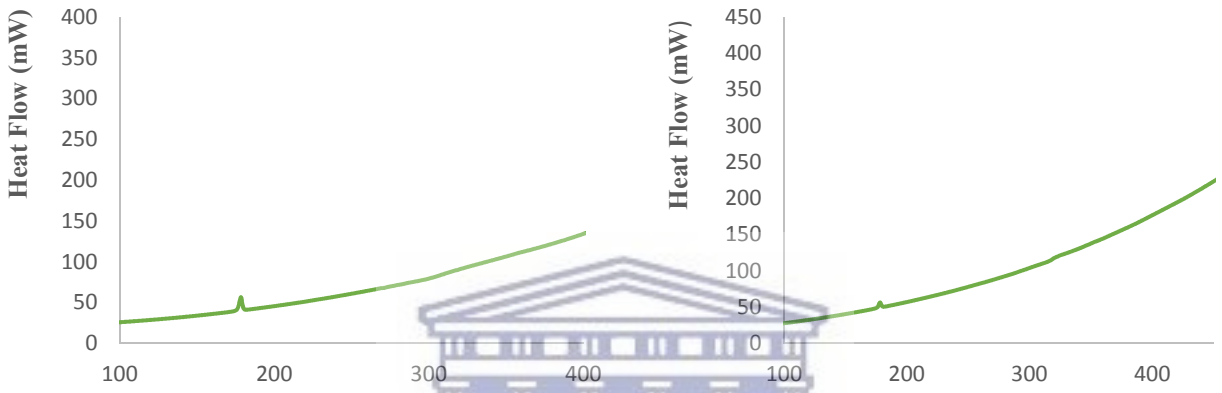


Figure 4.18-A DSC thermogram of lamivudine + unripe banana powder

Figure 4.18-B: DSC thermogram of lamivudine + ispaghula husk powder

4.5.2 Fourier transform infrared spectroscopy (FTIR)

This spectroscopic technique is sensitive to the structure and environment of organic compounds. Besides providing invaluable information on the solid-state behaviour of active pharmaceutical ingredients and their formulations, FTIR can be used as a compatibility screening tool since the vibrational changes serve as indicators of potential intermolecular interactions among the components of a formulation. Pharmaceutical interactions that results in dehydration, polymorphic changes, hydrate formation, desalting or transformation of amorphous to crystalline and vice versa during formulation can be detected using this tool. The presence of overlapping peaks on the spectra is however a major setback in this analysis.[52,53]

Lamivudine + unripe banana powder

No major shift in peaks of pure lamivudine was observed on the FTIR spectrum of combined lamivudine and UBP (Figure 4.17). The characteristic peak corresponding to the carbonyl group of the cystidine nucleus of lamivudine observed at 1634.93 cm^{-1} on the pure drug spectrum can be observed at 1635.85 cm^{-1} on the drug-excipient spectrum. The amino and hydroxyl functional groups signalled at 3195.40 cm^{-1} on the pure drug spectrum are presented at 3197.04 cm^{-1} on the drug-excipient spectrum. Asymmetrical and symmetrical stretching of the C-O-C functional group found in the oxathiolane ring of lamivudine which can be seen at 1285.96 cm^{-1} and 1158.65 cm^{-1} on the pure drug spectrum, is presented at 1286.13 cm^{-1} and 1157.48 cm^{-1} on the combined spectrum. It can thus be deduced that no incompatibilities exist between lamivudine and UBP since no major vibrational changes were observed on the combined spectrum.

Lamivudine + ispaghula husk powder

No major shift in peaks of pure lamivudine was observed on the FTIR spectrum of combined lamivudine and IHP (figure 4.19). The characteristic peak corresponding to the carbonyl group

of the cystidine nucleus of lamivudine observed at 1634.93 cm^{-1} on the pure drug spectrum can be observed at 1633.99 cm^{-1} on the drug-excipient spectrum. The amino and hydroxyl functional groups signalled at 3324.16 cm^{-1} and 3195.40 cm^{-1} on the pure drug spectrum are presented at 3325.19 cm-^{1} and 3197.21 cm^{-1} on the drug-excipient spectrum. Asymmetrical and symmetrical stretching of the C-O-C functional group found in the oxathiolane ring of lamivudine which can be seen at 1285.96 cm^{-1} and 1158.65 cm^{-1} on the pure drug spectrum, is presented at 1285.92 cm^{-1} and 1158.82 cm^{-1} on the combined spectrum. It can thus be deduced that no incompatibilities exist between lamivudine and IHP since no major vibrational changes were observed on the combined spectrum.

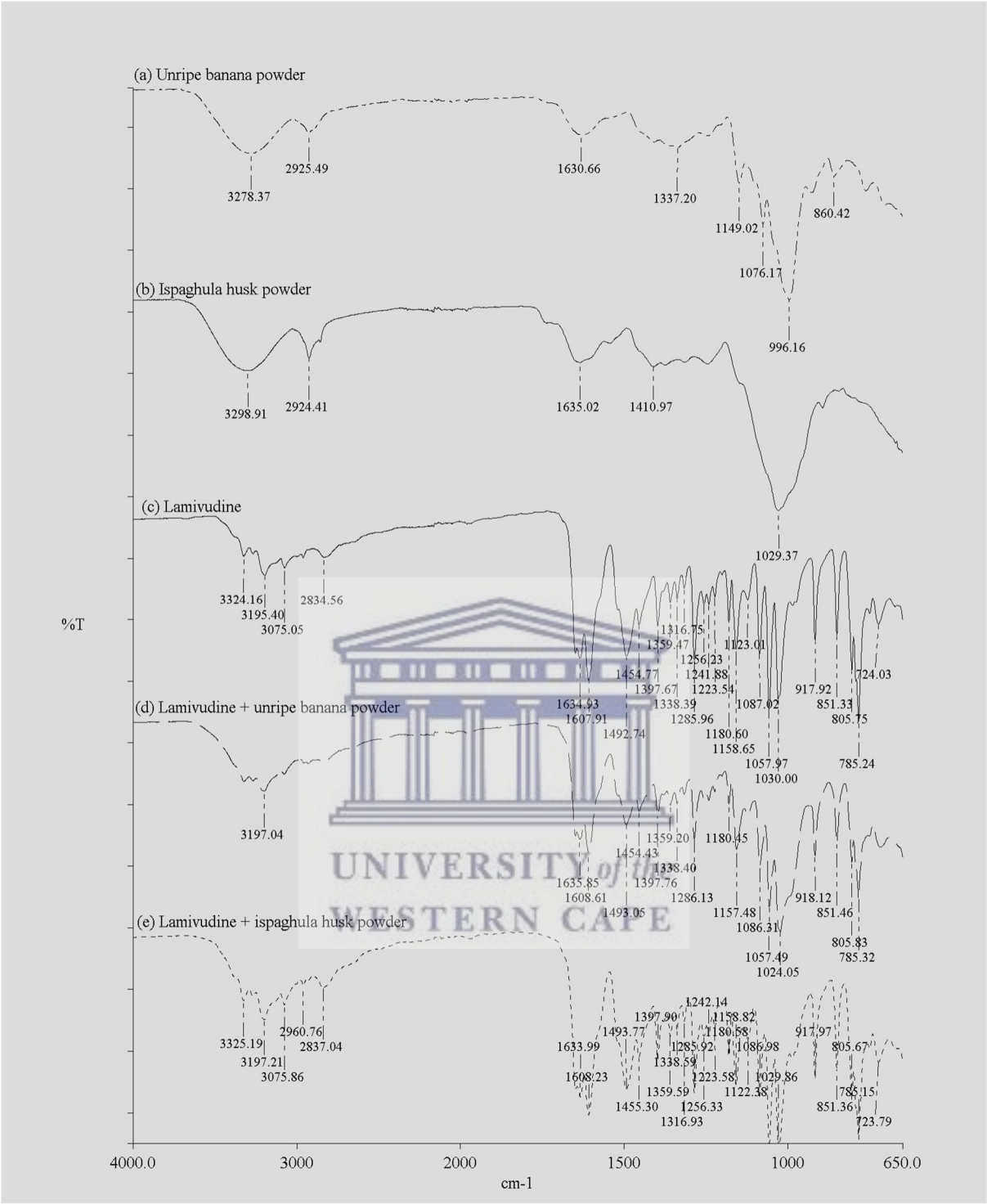


Figure 4.19: FTIR spectra of 1:1 mixtures of lamivudine and the natural polymers

4.6 Tableting

4.6.1 Powder blending

The active pharmaceutical ingredient lamivudine was blended with varying concentrations of the natural polymers. Magnesium stearate was added to the powder blends to enhance flowability. In an attempt to make the innovative product as cost effective as possible, no other excipient was used in this study. This approach highlights the uniqueness of this project since the use of the natural polymers as multipurpose excipients was investigated.

Compression

Twelve different powder blends were formulated and a batch of ten tablets was compressed for each formulation using different compression forces. The different formulations used are shown in table 4.15 below. The tablets were assessed for friability, resistance to crush and *in-vitro* dispersion time. The formulation which yielded the best product i.e. the optimum formulation was retained and upscaled.

Table 4.15: Different formulation blends prepared

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
L (mg)	150	150	150	150	150	150	150	150	150	150	150	150
B (mg)	3.06	3.06	8.01	17.04	27.11	46	-	-	-	-	-	-
I (mg)	-	-	-	-	-	-	3.06	3.06	8.01	17.04	27.11	46
M (mg)	-	3.125	3.22	3.41	3.61	4	-	3.125	3.22	3.41	3.61	4
S %	2	2	5	10	15	23	2	2	5	10	15	23

* L(lamivudine), B(Banana powder), I(Ispaghula husk powder), M(Magnesium stearate), S(superdisintegrant)

Formulation F1

This powder blend could not be compressed into a solid mass at any of the employed compression forces.

Formulation F2

A solid mass was obtained when compressed at 50 kN. The resultant tablet was very brittle and crumbled in the friabilator. The average crushing strength and dispersion time recorded was 5N and 10±3 seconds respectively.

Formulation F3

No significant improvement was observed with this formulation. The tablets compressed at 50 kN were slightly more resilient than the tablets compressed with the F2 formulation blend. Capping was observed, and the tablets crumbled in the friabilator within 5 revolutions.

Complete dispersion in water at 25 ºC occurred within 15±2 seconds. The highest crushing force observed was 7 N.

Formulation F4

Considerable improvement was observed with this formulation. The powder blend was compressed into a solid mass at 40, 45, and 50 kN. The most resilient tablets were obtained at 50 kN. The tablets were brittle and fragmented during the friability assessment within 5 revolutions. The highest crushing force observed was 17 N and dispersion in water occurred within 13±1 sec.

Formulation F5

The powder blend was compressed into a solid mass at 35 kN, the resultant tablet crumbled at the touch. Tablets with an average crushing strength of 25 N and a dispersion time of 25±2 sec were obtained at 40 kN. These tablets were fragmented during the friability test. Virtually all the tablets compressed at 45 kN and 50 kN were capped.

Formulation F6

As observed with formulation F5, the incidence of capping was high at the compression forces of 45 kN and 50 kN. The tablets compressed at 40 kN had an average crushing strength of 40±5 N and dispersion in water occurred within 45±3 seconds. A loss in mass of 18 % was recorded during the friability test.

Formulation F7

This powder blend could not be compressed into a solid mass at any of the employed compression forces.

Formulation F8

No improvement observed with this compression. No solid mass was obtained at any of the employed compression forces.

Formulation F9

Considerable improvement was observed with this formulation. Tablets were successfully compressed at 40, 45 and 50 kN. Compression forces 40 and 45 kN yielded very brittle tablets which crumbled at the touch. Relatively more resilient tablets were obtained at 50 kN with an average crushing strength of 10 N. The average dispersion time recorded was 20±2 seconds.

Formulation F10

A high incidence of capping was observed at 50 kN. Tablets with an average crushing strength of 15 N and an average dispersion time of 15±1 seconds were obtained at 40 kN. The tablets obtained at 35 kN were brittle and crumbled at the touch.

Formulation F11

An increase in crushing strength and dispersion time was observed with this formulation. The incidence of capping was high at 45 and 50 kN. Tablets with an average crushing strength of 18 N and an average dispersion time of 85±10 seconds were obtained at 40 kN. The tablets became tacky when in contact with water and constant agitation was needed for complete disintegration.

Formulation F12

Tablets compressed at 40 kN showed improved crushing strength and an increase in dispersion time as compared to the tablets compressed with the F11 formulation blend. A thick gelatinised mass was formed when the tablets were in contact with water consequently, vigorous agitation was required to completely disintegrate the tablets. The average crushing strength and

dispersion time recorded were 25 N and 165 ±20 seconds, respectively. The tablets were cleaved in the friabilator within 10 revolutions.

Observations

Using the natural polymers as multipurpose excipients in the different formulations made it impossible to assess their disintegrating properties below concentrations of 5% w/w since their binding effect was made manifest above this concentration. Owing to the poor compressibility of lamivudine, the properties of the tablets were highly dependent on the amount of natural polymer in the formulation blend. An equilibrium had to be reached between compression force and the percentage of superdisintegrant in the tablet since these two variables influenced tablet crushing strength and dispersion time. An increase in compression force led to an increase in crushing strength and an increase in the incidence of capping at higher compression forces. Tablets formulated with lower percentages of superdisintegrant were relatively more brittle as compared to tablets containing higher percentages. This suggests that the binding effect of the natural polymers increases with increasing concentrations. Also, an increase in the amount of superdisintegrant in the tablet led to a decrease in dispersion time up to a saturation point where dispersion time begun to increase. This correlation is depicted in figure 4.20 below.

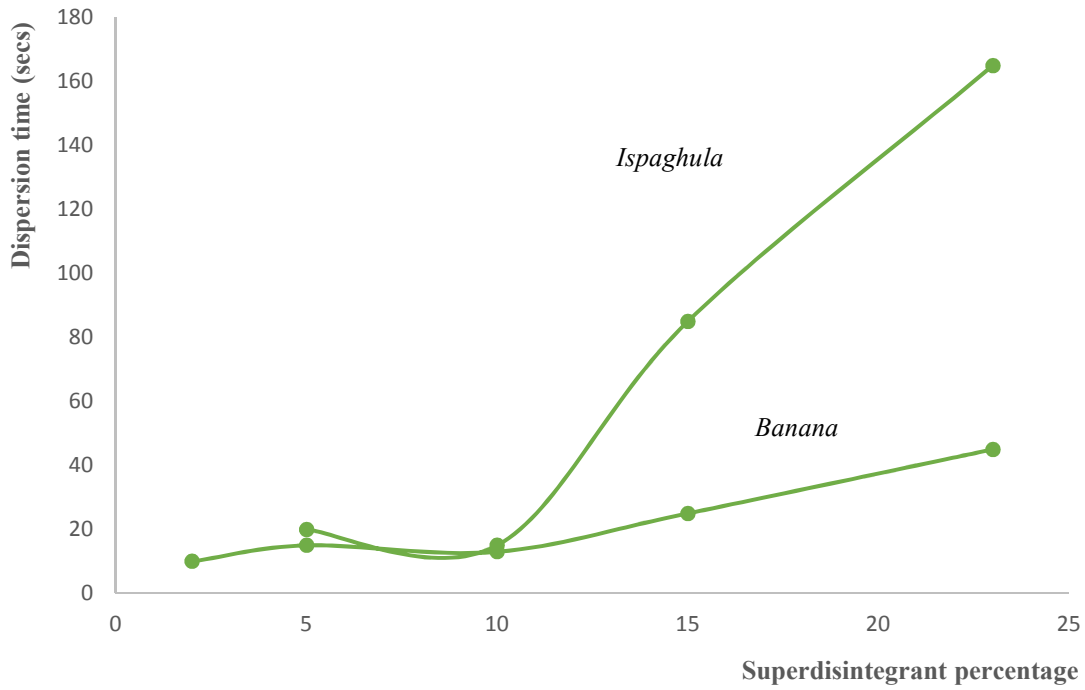
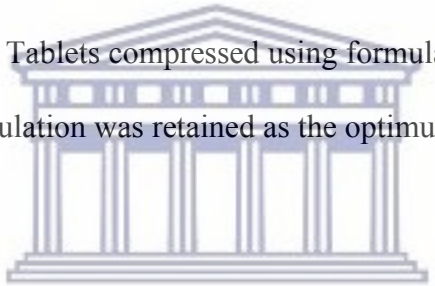


Figure 4.20: A plot of dispersion time against superdisintegrant percentage

Although it was clearly shown that the natural polymers used in this study possess substantial disintegrating properties, it was quite challenging to accurately quantify their disintegrating effect. In the case of UBP, formulation F2 which contained the least amount of superdisintegrant unexpectedly displayed the shortest *in-vitro* dispersion time. However, the tablets with the least amount of superdisintegrant were the most friable and displayed the lowest resistance to crush. This indicates that the tablets containing the lowest amount of superdisintegrant were relatively more porous due to their relatively low binding strength. Literature reports that porosity enhances tablet disintegration owing to the presence of loose interstices which allow the free circulation of water in and out of the tablet matrix.[54] Consequently, dealing with three different variables i.e. porosity, superdisintegrant concentration and tablet hardness which considerably influence tablet dispersion time rendered the task of assessing the disintegrating effect of the natural polymers strenuous. It would have been fitting to make use of a multipurpose tablet excipient such as mannitol in order to gain a

detailed insight of the disintegrating properties of the natural polymers over a wide range of concentrations. However, the use of another tablet excipient in this formulation would have defied the purpose of this study since the aim was to formulate a tablet at minimal cost with the least possible excipients. Also, the natural polymers were selected on the basis that they would play several roles in the formulation blends i.e. disintegration, binding and filling.

Although IHP proved to have better swelling properties than UBP which is an indication of superior disintegrating properties, it was shown that IHP cannot serve as a superdisintegrant at concentrations greater than 10% w/w due to the formation of a highly gelatinised structure which incumbers tablet disintegration by obstructing water permeation. Also, UBP was shown to display better binding properties than IHP and thus can serve as a superdisintegrant over a wider range of concentrations. Tablets compressed using formulation F6 at 40 kN yielded the best product, as such this formulation was retained as the optimum formulation.



4.7 Optimum formulation

Formulation F6 was retained as the optimum formulation. The master formula is presented below.

Master formula

Lamivudine 150 mg tablet

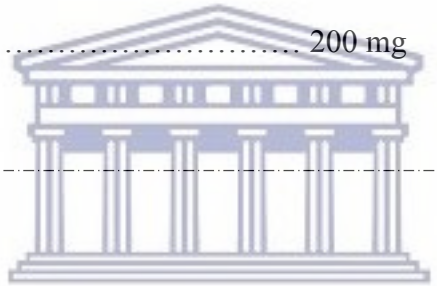
Lamivudine……. 150 mg

Unripe banana powder…… 46 mg

Magnesium stearate…….…… 4 mg

Talc ……..…… *qs*

Theoretical weight…… 200 mg



The master formula was upscaled and a batch of 60 tablets was compressed. The working formula is presented in the table below.

Table 4.16: Working formula of the optimum formulation

Ingredient	Master formula	Working formula	5 % excess	Final amount
Lamivudine	150 mg	9000 mg	450 mg	9600 mg
Banana	46 mg	2760 mg	138 mg	2944 mg
Mg stearate	4 mg	240 mg	12 mg	256 mg
Total				**12.8 g**

4.7.1 Micromeritic properties of the optimum formulation

The micromeritic properties of the optimum formulation blend were determined and the results obtained are displayed in table 4.17 below. Lamivudine was blended with UBP and the flow properties of the powder blend were assessed. Subsequently, magnesium stearate was added to the powder blend and the micromeritics properties of the final mixture were determined.

Table 4.17: Micromeritic properties of the optimum formulation blend

Property	Lamivudine +Banana	Lamivudine + Banana + Mg stearate
Angle of repose (º)	51	31
Bulk density (g/cm^3)	0.53	0.70
Tapped density (g/cm^3)	0.90	0.98
Carr's index	41.6	27.8
Hausner's ratio	1.7	1.4

Although the amount of powder used for this assessment was smaller than the recommended amount (30 g)[20] because of the limited amount of the active ingredient available, the results obtained nevertheless gave the formulator a general overview of the tabletting properties of the optimum formulation blend. The role of magnesium stearate as a flow enhancer was appraised. The lamivudine and UBP blend displayed a very poor flow propensity overall, inferior to the flow properties of the individual powders. The addition of magnesium stearate drastically improved the flow properties of the formulation. A good settling geometry was noted although the compressibility index and Hausner ratio remained poor.

4.7.2 Compression

The optimum formulation blend was compressed using four different compression forces. 40 kN was found to be ideal in terms of conferring sufficient resilience to the tablet and allowing for rapid tablet disintegration. A high incidence of capping was observed when compression forces above 40 kN were employed. Compression forces below 40 kN yielded brittle and less robust tablets. UBP was found to possess good tablet binding properties, a correlation was observed between tablet crushing strength and the amount of UBP in the tablet. Tablets with a higher concentration of dehydrated powder were found to be more resilient at any given compression force. This correlation is shown in figure below 4.21.

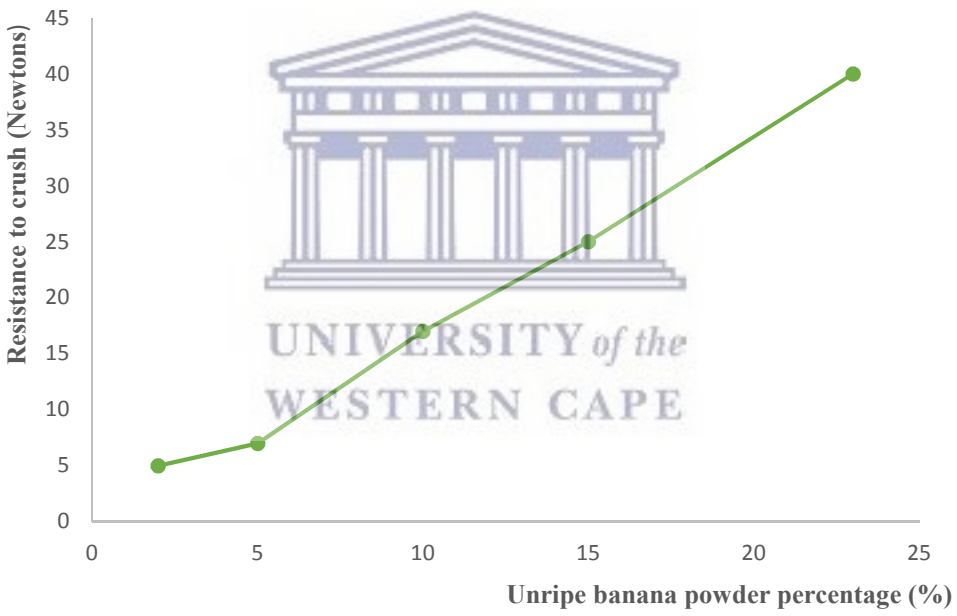
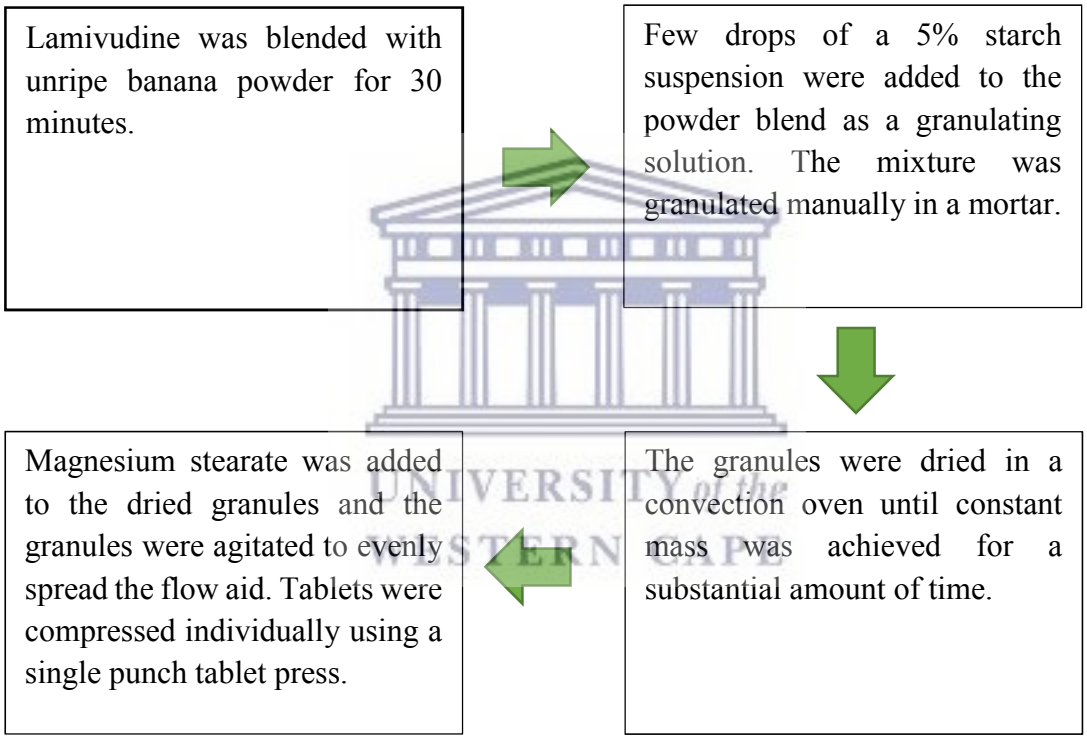


Figure 4.21: Plot of resistance to crush against superdisintegrant percentage

A fairly linear relationship was observed between tablet crushing strength and superdisintegrant percentage.

4.7.3 Wet granulation

Attaining a low friability percentage was a major challenge in this study, the average percentage mass loss recorded when the tablets were subjected to the friability test was 18%. This value exceeds by far the USP maximum friability limit of 1%.[20] Wet granulation was employed in an attempt to render the product less friable. Enough powder to compress 60 tablets was granulated. The procedure employed is shown below.



Theoretical granules weight:	12.544 g
Granules weight post drying:	12.167 g
Mass of Mg stearate added:	0.2483 g
Final weight:	**12.42 g**

4.8 Quality assessment of the innovative and branded tablets

The innovative tablets were evaluated as per the USP specifications and compared to a brand available on the South African market.

4.8.1 Organoleptic evaluation

Sensory appraisal of the tablets was effectuated to determine their overall aesthetic appeal. The results obtained are displayed in table 4.18 below and the tablets are shown in figure 4.22.

Table 4.18: Organoleptic properties of the innovative and branded tablets

No	Property	Innovative tablets	Branded tablets
1	Shape	Round	Rhomboid
2	Colour	Greyish-white	Creamy white
3	Odour	No distinct odour	No distinct odour
4	Surface texture	Smooth	Smooth

The tablets had an overall satisfactory physical appearance. No flaws or inconsistencies were detected on the tablets, and no repulsive odour was perceived.

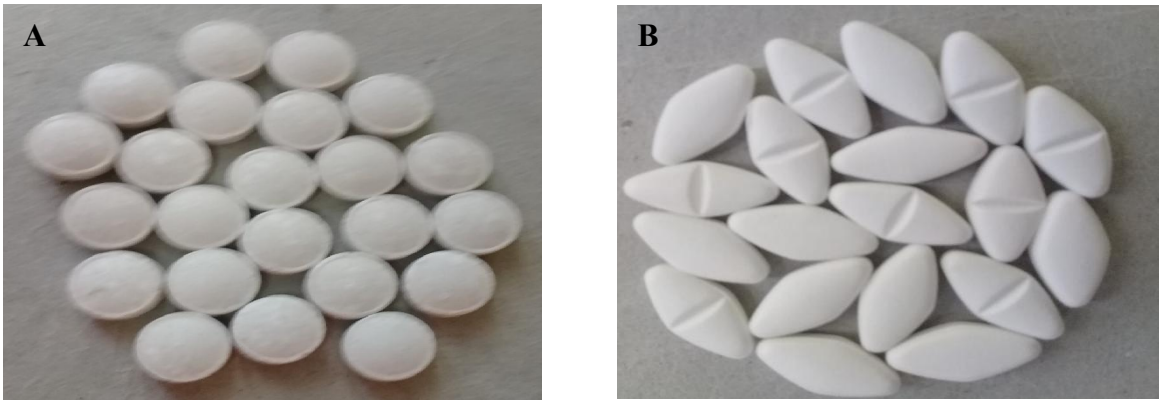


Figure 4.22: Photographs of the assessed tablets. A) Innovative B) Branded

4.8.2 Tablet mensuration

Metric assessment of the tablet was effectuated, and the results obtained are presented in table 4.19 below.

Table 4.19: Mensuration of the innovative and branded tablets

No	Property	Innovative tablets	Branded tablets
1	Diameter (mm)	8	14
2	Thickness (mm)	3	4
3	Average weight (mg)	215.0	333.33

Tablet size

The branded tablets were found to have a relatively larger diameter and thickness than the innovative tablets. This is evident considering the significant difference in mass between the branded tablets and the innovative tablets. Also, the trapezoidal shape of the branded tablets accounts for their larger size, since the longest axis length was recorded as the diameter. A smaller tablet size is beneficial for the manufacturer because relatively small packaging and less storage space are required.[55] Fast dispersible tablets designed with minimal ingredients are not only cost effective but are patient friendly since the amount of residual tablet fragments in the mouth which can cause poor mouth feel is considerably reduced.[56]

Weight uniformity

To assess the weight uniformity of the branded and the innovative tablets, the standard weight deviation from the mean weight of 20 tablets was calculated and expressed as a percentage. The results obtained are presented in table 4.20 below.

Table 4.20: Standard deviation from mean tablet weight

Parameter	Innovative tablets	Branded
Average tablet weight (g)	0.215	0.333
Standard deviation	0.0154	0.0091
% Standard deviation	7.2	2.7

The USP specifies that the percentage deviation from the mean tablet weight should not exceed 7.5% for tablets weighing between 80 and 250 mg, and 5% for tablets weighing more than 250 mg.[19] Although both the branded tablets and the innovative tablets met the USP prerequisites in their respective categories, the comparator displayed superior weight uniformity. This assessment is very crucial in ensuring the uniformity of the active pharmaceutical ingredient in the final product especially for drugs which have a narrow therapeutic index. This is also a reflection of the uniformity of the powder blend and the level of accuracy with which powders were weighed for individual tablets.

4.8.3 Friability

The percentage friability of the branded tablets and the innovative tablets (directly compressed and wet granulated) was determined and the results obtained are presented in table 4.21 below. The USP stipulates that the percentage loss in mass for a conventional tablet should not exceed 1% of the mean tablet weight.[20] The branded tablets met pharmacopoeial specifications with a 0.13% mass loss. Both the directly compressed and the wet granulated innovative tablets did not meet pharmacopoeial requirements. The directly compressed tablets displayed an extremely high friability of 18% while a mass loss of 2.1% was recorded for the wet granulated innovative tablets. Although the USP does not provide specific requirements for the friability assessment of fast dispersible tablets, a 2% mass loss is an acceptable friability percentage for

a fast dispersible tablet given that FDTs are manufactured to be more porous and less resilient than conventional tablets to allow for rapid tablet disintegration.[57] Consequently, adequate packaging should be used in order to minimise mechanical damage to the tablets. The drastic improvement observed with the wet granulated innovative tablets suggests that wet granulation was effective in optimising the directly compressed tablets thus yielding a more satisfactory product.

Table 4.21: Percentage friability of the branded and innovative tablets

Parameter	Branded tablets	Innovative (DC*)	Innovative (WG*)
Initial weight (g)	6.660	4.420	4.294
Final weight (g)	6.651	3.182	4.203
Percentage loss (%)	0.13	18	2.1

*DC: directly compressed, WG: wet granulated

4.8.4 Hardness

The robustness of the branded and innovative tablets was assessed by measuring the resistance to crush or the force in Newtons required to cleave or fragment a single tablet. Tablets need a certain amount of resilience to be able to withstand mechanical stress during manufacturing handling, packaging, transportation and storage. Twenty randomly selected tablets were assessed from the three formulations and the average crushing force was calculated. Generally, a crushing strength between 40 to 80 Newtons is acceptable for uncoated tablets.[20] As anticipated, the branded tablets were found to be the most resilient with an average crushing strength of 106.3 Newtons (table 4.22). Considering the difference in mass between the branded tablets and the innovative tablets, and the fact that conventional swallow tablets are generally more robust than fast dispersible tablets, it is admissible for the branded tablets to display a greater resistance to crush. Additionally, the larger diameter of the comparator also

contributes to its greater resistance to crush since larger tablets require a greater force to cause fracture.[58]

Surprisingly, the directly compressed innovative tablets inexplicably displayed a greater resistance to crush than the wet granulated innovative tablets although the former was extremely friable. The directly compressed innovative tablets had an average resistance to crush of 40.3 Newtons while the wet granulated tablets had an average resistance to crush of 20.7 Newtons. This was quite perplexing because wet granulation generally yields more robust tablets than direct compression for the same powder blend.[59] However, resistance to crush is not an absolute indication of tablet strength, since some powder blends when compressed into hard tablets tend to cap on attrition losing their upper portions.[60] This is the plausible explanation to the low resistance to crush displayed by the wet granulated tablets since most tablets fractured by capping when subjected to the hardness test. Also, monthly assessments of the wet granulated innovative tablets did not reveal any significant increase in friability or reduction in resistance to crush whereas the directly compressed innovative tablets were found to be completely fragmented within two months of storage in an airtight container with a desiccating agent. This suggests that for a poorly compressible powder blend, wet granulation is essential in producing a relatively more resilient and long-lasting product.

Table 4.22: Crushing strength of the comparator and innovative tablets in Newtons

Number	Branded	Innovative (DC*)	Innovative(WG*)
1	108	39	15
2	88	49	20
3	113	41	25
4	79	32	20
5	108	28	18
6	118	39	24
7	111	47	17
8	134	39	21
9	96	50	23
10	108	39	24
Mean	106.3	40.3	20.7

*DC: directly compressed, WG: wet granulated

4.8.5 Wetting time

The amount of time required for complete wetting of the different tablets is presented in table 4.23. The directly compressed innovative tablets achieved complete wetting within 10±2 seconds while an average wetting time of 13±2 seconds was recorded for the wet granulated innovative tablets. The branded tablets achieved complete wetting within 660±90 seconds. This assessment is however trivial for conventional tablets which are meant to be swallowed. In view of the results obtained for the innovative tablets, it can be deduced that UBP possesses outstanding wetting properties and hence good disintegrating properties.

Wetting is the ability of a liquid to remain in contact with a solid surface as a result of intermolecular interactions when the two surfaces come into contact.[61] Wetting time is dependent on the wettability of a solid i.e. the ability of a solid surface to reduce the surface tension of a liquid in contact with it in such a way that it spreads over the solid surface and adheres to it. Wettability is influenced by particle shape and morphology, hydrophilicity and

the contact angle between water and individual particles.[62] Generally, rough and round particles tend to be less hydrophobic than smooth and elongated particles, hence they display superior wetting properties.[63] Materials which are intrinsically hydrophilic tend to display superior wetting properties compared to hydrophobic materials due to favourable interactions with water molecules.[64] The angle of contact between water molecules and individual particles quantifies the wettability of a solid surface via Young's equation as shown in figure 4.23.

Young's equation

$$\gamma^{sv} = \gamma^{sl} + \gamma^{lv}\cos\theta$$

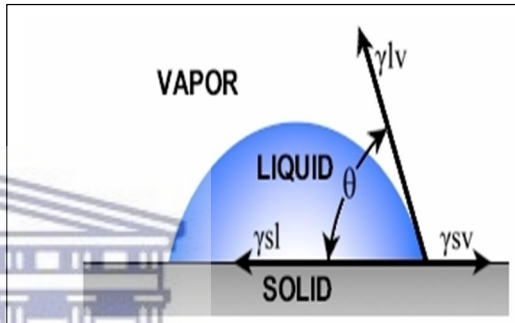
Where

θ is the contact angle

γ^{sv} is the solid surface free energy

γ^{sl} is the solid/liquid interfacial free energy

γ^{lv} is the liquid surface free energy



Depiction of contact angle

Figure 4.23: Young's equation and contact angle

Wettability increases with decreasing contact angle. A contact angle below 90º is favourable for wetting and indicates that water will spread over the surface considerably while a contact angle above 90º is an indication of poor wetting and minimal contact of the surface with water.[65]

Although the wetting time of a fast dispersible tablet is greatly determined by the wetting properties of the superdisintegrant, the wetting properties of the other constituents will also affect the time required for complete wetting.[54] The presence of hydrophobic constituents can increase the time required for complete tablet wetting by restricting surface interactions with

water. In the case of the innovative tablets, the presence of magnesium stearate could have delayed complete wetting of the tablets by restricting interactions with water molecules due to its hydrophobic nature.[66] However, considering the percentage of magnesium stearate used, the effect of such hydrophobic restrictions would have been inconsequential.

4.8.6 Water absorption ratio

This test assesses the water uptake behaviour of fast dispersible tablets. It is an indication of the efficiency of a superdisintegrant within the tablet matrix i.e. the swiftness at which a superdisintegrant draws water into the tablet matrix to create sufficient pressure to facilitate disintegration.[67] The average water absorption ratio of the directly compressed and wet granulated formulations was found to be 112±7 and 105±4, respectively. These results were deemed satisfactory as the amount of water absorbed by the tablets was sufficient to cause instantaneous disintegration by virtue of the swelling index of UBP (table 4.23).

Table 4.23: Wetting time and water absorption ratio of the different tablet formulations

Assessment	Branded tablets	Innovative (DC)	Innovative (WG)
Wetting time (s)	660±90	10±2	13±2
Water absorption ratio (%)	-	112±7	105±4

4.8.7 *In-vitro* dispersion

Table 4.24 presents the average time in seconds required for the different tablet formulations to breakdown into smaller fragments in deionised water and a 6.8 phosphate buffer solution at 21° C. This assessment is a preview of the pharmacopoeial disintegration test. It enables the formulator to estimate the time required for a tablet to disintegrate should a patient opt to disperse the dosage form in a small amount of water prior to administration. The average

dispersion time recorded for the branded tablets was 840±60 sec in deionised water and 980±40 sec in the phosphate buffer solution. The directly compressed innovative tablets displayed an average dispersion time of 45±3 sec in deionised water and 31±2 sec in the phosphate buffer solution. An average dispersion time of 57±4 sec in deionised water and 36±2 sec was recorded for the wet granulated formulation. The relatively faster dispersion of the directly compressed tablets is an indication of a very porous structure as a result of weaker binding forces that operate within the tablet matrix. The more porous a tablet is, the faster the dispersion due to the presence of more openings on the tablet surface which enhance permeation.[54] Wet granulation increases the binding forces between particles thereby reducing the interstices between them.[59] Consequently, it requires a longer time for the solvent to break through the tightly packed matrix.

Table 4.24*: In-vitro* dispersion times in seconds of the different tablet formulations

Medium	Branded	Innovative(DC)	Innovative(WG)
Deionised water	840±60	45±3	57±4
6.8 Phosphate buffer	980±40	31±2	36±2

The *in-vitro* dispersion timeline of the wet granulated innovative tablet is shown in figure 4.24 below. A tablet was placed in the centre of a petri dish containing deionised water and photographs were taken at different time intervals.

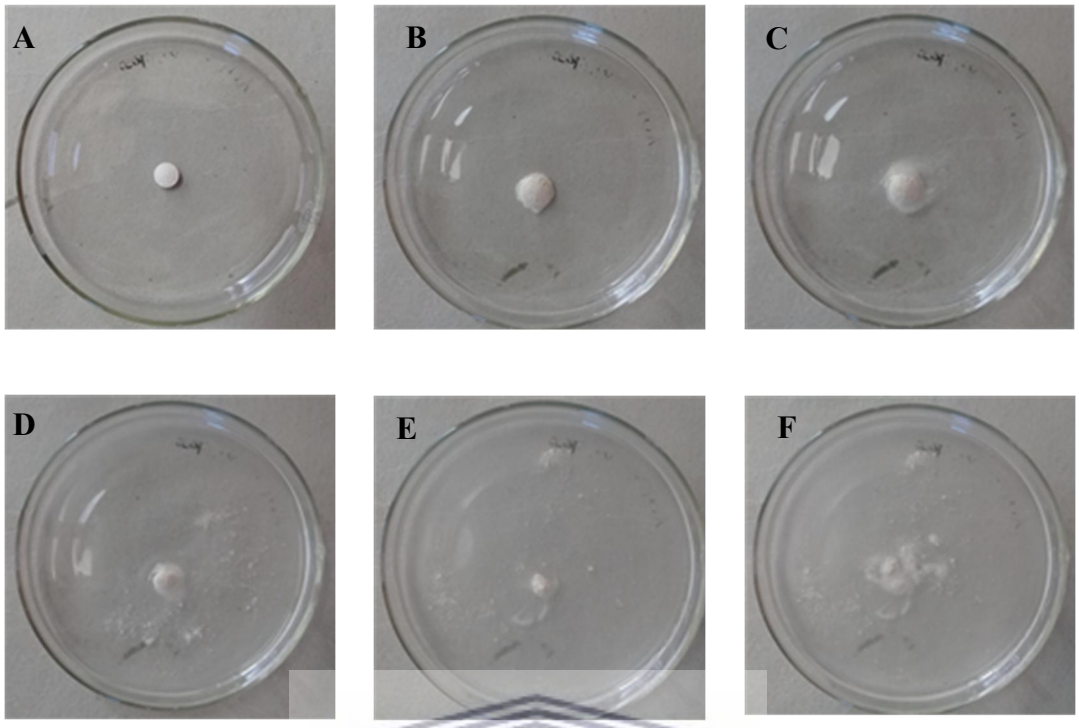


Figure 4.24: Dispersion timeline of the wet granulated innovative tablets

a) Tablet in dry petri dish **b)** Considerable wetting of the tablet can be observed at 10 sec **c)** Diffusion of the drug from the swollen tablet can be observed at 20 sec **d)** Gradual disintegration of the tablet at 30 sec **e)** Considerable disintegration of the tablet at 40 sec **f)** Complete dispersion of the tablet with swollen residues at 60 sec

4.8.8 Disintegration study

The average time required for the branded and innovative tablets to breakdown into smaller fragments in a neutral and buffered medium is presented in table 4.25 below. Both the branded and the innovative tablets met the USP prerequisites for the disintegration of uncoated tablets given that complete disintegration of the assessed tablets occurred within 30 minutes.[20] An average disintegration time of 780±26 sec in deionised water and 960±20 sec in the 6.8 phosphate buffer solution was recorded for the branded tablets. The innovative tablets disintegrated in both media within 60 seconds thus fulfilling the fundamental prerequisite for

fast dispersible tablets which entails instantaneous disintegration.[68] The disintegration of the branded tablets was relatively faster in water whereas the innovative tablets disintegrated faster in the phosphate buffer solution. This suggests that saliva will offer a propitious environment for the prompt disintegration of the innovative tablets at body temperature since it is a buffered medium.

Table 4.25: Average disintegration time in seconds for the comparator and wet granulated innovative tablets

Medium	Branded tablet	Innovative tablets
Deionised water	780±26	50±3
6.8 Phosphate buffer	960±20	35±2

Disintegration is the precursor and a key determining factor of dissolution. The faster the disintegration process the quicker the active pharmaceutical ingredient is released from the tablet matrix. The higher the extent of disintegration the more drug is released from the dosage form at a faster rate. Basically, the breakdown of a tablet into smaller components increases the available surface area for permeation and drug diffusion.[65,69] This is depicted by Noyes-Whitney's equation.

Noyes-Whitney equation[65]

$$\frac{dm}{dt} = \frac{A\ D\ (C_s - C_b)}{d}$$

Where

dm/dt = dissolution rate of the solute (kg. s^{-1})

m = mass of dissolved material (Kg)

t = time (s)

A = surface area of solute particle (m^2)

D = diffusion coefficient (m. s^{-1})

d = thickness of the concentration gradient (m)

C_s = particle surface concentration (moles/L)

C_b = concentration in the solvent (moles/L)

As shown in the above equation , an increase in surface area (A) leads to an increase in the rate at which the active pharmaceutical ingredient (API) goes into solution. This suggests that adding a superdisintegrant such as UBP into a tablet formulation should enhance disintegration as well as dissolution. The equation also shows that the rate of dissolution is decreased by the thickness of the concentration gradient(d). Consequently, superdisintegrants that have the propensity to form gelatinous structures in water such as IHP might decrease the rate at which the API is released into solution.

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Chapter 5

Drug release studies

This chapter gives an account of the HPLC method validation process and the outcome thereof, alongside a comparative assessment of the drug release patterns of the branded and innovative tablets.

5.1 HPLC method validation

Chapter 2 identified and discussed four articles as a reference point to design an HPLC method to analyse the drug release patterns of the branded and innovative tablets. The information sourced from these articles was compiled and used to develop a HPLC method elaborated in chapter 3. The method was assessed using standard validation parameters which include system suitability, specificity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ), robustness and linearity.

5.1.1 System suitability

The HPLC setup was assessed for resolution, column efficiency and repeatability to ensure the adequacy of the method for the analysis of lamivudine for both the branded and innovative formulations. The parameters investigated include theoretical plates, tailing factor and retention time.

Lamivudine standard concentrations prepared with deionised water (100, 150, 200, 250, 300, 350 ug/ml) were used. Injections were made in triplicate for each concentration and the mean value for each parameter was determined as presented in table 5.1 below.

Table 5.1: System suitability data (channel 285:10:400:10)

Conc. (ug/ml)	Theoretical plates (Foley-Dorsey)	Theoretical plate (Tangential)	Tailing factor	Retention time (Minutes)
100	1671.167	2328.461	1.434	0.988
150	1593.351	2155.967	1.459	0.991
200	1529.319	1925.282	1.457	0.993
250	1496.942	1620.485	1.421	0.996
300	1430.302	1478.414	1.418	0.997
350	1383.205	1333.941	1.380	1.000

Column efficiency

The efficiency of a column is depicted by the number of theoretical plates i.e. N = 16(tR/wb)2. The narrower the peak, the greater the number of plates. The greater the number of theoretical plates, the greater the resolving power (Figure 5.1)

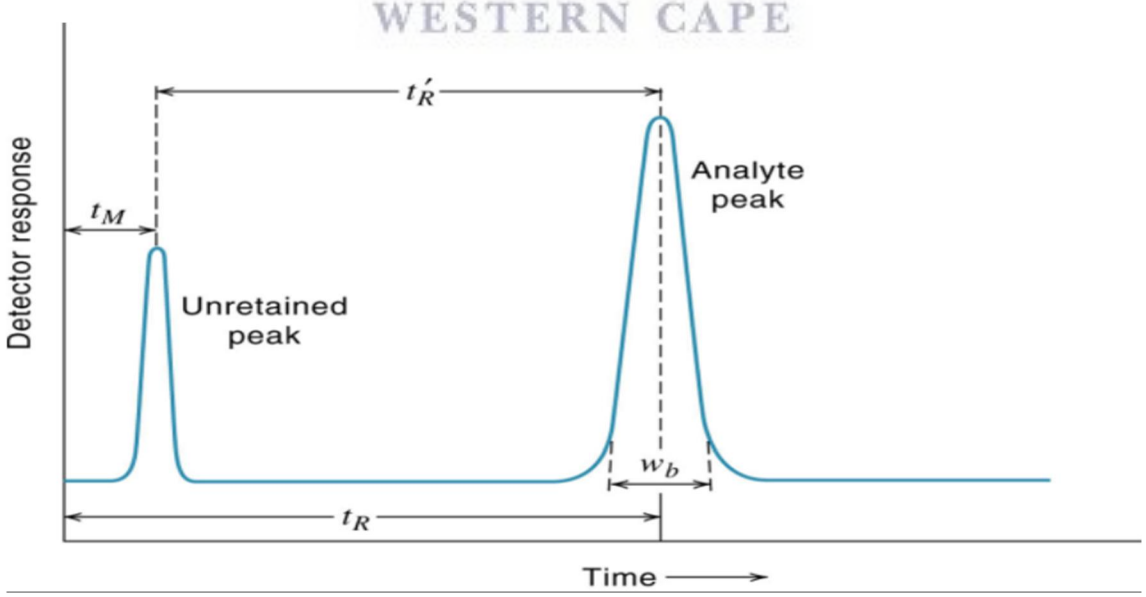

Figure 5.1: Determination of column efficiency[1]

The column efficiency of the standard samples of lamivudine was higher at lower concentrations. Inverse proportionality between the concentration of lamivudine and the number of theoretical plates was evident. It can thus be inferred that lower concentrations of lamivudine yield higher column efficiency. Consequently, lower concentrations of lamivudine were adequate for this analysis.

Tailing factor

Tailing factor measures and provides a means to quantify the extent of tailing of a peak. The results obtained from running lamivudine standard samples were found to be ideal as they did not exceed the non-ideal 2 ≤ value. The resultants peaks however did not depict the Gaussian peak since the tailing factor exceeded 1. Although the peaks deviated from the ideal Gaussian peak, they were still considered acceptable given that the tailing factors did not exceed 1.5.

The retention times recorded for the standard samples of lamivudine demonstrated an ideal system suitability. The mean retention time was found to be 0.99 minutes rounded up to 1. The relative standard deviation (RSD) was 0.45%. This result is considered ideal as it shows that the variation between the retention times of the different concentrations is trivial. This indicates that the chosen setup was consistent and reproducible for all the assessed concentrations.

A dissolution test using deionised water as medium was conducted and aliquots were withdrawn from a chosen vessel at predetermined time intervals (10, 20, 30, 40, 50, 60 minutes). The samples were used to obtain system suitability data for the branded and innovative tablets as shown in tables 5.2 and 5.3.

Table 5.2: System suitability data of the innovative samples (channel 285:10:400:10)

Parameters*	10	20	30	40	50	60
TP(FD)	3517.90	3798.66	3735.97	3713.54	3753.99	3757.93
TP(T)	6682.76	6561.45	6980.04	6964.09	6856.00	7190.34
Tailing factor	1.60	1.47	1.48	1.49	1.51	1.47
RT	1.003	0.99	0.99	1.000	1.001	1.004

Table 5.3: System suitability data of the branded samples (channel 285:10:400:10)

Parameters*	10	20	30	40	50	60
TP(FD)	4147.12	3819.60	3745.17	3601.10	3552.42	3656.16
TP(T)	8237.61	7592.29	6656.70	6459.73	6740.20	6455.74
Tailing factor	1.51	1.49	1.46	1.47	1.47	1.46
RT	1.003	1.006	1.005	0.987	0.987	0.990

* TP(FD): Theoretical plates (Foley-Dorsey), TP(T): Theoretical plates (Tangential), RT: Retention time (minutes)

The number of theoretical plates for both the innovative and branded samples were high, exceeding the minimum of 2000 plates hence depicting column efficiency. The number of plates further yielded sharper peaks and less peak separation. Consequently, it can be deduced that the column was highly efficient.

Ideally, a peak should adopt the Gaussian shape with a tailing factor of 1. Both the innovative and branded samples had tailing factors above 1 but not exceeding 2. The average tailing factor for the innovative samples was 1.504 while the average tailing factor for the branded samples

was 1.48. Tailing for both the innovative and branded samples is illustrated by figures 5.2 and 5.3.

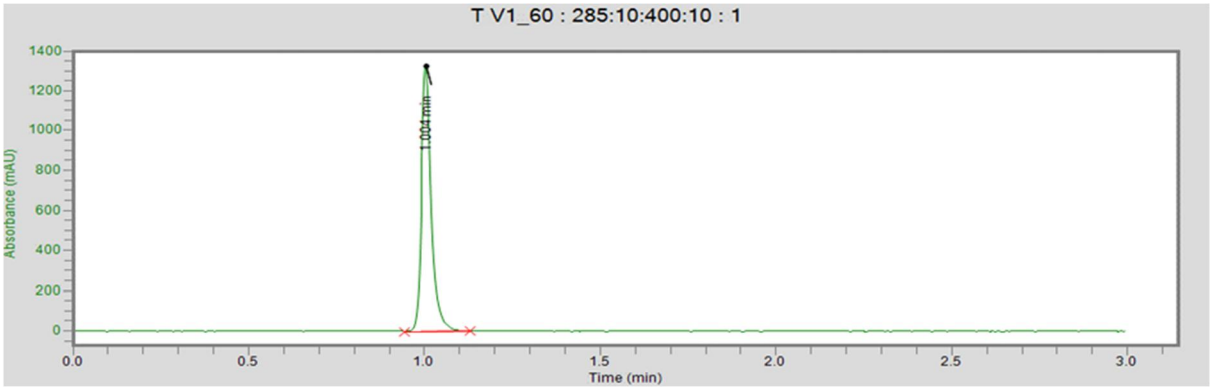

Figure 5.2: Graphical representation of tailing for the innovative sample at 60 minutes

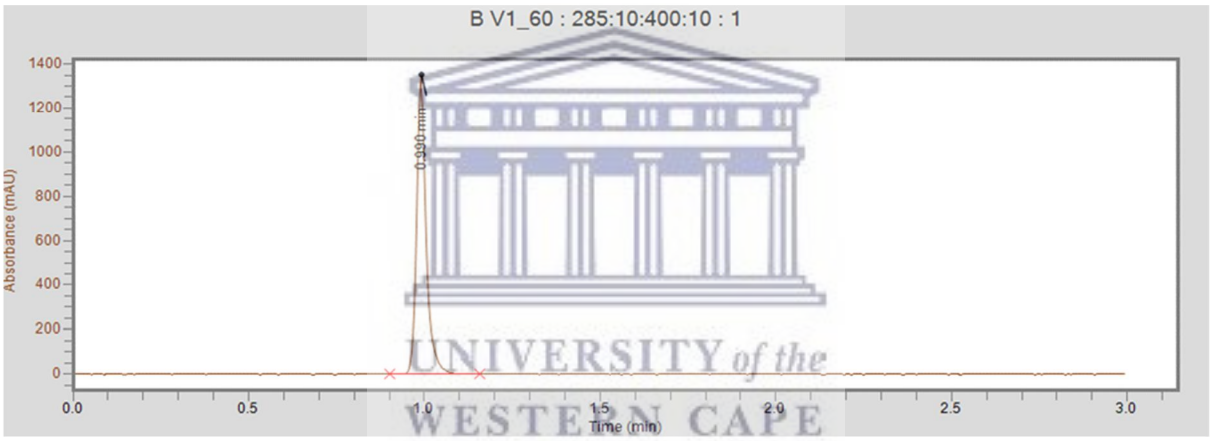

Figure 5.3: Graphical representation of tailing for the branded sample at 60 minutes

The retention times for both the innovative and branded samples were recorded and the mean value vas determined. The average retention time of the innovative samples was 1.00 while the average retention time of the branded samples was 0.99. The relative standard deviation of the retention times was 0.23% for the innovative samples and 0.95% for the branded samples. This indicates that deviation from the recorded set of data is inconsequential. Overall, it required 1 minute for the injected sample to be detected by the HPLC system. Consequently, the suitability of the setup was corroborated by the theoretical plate values, tailing factor, retention time and a lack of deviation from the data set.

Purity

The branded sample was found to have a purity of 1.26 while the purity of the innovative sample was 1.19. The closer the purity value is to 1 the purer the sample. Since the purity of the innovative sample is closer to the ideal value, it can be deduced that it is of superior purity than the branded sample i.e. fewer impurities were co-eluted with the active pharmaceutical ingredient (API). The innovative sample was expected to display an inferior purity since it contains a botanically sourced excipient. Instead, the branded sample displayed an inferior purity. The ideal Gaussian peak shape (Figure 5.4) was not depicted by the innovative samples since tailing was observed (tailing factor of 1.52) showing some of lamivudine solutes being retained by the stationary phase.

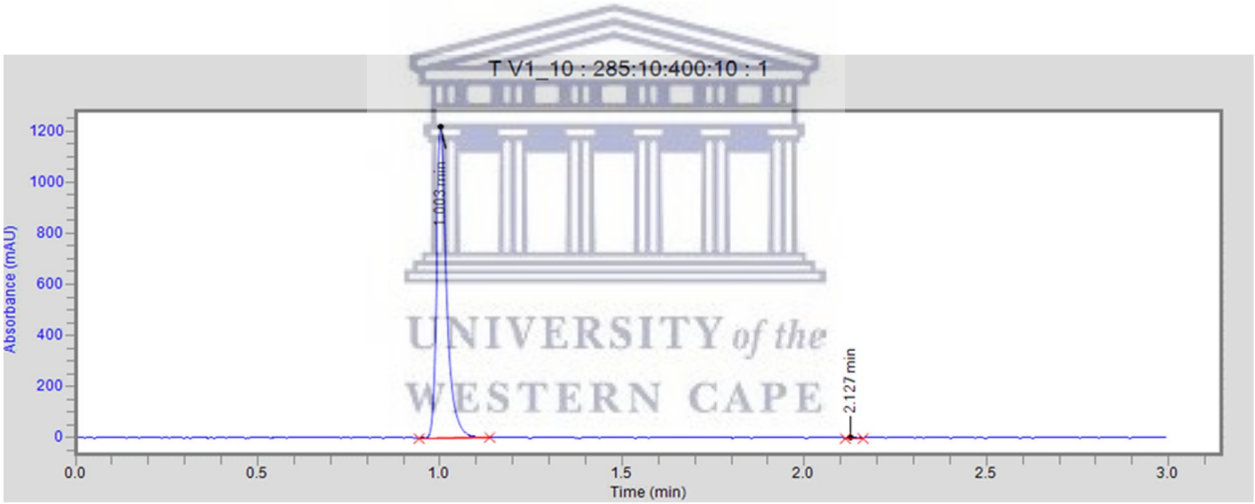

Figure 5.4: Non-ideal Gaussian peak shape for the innovative tablets with tailing factor of 1.52

The analysed peak of the branded tablets (Figure 5.5) also failed to depict the ideal Gaussian peak shape. Tailing of the peak was apparent where the tailing factor was found to be 1.44. The tailing factors of the innovative and branded samples are however acceptable since they did not exceed the maximum tailing factor of 1.5.

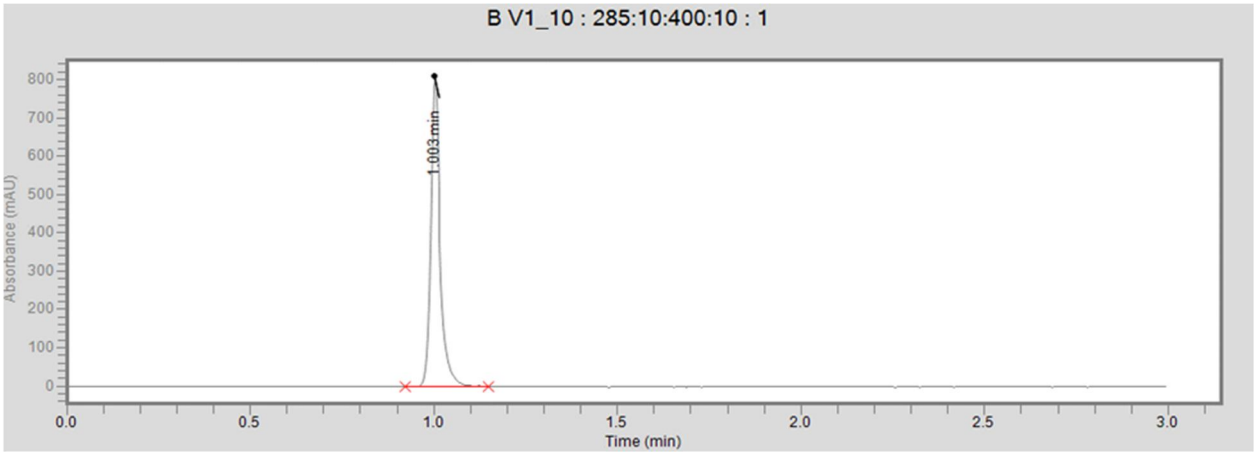

Figure 5.5: Non-ideal Gaussian peak shape for the branded tablets with tailing factor of 1.44

Tailing observed in both the innovative and branded lamivudine samples may be due to the utilisation of the C-18 column. In this column, pure hydrophobic retention mechanism is employed where half of the surface that constitutes silica is unbounded. The exposed silanol functional groups on the surface might have interacted with the samples. These functional groups are prone to interact with basic solutes such as lamivudine, hence prolonging the retention of these solutes on the stationary phase. Furthermore, the presence of trace metals in the silica matrix may have contributed to the tailing due to their ability to increase the acidity of the silanols, thereby enhancing interactions between silanols and lamivudine solutes.

5.1.2 Specificity

The impact inactive ingredients (excipients in both the innovative and branded samples) have on the assay of the API within the tablet formulation was assessed. The chromatograms revealed no interfering peaks within the region of lamivudine (Figures 5.2 and 5.3).

5.1.3 Accuracy

To determine the accuracy of a system, a minimum of nine determinations over a minimum of three concentration levels covering the specified range should be carried out. Due to unforeseen circumstances with regards to the apparatus used, the percentage of analyte recovered after spiking samples in a blank was unattainable. Consequently, attaining a reference and asserting the accuracy of the setup was not possible. Nevertheless, a mean recovery assay should be within the range of 100 +/- 5.0% at each concentration over the assessed range.

5.1.4 Precision

The precision of the method was determined by performing intra-day and inter-day studies, and the repeatability precision levels were evaluated. For intra-day studies, prepared samples were analysed in triplicate within the same day. For inter-day validation, concentrations were assessed within three separate days. The relative standard deviation percentage (% RSD) values obtained from the peak area of lamivudine were assessed and were found to be below 0.007 for all the six samples analysed (Table 5.4). This indicates that the method used was precise since a good repeatability was obtained.

Table 5.4 : Precision data for lamivudine standard concentrations

Lamivudine Con. (ug)	Mean absorbance	Standard deviation	RSD (%)
100	1918842.77	7320.51	0.004
150	2848465.44	18577.65	0.007
200	3665872.15	18678.00	0.005
250	4372885.94	36183.88	0.008
300	5034368.87	20294.57	0.004
350	5557221.95	19287.49	0.003
Total	23397657.11		

5.1.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and the Limit of Quantitation (LOQ) are parameters that are used to determine the smallest detectable concentration of an analyte. However, it is not a certainty with regards to stated experimental conditions. The most common techniques for estimating and determining detection and quantitation limit make use of the standard deviation of the blank, extrapolations from the calibration line at low concentrations, visual definition and signal-to-noise ratio. The signal-to-noise ratio method is carried out by measuring the peak-to-peak noise under the retention time. This measurement can be done by making use of an auto-integrator system of the apparatus, or by manually assessing the chromatogram.[1,2] The following equation is used to determine signal-to-noise values:

$$\mathbf{S/N} = \frac{\boldsymbol{H}}{\boldsymbol{h}}$$

H represents the signal peak height on the chromatogram generated by the analyte in solution. It is measured from the maximum of the peak to its baseline. The difference in height between the highest and lowest peak of the noise generated on the chromatogram is denoted by h.

Table 5.5: Limit of detection by signal-to-noise ratio

Injection No	H - Signal Height (mAU)	h - height of noise (mAU)	S/N Ratio
1	24,83	3,50	7,09
2	26,43	4.00	6,61
3	25,60	3,50	7,31

The mean LOD value was found to be 7,00 which is double the accepted value between 2 and 3. There are several factors that affect the LOD value generated from analysis of the chromatogram. However, these might not all be relevant to the experimental procedure conducted. The wavelength used in this analysis may not have been optimal based on the UV max of the analyte. The best wavelength for selectivity is not necessarily the most ideal wavelength for detector response since most organic compounds are known to have a strong absorbance around 200 nm. Consequently, the use of a wavelength less than the UV max may increase the response in some instances.[3] The S/N ratio could also be enhanced by increasing the amount of sample injected. Injection of samples may not always be complete and could be hindered due to air entrapment in vessels as well as inadequate closure of the HPLC vials.

5.1.6 Robustness

Robustness assesses the validity of a method by measuring how well the method withstands small changes made to the operating parameters. These parameters are changed one at the time and may include flow rate, wavelength and mobile phase composition.[1] No robustness test was conducted specifically to validate this method. The parameters that could have been varied to assess the robustness of this method include wavelength and mobile phase composition ratio. The innovative and branded samples were both assessed using wavelengths of 275 nm and 285 nm. Both samples had a mean retention time of one minute at both wavelengths.

Altering the mobile phase by changing the water to methanol ratio from 70:30 (%v/v) to 75:25 (%v/v) or 65:35 (%v/v) could have been effectuated to assess the robustness of this method

5.1.7 Linearity and range

Under the optimised conditions, a calibration curve was used for lamivudine. Six standard samples of different concentrations were prepared. The corrected peaks were used to construct the calibration curve and to determine linearity range, regression equation, correlation coefficient, slope, standard errors of slope and standard errors of intercept. The plot of absorbance of each sample against their respective lamivudine concentration (Figure 5.6) was found to be linear in the range of 100 ug/ml – 350 ug/ml, using regression analysis of the linear equation for lamivudine Y= mx + c with correlation coefficient of r^2= 0,99 Linearity data obtained from the measurements are given in Table 5.6.

Linearity data for Lamivudine

The linearity was determined by assessing different concentrations (100 ug/ml – 350 ug/ml) of the lamivudine standard solution. The calibration curve was constructed by plotting the concentration of standard samples against mean peak areas and the regression equation was computed. The parameters are shown in Table 5.6 below.

Table 5.6: Linearity data of lamivudine standards

No	Concentration (ug/ml)	Peak Area	Retention time
1	100	1918842.77	0.989
2	150	2858939.73	0.992
3	200	3676649.63	0.994
4	250	4353962.33	0.996
5	300	5023039.47	0.998
6	350	5548591.87	0.998

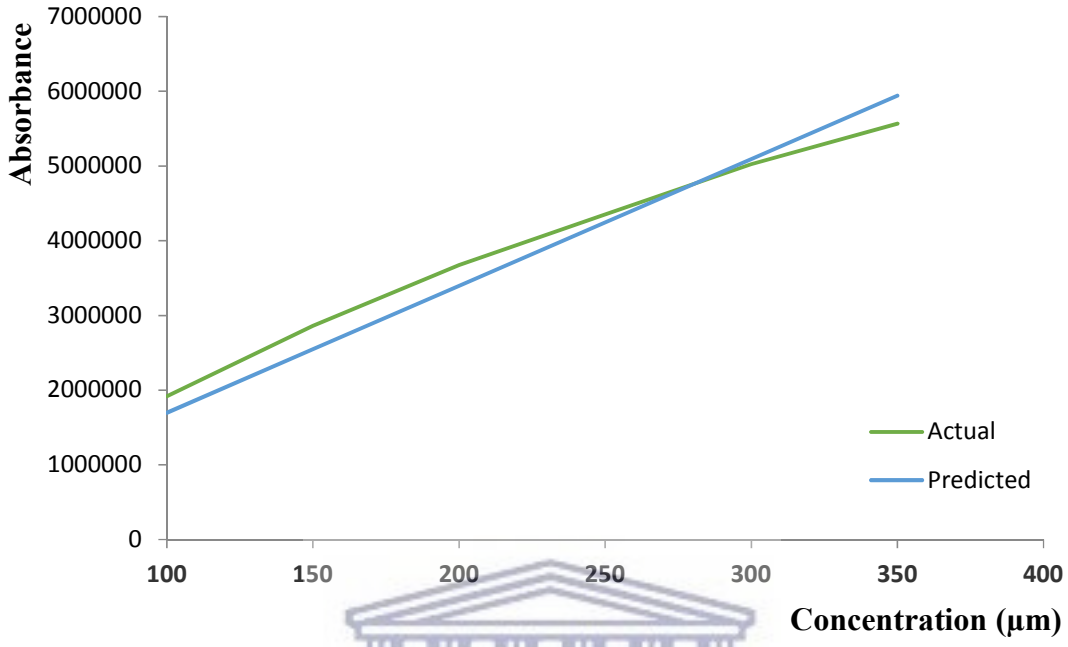

Figure 5.6: Regression curve of lamivudine standard solution

Table 5.7: ANOVA Statistical Analysis of lamivudine standard solution

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1E+14	1E+14	1308,986	3,48E-06
Residual	5	3,83E+11	7,65E+10		
Total	6	1,01E+14			

Where:

"df" - Are the degrees of freedom (df) associated with the sources of variance. The total variance has N-1 degrees of freedom. The df value corresponds to the number of estimated coefficients subtracted by 1. As the value of df rises, the likelihood of a significant test also rises. Hence the importance of sample size; the larger the sample size the easier it is to obtain a significant result.

"SS" – The sum of squares (SS) measures the total variation among the values.

"MS" - The Mean Squares (MS), is the SS divided by their respective df.

"F" - tests the overall significance of the regression curve. It tests the null hypothesis that all the coefficients are equal to zero. This tests the full data set against one with no variables and with the estimate of the dependent variable being the mean of the values of the dependent variable. The F value is the ratio of the mean regression SS divided by the mean error SS.

"Significance of F" - This indicates the probability that the Regression output could have been obtained by chance. A small Significance of F confirms the validity of the Regression output.

Table 5.8: Regression statistics of lamivudine standard solution

Regression Statistics	
Multiple R	0,995
R Square	0,999
Adjusted R Square	0,987
Standard Error	155343,27
Observations	6

Table 5.9: Standard curve parameters of lamivudine standard solution

	2	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	598379.15	178733.2	3.0	0.03	102136.3	1094622	102136.3	1094622
X Variable1	14646.30	742.68	19.72	3.9E-05	12584.28	16708.32	12584.28	16708.32

5.2 Dissolution

The validated HPLC method was used to analyse the drug release pattern of lamivudine from the branded and innovative tablets. The data obtained was used to plot a dissolution curve using extrapolated concentrations of the various aliquots withdrawn at the set time intervals (Figure 5.10). The release rate data was evaluated using different models namely Zero order, First order, Higuchi and Korsmeyer & Peppas function. Considering the fact that the drug release pattern of a conventional tablet (branded) was compared to that of a fast dispersible tablet (innovative), the purpose of this study was not to improve the drug release rate of the former, but instead to ascertain that the latter meets one of the key requirements of FDTs i.e. rapid dissolution.

The regression value of lamivudine was found to follow the Zero order release pattern. The assay showed that the innovative tablets attained complete dissolution within 10 minutes whilst the branded tablets attained complete dissolution within 30 minutes (Table 5.11). Both the branded and innovative tablets met the USP prerequisites for the dissolution of uncoated tablets. The USP stipulates that the time required for the complete dissolution of an assayed batch of uncoated tablets should not exceed 30 minutes.

The regression graph clearly depicts the direct proportionality between absorbance and lamivudine concentration. Higher concentrations of lamivudine yielded higher absorbance values and stronger peaks. The absorbance data of the branded samples clearly depicted this correlation as different absorbance values were obtained at the various time intervals. Conversely, the innovative samples had a narrow range of absorbance values due to the abrupt increase in concentration as a result of the complete release of the drug within 10 minutes. This abrupt release of the API from the innovative tablets can be attributed to the instantaneous disintegration of the tablets due to the presence of the superdisintegrant UBP.

Table 5.10: Dissolution rate profile of the branded and innovative tablets

Time (minutes)	Concentration (µM) Branded tablets	Concentration (µM) Innovative tablets
10	97.56	146.09
20	129.92	156.98
30	151.18	159.94
40	154.75	160.46
50	156.42	160.17
60	158.45	160. 02
Standard deviation	21.77	5.14

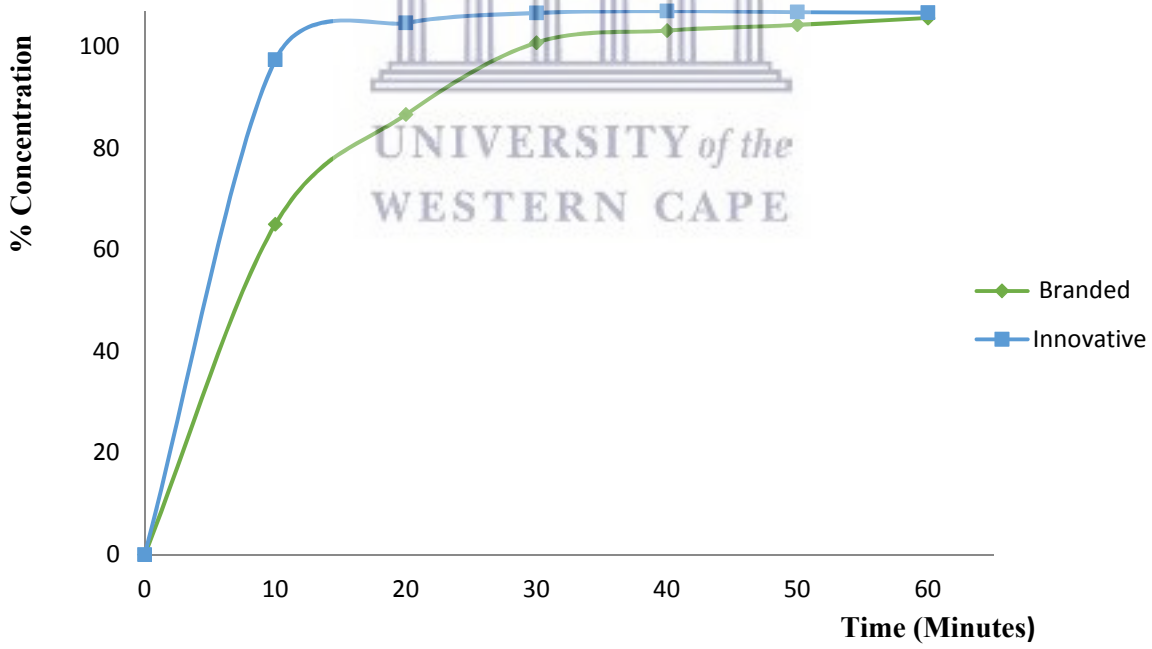

Figure 5.7: Dissolution curve of the branded and innovative tablets

The rapid dissolution of the innovative tablet was envisaged since it disintegrated in water instantly. As elucidated by Noyes-Whitney's equation, disintegration increases the surface area

of the solutes exposed to the medium which in turn increases the rate of dissolution as the API diffuses freely from the dissociated tablet matrix. It can thus be deduced that the rate and extent of disintegration is a reflection of the rate at which the API is released from the tablet matrix. The reverse is true for the branded tablet which had a lengthy disintegration and subsequently displayed a relatively slow dissolution rate. The two main advantages of rapid dissolution are quick onset of action and enhanced bioavailability. Quick onset of action implies that the therapeutic effect of the drug is initiated promptly since the drug is made available at the site of action swiftly while enhanced bioavailability indicates an increase in the amount of the drug available for absorption. While the former might not be very significant as far as antiretroviral therapy is concerned, the latter is a sought property for antiretroviral formulations since adequate amounts of drug absorbed into the body contribute to therapeutic success.

Drug release data can also be used by the formulator to assess the uniformity of the dosage form with respect to the amount of API present in each unit. This reflects the homogeneity of the powder blend and the accuracy with which the powder was weighed for each individual tablet. Substantial deviation from the set amount of API per dosage form unit poses the risk of underdosing or overdosing. While underdosing can lead to resistance and treatment failure, overdosing can lead to toxicity and unwanted effects of the drug especially for highly potent drugs and drugs with a narrow therapeutic index. Both the branded and innovative tablets attained 100 % drug release indicating the tablets assessed each contained the expected 150 mg of lamivudine.

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Chapter 6

Limitations and recommendations

This chapter highlights the main setbacks encountered in this project and suggests approaches that can be used to adequately optimise the formulated tablet into a marketable product.

6.1 Limitations

Several challenges were encountered throughout the completion of this project some of which were resolved in the process. For example, the issue of friability was overcome by making use of wet granulation. The nature of this project was self-limiting in that the parent powders displayed poor tableting properties with regards to flowability and compressibility. Also, the use of the natural polymers as multipurpose excipients in view of reducing the cost associated with formulation made it difficult to obtain a product of high integrity.

Another major setback was the tableting machine used for this project. A single punch manually operated tablet press was used to compress the innovative tablets (figure 6.1). This entailed that an adequate amount of powder had to be weighed accurately for each individual tablet. This process was lengthy and increased the risk of spillage, inaccurate powder measurement and demixing as a result of the powder blend standing still for a long period of time. These issues could affect the uniformity and integrity of the resultant tablets.

Figure 6.1: Tablet press used for compression

6.2 Recommendations

6.2.1 Using banana starch instead of unripe banana powder

Literature reports that unripe banana powder (UBP) possesses a myriad of phytochemicals and nutrients which are beneficial to the human body.[1] However, the presence of fibres, proteins, sugars, and other phytochemicals might adversely affect the flowability, compressibility and binding properties of the powder. Also, these phytochemicals offer a propitious environment for microorganisms to thrive since they serve as nutrients for bacteria and fungi. A high bioburden will thus affect the integrity and abridge the shelf life of the powder. Since starch accounts for more than 70% of UBP and is almost entirely responsible for the binding and disintegrating properties of the powder, it is well suited to make use of pure banana starch as an excipient rather than using the whole powder.[2] Starch can be extracted from banana powder by centrifugation or alkali extraction. The innovative tablets can be optimised by replacing banana powder with banana starch and comparatively assessing the two formulations.

6.2.2 Taste assessment and taste masking

Palatability plays a major role in ensuring adherence to a prescribed treatment for both paediatric and adult subjects. Tablets that are bitter or leave an unpleasant feel in the mouth are usually aversive to patients and are a major hindrance to treatment completion. Most drugs are intrinsically bitter and impart an unpleasant taste to the tablet into which they are formulated.[3] It is thus imperative to conduct a taste assessment on a newly formulated tablet especially for fast dispersible tablets given that they come into contact with the tongue for a substantial amount of time. Taste assessment can be carried out *in-vivo* by making use of healthy volunteers and *in-vitro* by making use of analytical gustatory tools such as the electronic tongue (E-tongue®). Although the former is an ideal and straight forward approach, it is not quite feasible at this level of research since it requires ethical approval and necessitates a large group

of volunteers to obtain sufficient data. An electronic tongue is an instrument designed to screen the taste attributes of oral formulations in a rapid timeframe. An electronic gustatory system is usually composed of varied taste sensors, a sample table, an amplifier and a computer for data recording. Taste sensors are capable of detecting the chemical compounds which are responsible for taste and elicit responses in a similar way human taste buds do (Figure 6.2).[4] It will be fitted to assess the innovative tablet with an electronic gustatory device to be cognisant of its palatability and be guided on how to improve its taste.

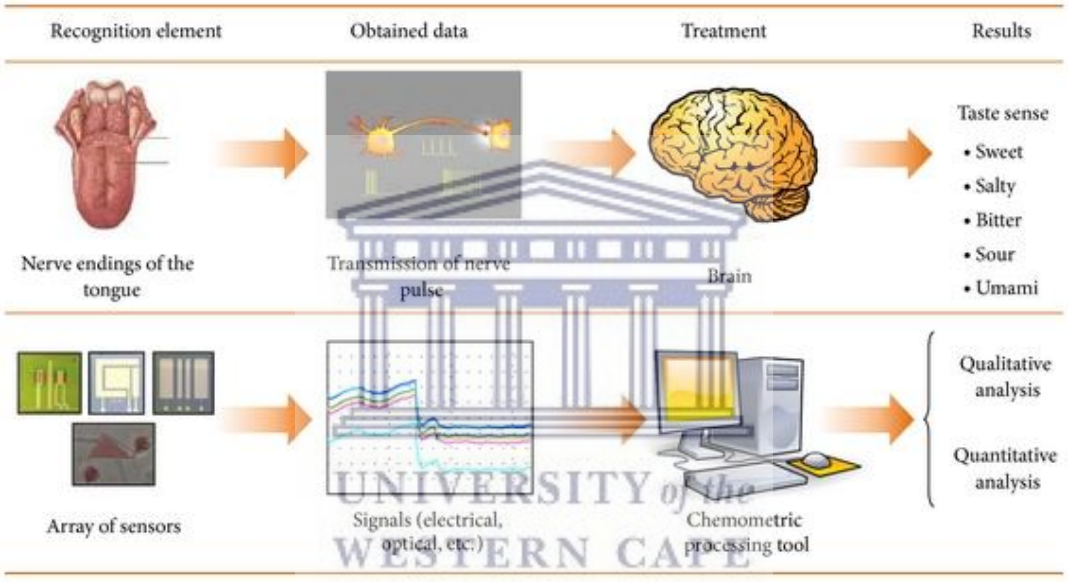

Figure 6.2: Comparison between the tongue and an electronic gustatory device

Coating, flavouring and taste-masking are the techniques commonly employed to camouflage or attenuate the unpleasant taste of drugs in tablets. Flavouring remains the most cost effective and straight forward technique consisting of incorporating a flavouring excipient into the powder blend prior to compression. Commonly used tablet flavouring agents in the pharmaceutical industry include banana, mint, strawberry and vanillin. Strawberry and vanillin are suitable flavouring agents for the innovative tablets since both flavours are tolerated by paediatrics as well as adult subjects.[5] Also, the addition of an excipient such as mannitol to

the innovative formulation could be beneficial. Mannitol is a multipurpose excipient which is commonly used in orodispersible tablets because of its pleasant mouth feel.[6]

6.2.3 Stability assessment of the innovative tablets

The purpose of stability testing is to provide evidence on how the quality of a drug or a dosage form varies with time under the influence of environmental factors such as light, humidity and temperature. This assessment enables the formulator to establish a shelf life for the product, recommend suitable packaging and storage conditions, and identify drug-excipient or excipient-excipient incompatibilities. Also, the information obtained from stability testing can be useful for the optimisation of the product.[7] It is thus imperative for the innovative tablets to be assessed for stability. An accelerated stability study as per the International Council for Harmonisation (ICH) guidelines can be conducted at 40 ºC/ 75RH over a period of 3 months. Tablets should be assessed for weight uniformity, hardness, friability, disintegration time, and dissolution at regular intervals and the data should be comparatively assessed. Thermal and spectroscopic tools should also be employed to detect any drug-excipient incompatibility and degradation.[8]

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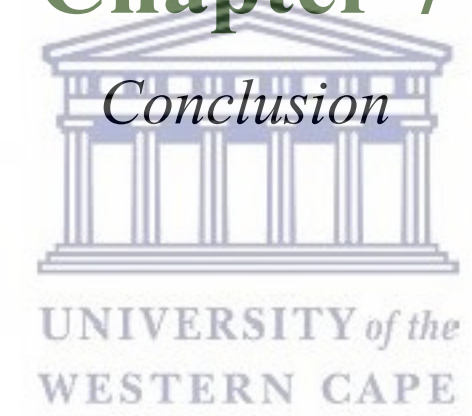
Chapter 7

Conclusion



Conclusion

The aim of this study which consisted in developing a cost effective fast dispersible tablet of lamivudine using alternative excipients and conventional techniques was successfully achieved. The innovative tablets displayed rapid disintegration (50 s in water, 35 s in 6.8 phosphate buffer solution) thus fulfilling the key requirement for fast dispersible tablets.

The natural polymers which were selected for this study were characterised and their ability to be used as multipurpose tablet excipients was assessed. Ispaghula husk powder albeit having a high swelling capacity, a sought characteristic for superdisintegrants, displayed poor binding properties and a narrow range of superdisintegrating activity since a drastic reduction in its disintegrating effect was observed at concentrations above 10 % w/w as a result of the formation of a gelatinous mass. This gelatinous mass impeded disintegration by serving as a barrier to the penetration of water into the tablet matrix. This suggests that Ispaghula husk powder may not be used as a multipurpose excipient in a tablet formulation but rather as a superdisintegrant at low concentrations amid other excipients serving as binders and fillers.

Unripe banana powder on the other hand displayed satisfactory disintegrating as well as binding properties. Although the disintegrating effect of unripe banana powder decreased with increasing concentration of the natural polymer subsequent to reaching its saturation point, an increase in its binding effect was observed and the final product nevertheless disintegrated rapidly. Consequently, it can be deduced that unripe banana powder can serve as a multipurpose excipient and be used at concentrations above 10% w/w without a substantial decrease in its super disintegrating effect or the risk of gel formation.

Ispaghula husk powder and unripe banana powder were found to be compatible with the active pharmaceutical ingredient lamivudine since no incongruity was observed on the DSC curves and the FTIR spectra of the 1:1 drug-excipient mixtures. However, stability studies under

accelerated conditions alongside antiviral testing should be undertaken to ascertain whether the natural polymers do not adversely impact the integrity and the therapeutic activity of lamivudine throughout the shelf life of the innovative tablets.

Lamivudine powder which was found to have poor material properties with regards to flowability and compressibility was successfully compressed with unripe banana powder and magnesium stearate using direct compression. However, the resultant tablets were highly friable and unevenly compressed. Wet granulation was shown to drastically reduce the friability of the innovative tablets whilst allowing the tablets to disintegrate rapidly. The innovative tablets displayed rapid disintegration and rapid dissolution as opposed to the branded tablets. This suggests that natural polymers with super disintegrating properties such as unripe banana powder are effective at reducing the disintegration time and dissolution time of tablets.

A HPLC method suitable for the assay of lamivudine in a tablet formulated with a natural polymer was validated and was successfully used for the drug release analysis of the innovative and branded tablets. The purity and absorbance of lamivudine were unaltered by the presence of unripe banana as revealed by the HPLC data.

Despite the numerous challenges encountered, the objectives of this study were met. With adequate optimisation, the innovative tablets can be introduced into the pharmaceutical market. This tablet is socioeconomically sound in that it is convenient, patient friendly and cost effective.