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Development and in vitro evaluation of immediate- and sustained release Rooibos (*Aspalathus linearis*) preparations

By

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DECLARATION

I, Johan Henry Neethling, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

6 November 2018

Signed **Date**

Abstract

Rooibos or rooibos tea is an herbal tea made dried leaves and stalks of the *Aspalathus linearis* plant that is endemic to the Cederberg Mountains in the Western Cape Province of South Africa. Rooibos is rich in polyphenols, caffeine free and low in tannins when compare to *Camellia sinensis* teas (Joubert *et al.*, 2008). Many health benefits have been associated with Rooibos mainly due to the polyphenol content. These include the modulation of the bodies redox status (Kucharská *et al.*, 2004), lipid oxidation (Marnewick *et al.*, 2011), cancer prevention in different cancer models including skin cancer (Marnewick *et al.*, 2005) and oesophageal cancer (Sissing *et al.*, 2011) as well as in *in vitro* assays (Baba *et al.*, 2009; Marnewick *et al.*, 2000; Snijman *et al.*, 2007), a reduction of risk factors for cardiovascular health (Marnewick *et al.*, 2011; Persson *et al.*, 2010),diabetes, inflammation reduction and modulation of metabolic processes (Beltrán-Debón *et al.*, 2011; Marnewick *et al.*, 2012; Schloms *et al.*, 2012).

Different methodologies are used to determine the *in vitro* anti-oxidant capacity of Rooibos. The Folin-Ciocalteu assay is used to determine the total polyphenols. The ferric reduction/antioxidant power assay (FRAP) and the Trolox equivalent antioxidant capacity assay (TEAC) measure the electron transfer reactions and the oxygen radical antioxidant capacity assay (ORAC) are used to measure the hydrogen transfer reactions. It has been determined that rooibos has a low bioavailability. The absorption of the Rooibos polyphenols is low (Del Rio *et al.*, 2013) and it is rapidly eliminated from the systemic circulation (Parisi *et al.*, 2014; Sissing *et al.*, 2011). The poor bioavailability and the rapid elimination of Rooibos require that Rooibos should be consumed regularly during the day for optimal health benifits.

Controlled release dosage forms can be used to maintain a stable drug concentration in the systemic circulation (Alderblom, 2007; Anal, 2007). The mini-tablet-in-capsule system is a versatile drug delivery system where 6 mm tablets are packed into size zero hard gelatine capsules. It allows for mini-tablets with

different release profiles to be packed into the gelatine capsule to manipulate the dissolution profile i.e. a loading dose and a maintenance dose.(Li and Zhu, 2004).

Different fillers were evaluated for both the immediate release and sustained release mini-tablets. Powder flow tests were performed on the excipients and the Rooibos extract to ensure reproducible tablet production. The physical properties of the mini-tablets were evaluated to ensure the tablets would fit into the gelatine capsules and would withstand the attrition during the manufacturing and packaging processes. The Rooibos extract and the mini-tablets were analysed the polyphenol content and antioxidant properties. The mini-tablet-in-capsules were subjected to a six month stability test.

This study designed and produced a mini-tablet-in-capsule system that that delivered the 400 mg Rooibos polyphenols in 8 hr in an almost linear fashion. The dissolution studies revealed the dissolution followed the Korsmeyer-Peppas model. The equation was $F=0.4826 \cdot t^{0.8572}$. The preliminary stability test showed the mini-tablet-in-capsule system was stable and should have at least an 24 month stability.

The photograph above shows the mini-tablet-in-capsule system with ten mini-tablets filled into a size zero hard gelatine capsule. Two mini-tablets were coated white to represent the two slow release mini-tablets.

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I wish to thank:

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DEDICATION

I wish to dedicate this dissertation to my parents, my wife and children.

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

The past decade has shown an exponential increase in the number of research studies reporting on the health benefits of the South African indigenous herbal tea, Rooibos (also referred to as Red Bush in other countries). However, when orally consumed some of the active ingredients of Rooibos (e.g., phenolic compounds) have relatively short plasma half-lives due to rapid elimination. A need therefore exists to develop a sustained release dosage form that is capable of releasing the phytochemical components of Rooibos over an extended period of time after oral administration in order to prolong the proposed health benefits to the consumer after a single dose. The preparation of a matrix-type tablet with modified release properties that contain a powdered Rooibos extract requires the correct formulation design. This study focused on the development of a sustained release Rooibos formulation in the form of a mini-tablet-in-capsule delivery system. In this innovative delivery system, certain mini-tablets were intended to provide immediate release (i.e. the loading dose) and the other mini-tablets were intended to provide sustained release (i.e. the maintenance dose) of the active constituents of the Rooibos extract after oral administration. The physical properties and dissolution profiles of different formulations of mini-tablet-in-capsule delivery systems containing rooibos extract were measured to identify the most suitable formulation for sustained release over a period of 8 h.

1.1.1 *Aspalathus linearis* (Rooibos)

Rooibos tea is the name of an herbal tea made from the leaves and stalks of *Aspalathus linearis* (Burm.f.) Dahlg.; family: Fabaceae; tribe: Crotalarieae, a plant that is endemic to the Cederberg Mountain region of the Western Cape, South Africa. Rooibos is rich in phenolic compounds and the only source of aspalathin, also the main phenolic constituent, which is oxidized to flavanones (namely

dihydro-iso-orientin and dihydro-orientin) during fermentation. These phenolic compounds are mainly responsible for the antioxidant properties of Rooibos (Joubert *et al.*, 2008; Koeppen and Roux, 1965; Marnewick, 2009).

Epidemiological studies have shown a link between the consumption of polyphenol rich foods and their health benefits, which includes the reduction in the risks associated with the development of cardiovascular disease, specific cancers and neurodegenerative diseases (Del Rio *et al.*, 2013). A number of *in vitro*, *in vivo* and human intervention studies on Rooibos have found similar health benefits including risk reduction for cardiovascular disease and cancer modulating properties (Marnewick, 2009).

Results from previous studies suggested that the Rooibos polyphenols are absorbed in the small intestine after oral administration, but does not collect in the bloodstream for long periods due to relatively rapid excretion via the renal system (Courts and Williamson, 2009; Stalmach *et al.*, 2009). Due to this relatively rapid excretion, it was advised that six cups of Rooibos should be taken at regular intervals during the day to exert its optimal health benefits (Marnewick *et al.*, 2011). Commercial products, such as tablets and capsules containing Rooibos extracts are available on the market, but no information regarding release of the active ingredient(s) (e.g., rate and extent) from these formulations have been reported. Dissolution data of these type of delivery systems is very important to prove the pharmaceutical availability of the active ingredients (Glube *et al.*, 2013) in order to predict levels in the blood stream to produce the intended pharmacological effect. When incorporating phytochemicals into solid oral dosage forms, it is essential that the correct formulation is designed to ensure an optimal rate of release of the active ingredient(s) in order to optimise the health benefits derived from the product.

1.1.2 Formulation of dosage forms

1.1.2.1 The function of dosage forms

Pharmacologically active compounds (for this study “drug” refers to the active ingredient(s) in Rooibos) are almost never administered in their pure form, but are

usually formulated in dosage forms that consist of both the active ingredient and adjuvants referred to as excipients (Hamman and Steenekamp, 2012; Hamman and Tarirai, 2006). Conventional dosage forms such as immediate release tablets were initially formulated to provide active pharmaceutical ingredients in sufficiently high concentrations in the areas where absorption can take place to result in therapeutic blood levels. The active ingredients are therefore released as quickly as possible after administration in the gastrointestinal track from where it is absorbed into the systemic circulation (Mounika *et al.*, 2015). However, any active ingredient absorbed into the systemic circulation is eliminated by means of metabolism and renal excretion and therefore its concentration will decrease to below therapeutic levels if another dose is not administered. Rapid elimination of any compound results in a short half-life of that compound. This also causes a relatively large fluctuation in the compound's concentration in systemic circulation between consecutive doses (Rowe, 2012).

1.1.2.2 Modified release dosage forms

Commonly employed solid oral modified release dosage forms include the following (Collet and Moreton, 2007):

- Matrix systems;
- Reservoir or membrane-controlled systems;
- Osmotic pump systems; and
- Gastric retentive systems.

Matrix systems can be divided into soluble (hydrophilic) and insoluble (hydrophobic) systems (Collet and Moreton, 2007). In soluble systems, the active ingredient(s) is released as the matrix is hydrating, which may result in swelling of the matrix and/or dissolution of the matrix. Soluble matrices can easily be manipulated by the addition of different excipients and/or the variation of particle size of the excipients to obtain a satisfactory release rate of the active ingredient(s). Insoluble matrices require the solvent to enter the matrix in order to dissolve the active component(s), which is released by capillary diffusion through pores, or due to erosion of the matrix. These insoluble matrices are easy to

manufacture, but the release of the active ingredient usually does not follow zero order kinetics and it can be difficult to control or regulate the drug release (Collet and Moreton, 2007).

Membrane-controlled systems comprise of tablets, granules or beads that are coated with a polymer film, which forms a surface membrane that controls the release of active ingredient(s) from the core. Solvent enters the core by diffusion through the membrane and dissolves the active ingredient(s). The dissolved active ingredient(s) moves out of the dosage form through the membrane by means of diffusion (Collet and Moreton, 2007).

Osmotic pump systems also make use of a membrane to control the release of the active ingredient(s). The active compound, mixed with a water soluble matrix, forms the core of the system. The semi-permeable membrane surrounding the core allows water to diffuse into the core to dissolve the soluble matrix. This results in an osmotic pressure build-up inside the membrane. The dissolved active(s) and matrix are then forced out through a small hole or orifice made in the membrane by means of laser drilling. The main benefit of this system is that the release can be controlled to give a zero order release profile that is not affected by the type of drug or the drug concentration. The disadvantages are that the coating must be free of any defects and the size of the laser-drilled hole must be accurate, resulting in a high cost of manufacture (Collet and Moreton, 2007).

Gastric retentive systems are designed to remain in the stomach, while releasing the active ingredient(s) slowly over an extended period of time. This can be accomplished by manipulating the size of the dosage form (e.g., a matrix that rapidly swells) or by using a floating dosage form. Gastric retentive systems cannot be used for compounds that are sensitive to the acid conditions in the stomach (Collet and Moreton, 2007).

1.1.2.3 Single-unit vs. multiple-unit dosage forms

Single-unit dosage forms consist of a single unit containing the complete dose of the active ingredient, usually a tablet or a capsule. Multiple-unit dosage forms

comprise of more than one sub-unit, each containing a portion of the dose. Examples of multiple-unit dosage forms include granules, beads, pellets, microspheres or mini-tablets, which are filled into hard gelatin capsules or sachets or can be compressed into tablets. The drug content of all the sub-units amounts to the total dose of the dosage form (Collet and Moreton, 2007; Mounika *et al.*, 2015; Solanki *et al.*, 2012). Hard gelatin capsules present a convenient packaging format for the sub-units of a multiple-unit dosage form. When the hard gelatin capsules are filled with the sub-units, it results in a more accurate and consistent dosing of the multiple-units. When this multiple-unit dosage form is administered via the oral route, the capsule shell dissolves in the stomach and releases the sub-units. Mini-tablet-in-capsule systems are considered novel dosage forms that merge the benefits of traditional tablet production with the benefits of multiple-unit dosage forms (Ishida *et al.*, 2008).

There are many advantages for multiple-unit dosage forms when compared to single-unit dosage forms (Solanki *et al.*, 2012):

- The risk of dose dumping or systemic toxicity is decreased substantially due to multiple-units each containing only a portion of the total dose.
- Drugs that are not chemically compatible in one dosage form, such as a tablet, can be included as different sub-units in a capsule.
- Drug release can easily be adjusted by utilizing different subunits with different compositions.
- Gastric emptying is more predictable due to the dispersion of the subunits in the gastrointestinal tract.
- The risk of local irritation is reduced due to even spreading of the sub-units over a relatively large area of the gastrointestinal tract.
- A decrease in the dosing frequency is possible when drug release is modified.
- An accurate dosing can be ensured by incorporating the sub-units into accurately filled capsules.
- Increased bioavailability.

- The increased surface area makes it ideal for surface coating to control the drug release.

Multiple-unit dosage forms also have some disadvantages, namely (Solanki *et al.*, 2012):

- The manufacture process and packaging (e.g., capsule filling) can be more complicated.
- The production cost is higher in some instances.
- The filling of the gelatin capsules is complicated further when different sub-units are used.
- An increase in the cost of surface coating agents due to a larger surface area.

1.1.2.4 The mini-tablet-in-capsule system as a specialized dosage form

Mini-tablet-in-capsule dosage forms are multiple-unit drug delivery systems where a number of mini-tablets are filled into hard gelatin capsules. This delivery system has many advantages over single-unit dosage forms as well as other multiple-unit dosage forms (Li and Zhu, 2004; Mounika *et al.*, 2015), which include the following:

- Mini-tablets with uniform size and shape as well as a smooth surface can be produced with relative ease by means of traditional tablet manufacturing processes.
- The tablets can easily be film-coated with polymers to further control the drug release rate.
- The drug dose and release profiles can easily be adjusted using different excipients.
- A lower degree of inter- and intra-subject bioavailability variation due to a more even dispersion of the units (i.e. the mini-tablets) in the gastrointestinal tract.
- The risk of dose dumping is decreased due to multiple-units each containing only a portion of the total dose.

- It is relatively easy to adjust the loading dose and maintenance dose of this dosage form.

1.2 Research Problem

The health benefits of Rooibos are well known, but several cups of this herbal tea need to be consumed at regular intervals (e.g., six cups evenly spread during a day) for optimum effects. This is mainly because the active ingredients (i.e. phenolic constituents) of Rooibos are relatively rapidly eliminated from the body. Because of this rapid elimination, the body's exposure to the beneficial components of Rooibos is short-lived and this may limit the proposed health benefits if it is not consumed regularly during the course of a day.

One way to overcome this problem is by designing a modified release dosage form that releases the active component(s) of Rooibos over an extended period of time. The research problem that was investigated in this study was therefore to identify and develop a suitable modified release, solid oral dosage form for a Rooibos extract that contains the beneficial phenolic constituents that are associated with this herbal tea.

1.3 Aim

The aim of this study was to develop a modified release, multiple-unit dosage form (i.e. a mini-tablet-in-capsule system) for a standardized powdered Rooibos extract that provides immediate release of a loading dose and sustained release of a maintenance dose over an extended period of time.

1.4 Study objectives

The following objectives were set for the study to address the main research aim:

- To evaluate a Rooibos extract by means of suitable analytical techniques in order to quantitatively determine its main phenolic composition.

- To develop a mini-tablet-in-capsule system containing 400 mg Rooibos extract, which is capable of releasing a loading dose (80 mg) rapidly, and a maintenance dose (320 mg) over a period of 8 h.
- To produce mini-tablets with acceptable physical as well as dissolution properties by using different excipients at different concentrations (e.g., fillers, disintegrants and lubricants).
- To evaluate the powder flow properties of the extract, excipients and the tablet mixture in order to confirm acceptable powder flow for mini-tablet production.
- To evaluate the physical and dissolution properties of the mini-tablet-in-capsule systems.

1.5 Dissertation layout

Chapter 2 includes an overview of the latest literature to sketch a detailed background of the properties and potential health benefits of Rooibos and polyphenol rich foods as well as the production, testing and properties of different dosage forms. The materials used and a detailed methodology are discussed in Chapter 3. Chapter 4 contains all the results obtained and the discussion of these results. The conclusions made from the results obtained and areas that require further study are discussed in Chapter 5.

CHAPTER 2: LITRATURE REVIEW

2.1 Rooibos

Rooibos, or Rooibos tea as it is also referred to, is an herbal tea enjoyed by many as a naturally caffeine free alternative to black tea. Rooibos is made by infusing the leaves and stalks of the plant *Aspalathus linearis* (Burm.f.) Dahlg.; family: *Fabaceae*; tribe: *Crotalarieae*. This indigenous plant is endemic to the Cederberg Mountain range in the Western Cape Province of South Africa. Rooibos is a rich source of a unique composition of phenolic compounds with the main phenolic compound being aspalathin. In fact, the compound aspalathin is unique to Rooibos. It is oxidized to the flavanones, dihydro-iso-orientin and dihydro-orientin, when fermented during the manufacturing process (Joubert *et al.*, 2008; Koeppen and Roux, 1965; Marnewick, 2009).

The leaves and stalks used to prepare Rooibos are cut into 3-4 mm lengths, crushed and allowed to be fermented by enzymes in the leaves before being dried in the sun (Bramati *et al.*, 2002). The fermentation process is responsible for the development of the characteristic colour, aroma and flavour (Joubert and Schultz, 2012; Marnewick, 2009; McKay and Blumberg, 2007).

2.1.1 Chemical composition of Rooibos

Rooibos is rich in phenolic compounds, contains no alkaloids such as caffeine and contains only low amounts of tannins when compared to *Camellia sinensis* teas (Joubert *et al.*, 2008). Bramati *et al.* (2002) reported the presence of phenolic compounds, in particular flavonoids, in Rooibos as determined by high performance liquid chromatography (HPLC) and mass spectroscopy (Table 1).

Table 1: Phenolic compounds detected in fermented Rooibos (Bramati *et al.*, 2002)

Phenolic sub-groups	Chemical compound
Flavonols	Quercetin Isoquercitrin Rutin
Aglycones	Luteolin Chrysoeriol
Dihydrochalcones	Aspalathin Nothofagin
Flavones	Orientin Isoorientin Vitexin Isovitexin
Phenolic acids	Caffeic acid Ferulic acid p-Coumaric acid p-Hydroxybenzoic acid Vanillic acid Protocatechuic acid Syringic acid

Flavonoids are the largest sub-group of phenolic compounds commonly found in many plants and can be divided into sub-groups. The polyphenol sub-groups listed in Table 1 form part of the flavonoid sub-group. The basic skeleton from which these subgroups are derived consist of a 3-ring structure as can be seen in Figure 1. This figure also shows the sub-groups with two compounds as examples of each these subgroups (Panche *et al.*, 2016).

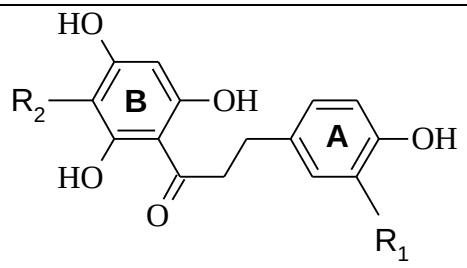
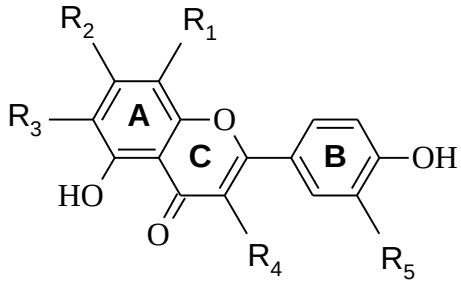
<table>
<tr><td> 
</td><td>Aspalathin</td><td>R1 = OH, R2 = C-β-D-glucosyl</td></tr>
<tr><td></td><td>Nothofagin</td><td>R1 = H, R2 = C-β-D-glucosyl</td></tr>
<tr><td rowspan="9"> 
</td><td>Orientin</td><td>R1= C-β-D-glucosyl, R2 = OH, R3 = H,
R4 = OH, R5 = H</td></tr>
<tr><td>Isoorientin</td><td>R1= H, R2 = OH, R3 = C-β-D-glucosyl,
R4 = H, R5 = OH</td></tr>
<tr><td>Vitexin</td><td>R1= C-β-D-glucosyl, R2 = OH, R3 = H,
R4 = H, R5 = H</td></tr>
<tr><td>Isovetexin</td><td>R1= H, R2 = OH, R3 = C-β-D-glucosyl,
R4 = H, R5 = H</td></tr>
<tr><td>Luteolin</td><td>R1 = H , R2 = OH, R3 = H, R4 = H,
R5 = OH</td></tr>
<tr><td>Chrysoeriol</td><td>R1 = H , R2 = OH, R3 = H, R4 = H,
R5 = OCH3</td></tr>
<tr><td>Quercetin</td><td>R1 = H , R2 = OH, R3 = H, R4 = OH,
R5 = OH</td></tr>
<tr><td>Isoquercitrin</td><td>R1 = H , R2 = OH, R3 = H, R4 = O-β-D-glucosyl, R5 = OH</td></tr>
<tr><td>Rutin</td><td>R1 = H, R2 = OH, R3 = H,
R4 = O-β-D-rutinosyl, R5 = OH</td></tr>
</table>

Figure 1: The basic structures of the flavonoids in the Rooibos extract (Joubert *et al.*, 2012). The second structure is a composite structure derived from more than one structure.

These compounds are known to have strong anti-oxidant properties, and associated health benefits (Panche *et al.*, 2016). The presence of these compounds in Rooibos may explain the bio-activity and health benefits of Rooibos (Breiter *et al.*, 2011; McKay and Blumberg, 2007). Flavonoids can be divided into different categories based on which carbon atom in the C ring is attached to the B ring and the amount of saturation and oxidation present. Isoflavones has the B ring linked to carbon 3 and neoflavonoids are linked to carbon 4. Those with the B ring attached to carbon 2 can be sub-divided into sub-groups. These sub-groups include flavones where a double bond is present between carbon 2 and 3 in the C

ring, flavonols where a double bond is present between carbon 2 and 3 and a hydroxyl group on carbon 3 is present in the C ring (Panche *et al.*, 2016).

Even though Rooibos contains many phenolic compounds, in this study we will focus on the polyphenol content as the total polyphenol assay was used to quantify the phenolic compounds. Polyphenols are metabolised in the intestines by means of type I metabolism and in the liver and kidneys by type II metabolism. The metabolism in the kidneys can explain why some metabolites are found in high concentrations in the urine but not in the serum. The metabolite that is formed by metabolism may not have the same properties *in vivo* as the parent compound before metabolism (Maqueda, 2012).

Besides the phenolic compounds Rooibos was also found to contain the following trace elements: Cu, Mn, Ca, Fe, K, Mg, Na, P (PO_4^{3-}) and Zn (McKay and Blumberg, 2007). However, concentrations of the trace elements are mostly below 1% of the recommended daily intake with the exception of Cu, Mn and Fe that are 7.8%, 5.5–7.3% and 1.7–2.2%, respectively.

Rooibos has also been found to contain very low concentrations of catechin, procyanidin B3 and profistinidin triflavanoid which confirms the low tannin content claim (Bramati *et al.*, 2002). Aspalathin (2´,3,4,4´,6´-pentahydroxy-3´-C-β-D-glucopyranosylhydrochalcone) is a unique phenolic compound only found in Rooibos as well as the main phenolic compound. It is classified as a dihydrochalcone C-glucoside (de Beer *et al.*, 2015). The structure of aspalathin is shown in Figure 2.

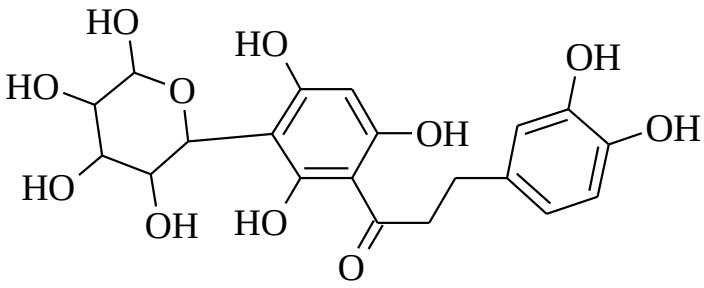


Figure 2: The structural formula for aspalathin (Jaganyi and Wheeler, 2003)

Aspalathin is found in relatively high concentrations in green- or unfermented Rooibos, but the concentration decrease during the fermentation process used to produce the fermented Rooibos that is normally consumed. The concentration of aspalathin in the unfermented Rooibos has been reported to be 14.65 g/kg in the dry tea leaves resulting in 77.3 g/kg soluble solids in the prepared tea. After fermentation the concentration decreases to 1.02 g/kg in the dry tea (Joubert, 1996) and between 3.49 and 8.31 g/kg in the soluble solids (Joubert *et al.*, 2008).

2.1.2 Health benefits

Nutraceuticals can be defined as functional foods used in the prevention and/or treatment of a disease or disorder (Kalra, 2003). Rooibos may be described as a nutraceutical if the health benefits are considered as discussed below in this section. Many of the health benefits of Rooibos were initially based on anecdotal evidence (Marnewick *et al.*, 2011). Since the early 1990's, many research studies have been performed to confirm some of these health benefits (Joubert *et al.*, 2008). Many of the claimed health benefits can be attributed to the polyphenols in Rooibos. Contrary to other dietary components such as vitamins, polyphenols are not essential for the short-term wellbeing of humans. However, evidence suggests ingestion of polyphenols can be associated with long-term favourable effects (Del Rio *et al.*, 2013). In a clinical study by Marnewick *et al* (2011), it was shown that the consumption of six cups of Rooibos over a six week period did not cause any of the liver or kidney markers to move outside their normal reference ranges, which indicated that the consumption of this quantity (i.e. six cups) of Rooibos a day can be regarded as safe.

However, it should be noted that individual cases of possible adverse effects caused by consuming excessive amounts of Rooibos by diseased or ill individuals has been reported in literature (Engels *et al.*, 2013; Reddy *et al.*, 2016; Sinisalo *et al.*, 2010). All cases reported elevation of liver functions that was reversed upon the discontinuation of Rooibos. This again stresses that 'more' does not equate to 'better' for you and that the consumer should pay attention to the manufacturer's recommendations.

The biological properties of polyphenols have been studied extensively. These characteristics include anti-oxidant, anti-inflammatory, cardioprotective and neuroprotective properties. Polyphenols are also claimed to constrain bacterial, fungal and viral infections and the development of tumours. Polyphenols are known to interact with proteins such as enzymes, tissue proteins and cell membrane receptors. These properties of polyphenols are the reason why it is suggested that they should be included in some pharmaceutical and biomedical products, for example, as additives for the protection against risk factors. Polyphenols can also be included in nutraceuticals or as food supplements or in cosmetics for their anti-aging properties (Parisi *et al.*, 2014).

2.1.2.1 Redox status

A number of studies have shown Rooibos to modulate the redox status *in vivo*, with, in certain cases, an accompanying reduction in oxidative damage to lipids. One of the earliest studies reported Rooibos to suppress the increase in lipid peroxidation in the brains of aging rats over a 21 month period. Suggesting that the flavonoid compounds in Rooibos crossed the blood brain barrier and acted as free radical scavengers in the brain (Inanami *et al.*, 1995). Another study reported to improve the redox status and reduced liver damage in rats caused by trichloromethyl and trichloromethylperoxyl radicals after exposure to carbon tetrachloride for 10 weeks. Lipid peroxidation was also reduced in these rats (Kucharská *et al.*, 2004). A clinical study performed by Marnewick *et al*. (2011) showed a significant decrease in marker of lipid oxidation (conjugated dienes and malondialdehydes) in the subjects when consuming Rooibos (six cups daily for a six week period). This study also had a significant improvement in the redox status of the glutathione as seen in the increase in the glutathione:glutathione disulphide (GSH:GSSG) ratio, an indication that Rooibos improved the redox status of the participants. Numerous studies have reported similar results (Hong *et al.*, 2014; Marnewick *et al.*, 2003; Marnewick *et al.*, 2011; Pantsi *et al.*, 2011).

2.1.2.2 Cancer prevention

Various extracts and purified phenolic compounds of Rooibos were investigated for their *in vitro* anti-mutagenic properties using the *Salmonella typhimurium* mutagenicity assay using TA98 and TA100 test strains with 2-acetylaminofluorene and aflatoxin B_1 as mutagens and (−)epigallocatechin gallate (EGCG) as a positive control. The individual phenolic compounds of Rooibos had varied anti-mutagenic responses with luteolin giving the highest response similar to that of EGCG (Snijman *et al.*, 2007; van der Merwe *et al.*, 2006), while aqueous extracts showed significant antimutagenic activity against the mentioned mutagens with unfermented Rooibos (Marnewick *et al.*, 2000). Baba *et al.* (2009) showed that Rooibos protected against DNA damage caused by oxidative stress in rats with dextran sodium sulphate induced colitis. Rooibos has also been reported to potentially protect against ionizing radiation. It was shown to suppress 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induced oncogenic transformation of mouse C3H10T12 cells. In addition it has also been shown that Rooibos can protect against the carcinogens in tobacco smoke (Gelderblom *et al.*, 2017).

Fumonisin are mycotoxins produced mainly by two fungi namely *Fusarium verticilioides* and *Fusarium proliferatum*. It has been reported that they contribute to the development of oesophageal cancer in humans. This was first confirmed in the Transkei region of the Eastern Cape where maze contaminated with fumonisin was consumed and a high rate of oesophageal cancer is prevalent. It is suggested that Rooibos may protect against these mycotoxins (Sissing *et al.*, 2011; Waśkiewicz *et al.*, 2012). It has also been reported that Rooibos can protect against fumonisin B1 induced liver cancer. This could in part be due to the reduction in oxidative stress and the induction of apoptosis in the precancerous lesions (Marnewick *et al.*, 2009).

Topical application of Rooibos have also been shown to decrease tumor genesis of mouse skin cancer initiated with the carcinogen, 7,12-dimethylbenz(a)anthracene, by reversing tumor promotion. This could be due to

the anti-oxidant activity of the Rooibos polyphenols and the disruption of the metabolism of the carcinogen (Marnewick *et al.*, 2005).

2.1.2.3 Cardiovascular health

A study using experimental rats has shown that the oral intake of Rooibos reduced cardiac injuries resulting from induced-ischaemia and increased aortic output after ischaemia. This was proposed due to the polyphenolic anti-oxidant present in Rooibos affecting the heart muscle and inducing vasodilatory effects (Pantsi *et al.*, 2011). In a clinical study, Rooibos caused a significant decrease in oxidative damage to lipids and a significant, favourable improvement of the lipid profile and redox status of the study participants, which can be beneficial in terms of preventing certain negative effects or risk factors over the long term (e.g., atherosclerosis) (Marnewick *et al.*, 2011). There is evidence of a link between inflammation and coagulation and vascular diseases. Rooibos polyphenols have been shown to have anti-oxidant and anti-inflammatory properties. These compounds also have anti-coagulation effects, which could help in the prevention and treatment of vascular diseases (Ku *et al.*, 2015b). In addition, it has also been shown that Rooibos polyphenols decrease vascular inflammation caused by high plasma glucose levels, reducing the risk of complications, such as atherosclerosis, due to increased glucose levels in diabetic candidates (Ku *et al.*, 2015a). It has also been reported that Rooibos modulates the activity of the angiotensin-converting enzyme (ACE). This enzyme is a key factor involved in the development of cardiovascular disease (Persson *et al.*, 2010). Dludla and co-workers reported that Rooibos helped to protect cardiomyocytes from oxidative stress *ex vivo* in rats (Dludla *et al.*, 2014).

2.1.2.4 Neurologic protection

It has been suggested that exposure to physical or psychological stresses over a period of time can lead to the production and accumulation of free radicals and other oxidizing species. These can result in oxidative damage to lipids, proteins and DNA. In the brain, this damage can lead to neurological diseases. The brain is very sensitive to oxidative damage due to the low levels of anti-oxidant enzymes

present in the brain and high levels of reactive oxygen species being produced by brain metabolism and function. In a 21 month experiment on rats, it was shown that Rooibos maintained the GSH:GSSG ratio and limited the lipid peroxidation of the rat brain when compared to rats that did not get the Rooibos supplementation (Hong *et al.*, 2014).

Luteolin, one of the phenolic compounds found in Rooibos, has been shown to have neuroprotective properties. Extracts, high in luteolin, from *Eclipta alba* has been used in the treatment of epilepsy, where it reduces the inflammation that triggers potential epilepsy causing receptors. Luteolin also has positive effects on autism by reducing inflammation and decreasing the activation of mast cells in the brain. It was also reported to retard the progression of Alzheimer's and Parkinson's disease by scavenging of free radicals in the brain, thereby reducing inflammation. Cognitive decline associated with diabetes can be attributed to oxidative stress and neural inflammation. It has been noted that flavonoids, such as luteolin, can decrease the cognitive decline in these cases. Luteolin has shown promise in the treatment of multiple sclerosis by demonstration of protective immunomodulatory effects (Nabavi *et al.*, 2015).

2.1.2.5 Diabetes

Rooibos, and particularly aspalathin have been shown to have hypoglycaemic and anti-diabetic properties resulting in an improvement of glucose tolerance tests. This can be attributed to an insulin independent increase in the amount of glucose absorbed by muscle cells in the body (Kawano *et al.*, 2009). Muscle cells play a major role in the removal of glucose from the systemic circulation and Rooibos improves this absorption even in insulin resistant cells (Mazibuko *et al.*, 2013). Diabetic cardiomyopathy is a disease that affects the heart muscle of diabetic patients. This condition is caused by increased levels of free radicals in the tissue. An *ex vivo* study showed that Rooibos can protect cardiomyocytes against increased free radical levels (Dludla *et al.*, 2014).

2.1.2.6 Inflammation

It has been shown in rats that Rooibos polyphenols can decrease systemic vascular inflammation (Kwak *et al.*, 2015). Another study in rats showed that Rooibos may reduce inflammation potentially due to its anti-oxidant properties (Baba *et al.*, 2009). However, no significant changes were reported in the C-reactive protein (CRP) levels, in the clinical trial by Marnewick *et al.* (2011), between the control and the Rooibos phase, indicating that Rooibos had no effect on this inflammatory marker.

2.1.2.7 Metabolic processes

Rooibos has been reported to modulate metabolic processes *in vivo*. In hyperlipidemic mice, that were fed a high fat diet, Rooibos lowered the cholesterol, triglycerides and free fatty acids in the systemic circulation although it had little effect on the mice that were fed normal chow. This indicates that Rooibos has an effect on specific biochemical pathways. A glucose tolerance test formed part of this study and showed that the hypolipidemic effect was not due to insulin resistance (Beltrán-Debón *et al.*, 2011). It was also reported that Rooibos had no adverse effects on the iron status of the participants when comparing serum iron, ferritin, transferrin, total iron binding capacity and percentage iron saturation in this clinical study (Marnewick *et al.*, 2012). Rooibos also has an anti-spasmodic effect by opening the potassium (K^+) channels in the intestinal tissues (Gilani *et al.*, 2006). Studies on different animals have shown that the opening of the K^+ channels by the Rooibos polyphenols also results in a bronchodilatory and an anti-hypertensive effect (Khan and Gilani, 2006). Steroid hormones are responsible for hormonal homeostasis. Imbalances in hormone levels have been a known contributor to diseases such as hypertension, metabolic syndrome, cardiovascular disease and diabetes. A study by Schloms *et al.* (2012) reported that Rooibos can assist with hormonal homeostasis by decreasing the production of stress hormones. Flavonoids also exhibited phytoestrogenic properties that can lead to a decrease in risk for osteoporosis, breast cancer and cardiovascular disease (Schloms *et al.*, 2012).

2.1.2.8 Antimicrobial treatments

Rooibos has been shown to have no negative effect on the activity of commercial anti-microbial treatments. In some cases, it had positive additional effects. This indicates that Rooibos consumption while taking anti-microbial preparations won't affect the efficacy of the treatment (Hübsch *et al.*, 2014).

2.1.3 Bioavailability studies

2.1.3.1 Bioavailability of polyphenols

It has been reported that despite the potential health benefits of polyphenols, these compounds are sensitive to environmental factors that can potentially decrease their effectiveness. Factors include light, heat, low water solubility in their free form and possible degradation and oxidation in water. This coupled with poor absorption in the intestine and a rapid elimination from the systemic circulation results in low plasma levels and poor bioavailability (Parisi *et al.*, 2014). Polyphenols in the systemic circulation were mostly found to be the phase II metabolites of the parent compounds ingested. Most of the polyphenols consumed were passed either as the compound or its metabolites into the colon where it was broken down to small phenolic acids and smaller aromatic compounds by the normal flora. Polyphenols and there glucuronide, methyl and sulphate conjugates are rapidly eliminated via the kidneys. For this reason, monitoring the metabolites in the urine is the best technique to estimate the bioavailability of polyphenols (Del Rio *et al.*, 2013; Stalmach *et al.*, 2009). A study using luteolin indicated that its absorption predominantly takes place in the ileum and colon (Nabavi *et al.*, 2015).

2.1.3.2 Polyphenols as bioactives

In the last decade questions have been asked about the low bioavailability of polyphenols and the resulting effects *in vivo*. Most anti-oxidants are poorly absorbed, rapidly conjugated and rapidly eliminated via the urinary system. The view that polyphenols act as anti-oxidants and free radical scavengers *in vivo* could not explain the effects of these compound and their metabolites at the low

concentrations found in circulation (Croft, 2016; Forman and Ursini, 2014; Schaich *et al.*, 2015). Forman and Ursini (2014) and Scaich et al. (2015) described how anti-oxidants activated signal transduction pathways and assisted with the regulation of gene expression, resulting in the regulation of detoxifying and anti-oxidant enzymes and their substrates. Anti-oxidants can thus be seen to optimize the cellular defence mechanisms and this could result in a decrease in inflammation and diseases associated with inflammation and oxidative stress. Anti-oxidants are often converted into their oxidised form when triggering these pathways. From this it can be deduced that different anti-oxidants, *in vivo*, work together with each other as well as with proteins and enzymes to produce their bio-effectiveness (Stocker, 2016). It has been suggested that polyphenols in foods should not be referred to as anti-oxidants, but rather as bioactives (Croft, 2016), as they exhibit very little anti-oxidant activity *in vivo*.

2.1.3.3 Bioavailability of Rooibos

In clinical studies, it has been determined that Rooibos has a low bioavailability. Only 0.26% of the total Rooibos flavonoids consumed by the participants could be detected in the plasma (Breiter *et al.*, 2011). The amount detected was reduced when isolated flavonoids were used at the same concentrations. No change in the plasma antioxidant capacity was observed by the oxigen radical absorbance capacity (ORAC) assay. This leads to the assumption that the measurement of the effectiveness of the Rooibos flavonoids should be assessed by investigating not the measurement of the flavonoids or the anti-oxidant capacity but rather by monitoring endogenous markers such as the redox status of glutathione (GSH:GSSG ratio) or malondialdehyde levels (Breiter *et al.*, 2011). In another clinical study it was reported that 0.35% of the aspalathin content of a green Rooibos extract was recovered as metabolites in the urine. In a study on pigs fed about 400x the amount consumed in the studies above over an 11 day period, the recovery of Rooibos polyphenols varied between 0.18% and 0.87%. This suggests that the absorption is relatively low even when high concentrations are administered (Del Rio *et al.*, 2013; Stalmach *et al.*, 2009). Aspalathin exposed to

simulated gastric juice for 2 h had a 100% recovery, indicating that it is stable at the low pH levels of the stomach (Del Rio *et al.*, 2013).

2.1.4 *In vitro* anti-oxidant capacity of Rooibos

The different techniques used to analyse Rooibos can either be used to determine the total polyphenols, the specific flavonoids or the different anti-oxidant activity markers (Joubert and Schultz, 2012). However, the latest views of the *in vitro* anti-oxidant activity test are that they have limited correlations to the *in vivo* activity. The concentrations of the anti-oxidants used in these tests are much higher than the levels found in the systemic circulation and the sterically hindered stable radicals used differed from the small readily accessible radicals found *in vivo*. These tests also don't account for lipid peroxidation that has a major effect *in vivo* (Schaich *et al.*, 2015). These assays should then rather be limited to describing a polyphenol rich pant extract or used as a quality parameter for phytochemical extracts.

Total polyphenols can be assayed using a modified Folin-Ciocalteu's colorimetric method described by Singleton and Rossi (Belwal *et al.*, 2016). Gallic acid is used as a standard and the results are expressed as mg gallic acid equivalents.

The analysis to determine the individual flavanones can be performed by high performance liquid chromatography (HPLC) with either a diode array detector or mass spectrometer (MS) (Joubert, 1996). The LC-MS allows for the identification of the flavanones based on their molecular mass and mass spectrum. It also has a lower detection limit (Iswaldi *et al.*, 2011).

The methods of analysis of anti-oxidant activity usually measure either electron transfer or hydrogen atom transfer. The FRAP assay (Ferric reduction/antioxidant power assay) and the TEAC assay (Trolox equivalent antioxidant assay) measure electron transfer whereas the ORAC assay (oxygen radical antioxidant capacity assay) measures the transfer of a hydrogen atom (Zulueta *et al.*, 2009). The FRAP assay measures the ability of a substance to reduce Fe^{III} to Fe^{II}. The formation of Fe^{II} tripyridyltriazine is monitored spectrophotometrically at 593 nm.

The result is reported as a dose-response relationship to either ascorbic acid, uric acid, Trolox or albumin (Benzie *et al.*, 1999; Benzie and Strain, 1996). The TEAC assay measures the decrease in the ATBS radical (2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) due to the scavenging effect of the anti-oxidants in the sample. The result is reported as a dose-response relationship to Trolox (µM Trolox equivalents). For the ORAC assay, fluorescein is used as a fluorescent probe. The peroxyl radical induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) damages the fluorescein resulting in a decrease in fluorescence. The rate of decrease in fluorescence is monitored. Anti-oxidants in the sample will scavenge the radicals and in doing so decrease the rate at which the fluorescence decrease. The result is reported as a dose-response relationship to Trolox (µM Trolox equivalents) (Ou *et al.*, 2001). These *in vitro* assays serve the purpose of quantifying or describing a complex phytochemical extract, but are not recommended for use when describing the *in vivo* activity.

2.2 Dosage forms

Pharmacologically active compounds (or drugs) are usually not administered in their pure form, but are administered in formulated preparations or dosage forms to produce acceptable medicinal products (Hamman and Steenekamp, 2012; Hamman and Tarirai, 2006). These formulations can vary from a simple solution to a complex drug delivery system in which the drug compound is mixed with functional excipients that play specific pharmaceutical roles in the drug delivery process. In modern dosage forms, excipients are included to optimize the bioavailability of the active compounds and to improve the manufacturing process (York, 2013).

Different types of dosage forms are used to administer pharmacologically active compounds, which are designed based on the route of administration i.e. oral, mucosal, topical, transdermal, nasal, inhalation or parenteral (injections) and target population (i.e. paediatric, geriatric, critically ill or chronically ill patients). The following are some of the most commonly used dosage forms (USP, 2016): aerosols, capsules, dry powder inhalers, emulsions including creams and lotions,

foams, medical gasses, gels, granules, medicated gums, implants, liquids, lozenges, ointments, pastes, transdermal patches, pellets, pills, plasters, powders, medicated soaps and shampoos, solutions, sprays, suppositories, suspensions, tablets and tapes.

2.2.1 Tablets

Tablets have many advantages and serve as one of the most commonly used solid oral dosage forms for several reasons, which include (Alderblom, 2007):

- Tablets have superior chemical and physical stability compared to that of liquid dosage forms.
- Tablets are versatile dosage forms.
- Tablets are convenient to handle and can easily be self-administered.
- Tablets offer an accurate dose per unit, which is easily produced in a highly repeatable way.
- Tablets can be produced for a specific delivery site in the gastrointestinal tract (e.g., enteric coating to release beyond the stomach or colon targeting).
- Tablets are produced in a cost effective manner.
- Tablet production can easily be monitored with good quality control.

However, tablets do have some disadvantages as a dosage form. For example, poor bioavailability may result from poorly water soluble drugs and poorly permeable drugs. Some drugs can cause local irritation in the gastrointestinal mucosa if released in relatively high concentrations (Alderblom, 2007).
It is generally accepted that tablets should have the following quality attributes (Alderblom, 2007):

- Tablets should have a consistent mass, size and neat appearance.
- The tablet should contain the specified dose within acceptable limits.
- Drug release from the tablet should be reproducible and should occur in a controlled manner.

- Tablets should be free from contaminants, excipients and microorganisms that can be harmful to the patient.
- Tablets should be mechanically strong enough to resist breaking and wearing during processing, packaging and handling.
- Tablets should be stable, chemically, physically and microbiologically, for the shelf life of the product.

2.2.1.1 Types of tablets

Many different types of tablets have been described, which can be divided into three categories based on their dissolution properties, namely immediate release, sustained/extended release and delayed release tablets (Figure 3). Immediate release tablets can be sub-divided into disintegrating, chewable, effervescent, sublingual and buccal tablets. These tablets disintegrate/dissolve rapidly and release the active compound directly after administration to become immediately available for absorption. Sustained/extended release tablets release the active component(s) relatively slowly over an extended period of time starting directly after administration. Delayed release tablets only start to rapidly release the drugs after a lag phase has occurred after administration, which can be released in a specific location in the gastro-intestinal tract (Alderblom, 2007; Anal, 2007).

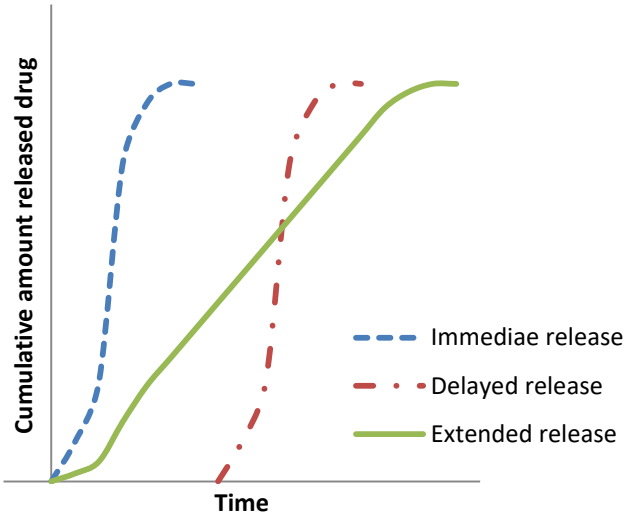


Figure 3: A graphic representation showing the difference in the dissolution profiles of immediate release-, delayed release- and extended release dosage forms (Alderblom, 2007)

2.2.1.1.1 Immediate release tablets

As mentioned before, immediate release tablets are formulated to release the drug load rapidly after administration (Alderblom, 2007). Some of the different strategies are applied when producing immediate release tablets are described below.

Disintegrating type immediate release tablets are the most commonly employed type of tablets, which are designed to provide rapid disintegration after being swallowed. This rapid disintegration results in smaller particles with an increase in surface area and an increase in dissolution rate of the drug. These tablets provide a relatively fast onset of action that can be used for management of moderate pain (Alderblom, 2007; Conway, 2007).

Effervescent type immediate release tablets are designed to be placed in a glass of water before administration. A mixture of a carbonate or bicarbonate salt and an acid is incorporated into the formulation of the effervescent tablet, which results in carbon dioxide being liberated that causes the tablet to break up into small

particles. The active ingredients are released and get dissolved in the water before being administered. These tablets are used for drugs that require rapid emptying from the stomach or where local high drug concentrations can cause irritation of the mucosa of the gastro-intestinal tract. Flavourants and colourants are often added to these formulations to provide an acceptable taste of the solution that is swallowed and also to mask the bad taste of certain drugs (Alderblom, 2007; Conway, 2007).

Chewable type immediate release tablets are formulated to be fragmented by the mechanical action of the teeth during chewing. The active(s) usually do not dissolve completely in the mouth, but dissolution of the particles takes place in the stomach or small intestine. These tablets are often used for children and the elderly that cannot swallow whole tablets. Flavourants and colourants can be added to make the tablets more appealing (Alderblom, 2007; Conway, 2007).

Sublingual and buccal type tablets are usually rapid disintegrating dosage forms (within seconds) that are used for rapid systemic drug delivery directly from the mouth, which avoids the first pass metabolism of the liver. These tablets are usually small and porous that allows for rapid disintegration and dissolution. Sublingual tablets are placed under the tongue and buccal tablets between the teeth and the cheek mucosa (Alderblom, 2007; Conway, 2007).

2.2.1.1.2 Sustained release tablets

Sustained release type tablets are dosage forms that release the drug load slowly over an extended period of time. Matrix type tablets are often employed to achieve sustained drug release kinetics. In many cases, direct compression can be used for the production of these matrix type tablets. Matrix type tablets release the drug by one of the following mechanisms: diffusion, degradation or erosion (Bose *et al.*, 2013). The aim with sustained release dosage forms is to maintain the drug at therapeutic levels in the systemic circulation for a longer time than is possible with immediate release formulations. This is important for drugs with relatively short half-lives and can assist to overcome fluctuations in the

concentration of the drugs in the systemic circulation between drug doses (Qazi *et al.*, 2013; Rowe, 2012).

2.2.1.1.2.1 Diffusion-controlled tablets

Diffusion-controlled drug delivery systems can be divided into matrix systems and reservoir systems. Matrix tablets comprise of a porous solid matrix, which is insoluble. The drug molecules dispersed in the outer layers dissolve first upon infiltration of solvent molecules into the matrix. As the liquid penetrates deeper into the matrix, the drug molecules in the inner layers of the matrix are dissolved and move to the surface by means of diffusion. The length of the distance over which the molecules diffuse determines the dissolution/release rate (Alderblom, 2007; Anal, 2007).

Reservoir tablets consist of a matrix core covered by a membrane (e.g., polymeric film). When the tablet is exposed to a liquid, the liquid molecules diffuse through the membrane into the matrix and dissolve the active(s). The concentration gradient across the membrane result in the diffusion of the dissolved drug molecules through the membrane to the outside (Alderblom, 2007; Anal, 2007).

2.2.1.1.2.2 Dissolution-controlled tablets

This type of extended release tablet relies on the rate at which the drug molecules/matrix dissolves. A slower dissolving derivative of the drug is sometimes incorporated into a slowly dissolving matrix. The dose can also be divided into smaller multiple-units such as beads. Portions of the different units can each be coated with a coating material that has different solubilities or different thickness before being compressed into tablets (Alderblom, 2007; Anal, 2007).

Compressed lozenges are an example of dissolution-controlled tablets that slowly dissolve in the mouth, releasing the active(s) in the saliva. Compaction at very high pressure without any disintegrants results in a tablet with a high mechanical strength and low porosity that will resist disintegration and that dissolves slowly. It can be used for systemic drug delivery or for local treatment in the mouth or

throat. Lozenges are often used for the delivery of local anaesthesia, antibiotics and antiseptic drugs to the mouth and throat. It is important that the fillers and binders used in the formulation of lozenges have a pleasant taste and thus flavours are usually added (Alderblom, 2007).

2.2.1.1.2.3 Erosion-controlled tablets

Erosion-control prolonged release tablets have the active components in a matrix that slowly erode when exposed to gastric juice or intestinal fluid. The eroded fragments can release the active compound rapidly or the release can be extended even more by incorporating other prolonged release mechanisms such as diffusion control release. Due to the potential combination of different release mechanisms, the erosion-controlled system can obtain a near zero order dissolution profile (Alderblom, 2007; Anal, 2007).

2.2.1.1.2.4 Osmosis-controlled drug delivery systems

In osmosis-controlled drug delivery systems, the tablet core is coated with a semi-permeable membrane into which a very small hole has been drilled by means of a laser. The membrane allows water molecules through to penetrate the matrix and the water then dissolves the active compounds in the tablet core. This results in an increased osmotic pressure inside the membrane, which forces the dissolved molecules through the hole in the membrane. The release rate is controlled by the amount of water that is allowed to diffuse into the tablet through the semi-permeable membrane and the size of the hole. Excipients that swell when hydrating can also be added to the tablet matrix to maintain the pressure required to release the dissolved compounds from the membrane. This technology is very expensive, but it is capable of producing zero order drug release kinetics (Alderblom, 2007).

2.2.1.1.3 Delayed release tablets

Delayed release tablets release the drug at a certain period of time after administration or at a specific site in the gastrointestinal tract. This is accomplished by coating the tablets with a polymer that is poorly soluble or by

coating the tablet with a coating material that has a pH dependant solubility. Enteric coated tablets are an example of a dosage form that is used to protect active compounds that are susceptible to degradation in an acidic environment. An enteric coating is insoluble in the acidic environment in the stomach, but dissolves in the small intestine where the pH of the environment is higher (Anal, 2007).

2.2.2 Different dosage forms utilised for modified drug release

Modified release drug delivery systems refer to dosage forms that release the active components at a pre-determined rate (e.g., sustained release over an extended period of time) and/or at a pre-determined site in the gastro-intestinal tract (Collet and Moreton, 2007). Tablets are commonly used as modified release drug delivery systems.

The rationale behind the design of modified release dosage forms include the following (Anal, 2007):

- To decrease the toxicity and risk of adverse effects of drugs that are commonly dumped in high concentrations by immediate release dosage forms.
- To optimize drug utilisation by increasing the duration of action of a drug after a single administration.
- To release the drugs in an area of the gastrointestinal tract where maximum absorption can take place.
- To maintain the drug at a therapeutic level in the systemic circulation for an extended period of time.
- To improve patient compliance by reducing the number of doses required for effective treatment.

2.2.2.1 Single-unit vs multiple-unit dosage forms

Single-unit dosage forms can be defined as drug delivery systems that contain the full dose in one unit, for example a tablet. Multiple-unit dosage forms consist of

sub-units such as pellets, beads, granules, microspheres or mini-tablets that each contains a portion of the total dose. The sub-units are combined into a single dosage form by different mechanisms such as packing into sachets, filling into hard gelatin capsules or compressing into tablets (Collet and Moreton, 2007; Mounika *et al.*, 2015; Solanki *et al.*, 2012).

Many studies have described the advantages and disadvantages of multiple-unit dosage forms compared to single-unit dosage forms. The advantages include (Chen *et al.*, 2006; Gaber *et al.*, 2015; Solanki *et al.*, 2012):

- A decreased risk of dose dumping since each sub-unit contains only a part of the total dose.
- Drugs that are incompatible in a single-unit dosage form (e.g., conventional tablet) can be incorporated in different sub-units (e.g., beads or mini-tablets) and then combined into a dosage form (e.g., multiple-unit particulate tablet or capsule).
- The overall drug release rate can be manipulated by using a combination of sub-units, each with a specific release rate.
- Gastric emptying is more predictable and reproducible, which results in better dispersion of the sub-units in the gastro-intestinal tract with less inter- and intra-subject variability in terms of bioavailability.
- The risk of local tissue irritation is reduced due to the better dispersion and spreading of the sub-units over a larger part of the gastro-intestinal tract.
- Modified release sub-units can result in a reduction of the dosing frequency.
- Accurate dosing can be achieved.
- The increased surface area of the sub-units makes it ideal for surface coatings to control drug release rate.
- The bioavailability of the active compounds is potentially increased.

The disadvantages of multiple-unit dosage forms include (Solanki *et al.*, 2012):

- The manufacture process and packaging can become complicated with increased costs involved.

- Filling of hard gelatin capsules with different sub-units (e.g., different coated beads) require specialised equipment that is expensive and require trained personnel to operate it.
- The relatively high surface area requires a large quantity of coating materials.

2.2.2.1.1 Multiple-unit pellet systems

Multiple-unit pellet systems (MUPS) consist of beads compressed into tablets or loaded into hard gelatine capsules. The tablets or capsules are administered via the oral route, which disintegrate in the stomach thereby releasing the sub-units intact (Nguyen *et al.*, 2012). MUPS are dosage forms with accurate and consistent dosing of the sub-units (Mounika *et al.*, 2015).

2.2.2.1.2 Mini-tablet-in-capsule systems

Mini-tablet-in-capsule systems merge the benefits of traditional tablet production methods with the benefits of multiple-unit dosage forms. Mini-tablets are produced using normal tablet production methods and are then loaded into hard gelatine capsules (Ishida *et al.*, 2008). This unique drug delivery system has several benefits over conventional single-unit dosage forms as well as over other multiple-unit dosage forms including the following (De Brabander *et al.*, 2000; Keerthi *et al.*, 2014; Li and Zhu, 2004; Mounika *et al.*, 2015):

- Mini-tablets are easily produced in bulk by normal tableting methods, which provide sub-units that are uniform in shape, dose and size.
- The mini-tablets can easily be film coated due to their smooth surface.
- The drug release rate can easily be modified with the use of different excipients.
- The inter- and intra-subject variability in the bioavailability is decreased due to the even distribution of the sub-units in the gastro-intestinal tract.
- Due to the increase in the density of the powders during compaction, a higher dose load is possible per capsule.

- It is relatively easy to manipulate the bi-phasic release by changing the amount of loading and maintenance dose sub-units packed in the dosage form.
- Mini-tablets require no solvents in the production process.

2.2.3 Tablet formulation and production

The development process of new tablets should ensure that the chemical properties of the active ingredient(s) are not affected by the excipients or by the production method. The tablets should meet the specifications in terms of physical properties, drug content and drug release/dissolution (Alderblom, 2007).

The pharmacopoeias list a number of tests and specifications that tablets must comply to in order to ensure consistent production and quality control. Some of these tests are performed on the components before tablet compression and some after the tablets are produced. It is important to determine the technical properties of the powder mixture to ensure successful production (also referred to as tableting). This includes the fact that powder mixtures should be homogeneous and should not segregate during the tableting process, have acceptable powder flow and compression properties (Alderblom, 2007).

2.2.3.1 Excipients in tablet formulation

Initially, excipients were described as “additives used as a medium for giving a medicament substance”, which can be interpreted as inert chemical substances giving the correct volume and mass to the dosage form (e.g., tablet). The International Pharmaceutical Excipients Council defines excipients as “substances, other than the active drug substances of finished dosage form, which have been appropriately evaluated for safety and are included in a dosage form to aid the processing of the drug delivery system during its manufacture, protect the active compound, support the tablet, enhance stability and bioavailability, patient acceptability and assist in product identification” (Virendrakumar *et al.*, 2015). However, progress in technology has led to the development of excipients that fulfil specific functions in modern dosage forms. These functions range from

improving the manufacturability to enhanced delivery of the active ingredient (Abrantes *et al.*, 2016).

Excipients can be divided into different categories based on their function in the tablet formulation (Alderblom, 2007; Conway, 2007). Fillers or diluents are added to the powder mixture to add bulk in terms of volume and mass to ensure the desired size of the tablet is achieved. Fillers should be chemically inert, non-hygroscopic and biocompatible with the drug, have good compatibility properties, have an acceptable taste and be cost-effective. In some cases, a single filler cannot fulfil all the needs and more than one filler can then be used in a single tablet formulation. Common fillers include sugars, cellulose, calcium carbonate and calcium phosphate (Alderblom, 2007; Conway, 2007).

Disintegrants are added to the tablet formulation to assist with the breakup of the tablet after administration when it is exposed to liquid in the gastrointestinal tract. This breakup results in small particles that allow for faster dissolution. Disintegrants have different mechanisms of action to cause the breakup of tablets, which include swelling, exothermic wetting reactions, gas production, particle repulsion and particle deformation recovery. Common disintegrants used include starches, cellulose, crosslinked polyvinylpyrrolidone, sodium starch glycolate, sodium carboxymethyl cellulose and carbonate and bicarbonate salts (Alderblom, 2007; Conway, 2007).

Binders are added to the tablet formulation to ensure that the final product has adequate mechanical strength. Binders can be divided into dry binders (powder form) and liquid binders (solutions of binders in appropriate solvents). Dry binders are added to the powder mixture before direct compaction or before granulation. Liquid binders are added to the powder mixture during granulation. Common binders used are starches, sucrose, gelatin and polymers such as polyvinylpyrrolidone, polyethylene glycol and cellulose derivatives such as hydroxypropyl methylcellulose (Alderblom, 2007; Conway, 2007).

Glidants are added to the tablet formulation to improve the powder flow properties. They are often added to direct compaction tablet formulations, but can also be

added during granulation. Commonly used glidants include colloidal silica, talc and magnesium stearate (Alderblom, 2007; Conway, 2007).

Lubricants reduce friction between the solid powder particles and the die and punches of the tablet press during compaction and ejection of the tablet. This can be achieved by either fluid lubrication or boundary lubrication. Fluid lubrication requires a fluid layer between the solid and the die surface. This type of lubricant is not often used in tablet production. Boundary lubricants are usually very fine powders that form a thin surface layer that reduce the shear forces between the tablet and the die. Stearic acid and magnesium stearate are the most commonly used lubricants. Polyethylene glycol and the salts of soaps are also used but have a lower ability to reduce friction (Alderblom, 2007; Conway, 2007).

During the tableting process, powder can adhere to the punch surfaces as a result of the moisture content of the powders, which is commonly referred to as sticking or picking. This can be overcome by the addition of an anti-adherent such as magnesium stearate, talc, starch and cellulose (Alderblom, 2007; Conway, 2007). Sorbents are used to absorb fluids, mostly oils or oil-drug solutions to form dry powders that can be used in the tableting process. Microcrystalline cellulose and silica are often used as sorbents. Flavourants can be added to the tablet formulation or the tablet can be coated with a flavouring agent to mask an unpleasant flavour of a tablet. Colourants are usually coated onto a tablet for identification purposes and to make the tablets more visually appealing (Alderblom, 2007; Conway, 2007).

2.2.3.2 Powder flow tests

The flow properties of the powder ingredients of a tablet formulation such as the active(s) and excipients play a critical role in the production of acceptable tablets. If the powders don't flow well, it will negatively affect the filling of the die, which will result in an inconsistent tablet mass. Some excipients may be added to tablet formulations to enhance the flow properties of the powder mixture. Wet or dry granulation can also be employed to enhance the flow properties. The powder

mixture should also contain sufficient lubricants to minimise friction (Alderblom, 2007).

2.2.3.2.1 Angle of repose

The angle of repose is determined by allowing a powder to flow through an orifice onto a solid horizontal surface. The angle between the solid surface and the powder cone surface is then calculated by measuring the height and the base of the cone. This parameter gives an indication of the physical properties and interactions between the powder particles. This angle is influenced by the friction or resistance to flow between the particles of a powder. The result can be interpreted using Table 2.

Table 2: Description of powder flow properties based on the angle of repose (USP, 2016)

Description of flow properties	Angle of repose (degree)
Excellent	25 – 30
Good	31 – 35
Fair	36 – 40
Passable	41 – 45
Poor	46 – 55
Very poor	56 – 65
Very, very poor	>66

Some powders do not form a cone when flowing through an orifice onto a solid surface and for these powders, the angle of repose is not a good indicator of flow properties. If the angle of repose indicates passable or poor powder flow properties, the formulation might need glidants or granulation to improve the flow of the tablet formulation (USP, 2016).

2.2.3.2.2 Critical orifice diameter

The critical orifice diameter (COD) is measured using a series of tight fitting brass disks that are stacked on top of each other. The inside of the stacked disks form a smooth taper from 32 mm to 1.5 mm. The sample is poured into the tapered hole while the stack is placed in a level surface. The stack is lifted and the rings are removed from the bottom one at a time until the powder starts to flow. The diameter of the bottom ring is recorded. This gives an indication of the smallest diameter hole that the powder will flow through. The United States Pharmacopeia (USP, 2016d) states in section 1174 that there is no reference to compare powder flow measured by COD to, since it is dependent on the equipment used in the analysis. It is generally accepted that the smaller the orifice the better the flow properties.

2.2.3.2.3 Flow rate

Flow rate can be defined as the time it takes for a specific quantity of powder to flow through an opening of specific size. This is a commonly performed assay, but because of the large number of variables there is no reference range specified in the pharmacopoeias. The test is used more as a comparative test. The results are influenced by the diameter and shape of the orifice, the wall friction of the hopper and the height and diameter of the powder bed (Copley, 2008).

2.2.3.2.4 Bulk and tapped volume or density and compressibility index and Hausner ratio

Bulk density is a term that describes the ratio of the mass of an untapped powder bed to its volume. The volume is made up of the volume of the particles and the space between them. Tapped density refers to the change in volume of the same mass of powder in a container after it was tapped. The tapping of the powder results in a close spacing of the particles resulting in a decrease in volume. To measure tapped density, a known mass of powder sample is added into a 250 ml measuring cylinder. The volume is recorded. The cylinder is then fitted into the

tapped density apparatus. The powder is tapped and the tapped volume is then recorded (USP, 2016d)

The specification for an apparatus that is used to determine bulk and tapped density is shown in Figure 4 (USP, 2016d). The apparatus will tap the flask from a 3 mm height at a rate of 250 taps per min for 5 min. After 1250 taps, the tapped volume is recorded (USP, 2016d).

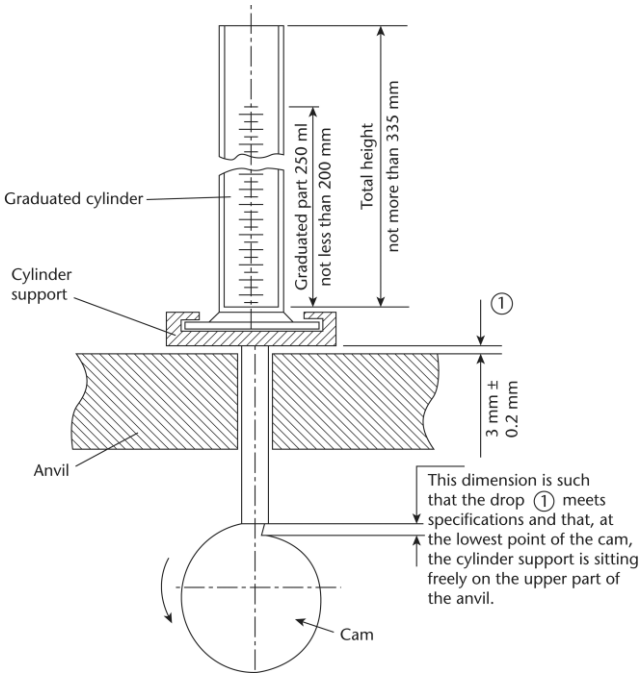


Figure 4: A graphic representation showing the dimensions of a tapped density apparatus (USP, 2016d)

The Hausner ratio and the compressibility index are calculated from the bulk and tapped density. Since the interactions between the particles that affect the compression or settling of the particles also affect the ability of the particles to flow freely, the compressibility index and Hausner ratio give a good indication of the flow properties of a powder.

Table 3 shows the relationship between the compressibility index and Hausner index and the flow properties of a powder. (USP, 2016d)

Table 3: The relationship between powder flow and compressibility index and Hausner ratio (USP, 2016d).

Flow	Compressibility index (%)	Hausner ratio
Excellent	≤ 10	1.00 – 1.11
Good	11 – 15	1.12 – 1.18
Fair	16 – 20	1.19 – 1.25
Passable	21 – 25	1.26 – 1.34
Poor	26 – 31	1.35 – 1.45
Very poor	32 – 37	1.46 – 1.59
Very, very poor	≥ 38	≥ 1.60

2.2.3.3 Tablet production

Tablets are produced by the compression of a powder mixture consisting of drug and excipients into solid mass of dimensions determined by the size of the die and punches used in the tablet press. This can be achieved by direct compression of the powder mixture or by prior granulation before compaction (Santos and Sousa, 2007).

During compression, pressure is applied to the powder mixture by the punches whereby the volume of the powder mixture in the die is reduced. The initial reduction in powder mixture volume can be attributed to rearrangement of the powder particles resulting in a decrease in the spaces between the particles and thus the porosity. In the next phase, the powder particles deform, which can be due to the fracturing of the particles into smaller particles or a change in the shape of the particles. The change in shape can be temporary and in this case the particles revert to the original shape once the pressure is released, which is known as elastic deformation. Elastic deformation will not result in cohesion between the particles. If however, the change in shape is permanent and the changed shape does not revert, it is known as plastic deformation and this result in cohesion between the particles (Santos and Sousa, 2007).

Direct compression of tablets requires that the drug and excipient powder mixture be compactible without prior formation of granules. Compactibility of a powder mixture can be described as the ability of the powder mixture to form tablets when external pressure is applied such as compression in a tablet press. Thorough mixing of drugs and excipients need to be performed before compaction in the tablet press (Santos and Sousa, 2007).

Production of tablets by means of direct compression have advantages such as; fewer steps are required in the production process, thus reducing cost and production time and the need for liquids and heat is eliminated. The disadvantages include that more quality control tests may be required, more expensive excipients may have to be used, excipient and drug powders must have relatively large particle sizes and have good powder flow properties, and the excipients and drugs must form a homogenous mixture that is not prone to segregation during production. Tablets with a high dose concentration may present challenges for direct compression, especially if the drug has poor compactibility (Alderblom, 2007).

Granulation can be divided into wet-granulation and dry-granulation. The wet granulation process involves wetting the drug and excipient powder mixture with a liquid during agitation followed by drying. The product must then be sieved to a uniform size. In dry granulation, the drug and excipient powder mixture is mixed and compressed with rollers followed by milling before tablet production can take place. The aim of powder granulation is to improve the bulk density of the powder mixture, the flow properties, homogeneity of the powder and the compactibility, while decreasing segregation of the powder components (Alderblom, 2007; Conway, 2007).

2.2.3.4 Tablet press tooling

The tooling used in a tablet press includes a die and the punch set (consisting of both the upper and lower punch). The die is responsible for the shape of the tablet (e.g., circular, oval or oblong). The punches are responsible for the top and bottom shape of the tablet (e.g., flat, concave or convex with or without bevelled

edges). Different size tooling sets are used for different sized tablets (Alderblom, 2007; Santos and Sousa, 2007). Because of the high pressures used in the tableting process, the tooling is made from special types of steel that may be coated with a hard chrome coating to reduce the wear on the components and protect against corrosion (Santos and Sousa, 2007).

2.2.3.4.1 Tableting

The compression of a powder mixture into tablets takes place between two punches in a die. The position of the bottom punch can be adjusted to change the powder fill volume in the die and in doing so, the mass of the tablet is changed to the desired weight. The top punch can be adjusted to regulate the pressure at which the powder mixture is compressed to produce tablets with the desired strength or hardness (Alderblom, 2007).

The tableting process comprises of tree basic steps (Alderblom, 2007), which include filling of the die, compaction and tablet ejection. During the first step, the lower punch moves down to the set position and the filling shoe moves over the die and allows powder to flow into the die under gravity. The filling shoe then moves away. During the second step, the upper punch moves down into the die and compresses the powder. After the powder is compressed and the tablet is formed the upper punch moves back up. During the third step, the lower punch moves up until it is level with the upper surface of the die and the tablet is pushed away from the die by the filling shoe.

During the tableting process technical problems may arise, which should be monitored during production. Aspects that need to be monitored include mass and dose variation, mechanical strength of the tablets, capping or lamination of the tablets, powder sticking to the punches and high friction when ejecting the tablets (Alderblom, 2007). Some problems during tableting may be caused by a powder mixture that is not homogenous, settling of the powder or poor powder flow and the tooling or the conditions set on the tablet press such as too high production speed or too high pressure during compaction. Insufficient amounts of

lubricant or anti-adherents may result in powder sticking to the punches, causing an increase in the required ejection force (Santos and Sousa, 2007).

2.2.3.4.2 The tablet press

The single-punch type tablet press (eccentric press) and the rotary type tablet press (multi-station press) are commonly used for tablet production. Hydraulic presses are sometimes used in experimental research and development work (Alderblom, 2007; Santos and Sousa, 2007).

The single-punch tablet press consists of one die and one upper-and lower punch set. The powder is housed in a hopper during tablet compression. Single-punch tablet presses can usually produce up to 200 tablets per min. It is thus useful for the production of small batches of tablets (Alderblom, 2007). The Korsch XP1 is a single-punch tablet press that was used in this study (Figure 5).

Figure 5: The Korsch Xp1 singe punch tablet press used in this study (Korcsch, 2017)

The rotary tablet press is used for large scale production of tablets. It comprises of a large circular table with multiple die and punch sets. The table and the dies rotate in a circle together with the punches. The vertical positions of the punches change as the table rotate. The powder is housed in a hopper that allows the powder to flow into the die when the die moves during rotation of the table. The punches compress the powder as the table rotates. The lower punch moves up to eject the tablet and a scraper pushes the compressed tablets from the table into a collecting bin. Since multiple die and punch sets are used, the production rate is increased considerably and some of these tablet presses can produce up to 30 000 tablets per min (Alderblom, 2007).

2.2.3.5 Evaluation of the physical properties of tablets

After production of a batch of tablets, a random sample is selected to test the physical properties of the tablets to ensure they conform to the specifications as specified by the pharmacopoeia. Measurements include the diameter, thickness, breaking force, friability, disintegration and mass variation. The criteria for these tests as described by the United States Pharmacopoeia (USP, 2016) are discussed below.

2.2.3.5.1 Diameter and thickness

The diameter and thickness of ten tablets should be measured using a vernier calliper or a micrometer. It can also be measured together with the breaking strength in some automated instruments. This measures the physical size of the tablet that can be affected by variation in the compression of the tablet or variable powder flow when filling the die or variation of particle size and composition of the powder mixture. A variation in the diameter and thickness of up to 5% is acceptable (Lee, 2007).

2.2.3.5.2 Hardness

The hardness or breaking strength of ten randomly selected tablets is measured by measuring the force required to fail or break each tablet. This gives an indication how the tablets will withstand mechanical forces during the coating,

printing and packaging processes (Alderblom, 2007). Depending on the application, a hardness of between 20 N and 130 N is acceptable. Tablets with a low hardness must withstand the friability test to be acceptable and high hardness tablets may be prone to capping (Alderblom, 2007).

2.2.3.5.3 Friability

The friability test measures the percentage weight loss of tablets during tumbling in a rotating cylinder for a specified time at a specified rotation speed. Ten randomly selected tablets are dusted, weighed and placed in the cylinder of a friability tester. The cylinder should be made from a clear non-static acrylic polymer with a diameter of 300 mm and a width of 38 mm. An arc shaped partition (baffle) should be fitted inside the cylinder (Figure 6). When the cylinder turns, the partition causes the tablets to tumble. The cylinder is turned at 25 rpm for 4 min resulting in the tablets being tumbled 100 times. The tablets are removed dusted again and the mass measured. The percentage weight loss is calculated from the data. The tablets fail this test if there are any cracks visible on any tablet or if the mass loss is more than 1.0 % (USP, 2016c). Friability is a measure of how the tablets will withstand attrition during and after production.

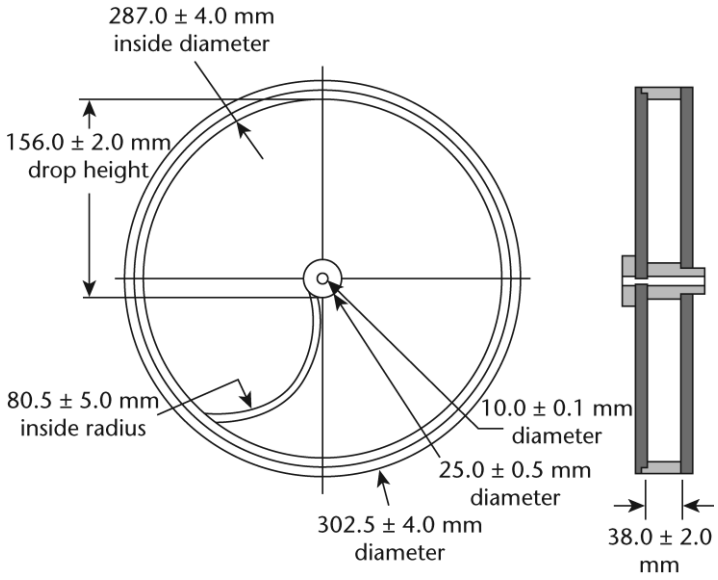


Figure 6: Schematic illustration showing the dimensions of the cylinder used in the friability tester (USP, 2016c)

2.2.3.5.4 Disintegration

The disintegration test is used to test if an immediate release tablet is capable of disintegrating within the required time as specified in the monograph. This test only measures if the tablet disintegrates and not if the drug is released or dissolved. The test must be performed on at least six tablets. Each tablet is placed in an individual basket with a sieve bottom and placed in the apparatus. Figure 7 is a schematic illustration of the disintegration apparatus as described in the pharmacopoeia (USP, 2016). It also shows the specifications of the disks that can be used for floating dosage forms. The baskets are moving inside a beaker filled with liquid at a rate of 30 strokes per min. Tablets are visually examined to see whether there are tablet fragments left or not on the sieve after 15 min (USP, 2016). The tablet has failed the test if an immediate release tablet didn't disintegrate completely in the prescribed time or if a sustained release tablet does disintegrate completely in this time (USP, 2016e).

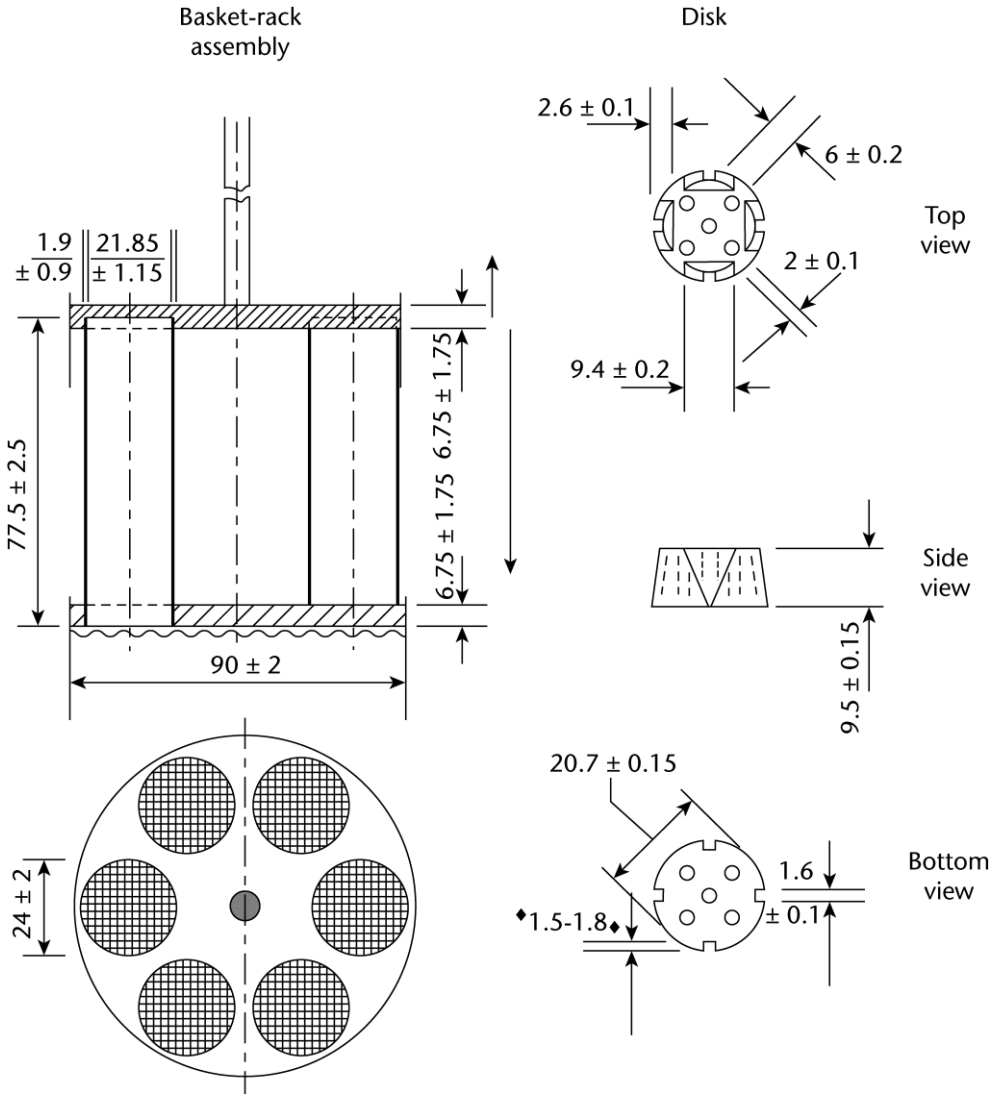


Figure 7: Schematic illustration of a disintegration tester showing the dimensions (mm) of the basket assembly (USP, 2016e)

2.2.3.5.5 Mass variation

Mass variation is used to determine if the difference in the mass of individual tablets in the batch is within an acceptable range. A minimum of 20 tablets must be dusted and weighed. The difference in mass between each individual tablet and the average mass is divided by the average mass and expressed as a percentage mass variation. The acceptable range varies for different formulations based on the mass of the tablet and is summarised in

Table **4** (USP, 2016b).

Table 4: Mass variation specifications for tablets with different average masses (USP, 2016b).

Average mass of the tablets	Percentage difference allowed
< 130 mg	10 %
130 to 324 mg	7.5 %
> 324 mg	5 %

2.2.3.6 Dissolution testing

For the active ingredient in a solid oral dosage form to be absorbed after administration, it must first dissolve in the gastro-intestinal fluid. Dissolution of the drug from the dosage form provides a concentration gradient in the liquid phase between the surface of the absorbing mucosa and the blood surrounding the gastro-intestinal tract. Dissolution continues to replace the drug molecules that are removed by means of absorption (York, 2013).

Dissolution testing aims to monitor the cumulative release of the active ingredient as a function of time. The data obtained is used to construct a dissolution curve or to calculate the rate of release of the active(s) from the dosage form. It is used extensively in the development of drug formulation, comparison of generic dosage forms and in the quality control processes. The data obtained can be used to predict the bioavailability of the compounds (if the rate of absorption is dissolution dependent). The dissolution data can also be used for *in vitro/in vivo* correlations, which can be used to better describe bioavailability (Anand *et al.*, 2011; Collet and Moreton, 2007).

The USP describes seven different types of dissolution apparatuses. The different apparatuses were designed to accommodate specific applications.

Table **5** lists the different dissolution apparatuses and the application of each (Uddin *et al.*, 2011).

Table 5: The seven different dissolution apparatuses and the intended application for each (Uddin *et al.*, 2011)

United States Pharmacopeia apparatus		**Specific application**
Apparatus 1	Basket apparatus	Immediate release oral solid dosage form
Apparatus 2	Paddle apparatus	Tablets, capsules and suspensions (floating solids requires a sinker)
Apparatus 3	Reciprocating cylinder apparatus	Extended release products Modified release beads Beads in capsules
Apparatus 4	Flow-through cell apparatus	**Solids:** tablets, capsules, implants, powders and granules **Semi-solids:** suppositories, soft gelatin capsules and ointments **Liquids:** suspensions
Apparatus 5	Paddle-over disk apparatus	Transdermal patches
Apparatus 6	Cylinder apparatus	Transdermal patches
Apparatus 7	Reciprocating holder apparatus	Small transdermal patches

Dissolution apparatus 2 (paddle) is most widely used for tablets and capsules (Chevalier *et al.*, 2009). Variation in results can occur due to the position of the tablets in the dissolution vessel resulting from a difference in the hydrodynamic effects and the fluid sheer forces in the different positions in the vessel (Missaghi and Fassihi, 2005). The USP describes the paddle apparatus as a 1000 ml glass vessel with a semi-hemispherical bottom with an inside diameter of between 98 and 106 mm. The vessel is housed in a water bath maintained at 37 °C. The dimension of the paddle should be a trapezoid made of 3 to 5 mm thick stainless steel. The top of the trapezoid should be 75 mm and the bottom 42 mm. The height of the trapezoid should be 19 mm ± 0.5 mm. The diameter of the paddle

shaft is 9.4 to 10.1 mm. The paddle is inserted to a depth of 25 mm from the bottom of the vessel (USP, 2016a). The stirring rate or agitation is usually 50 rpm but can be increased to 75 or 100 rpm, if required (USP, 2016a).

The reaction vessel is filled with 600 ml 0.1 M HCl and allowed to equilibrate to 37 °C. The dosage form should be introduced while stirring at the prescribed rate (50 rpm). Samples are taken at predetermined times and the volume removed should be replaced with the same volume of 0.1 M HCl. After 2 h the pH is increased by the addition of 300 ml 0.2 M Na_3PO_4 and adjusted to 6.8 by the addition of 2 M HCl or 2 M NaOH. The volume of sample should now be replaced by the same volume of pH 6.8 buffer. Samples are taken for 6 h after adjusting the pH. All samples should be filtered before analysis. This is a modified method based on the USP as used at North West University.

As described in the following chapter (Chapter 3: Materials and Methods), the study presented here followed the mini-tablet-in-capsule system approach and the application of the various testing parameters mentioned in the literature review to determine the properties of the products produced.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

In this study a commercially available Rooibos extract made by Rooibos LTD was used. This extract is available in large quantities and it has a consistent composition between different batches. To create the sustained release tablet, it was decided to evaluate Kolidon SR®, Retalac®, hydroxypropyl methyl cellulose (HPMC) and a Chitosan-HPMC combination as possible rate controlling polymer excipients. Excipients available at the North-West University, Potchefstroom Campus, School of Pharmacy, Department of Pharmaceutics were used to produce the immediate release and sustained release mini-tablets.

3.2 Materials

The spraydried rooibos extract powder used in this study was donated by Rooibos Limited (Clanwilliam, South Africa). All chemicals used in the assays and tablet production were of analytical (AR) grade obtained from Associated Chemical Enterprises (Johannesburg, South Africa), Merck Millipore (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, USA). The pharmaceutical excipients were supplied by the companies listed in Table 6.

Table 6: List of excipient suppliers

Excipient	Supplier
Avicel PH101	FMC International (Philadelphia, USA)
Emcompress	JRS PHARMA GmbH & Co (Rosenberg, Germany)
Tablettose	Meggle (Wasserburg, Germany)
$NaHCO_3$	Merck Millipore (Darmstadt, Germany)
Ac-Di-Sol	FMC International (Philadelphia, USA)
Explotab	JRS PHARMA GmbH & Co (Rosenberg, Germany)
Magnesium Stearate	E.I. Rogoff (Germiston, South Africa)
Chitosan	Warren Chemicals (Cape Town, South Africa)
HPMC	Shin-Etsu Chemical Company (Tokyo, Japan)
Kolidon SR	BASF (Ludwigshafen, Germany)
Retalac	Meggle (Wasserburg, Germany)
Kolidon VA64	BASF (Ludwigshafen, Germany)

3.3 Chemical characterisation of the Rooibos extract and *in vitro* anti-oxidant capacity determinations

The *in vitro* anti-oxidant content and capacity assays were performed in the Oxidative Stress Research Centre at the Cape Peninsula University of Technology. The assays/methods used in this study are the same standard assays/methods used in this laboratory for other studies and contractual work.

3.3.1 Total polyphenols assay

The total polyphenols assay is a modified method based on the method of Singleton and Rossi, (1956) as published by Belwal *et al,* (2016). In this analysis

the Folin Ciocalteu reagent was used (together with gallic acid as the standard) to measure total polyphenols in samples using 96 well plates and a UV/Vis plate reader.

The reagents were prepared as described below. A working Folin Ciocalteu reagent was prepared by dilution of the Folin Ciocalteu reagent (Sigma-Aldrich) in a ratio 1:10 with distilled water. A 7.5% w/v sodium carbonate solution was prepared by dissolving 7.5 g of Na_2CO_3 (Sigma-Aldrich) in distilled water and made up to a final volume of 100 ml. Gallic acid standards were prepared in 10% v/v ethanol solution with a concentration range of 0 to 500 mg/l and used to prepare the standard curve.

The assay was performed by placing 25 µl of the blank, standard or sample into the respective wells of a 96 well plate. A volume of 125 µl of working reagent was added to each well and incubated at room temperature for 5 min. Then, 100 µl Na_2CO_3 was added to each well and incubated for 2 h at room temperature. The absorbance of each well was measured at 765 nm on a Thermoscientific Multiskan Spectrum plate reader. The results were expressed as gallic acid equivalents.

3.3.2 Trolox Equivalent Antioxidant Capacity (TEAC) assay

The TEAC assay measures the ability of anti-oxidants to scavenge the 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical. The method used in this study was based on the method previously described by (Re *et al.*, 1999).

The working reagent was prepared 24 h in advance to allow for the formation of the $ABTS^{\bullet+}$ radical. A 7 mM solution of ATBS diammonium salt (Sigma-Aldrich) and a 140 mM potassium peroxodisulphate (Sigma-Aldrich) solution were prepared. A volume of 88 µl of the potassium peroxodisulphate solution was added to 5 ml of the ATBS solution and stored in the dark for 24 h at room temperature. This solution was diluted with absolute ethanol to obtain an absorbance of 2 at 734 nm just before use. Trolox (Sigma-Aldrich) standards

were prepared for a standard curve with concentrations between 0 and 500 µM in ethanol, while ethanol was used as a blank solution.

A volume of 25 µl of the blank, standard and samples were aliquoted into the wells of a 96 well plate. Then, 300 µl of the working reagent was added to each well. The plate was then incubated at room temperature in the dark for 30 min. The absorbance was determined at 734 nm in a plate reader. The results were reported as µmol Trolox/g.

3.3.3 The Ferric Reducing Ability of Plasma (FRAP) assay

The FRAP assay as described by Benzie *et al.* (1996 and 1999) measures the ability of anti-oxidants to reduce Fe(III) to Fe(II). The Fe(III) tripyridyltriazineis (TPTZ) solution is colourless, but changes to blue when oxidized to the Fe(II)TPTZ at pH 3.6.

The amount of Fe(II) TPTZ formed was spectrophotometrically determined at a wavelength of 593 nm. The working reagent was made by preparing the following solutions: 300 mM pH 3.6 acetate buffer, 10 mM TPTZ (Sigma-Aldrich) in 40 mM HCl, and 20 mM iron(III)chloride hexahydrate. The reagents were mixed in a ratio of 10:1:1:2 in terms of acetate buffer:TPTZ:$FeCl_3$·$6H_2O$:distilled water. Ascorbic acid (Sigma-Aldrich) solutions with a concentration of 0 to 1000 µM were used as standards. A volume of 10 µl of the blank, standard or sample was added into the wells of a 96 well plate. Then 300 µl of the working reagent was added to each well. The 96 well plate was incubated at 37 °C in the dark for 30 min. The absorbance was read on a Thermoscientific Multiskan Spectrum plate reader at a wavelength of 593 nm. The results were reported as µmol ascorbic acid equivalents/g.

3.3.4 The Oxygen Radical Absorbance Capacity (ORAC) assay

The ORAC assay measures the ability of a sample to scavenge the 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) radicals. The method described is based on the methods previously published by Ou *et al*. (2001). The reagents

required for the assay are: 75 mM pH 7.4 phosphate buffer, 0.036 ppb fluorescein (di-sodium salt) (Sigma-Aldrich) in phosphate buffer, 0 - 400 µM Trolox (Sigma-Aldrich) standards in phosphate buffer and a 25 mg/ml AAPH· (Sigma-Aldrich) solution in phosphate buffer to be prepared just before use. A volume of 12 µl blank, standard and samples was pipetted into the wells of a 96 well fluorescence plate (black plate). A volume of 138 µl fluorescein solution was added to each well. A volume of 50 µl of the AAPH·solution was added to each well. The decrease in fluorescence was measured using a 485 nm excitation filter and a 535 nm emission filter for a period of 2 h using a Thermoscientific Floroskan Asent plate reader. The area under the curve was used to calculate the ORAC values for the samples. Results were reported as µmol Trolox equivalents/g.

3.3.5 Analysis of the main individual polyphenol content

The main individual polyphenol make-up were analysed by HPLC. The HPLC analyses were performed using an Agilent 1200 HPLC system (Santa Clara, USA) equipped with an auto-sampler and a diode array detector. A YMC-Pack Pro C18 RS (5 µm) 150 X 4.6 mm (YMC America, Allentown, USA) reverse phase column was used for the separation of the compounds with a 10 µl injection volume. The mobile phase employed was a gradient (Table **7**) of (A) 2% v/v formic acid and (B) 100% methanol (Merck) at a flow rate of 0.5 ml/min. The gradient composition is shown in Table 7. Samples were filtered with 0.22 µm syringe filters before being diluted with distilled water. The chromatograms were constructed by monitoring the absorbance at 287 nm and 360 nm (Joubert, 1996).

Table 7: Solvent gradient used during HPLC analysis of Rooibos extract

Time (min)	% A	% B
0	80	20
5	80	20
25	75	25
40	70	30
50	60	40
60	50	50
70	40	60
80	20	80
90	40	60
110	80	20
125	80	20

3.4 Powder flow test for selected tablet excipients

The powder flow tests were performed using the standard methodology used at the University of the North West, Department of Pharmaceutics. These methods are based on section 1174 of the United States Pharmacopoeia (USP, 2014). The assays were performed on all the major excipients, the Rooibos extract and the final immediate release and sustained release formulations.

3.4.1 Angle of repose

The angle of repose was measured by allowing 50 g of the powder (including excipients, Rooibos extract and final formulations) to be tested to flow from a funnel onto a solid surface. **Figure 8** shows a schematic diagram of the apparatus used in this measurement. The height and the diameter of the base of the powder

cone that formed were recorded. The angle of repose (α) was calculated using the following Equation 1:

Equation 1

$$\tan \alpha = \frac{\text{height}}{\text{0.5 x diameter of base}}$$

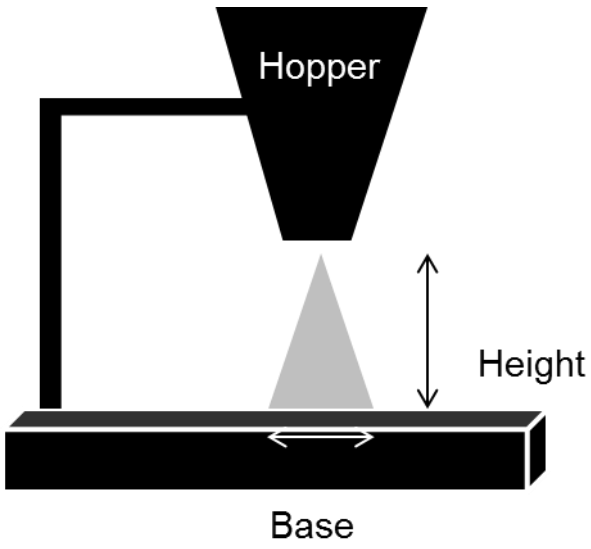


Figure 8: Schematic diagram of the apparatus used in the angle of repose measurement

3.4.2 Critical orifice diameter

The apparatus used to measure the critical orifice diameter (COD) consisted of a series of disks that when placed on top of each other formed a smooth cone on the inside when placed in the correct order (Figure 9). The diameter of the cone varied from 1.5 mm at the bottom to 32 mm at the top. A sample of the powder (including excipients, Rooibos extract and final formulations) to be tested was poured into the cone. The disks were removed one by one from the bottom until the powder flowed out of the orifice; the size of the orifice when the powder flowed out was recorded as the COD.

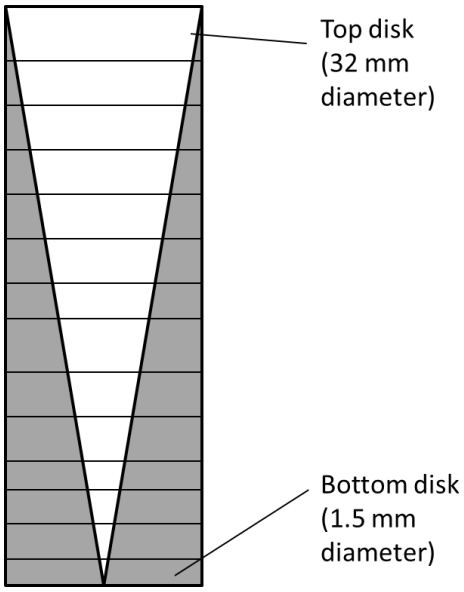


Figure 9: Schematic diagram of the apparatus used in the critical orifice diameter measurements

3.4.3 Powder flow rate

The flow rates of the powders were measured using an Erweka GTC powder flow meter (Heidenstam, Germany). A mass of 50 g of each powder (including excipients, Rooibos extract and final formulations) was placed in the hopper. The time it took for the powder to flow through a 25 mm orifice was recorded. The instrument calculated the flow rate and expressed it in g/s.

3.4.4 Hausner ratio and compressibility index

To determine the Hausner ratio and compressibility index for the powders (including excipients, Rooibos extract and final formulations), it was required to measure the bulk and tapped density of the powders. The bulk density was measured by pouring 50 g of powder into a 250 ml measuring cylinder readable to an accuracy of 2 ml. The volume was recorded as the volume of the bulk powder. The cylinder was then installed in an Erweka SVM223 tapped density apparatus (Heidenstam, Germany). The cylinder was tapped from a height of 3 mm for 5 min at 250 taps per min. After 1250 taps the tapped volume was recorded. The

Hausner ratio and compressibility index values were calculated using equations 2 and 3 as listed in USP (2016c):

Equation 2

$$\text{Hausner ratio} = {}^{V_i}/_{V_f}$$

Equation 3

$$\text{Compressiblilty index} = 100(V_i\text{-}V_f)/V_i$$

Where V_i is the bulk volume and V_f is the tapped volume.

3.5 Tablet formulation and production

The aim was to produce a mini-tablet-in-capsule system that can deliver 400 mg Rooibos extract over an 8 h period with an initial loading dose of 80 mg that is immediately released followed by a zero order or close to zero order release of a maintenance dose of 320 mg. The mini-tablet-in-capsule system was designed to contain two immediate release tablets and eight sustained release tablets each containing 40 mg Rooibos extract. Different immediate release tablets were formulated using different fillers namely Avicel®, Emcompress®, Tablettose® and sodium bicarbonate and different disintegrants namely Explotab® and Ac-Di-Sol® while magnesium stearate was used as a lubricant. The different sustained release tablets were formulated with Kolidon SR®, Retalac®, HPMC or a 50/50 mixture of HPMC and chitosan as rate controlling polymer excipients, Kolidon VA 64® was used as a binder and magnesium stearate as lubricant.

3.5.1 Mini-tablet formulation design

Different immediate release mini-tablets were produced using different combinations of excipients as shown in Table 8 and these tablets were evaluated in terms of physical properties as well as disintegration and dissolution profiles.

Table 8: Composition (% w/w) of different immediate release tablet formulations tested

Tablet ID	Rooibos extract	Magnesium stearate	Avicel®	Emcompress®	Tablettose®	$NaHCO_3$	Explotab®	Ac-Di-Sol®
A-1	61.5 %		38.5 %					
A-2	66.8 %		30.5 %					2.7 %
T-1	66.6 %	0.2 %			28.2 %			5 %
T-2	66.5 %	0.2 %			23.3 %			10 %
T-3	66.7 %	0.15 %			32.15 %			1 %
T-4	66.7 %	0.15 %			29.15 %		4 %	
T-5	66.7 %	0.15 %			25.15 %		8 %	
M-1	66.7 %	0.1 %		32.3 %				
M-2	66.7 %	0.1 %		31.3 %				1 %
M-3	66.7 %	0.1 %		24.3 %			8 %	
M-4	66.7 %	0.1 %		23.3 %			8 %	1 %
N-1	66.7 %	1 %				24.3 %	8 %	
FIR*	66.7 %	0.5 %				24.8 %	8 %	

*** FIR – Final Immediate Release Formulation**

The different sustained release tablets were formulated as outlined in Table 9. The formulations were specifically evaluated in terms of dissolution to find a matrix type mini-tablet that released at least 90 % of the Rooibos extract content within a period of 8 h, but at a slower rate than the immediate release tablets.

Table 9: Composition (% w/w) of sustained release matrix type mini-tablet formulations tested

Tablet ID	Rooibos extract	Magnesium stearate	Kolidon VA64®	HPMC®	Kolidon SR®	Chitosan	Retalac®	Silica	Talc
C-1	66.7 %	0.25 %	3.5 %	12.5 %		12.5 %			4.55%
H-1	61.5 %	0.2 %	3.5 %	32.3 %					2.5 %
K-1	66.6 %	0.1 %	3.3 %		30 %				
K-2	61.2 %	0.1 %	3.5 %		35.2 %				
K-3	75 %	0.1 %	3.5 %		21.4 %				
K-4	80 %	0.1 %	3.5 %		16.4 %				
K-5	80 %	0.25 %	3.5 %		16.25 %				
R-1	61.5 %	0.1 %	3.5 %				34.9 %		
R-2	61.5 %	0.1 %	3.5 %				33.9 %	1 %	
R-3	75 %	0.1 %	3.5 %				20.4 %	1 %	
R-4	80 %	0.1 %	3.5 %				15.4 %	1 %	
R-5	80 %	0.25 %	3.5 %				15.25 %	1 %	
FSR *	80 %	0.25 %	3.5 %				15.25 %	1 %	

*** FSR Final sustained release formulation**

3.5.2 Mini-tablet production

The mass of Rooibos extract and excipients were calculated for the number of tablets to be produced for each formulation. The amount of extract and excipients were weighed and transferred into a vessel. The powder mixture was mixed in a Turbula® mixer (Willy A Bachofer, Switzerland) for a period of 2 min. A Korsch XP1 single stage tablet press (Korsch AG, Germany) was used with a 6 mm die and flat punches. The position of the bottom punch was set in such a way to obtain the intended mass and the travel of the top punch was adjusted to regulate

the hardness and thickness of the tablet. Once the setup was complete for the correct mass and hardness of the mini-tablets, the tablet press was operated on automatic mode to produce tablets at a rate of 30 tablets per min.

Based on preliminary data, it was decided that the hardness of the immediate release mini-tablets had to be between 35 and 55 N and the sustained release mini-tablets between 70 and 100 N. The thickness of the tablets had to be below 1.85 mm in order to be able to fill 10 tablets into a size zero hard gelatine capsule.

3.6 In process tablet testing

During the production process, the mini-tablets produced were monitored for mass, thickness and hardness by randomly selecting a tablet and testing these parameters. After the batch was produced, the tests described below were performed on randomly selected mini-tablets from each formulation.

3.6.1 Hardness, diameter and thickness

Ten randomly selected mini-tablets were placed in an Erweka TBH 425 TD hardness tester (Heusenstamm, Germany). The tablets were placed on their flat sides and a piston moved down to measure the thickness of each tablet. Then the jaw moved at a constant speed and pushed each tablet against the anvil in order to measure the diameter. The jaw then moved forward at a constant speed and applied pressure to the tablet until it broke, and the force required for the tablet to break was registered. The results were reported as millimetres (mm) for the diameter and thickness and in newton (N) for hardness.

3.6.2 Friability

Ten randomly selected mini-tablets were dusted with a soft brush to remove any dust. The tablets were weighed and placed in the drum of an Erweka TAR220 friability tester (Heusenstamm, Germany). The drum was rotated at 25 rpm for 4 min as described in the USP (2016c) section 1216. The tablets were removed and dusted to remove any dust that formed during the tumbling and they were

weighed again. Equation 4 was used to calculate the friability. The friability should be no more than 1 % according to section 1216 of the USP (USP, 2014).

Equation 4

$$\text{Friability } (\%) = \frac{\text{Mass before (g)- Mass after (g)}}{\text{Mass before(g)}} \text{X100}$$

3.6.3 Mass variation

The mass variation was determined by weighing 20 randomly selected mini-tablets individually, calculating the average mass and comparing the individual tablet mass to the average. The mass variation should be less than 10% if the average mass of the tablets are less than 130 mg (USP, 2014).

3.6.4 Disintegration

Six randomly selected mini-tablets were placed in the baskets of an Erweka ZT232 disintegration tester (Heidenstamm, Germany). The water bath of the apparatus was kept at 37 °C. A beaker with a volume of 1 L was filled with deionized water. The volume of distilled water was adjusted so that the tablets were submerged during the up-stroke and the top sieve of the basket was not submerged during the down-stroke when placed in the water bath. The volume of distilled water was adjusted to ensure that the bottom of the basket was at least 15 mm submersed during the upwards stroke and the top of the basket was not submersed during the downward stroke. The basket was cycled up and down at a rate of 30 strokes per min. The time was recorded until no visual fragment of the tablet was visible on the sieve in each cylinder. For immediate release mini-tablets, the tablet ideally should disintegrate in less than 15 min and for sustained release mini-tablets, the tablet should not disintegrate within 15 min. The timing of the immediate release tablets were stopped after 60 min.

3.6.5 Dissolution

Dissolution studies were performed using a Distek, Ink., Distek 2500 (NJ, USA) dissolution system in the USP apparatus II (paddle) configuration. All screening studies were performed in triplicate and the final products were assayed six fold. The stirring rate was 50 rpm and the system was maintained at 37 °C. The initial dissolution medium was 600 ml, pH 1 (0.1M) HCl solution for two hours. The pH was then increased by adding 300 ml 0.2 M tri-sodium phosphate. If necessary, the pH was adjusted to 6.8 by the addition of a sufficient quantity of either 2 M HCl or 2 M NaOH. Samples were manually taken at predetermined intervals and the volume of solution removed was replaced with either 0.1 M HCl or phosphate buffer (USP, 2014). The samples were filtered through a 0.45 µm syringe filter to remove any potential fragment of the undissolved tablets. These samples were analysed for total polyphenols and a dissolution profile was created by plotting percentage of the polyphenols released as a function of time.

The drug release kinetics were quantitatively analysed by fitting the dissolution data to different mathematical models used in the pharmaceutical industry. The models chosen for mathematical fitting were the zero order-, first order-, Higuchi-, Kosmeyer-Peppas- and Hixon-Crowell models (Gouda *et al.*, 2017).

The freely available add-in program in Microsoft Excel, called DDSolver, was used to facilitate the modelling of dissolution data. This software applies non-linear fitting methods and has a built-in model library containing forty different dissolution models. The DDSolver program uses the non-linear least-squares curve-fitting technique, which minimizes the sum of squares (SS) or optionally the weighted sum of squares (WSS) (Zhang *et al.*, 2010).

The DDSolver program provides a number of statistical criteria for evaluating the goodness of fit of a model to the dissolution data, which include the correlation coefficient (R_obs–pre), the coefficient of determination (Rsqr, R^2, or COD), the adjusted coefficient of determination (Rsqr_adj or R^2adjusted), the mean square error (MSE), the standard deviation of the residuals (MSE_root or Sy.x), SS (sum of squares), WSS (weighted sum of squares), the Akaike Information Criterion

(AIC), and the Model Selection Criterion (MSC). Among these criteria, the most popular ones are R^2, ACI and MSC (Zhang *et al.,* 2010). The R^2 values will be used to determine the best fit and AIC and MSC values will be used to confirm the best fit or predict another model that would better describe the dissolution curve.

The formulas for the mathematical models as used by DDSolver are given in equations 5-8:

Equation 5 Zero-order Model equation

$$F = k0 \cdot t$$

Equation 6 First-order model equation

$$F = 100 \cdot (1 - e^{-k1t})$$

Equation 7 Higuchi model equation

$$F = kH \cdot t^{0.5}$$

Equation 8 Hixson-Crowell equation

$$F = 100 - [1 - (1 - kHC \cdot t)^3$$

Equation 9 Korsmeyer-Peppas equation

$$F = kKP \cdot t^n$$

The value of n in the Korsmeyer-Peppas model indicates the type of release profile. If n = 0.5, it is said to be a Fickian diffusion or diffusion controlled release profile. If 0.5<n<1, it is an anomalous diffusion or non-Fickian transport i.e. a combination of diffusion and erosion controlled release. If n = 1, it is said to be Case II transport or zero order. If the value of n = 1 in the Korsmeyer-Peppas equation, the the equation is the same as that of the zero order model ($F = kKP \cdot t^n$ but when n =1 then $F = kKP \cdot t$). If n>1, it is said to be Super Case II or erosion a controlled release profile (Holowka and Bhatia, 2014).

3.6.6 Analysis of Rooibos extract content in the mini-tablets

Immediate release and sustained release mini-tablets were crushed and dissolved in 50 ml warm water before being analysed for the total polyphenol content, anti-oxidant capacity as well as determining the main individual polyphenol concentrations. The dissolved tablets were aliquoted and diluted with distilled water for each assay. The total polyphenol assay and anti-oxidant capacity assays were performed using the same methodology that was used for the analysis of the Rooibos extract in section 3.3.1.

The main individual polyphenol make-up of the immediate release and sustained release mini-tablets were analysed by HPLC. The analysis was performed as described in section 3.3.5.

3.6.7 Stability testing

Stability studies were performed to show that the active components would remain in the specified range during storage over a specified period. This testing is performed under different conditions for products used in different climatic zones around the world. South Africa falls within the Subtropical and Mediterranean climatic zone and testing was therefore performed at 25 °C and 60 % relative humidity (Medicines Control Council, 2012). Filled gelatine capsules were placed in two glass jars (25 in each). One jar was placed in an incubator at 25 °C and 60% relative humidity and the other in an incubator at 40 °C and 75 % relative humidity. The samples placed in the 40 °C incubator was stored under stressed conditions and were expected to show a more rapid decrease in the active compounds. Two random samples were collected from each jar once a week for the first month, and then once a month for the following six months. The samples were analysed by means of the total polyphenols assay and the TEAC assay to monitor the anti-oxidant content and activity respectively. The total polyphenols assay would measure if any breakdown of the active components occurred and the TEAC assay would indicate if the anti-oxidant capacity of the tablets were influenced. This method was based on the guidelines set out by the South African

Health Products Regulatory Authority (formerly the Medicine Control Council of South Africa's) regulations, but for this study it was not possible to adhere to all the requirements prescribed to determine the shelf life of a new product (Medicines Control Council, 2012). These include the three batches of 50 000 or more units to be produced and testing to be performed for a 12 month period.

3.6.8 Statistical analysis of data

The data analysis was performed using Microsoft Excel. The dissolution data was analysed using the DD Solve add-in in Microsoft Excel as described in section 3.6.5.

CHAPTER 4: RESULTS and DISCUSSION

4.1 Chemical characterization of the Rooibos extract and *in vitro* anti-oxidant analyses

The biochemical analysis of the Rooibos extract was performed to determine the total polyphenols, FRAP, TEAC and ORAC.

Table 10: The results for the TEAC, ORAC and FRAP anti-oxidant assays performed on the Rooibos extract (n = 3).

	Total polyphenols (mg GAE/g)	TEAC (µmol TE/g)	ORAC (µmol TE/g)	FRAP (µmol AAE/g)
Average	325.98	2237.20	4719.88	1275.60
Standard deviation (SD)	4.86	205.88	354.33	43.72

The aim was to produce capsules that each contains roughly 120 mg gallic acid equivalence (GAE) polyphenols (one cup contains ± 65 mg GAE). Based on the total polyphenol assay results of 325.89 mg GAE/g for the Rooibos extract, it was decided to use 40 mg of the rooibos extract per mini-tablet (10 mini-tablets per capsule).

The antioxidant capacity assays were performed to evaluate the ability of the Rooibos extract to partake in hydroxyl scavenging and electron transfer reactions. FRAP and TEAC assays are used to measure the electron transfer properties of the extract. The results, seen in Table 10, for these assays proved the ability of the Rooibos extract to quench the free radicals by electron transfer reactions. The results for the ORAC assay in Table 10 shows the ability of the Rooibos extract to scavenge the hydroxyl radicals. These results confirm that the Rooibos extract has beneficial antioxidant properties.

The Rooibos extract was analysed to determine the individual phenolic components by HPLC. All the phenolic compound elution was monitored at 287 nm except aspalathin at 360 nm. The results can be seen in Table 11 and the chromatograms in Figure 10 and Figure 11.

Table 11: HPLC analysis results for the Rooibos extract showing individual polyphenols.

	Aspalathin µg/g	Orientin µg/g	Isoorientin µg/g	Isovetixin µg/g	Vetexin µg/g	Hyperoside µg/g	Quercetin µg/g	Luteolin µg/g
Rooibos extract	1347.56	587.54	927.25	137.41	194.30	848.75	58.23	27.09

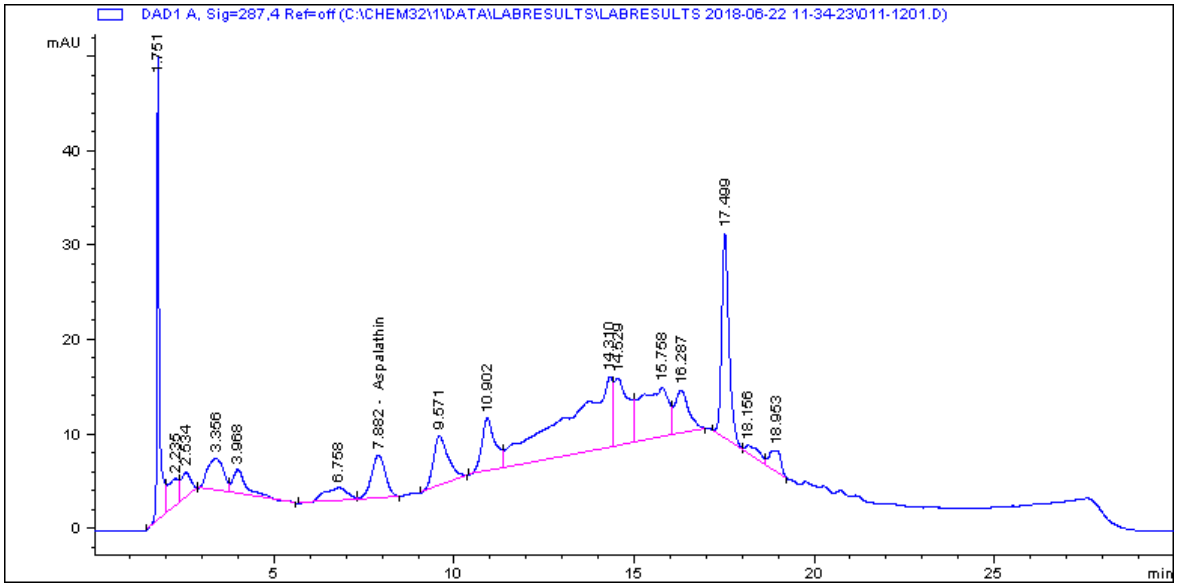


Figure 10: A chromatogram for the analysis of the Rooibos Extract monitored at 287 nm

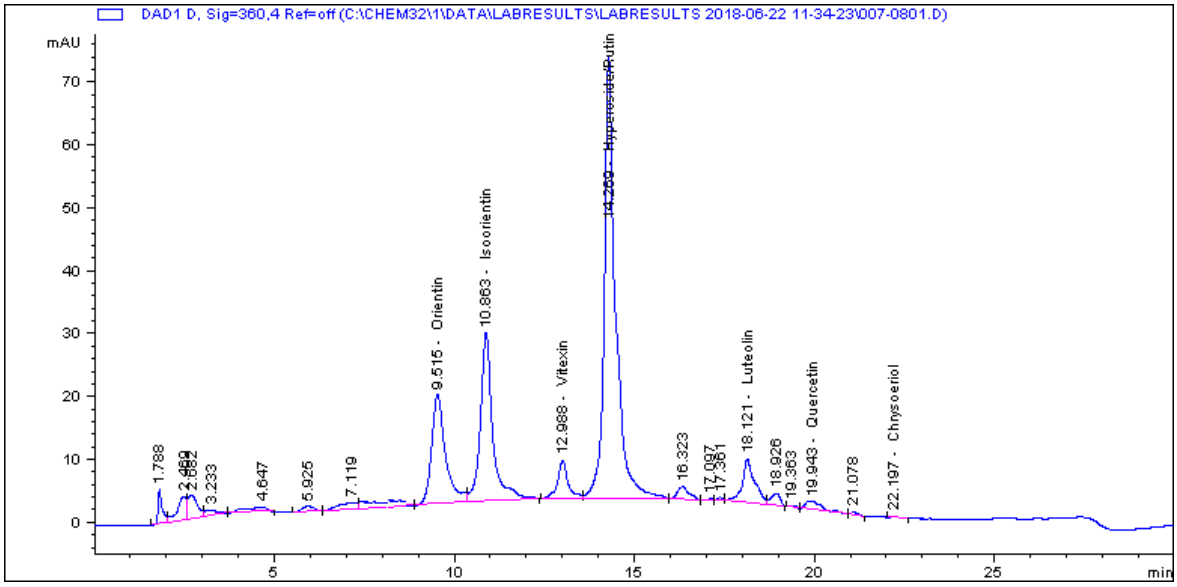


Figure 11: A chromatogram for the analysis of the Rooibos Extract monitored at 360 nm

4.2 Powder flow tests of selected tablet excipients

The results of the different powder flow tests for each excipient should be used together to evaluate the powder flow properties of the excipient. The powder flow properties were measured for both the Rooibos extract (i.e. the active ingredient) and all excipients with a concentration of more than 10% (w/w) in the final formulation as well as the final powder mixtures of both the sustained and immediate release mini-tablets.

4.2.1 Angle of Repose

Table 12 shows the average angle of repose values for the Rooibos extract, excipients and the powder formulations. The powder flow property category scale as shown previously in Table 2 (p35) was applied to the results to evaluate the powder flow properties.

Table 12: The angle of repose values of the selected excipients and powder mixtures of the final mini-tablet formulations

Material	n	Angle of Repose	Standard deviation	Flow property
Rooibos extract	3	21.35	0.355	Excellent
Avicel®	3	19.40	0.950	Excellent
Chitosan	3	35.11	0.736	Fair
Emcompress®	3	21.36	1.689	Excellent
HPMC	3	28.34	1.869	Excellent
Kolidon SR®	3	11.10	0.666	Excellent
Retalac®	3	25.36	2.172	Excellent
Sodium bicarbonate	3	21.15	0.787	Excellent
Tablettose®	3	22.91	0.697	Excellent
Immediate Release formulation	3	19.11	0.134	Excellent
Sustained Release formulation	3	15.30	0.237	Excellent

The powder cone can easily be distorted for some powders and requires special care when creating the cone. If the cone is distorted, the angle of repose doesn't give a true reflection of the powder flow properties. The Rooibos extract and the formulations containing the Rooibos extract didn't form a proper cone in this test. The cone tended to implode towards the top, which made this an inadequate method to predict the flow properties of the powder. All the other excipients exhibited excellent flow properties according to the angle of repose results except for chitosan, which was fair.

4.2.2 Critical Orifice Diameter

Table 13 contains the diameter of the orifice where the powder started to flow continuously from the cone.

Table 13: The critical orifice diameter values of the selected excipients and powder mixtures of the final mini-tablet formulations

	Critical orifice (mm)
Rooibos extract	16
Avicel®	3
Chitosan	24
Emcompress®	3
HPMC	20
Kolidon SR®	3
Retalac®	3
Sodium bicarbonate	8
Tablettose®	8
Immediate Release formulation	12
Sustained Release formulation	12

The USP states that the flow through a critical orifice is a very good technique to determine powder flow properties, but there are no criteria (or benchmark) to compare the results of this test to describe the powder flow properties. The results of this test as shown in Table 13 show that Chitosan, HPMC and the Rooibos extract only started to flow through larger orifices. The apparatus used

had a relatively narrow cone when compared to the apparatus used in the flow rate measurement. This could explain why some powders flowed through a smaller orifice in this test, but required tapping to initiate flow when measuring the flow rate.

4.2.3 Powder flow rate

The results for the flow rate of the Rooibos extract and the excipients are given in Table 14. The flow rate was measured through a 25 mm orifice. Some of the powders had to be tapped to initiate flow. Once the flow was established the powders flowed freely. This could be as a result of the wider angle of the funnel used in this apparatus.

Table 14: The flow rate of the selected excipients and powder mixtures of the final mini-tablet formulations

	Flowrate (g/s)
Rooibos extract	17.9 *
Avicel ®	35.7
Chitosan	8.9 *
Emcompress ®	71.4
HPMC	19.9
Kolidon SR ®	27.1
Retalac ®	38.5
Sodium bicarbonate	83.3
Tablettose ®	35.7
Immediate Release formulation	16.7 *
Sustained Release Formulation	17.2 *

*** - Samples had to be tapped gently to initiate flow**

The results indicate that the Rooibos extract powder and the powders of the tablet formulations containing the extract had a slower flow rate in comparison to most of the other excipients. This may indicate that the powders of the different tablet formulations might not flow sufficiently during tablet production. The mass variation test on the finished tablets would indicate if the flow rate was a problem and if a glidants (i.e. silica or talk) would be needed.

4.2.4 Bulk and tapped volume

The bulk and tapped volume results are given in Table 15. These values were used to calculate the Hausner ratio and compressibility index values, as shown in Table 16 and Table 17.

Table 15: The bulk and tapped volumes of the selected excipients and powder mixtures of the final mini-tablet formulations

	Bulk volume (ml)	Tapped volume (ml)
Rooibos extract	160	120
Avicel ®	130	102
Chitosan	242	170
Emcompress®	54	43
HPMC	126	94
Kolidon SR®	98	92
Retalac ®	160	122
Sodium bicarbonate	48	37
Tablettose®	70	56
Immediate Release matrix	104	80
Sustained Release matrix	112	86

Table 16: The Hausner ratio of the selected excipients and powder mixtures of the final mini-tablet formulations

	Hausner ratio	Flow properties
Rooibos extract	1.33	Passable
Avicel®	1.27	Good
Chitosan	1.42	Poor
Emcompress®	1.26	Good
HPMC	1.34	Passable
Kolidon SR®	1.06	Excellent
Retalac®	1.31	Passable
Sodium bicarbonate	1.30	Passable
Tablettose®	1.25	Good
Immediate Release matrix	1.30	Passable
Sustained Release matrix	1.17	Good

The Rooibos extract powder and all the excipients (except chitosan) exhibited Hausner ratio values below 1.4, indicating powder flow properties that ranged from poor to excellent. According to the Hausner ratio value, Rooibos extract powder exhibited passable flow properties.

Table 17: The compressibility index for the excipients and powder mixtures of the final mini-tablet formulations as well as the predicted powder flow properties.

	Compressibility index	Flow properties
Rooibos extract	25.0	Passable
Avicel ®	21.5	Good
Chitosan	29.8	Poor
Emcompress ®	20.3	Good
HPMC	25.4	Poor
Kolidon SR ®	6.12	Excellent
Retalac ®	23.8	Passable
Sodium bicarbonate	22.9	Good
Tablettose ®	20.0	Good
Immediate Release	23.1	Passable
Sustained Release matrix	14.2	Good

The compressibility index values indicated similar the powder flow properties as those indicated by the Hausner ratio values. Both chitosan and HPMC showed poor flow properties, while all the other powders ranged between passable and excellent flow properties.

4.3 Physical properties of the tablets

The in-process physical properties of the mini-tablets that were measured included mass, thickness, diameter and hardness during production, while other physical properties were measured after the production process.

4.3.1 Hardness, thickness and diameter

Table 18 and Table 19 show the results of the hardness, diameter and thickness for the different immediate and sustained release mini-tablet formulations, respectively.

Table 18: The hardness, diameter and thickness of the immediate release mini-tablet formulations (average values with SD in brackets)

Tablet ID	n	Hardness (N)	Diameter (mm)	Thickness (mm)
A-1	6	86.8 (4.26)	5.98 (0.020)	1.82 (0.002)
T-1	6	33.8 (2.93)	5.96 (0.004)	1.74 (0.297)
M-1	6	47.2 (1.47)	5.99 (0.012)	1.63 (0.028
N-1	6	36.3 (2.00)	6.00 (0.009)	1.58 (0.029)
Immediate release final formulation	10	48.0 (2.40)	5.98 (0.010)	1.51 (0.036)

Table 19: The hardness, diameter and thickness of the sustained release tablet formulations (average values with SD in brackets)

Tablet ID	n	Hardness (kN)	Diameter (mm)	Thickness (mm)
C-1	6	67.5 (2.95)	5.96 (0.015)	1.81 (0.089)
H-1	6	78.0 (5.48)	5.96 (0.005)	1.88 (0.017)
K-1	6	79.3 (3.44)	5.97 (0.010)	1.87 (0.023)
K-2	6	95.8 (4.00)	5.97 (0.004)	1.95 (0.031)
K-3	6	80.2 (5.27)	5.95 (0.016)	1.61 (0.032)
K-4	6	65.3 (4.18)	5.94 (0.012)	1.46 (0.014)
K-5	6	64.8 (3.37)	5.95 (0.12)	1.45 (0.023)
R-1	6	71.0 (5.76)	5.95 (0.004)	1.87 (0.013)
R-2	6	86.0 (0.01)	5.96 (0.009)	1.87 (0.014)
R-3	6	67.1 (4.49)	5.95 (0.005)	1.55 (0.014)
R-4	6	69.2 (3.87)	5.95 (0.009)	1.48 (0.005)
R-5	6	73.3 (4.37)	5.96 (0.005)	1.50 (0.013)
Sustained release final batch	10	78.6 (6.45)	5.96 (0.013)	1.52 (0.090)

The hardness of the mini-tablets was within the range set out for this study. This would indicate that the tablets would withstand the post-production handling. All mini-tablets were below 1.9 mm in thickness allowing 10 mini-tablets to fit in the size zero hard gelatine capsules.

4.3.2 Friability

Friability testing was performed on 10 randomly selected tablets and the results are given in Table 20 and Table 21 for the immediate release and sustained release mini-tablets, respectively.

Table 20: Friability testing results of the immediate release mini-tablet formulations

Tablet ID	n	% friability	Acceptable
A-1	10	0.073	√
T-1	10	0.527	√
M-1	10	0.338	√
N-1	10	0.665	√
Immediate release final formulation	10	0.454	√

Table 21: Friability testing results of the sustained release mini-tablet formulations

Tablet ID	Number of tablets used	% friability	Acceptable
C-1	10	0.299	√
H-1	10	0.031	√
K-1	10	0.111	√
K-2	10	0.046	√
K-3	10	0.323	√
K-4	10	0.612	√
K-5	10	0.403	√
R-1	10	0.045	√
R-2	10	0.164	√
R-3	10	0.185	√
R-4	10	0.377	√
R-5	10	0.676	√
Sustained release final formulation	10	0.055	√

All formulations had an acceptable friability of below 1 % as specified in the USP. This, together with the hardness results, confirmed that the mini-tablets will withstand post-manufacture handling.

4.3.3 Mass variation

The mass variation results indicate that most formulations had acceptable mass variation and that only a few individual tablets had a variation of more than 10%. From this it can also be deduced that the variation in active ingredient(s) composition of the mini-tablets should be relatively low within the same formulation. The results for the immediate release and sustained release formulations are summarised in Table 22 and Table 23.

Table 22: Mass variation of the immediate release formulations indicating that variation was acceptable (n=20).

Formulation	Average mass (g)	Lowest mass (g)	Highest mass (g)	No out of range	Acceptable
Avicel®	0.0683	0.0697	0.0673	0	Yes
Tablettose®	0.0645	0.0648	0.0636	0	Yes
Emcompress®	0.0662	0.0675	0.0639	0	Yes
$NaHCO_3$	0.0641	0.0700	0.0614	2	Yes
Immediate release	0.0616	0.0652	0.0597	1	Yes

Table 23: Mass variation of the sustained release formulations indicating that variation was acceptable (n=20).

Formulation	Average mass (g)	Lowest mass (g)	Highest mass (g)	No out of range	Acceptable
Chitosan 1	0.0635	0.0650	0.0623	0	Yes
HPMC 1	0.0655	0.0662	0.0646	0	Yes
Kolidon SR® 1	0.0631	0.0664	0.0618	1	Yes
Kolidon SR® 2	0.0644	0.0658	0.0632	0	Yes
Kolidon SR® 3	0.0554	0.0567	0.0542	0	Yes
Kolidon SR® 4	0.0506	0.0523	0.0496	0	Yes
Kolidon SR® 5	0.0521	0.0525	0.0516	0	Yes
Retalac® 1	0.0666	0.0678	0.0642	0	Yes
Retalac® 2	0.0671	0.0679	0.0658	0	Yes
Retalac® 3	0.0583	0.0546	0.0517	0	Yes
Retalac® 4	0.0528	0.0534	0.0520	0	Yes
Retalac® 5	0.0533	0.0538	0.0523	0	Yes
Sustained release	0.0550	0.0568	0.0542	0	Yes

4.3.4 Disintegration

Table 24 lists the disintegration times for the different immediate release mini-tablet formulations. The initial Avicel® formulation (A1) exhibited a very long disintegration time and was therefore not acceptable. The tablets formed a jelly-like layer around it during the disintegration test. This jelly-like layer prevented water penetration and thus inhibited the disintegration of the tablets as the tablets dissolved rather than disintegrating.

It was decided to use disintegration time to optimize the immediate release tablets. The optimum formulation would be tested to determine the dissolution rate. AC-DI-SOL® is a disintegrant used in the pharmaceutical industry to increase disintegration of tablets and capsules (Rowe *et al.*, 2006). A concentration of 2.7% (w/w) AC-DI-SOL® was added to the Avicel® formulation resulting in a slight improvement in the disintegration time, but none of the tablets disintegrated within 30 min.

Emcompress® was used as an alternative excipient to replace Avicel®. It is water insoluble and produces dense, brittle tablets (Anon, 2016). Emcompress® was firstly used without any disintegrants. This resulted in tablets with a disintegration time of between 26 and 43 min, with 66% of the tablets disintegrating in less than 30 min. It was envisaged that the addition of disintegrants would speed up the disintegration of the tablets. The addition of 1% (w/w) AC-DI-SOL® increased the disintegration time to between 37 and 42.5 min. It was decided to investigate if another disintegrant may improve the disintegration of the tablets. Explotab® is referred to as a "super disintegrant" and is used in tablets and capsules as a powerful disintegrant (Rowe *et al.*, 2006). Similarly, the addition of 8% by weight Explotab® instead of AC-DI-SOL® also increased the disintegration time to between 34 min and two tablets not disintegrating in 60 min. A combination of 8% (w/w) Explotab® and 1% (w/w) AC-DI-SOL® had a similar disintegration time to that of the Explotab® formulation. These results indicated that Emcompress®, even with disintegrants added, was not an ideal filler for the production of acceptable immediate release Rooibos tablets.

The Emcompress® was replaced by Tablettose®, which according to the manufacturer rapidly disintegrates (Anon, 2017). The AC-DI-SOL® ratio was increased to 5 % (w/w). This resulted in a better disintegration rate, but still three of the six tablets did not disintegrate within 30 min. The AC-DI-SOL® ratio was increased to 10 % w/w resulting in none of the tablets disintegrating in 30 min. This indicated that an increase in AC-DI-SOL® did not decrease the disintegration time. The AC-DI-SOL® concentration was further decreased to 1 % w/w, which resulted in all six tablets disintegrating between 26.5 and 28.5 min. This was an

improvement, but still did not produce tablets that could disintegrate within the generally acceptable time of 15 min.

The AC-DI-SOL® was replaced by 4 % (w/w) Explotab® in the previously discussed formulation. This resulted in a disintegration time of between 25 and 38 min. The Explotab® was increased to 8 % (w/w), but there was a relatively low decrease in the disintegration time (26 to 33 min). These results suggested that a combination of Tablettose® and Explotab® or AC-DI-SOL® was not an ideal combination to decrease the disintegration time.

From the results of the dissolution studies performed on the initial formulations and the Emcompress® (M 1), it could be seen that the dissolution rate increased more rapidly once the pH of the solution was increased. It was then decided to use sodium bicarbonate as an excipient to increase the pH around the surface tablet in an attempt to prevent the jelly like layer and thus improve the penetration of water into the tablet. This would allow the disintegrant to disintegrate the tablets more efficiently and in doing so speed up the disintegration of the tablets. A formulation was prepared using 24.3% w/w sodium bicarbonate, 8% (w/w) Explotab® and 1% (w/w) magnesium stearate. The mini-tablets disintegrated in less than 30 min, which was still more than the required 15 min. It was, however, decided to conduct a dissolution study on this formulation and if the bulk of the payload was released within the first 2 h (i.e. acidic phase) it would be used as the immediate release formulation.

Table 24: The disintegration times for the immediate release mini-tablet formulations

Tablet ID	Disintegration time (min)					
	1	2	3	4	5	6
A-1	38	45	45	>60	>60	>60
A-2	31.5	37	48	53	53	>60
T-1	26	30	30	31	38	43
T-2	33	35	36	36	38	38
T-3	26.5	27	28	28	28	28.5
T-4	25	27	28	29	36	38
T-5	26	28	29	30	33	33
M-1	26	26	28	29	31	47
M-2	37	38	39.5	40	42	42.5
M-3	34	36	38	54	>60	>60
M-4	33	34	59	59	>60	>60
N-1	9	14	16	16	25	27
Immediate release final formulation	12	13	17	18	21	28

Table 25 contains the disintegration times for the sustained release mini-tablet formulations, showing all had a disintegration time in excess of 30 min.

Table 25: The disintegration times for the sustained release formulations.

Tablet ID	Disintegration time (min)					
	1	2	3	4	5	6
C-1	>30	>30	>30	>30	>30	>30
H-1	>30	>30	>30	>30	>30	>30
K-1	>30	>30	>30	>30	>30	>30
K-2	>30	>30	>30	>30	>30	>30
K-3	>30	>30	>30	>30	>30	>30
K-4	>30	>30	>30	>30	>30	>30
K-5	>30	>30	>30	>30	>30	>30
R-1	>30	>30	>30	>30	>30	>30
R-2	>30	>30	>30	>30	>30	>30
R-3	>30	>30	>30	>30	>30	>30
R-4	>30	>30	>30	>30	>30	>30
R-5	>30	>30	>30	>30	>30	>30
Sustained release final formulation	>30	>30	>30	>30	>30	>30

All the sustained release formulations had disintegration times of more than 30 min, indicating that the mini-tablets should not disintegrate quickly after administration, which could contribute to a sustained release profile.

4.4 Dissolution studies

The dissolution results are presented as the percentage of total polyphenols released from selected mini-tablet formulations or mini-tablet-in-capsule systems plotted as a function of time in line graphs. All tests were performed in triplicate with the exception of the final formulations for which the tests were performed on

six capsules. The dissolution results were fitted to five mathematical models commonly used to describe the dissolution profiles. R^2 was used to determine the best fit.

Initially, six formulations were prepared and dissolution tests were performed to determine which formulation would show the most potential to modulate the release of the Rooibos polyphenols over an extended period of time. The formulations were those containing chitosan, HPMC, Kolidon SR®1 and 2 and Retalac® 1 and 2. The dissolution studies of these six formulations were performed on eight sustained release tablets and two Avicel® immediate release tablets. These dissolution assays were performed before Avicel® was rejected as the immediate release filler. The decision was taken that the dissolution studies of the revised formulations would be performed using only that formulation. Once the final immediate release and sustained release formulations were complete the combination would be tested in a dissolution assay.

4.4.1 Chitosan

The percentage release of total polyphenols plotted as a function of time from the mini-tablet-in-capsule system that contained two Avicel® immediate release mini-tablets and eight maintenance dose mini-tablets containing chitosan is shown in Figure 12.

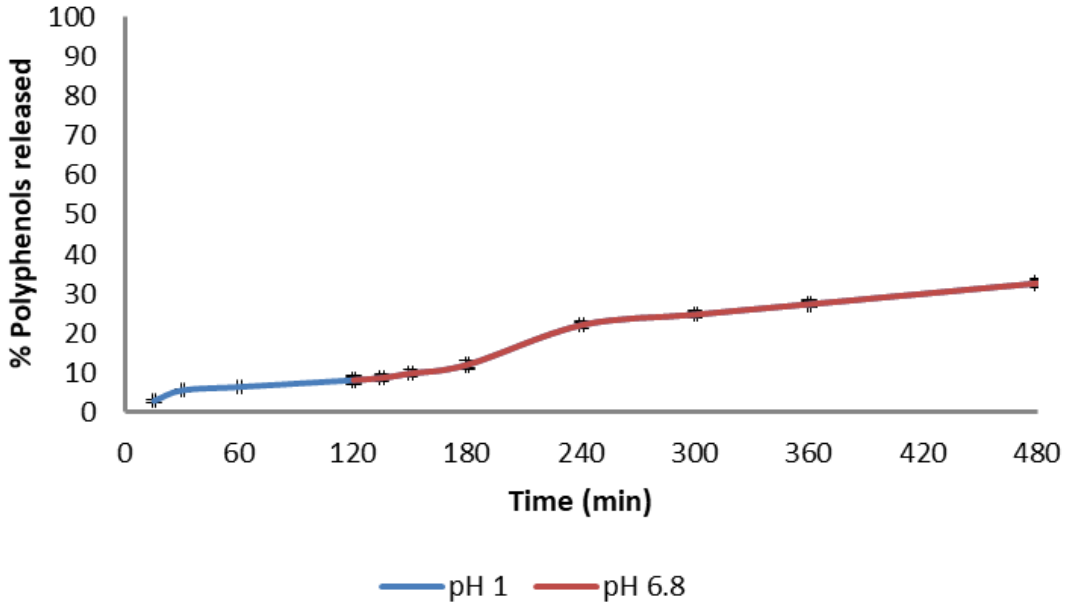


Figure 12: Dissolution profile for the mini-tablet-in-capsule system containing two Avicel® 1 immediate release mini-tablets and eight Chitosan 1 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the pH neutral buffered phase (simulated small intestine conditions).

The mathematical fit of the dissolution data showed the best fit to the first order model (R^2 closest to 1.0) (

Table **26**). Similar R^2 values were obtained after fitting of different mathematical models to the dissolution profile of the mini-tablets containing chitosan, indicating a non-specific release profile of polyphenols from this mini-tablet-in-capsule formulation. The first order model has the lowest value for ACI and the highest for MSC, confirming that the first order is the best fit for the chitosan containing formulation.

Table 26: R^2, ACI and MSC values for the mathematical fit of the dissolution data of the Chitosan 1 formulation

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9448	46.48	2.72	k0 = 0.0741
First order	0.9523	44.88	2.86	k1 = 8.577E-04
Higuchi	0.8157	59.75	1.51	kH = 1.2364
Korsmeyer-Peppas	0.9514	47.08	2.66	kKP = 0.1336 n = 0.8981
Hixson-Crowell	0.9519	44.98	2.85	kHC = 2.728E-04

The dissolution results for the chitosan formulation resulted in a 32.5 % of polyphenols released in an 8 h period.

4.4.2 Hydroxypropyl methylcellulose

The percentage release of total polyphenols plotted as a function of time from the mini-tablet-in-capsule system that contained two Avicel mini-tablets and eight HPMC mini-tablets is shown in Figure 13. The dissolution profile in Figure 13 shows a relatively slow release over the first 2 h followed by a slightly faster release. No burst release effect was observed and the total polyphenol release was only 34% after 8 h.

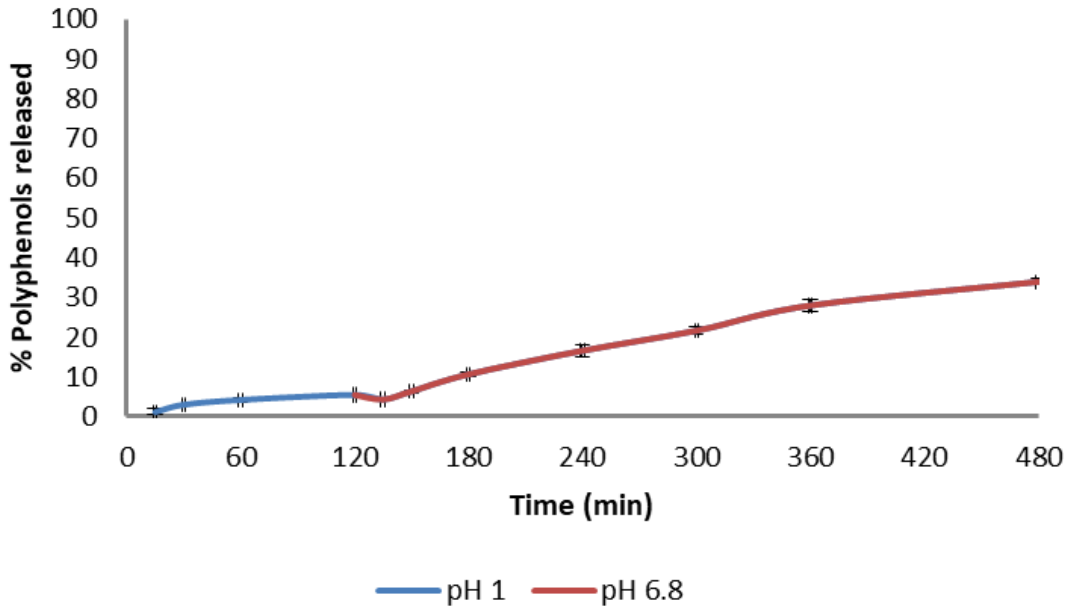


Figure 13: Dissolution profile for the mini-tablet-in-capsule system containing two Avicel® 1 immediate release mini-tablets and eight HPMC 1 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) of the HPMC containing mini-tablet-in-capsule formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 values (Table 27) and it has the lowest value for ACI and the highest for MSC, confirming that the Korsmeyer-Peppas model is the best fit for the HPMC containing formulation. The value for n is 1.24 indicating an erosion controlled release kinetics. The total % dissolution of polyphenols from the formulation after 8 h was only 34%, which is below expectation and the target value. This mini-tablet-in-capsule formulation was therefore not considered acceptable for the purpose of providing a complete dose of Rooibos polyphenols within an 8 h period.

Table 27: R^2 values for the mathematical fit of the dissolution data of the HPMC 1 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9439	48.6955	2.6979	k0 = 0.0687
First order	0.9200	52.5936	2.343551	k1 = 7.734E-04
Higuchi	0.6948	67.31801	1.004965	kH = 1.104
Korsmeyer-Peppas	0.9666	44.9697	3.03663	kKP = 1.673E-02 n = 1.243
Hixson-Crowell	0.9287	51.32037	2.459296	kHC = 2.482E-04

4.4.3 Kolidon SR® 1

The percentage release of total polyphenols plotted as a function of time from the mini-tablet-in-capsule system that contained two Avicel® immediate release mini-tablets and eight maintenance dose mini-tablets made of 30% (w/w)Kolidon SR® is shown in Figure 14. The dissolution profile in Figure 14 shows a relatively slow release over the first 2 h (acidic environment) followed by a slightly faster release over the next 6 h (neutral environment). No burst release effect was observed and the total polyphenol release was only 40% after 8 h. This was a slight improvement on the chitosan containing formulation, but the total release was still below the target value.

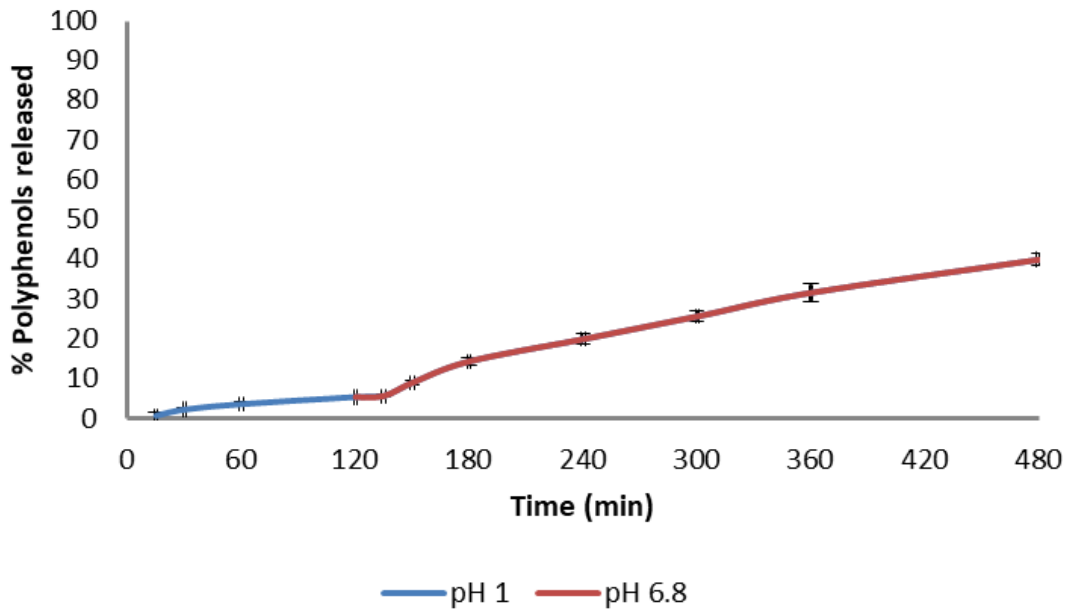


Figure 14: Dissolution profile for the mini-tablet-in-capsule system containing two Avicel® 1 immediate release mini tablets and eight Kolidon SR® 1 sustained release mini tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Kolidon SR® 1 mini-tablet-in-capsule formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 28). However, similar R^2 values were obtained for the first-order model (Table 28). The Kolidon SR® 1 fit had value for lowest ACI and the highest for MSC for the Korsmeyer-Peppas model, confirming that the Korsmeyer-Peppas model is the best fit for the Kolidon SR® 1 formulation. The value for n is 1.20 indicating an erosion controlled release kinetics.

Table 28: R^2 values for the mathematical fit of the dissolution data of the Kolidon SR® 1 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9629	48.6955	2.6979	k0 = 0.0816
First order	0.9774	52.5936	2.3436	k1 = 9.434E-04
Higuchi	0.7181	67.3180	1.0050	kH = 1.313
Korsmeyer-Peppas	0.9790	44.9697	3.0366	kKP = 2.582E-02 n = 1.198
Hixson-Crowell	0.9471	51.3204	2.4593	kHC = 3.00E-04

4.4.4 Kolidon SR® 2

The percentage release of total polyphenols plotted as a function of time from the mini-tablet-in-capsule system that contained two Avicel® immediate release mini-tablets and eight maintenance dose mini-tablets made of 35.2% (w/w) Kolidon SR® is shown in Figure 15. The dissolution profile in Figure 15 shows a relatively slow release over the first 2 h (acidic environment) followed by a slightly faster release over the next 6 h (neutral environment). No burst release effect was observed and the total polyphenol release was only 33.6% after 8 h. This was a decrease in the total amount released when compared to the Kolidon SR® 1 formulation, indicating that an increase in the Kolidon SR® concentration in the formulation results in a lowered the rate of dissolution.

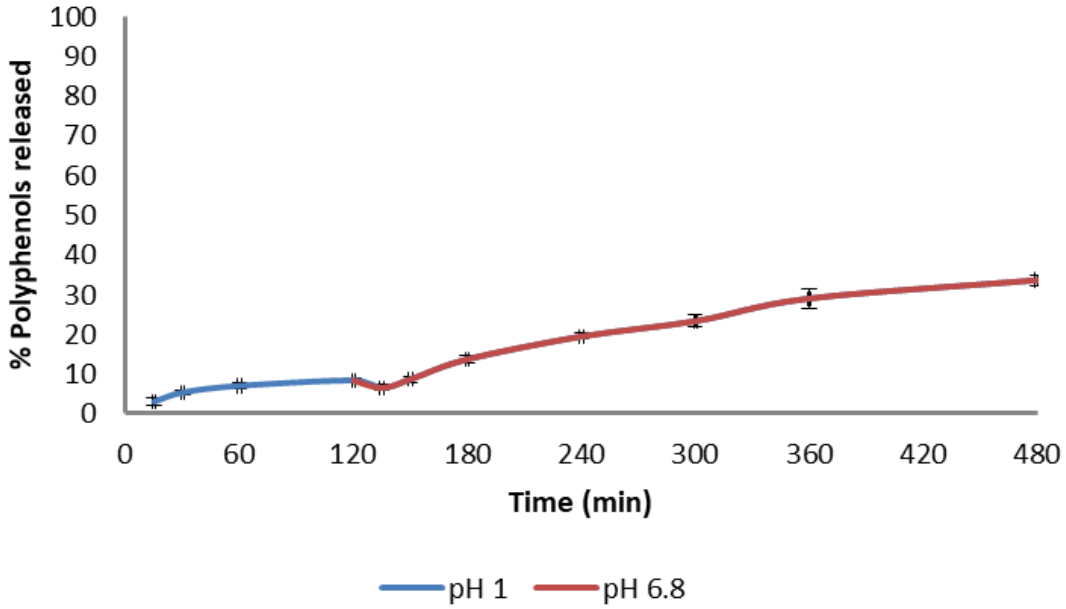


Figure 15 Dissolution profile for the mini-tablet-in-capsule system containing two Avicel® 1 immediate release mini-tablets and eight KolidonSR® 2 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Kolidon SR® 2 mini-tablet-in-capsule formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 29). However, similar R^2 values were obtained for the zero-order and Hixon-Crowell models (Table 29). The Kolidon SR® 2 fit had the lowest value for ACI and the highest for MSC for the zero order models, indicating that the zero order- and Korsmeyer-Peppas models is both a good fit for the Kolidon SR® 2 formulation. The value for n is 0.96 in the Korsmeyer-Peppas model indicating analogues release kinetics. The value is close to 1 indicating that the release is close to zero order which explain the ACI and MSC values.

Table 29: R^2 values for the mathematical fit of the dissolution data of the Kolidon SR® 2 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9522	45.4141	2.8592	k0 = 0.0742
First order	0.9488	46.1633	2.7910	k1 = 8.548E-04
Higuchi	0.7907	61.6595	1.3823	kH = 1.2291
Korsmeyer-Peppas	0.9529	47.2562	2.6917	kKP = 9.088E-02 n = 0.9649
Hixson-Crowell	0.9517	45.5282	2.8488	kHC = 2.721E-04

4.4.5 Retalac ® 1

The percentage release of total polyphenols plotted as a function of time from the mini-tablet-in-capsule system that contained two Avicel® immediate release mini-tablets and eight maintenance dose mini-tablets made of 34.9% (w/w) Retalac® is shown in Figure 16. The dissolution profile in Figure 16 shows a relatively slow release over the first 2 h (acidic environment) followed by a slightly faster release over the next 6 h (neutral environment). No burst release effect was observed and the total polyphenol release was only 46% after 8 h. This was an improvement on the chitosan containing formulation, but the total release was still below the target value.

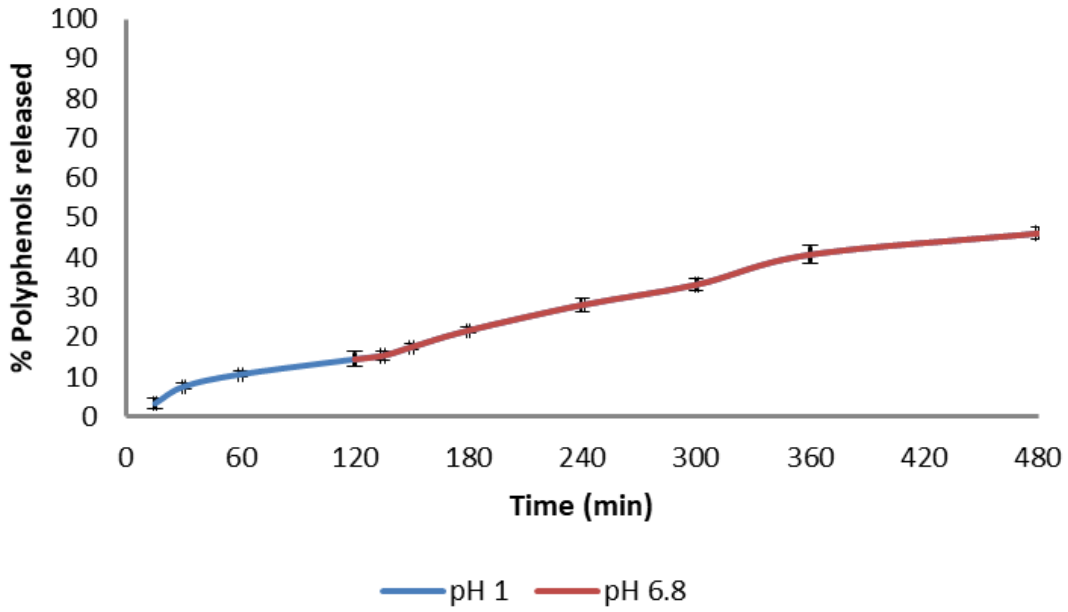


Figure 16: Dissolution profile for the mini-tablet-in-capsule system containing two Avicel® 1 immediate release mini-tablets and eight Retalac® 1 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Kolidon SR® 1 mini-tablet-in-capsule formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 30). However, similar R^2 values were obtained for the first-order model (Table 30). The Retalac® 1 fit had the lowest value for ACI and the highest for MSC for the Korsmeyer-Peppas model, indicating Korsmeyer-Peppas model is the best fit for the Retalac® 1 formulation. The value for n is 0.79 in the Korsmeyer-Peppas model indicating analogues release kinetics.

Table 30: R^2 values for the mathematical fit of the dissolution data of the Retalac® 1 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9509	51.8932	2.8317	k0 = 0.1082
First order	0.9818	40.9459	3.8269	k1 = 1.361E-03
Higuchi	0.8930	60.4537	2.0535	kH = 1.8274
Korsmeyer-Peppas	0.9857	40.3304	3.8829	kKP = 0.3645 n = 0.7894
Hixson-Crowell	0.9771	43.4988	3.5949	kHC = 4.217E-04

4.4.6 Retalac ® 2

The percentage release of total polyphenols plotted as a function of time from the mini-tablet-in-capsule system that contained two Avicel® immediate release mini-tablets and eight contained maintenance dose mini-tablets made of 33.9% (w/w) Retalac® is shown in Figure 17. The dissolution profile in Figure 17 shows a relatively slow release over the first 2 h (acidic environment) followed by a slightly faster release over the next 6 h (neutral environment). No burst release effect was observed and the total polyphenol release was only 50% after 8 h. This was a slight improvement on the Retalac® 1 formulation, indicating that the addition of silica improve the dissolution as suggested by the manufacturer.

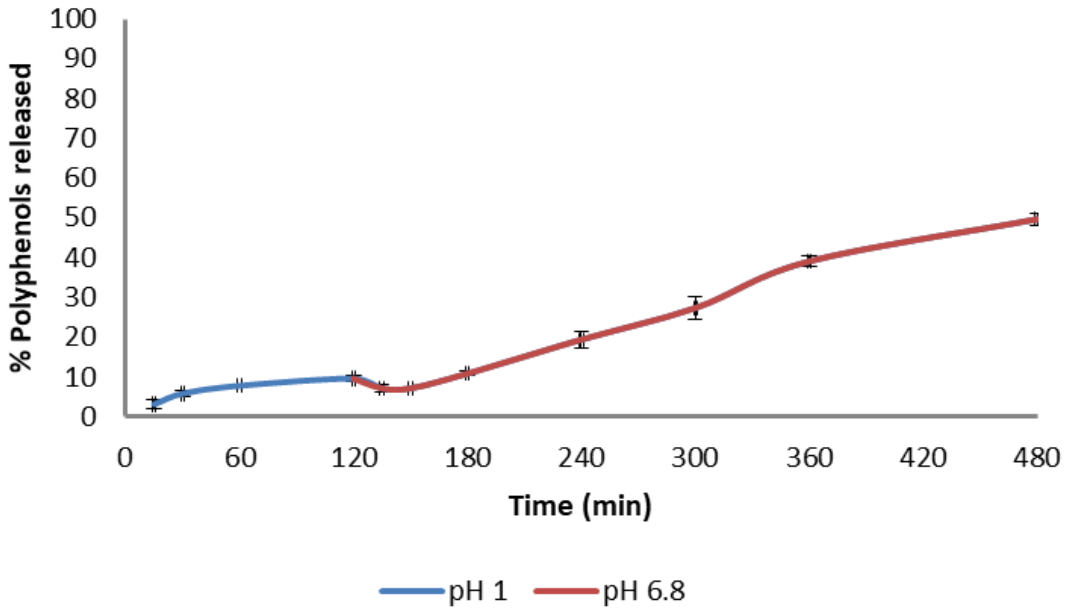


Figure 17: Dissolution profile for the mini-tablet-in-capsule system containing two Avicel® 1 immediate release mini-tablets and eight Retalac® 2 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Kolidon SR® 2 mini-tablet-in-capsule formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 31). This was confirmed by the lowest value for ACI and the highest value for MSC. The n value is 1.35 indicating an erosion controlled release.

Table 31: R^2 values for the mathematical fit of the dissolution data of the Retalac® 2 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9184	59.8442	2.3246	k0 = 0.0947
First order	0.8698	64.9864	1.8572	k1 = 1.106E-03
Higuchi	0.6609	75.5185	0.8997	kH = 1.5179
Korsmeyer-Peppas	0.9574	54.6986	2.7924	kKP = 1.272E-02 n = 1.3450
Hixson-Crowell	0.8868	63.4546	1.9964	kHC = 3.517E-04

Due to the higher percentage of polyphenol release obtained with the Kolidon SR® and Retalac ® containing formulations, they were chosen to be further optimised. The Kolidon SR® 1 had released a higher percentage of the Rooibos polyphenols in the 8 hours when compared to the Kolidon SR® 2, which indicated that a decrease in the quantity of Kolidon SR® in the formulation could increase the dissolution rate and also the total amount released over a period of 8h.

4.4.7 Emcompress® 1

The percentage release of total polyphenols plotted as a function of time from matrix type mini-tablets (i.e. for loading dose) containing 32.3% (w/w) Emcompress® is shown in Figure 18. A burst release effect can clearly be observed when the pH was changed from acidic (pH 1) to neutral (pH 6.8) during the time period of 120 -160 min.

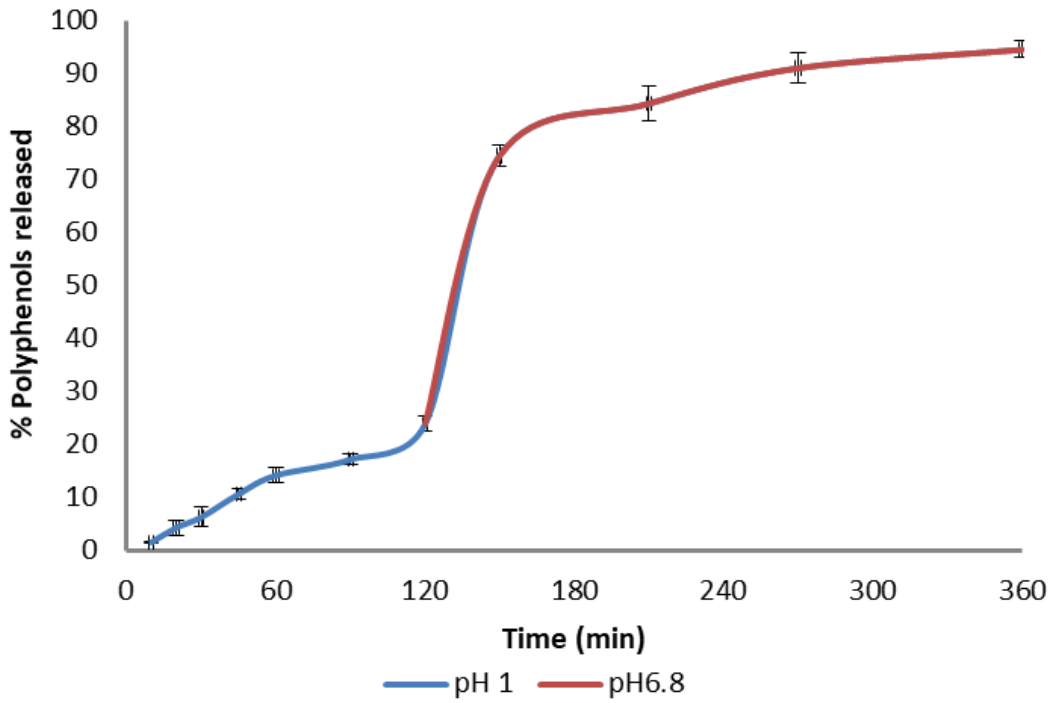


Figure 18: Dissolution profile for Emcompress® 1 immediate release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Emcompress® 1 mini-tablet formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 32). However, similar R^2 values were obtained for the zero-order and Hixson-Crowell models (Table 32). The Emcompress® 1 fit had the lowest value for ACI and the highest for MSC for the zero order and Hixon-Crowell models, indicating that the zero order-, Hixon-Crowell and Korsmeyer-Peppas models is a good fit for the Emcompress® 1 formulation. The value for n is 0.93 in the Korsmeyer-Peppas model indicating analogues release kinetics. The value is close to 1 indicating that the release is close to zero order which explain the ACI and MSC values.

Table 32: R^2 values for the mathematical fit of the dissolution data of the Emcompress® 1 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.8768	84.6759	1.9119	k0 = 0.3132
First order	0.8415	93.1620	1.1404	k1 = 4.4602
Higuchi	0.7335	87.4481	1.6599	kH = 5.317E-03
Korsmeyer-Peppas	0.8796	86.4177	1.7535	kKP = 0.4675 n = 0.9275
Hixson-Crowell	0.8737	84.9487	1.8871	kHC = 1.559E-03

A total of 97.8 % of the polyphenols was released in the 4 h following the change in pH. This indicated that the dissolution of the Rooibos polyphenols from this particular formulation was pH dependent.

4.4.8 Sodium bicarbonate

The percentage release of total polyphenols plotted as a function of time from loading dose mini-tablets containing $NaHCO_3$ as the main excipient is shown in Figure 19. The dissolution profile in Figure 19 shows a faster, steady release over the first 2 h (acidic environment). This was a major improvement over the Emcompress® 1 formulation that only released about 20 % of the payload to the $NaHCO_3$ releasing 57% in the acid solution within the 2 h period.

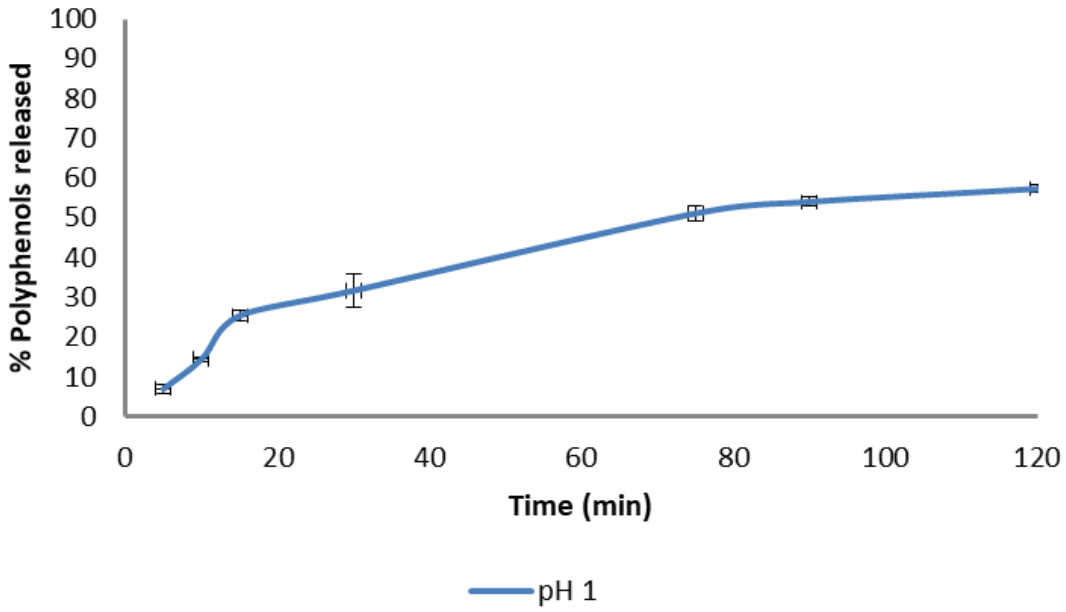


Figure 19: Dissolution profile for Sodium bicarbonate immediate release mini-tablets. The blue line represents the acid phase (simulated stomach conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the $NaHCO_3$ 1 mini-tablet formulation showed the best fit to the Korsmeyer-Peppas and Higuchi models according to the R^2 value (Table 33). However, the lowest value for ACI and the highest value for MSC were for the Higuchi model, indicating that this model has the best fit (Table 33). The value for n in the Korsmeyer-Peppas fit is 0.5043, indicating that it is a diffusion controlled release. The Higuchi model is used to describe a diffusion controlled release prolife.

Table 33: R^2 values for the mathematical fit of the dissolution data of the $NaHCO_3$ 1 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.6718	48.7437	0.8283	k0 = 0.5824
First order	0.8539	43.0763	1.6379	k1 = 9.182E-03
Higuchi	0.9678	32.4843	3.1511	kH = 5.5639
Korsmeyer-Peppas	0.9679	34.4752	2.8667	kKP = 5.4630 n = 0.5043
Hixson-Crowell	0.8028	45.1756	1.3380	kHC = 2.630E-03

This result confirmed once again that the dissolution of the Rooibos polyphenols is pH dependent and that by increasing the pH surrounding the mini-tablet the dissolution rate was increased. This formulation was selected as the immediate release formulation.

4.4.9 Kolidon SR® 3

The percentage release of total polyphenols plotted as a function of time from maintenance dose mini-tablets made of 21.4 % (w/w) Kolidon SR® is shown in Figure 20. The dissolution profile in Figure 20 shows a relatively slow release over the first 2 h (acidic environment) followed by a faster release over the next 6 h (neutral environment). The total polyphenol release was only 71% after 8 h. This was a major increase in the total amount of polyphenols released when compared to the Kolidon SR® 1 formulation.

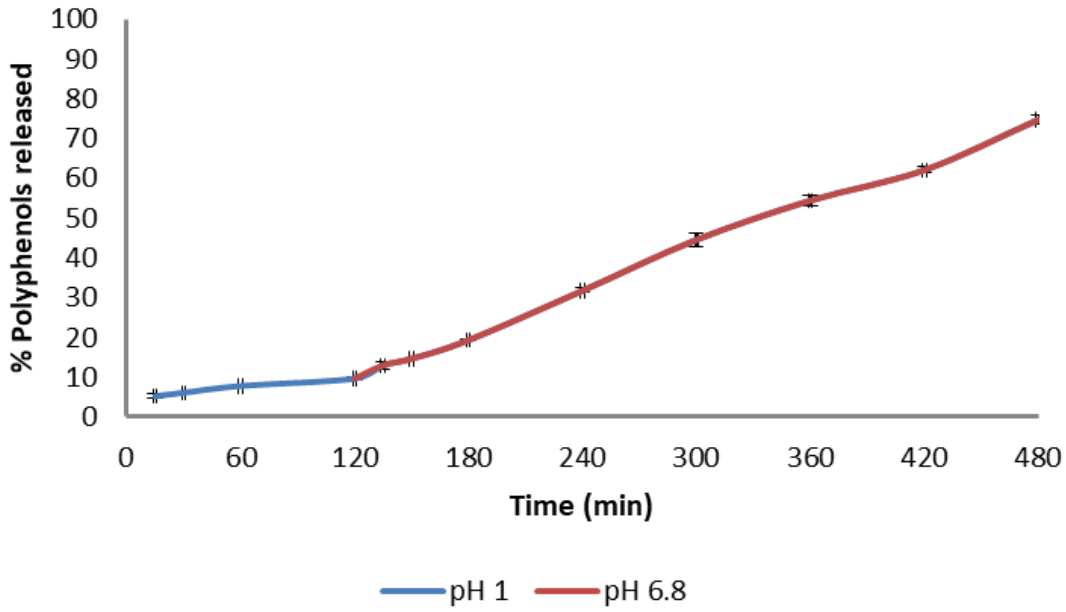


Figure 20: Dissolution profile for Kolidon SR® 3 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Kolidon SR® 3 mini-tablet formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 34). The lowest value for ACI and the highest value for MSC were for the Korsmeyer-Peppas model, confirming that this model has the best fit (Table 34). The value for n in the Korsmeyer-Peppas fit is 1.29, indicating that it is an erosion controlled release.

Table 34: R^2 values for the mathematical fit of the dissolution data of the Kolidon SR® 3 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9611	68.4655	3.0812	k0 = 0.1442
First order	0.8871	81.2657	2.0146	k1 = 1.904E-03
Higuchi	0.7197	92.1777	1.1052	kH = 2.4174
Korsmeyer-Peppas	0.9873	57.0375	4.0336	kKP = 2.632E-02 n = 1.2898
Hixson-Crowell	0.9138	78.0219	2.2849	kHC = 5.820E-04

4.4.10 Retalac® 3

The percentage release of total polyphenols plotted as a function of time from maintenance dose mini-tablets made of 20.4 % Retalac® is shown in Figure 21. The dissolution profile in Figure 21 shows a relatively slow release over the first 2 h (acidic environment) followed by a faster release over the next 6 h (neutral environment). The total polyphenol release was only 64% after 8 h. This was an increase in the total amount of polyphenols released when compared to the Retalac® 2 formulation.

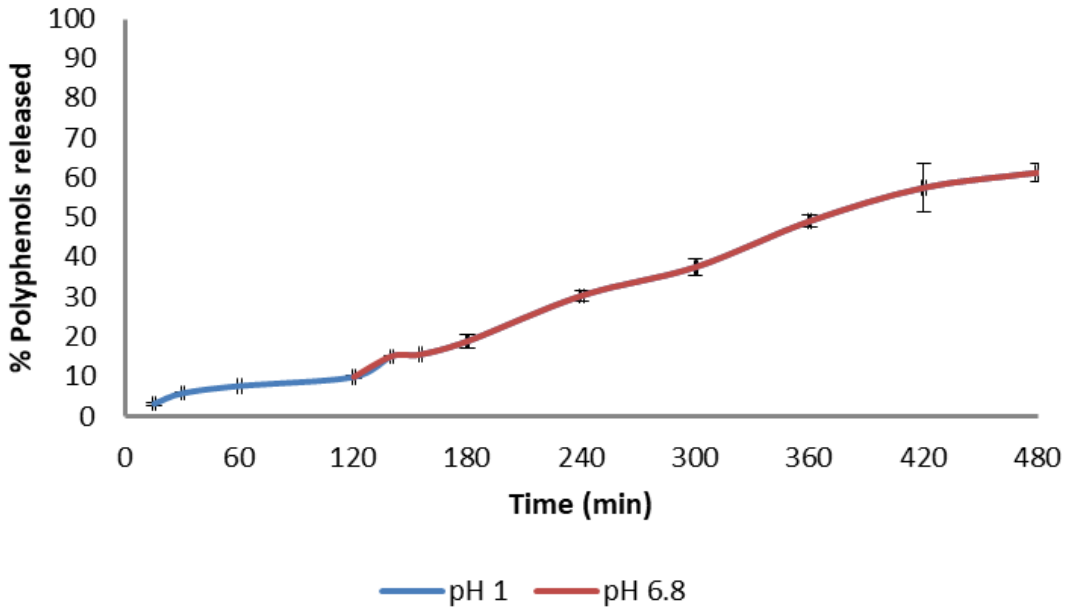


Figure 21: Dissolution profile for Retalac® 3 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Retalac® 3 mini-tablet formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 35). However, similar R^2 values were obtained for the first-order and Hixon-Crowell models (Table 35). The lowest value for ACI and the highest value for MSC were for the Korsmeyer-Peppas model, confirming that this model has the best fit (Table 35). The value for n in the Korsmeyer-Peppas fit is 1.15, indicating that it is an erosion controlled release.

Table 35: R^2 values for the mathematical fit of the dissolution data of the Retalac® 3 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.7970	57.1548	3.6968	k0 = 0.1283
First order	0.9332	71.0377	2.5399	k1 = 1.660E-03
Higuchi	0.7659	86.0916	1.2854	kH = 2.1751
Korsmeyer-Peppas	0.9872	53.1976	4.0265	kKP = 5.411E-02 n = 1.1475
Hixson-Crowell	0.9516	67.1819	2.8612	kHC = 5.102E-04

These formulations (K3 and R3) with the increased percentage Rooibos extract (decrease percentage Kolidon SR® and Retalac®) substantially increased the dissolution rate of the respective formulations. Based on these results, two formulations with a lower percentage of Kolidon SR® and Retalac® were prepared.

4.4.11 Kolidon SR® 4

The percentage release of total polyphenols plotted as a function of time from maintenance dose mini-tablets made of 16.4 % (w/w) Kolidon SR® is shown in Figure 22. The dissolution profile in Figure 22 shows a relatively slow release over the first 2 h (acidic environment) followed by a faster release over the next 6 h (neutral environment). The total polyphenol release was 96% after 8 h. This was a major increase in the total amount of polyphenols released when compared to the Kolidon SR® 3 formulation since the bulk of the payload was released.

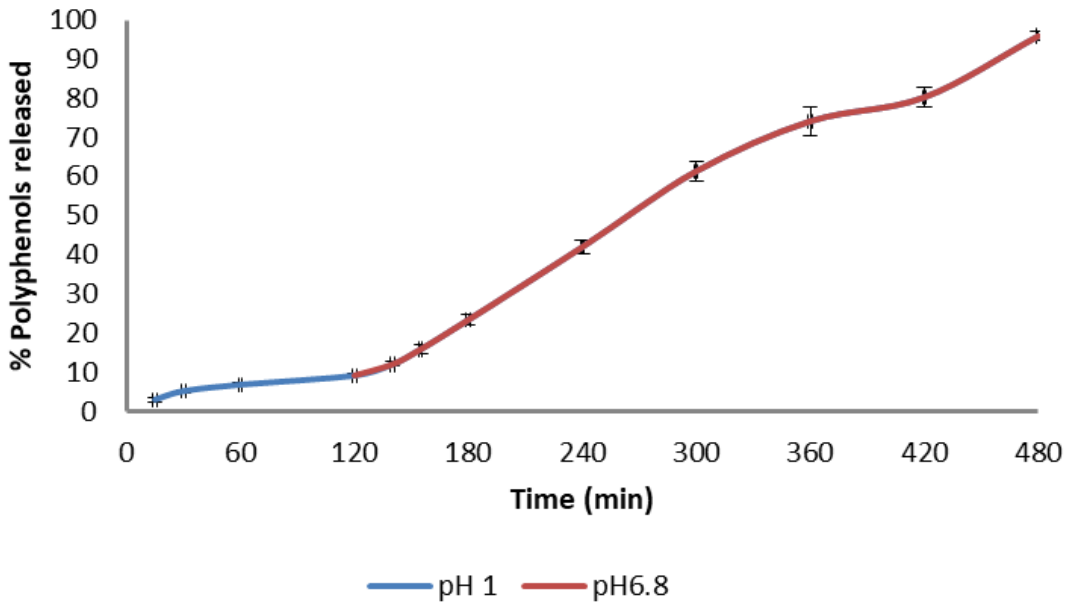


Figure 22: Dissolution profile for Kolidon SR® 4 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Kolidon SR® 4 mini-tablet formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 36). The lowest value for ACI and the highest value for MSC were for the Korsmeyer-Peppas model, confirming that this model has the best fit (Table 36). The value for n in the Korsmeyer-Peppas fit is 1.37, indicating that it is an erosion controlled release.

Table 36: R^2 values for the mathematical fit of the dissolution data of the Kolidon SR® 4 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9370	81.9349	2.5981	k0 = 0.1865
First order	0.8194	94.5732	1.5449	k1 = 2.640E-03
Higuchi	0.6818	101.3700	0.9785	kH =3.0942
Korsmeyer-Peppas	0.9771	71.8190	3.4411	kKP = 2.077E-02 n = 1.3740
Hixson-Crowell	0.8594	91.5663	1.7955	kHC =

4.4.12 Retalac® 4

The percentage release of total polyphenols plotted as a function of time from maintenance dose mini-tablets made of 15.4 % (w/w) Retalac® is shown in Figure 23. The dissolution profile in Figure 23 shows a relatively slow release over the first 2 h (acidic environment) followed by a faster release over the next 6 h (neutral environment). The total polyphenol release was 105% after 8 h. This was a major increase in the total amount of polyphenols released when compared to the Retalac® 3 formulation since the bulk of the payload was released.

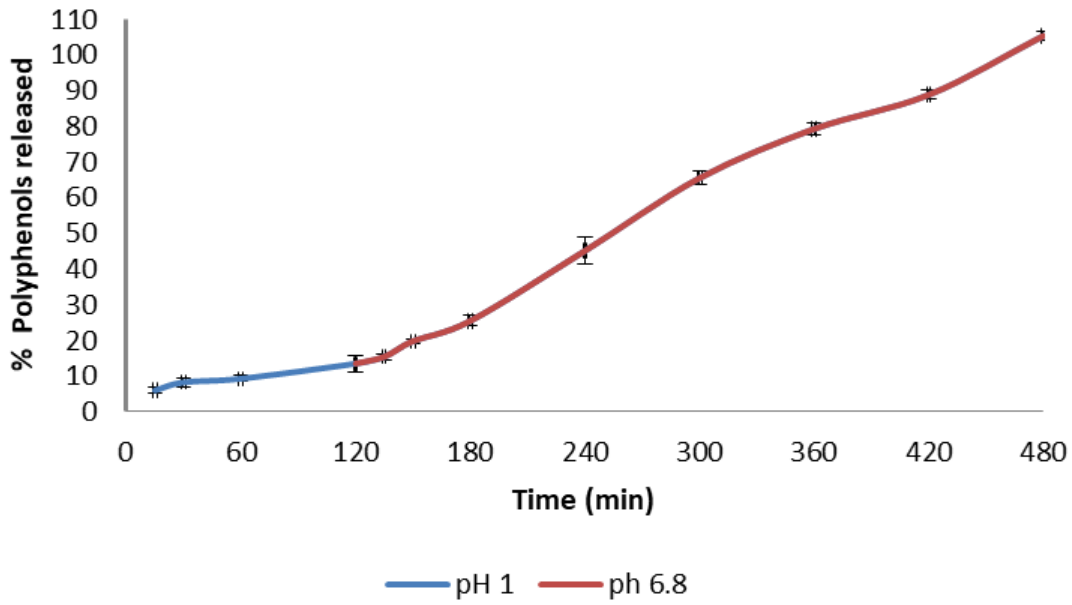


Figure 23: Dissolution profile for Retalac® 4 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Retalac® 4 mini-tablet formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 37). The lowest value for ACI and the highest value for MSC were for the first order model, indicating that this model has the best fit (Table 37). The value for n in the Korsmeyer-Peppas fit is 0.96, indicating that it is an analogues diffusion release.

Table 37: R^2 values for the mathematical fit of the dissolution data of the Retalac® 4 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9519	45.4141	2.8592	k0 = 7.419E-02
First order	0.8144	46.1633	2.7910	k1 = 8.548E-04
Higuchi	0.7036	61.6595	1.3823	kH = 1.2291
Korsmeyer-Peppas	0.9840	47.2562	2.6917	kKP = 9.088E-02 n = 0.9649
Hixson-Crowell	0.8600	45.5282	2.8488	kHC = 2.721E-04

The formulations containing either 16.4 % Kolidon SR® or 15.4 % Retalac® released the bulk of their payloads in the eight hour dissolution. Production scale batches (5 000 tablets each) of these two formulations were prepared. Both formulations showed picking, which could be the result of heat build-up in the punches and the die during the increased run time. One way to overcome this is to increase the amount of lubricant in the formulation (Conway, 2007). The amount of magnesium stearate was adjusted from 0.1 % to 0.25 % (w/w). These formulations were prepared to evaluate the effect of the increased magnesium stearate on the dissolution of the formulations since magnesium stearate is hydrophobic and decrease the rate of dissolution drugs in solid dosage forms (Rowe *et al.*, 2006).

4.4.13 Kolidon SR® 5

The percentage release of total polyphenols plotted as a function of time from maintenance dose mini-tablets made of 16.25 % (w/w) Kolidon SR® is shown in Figure 24. The dissolution profile in Figure 24 shows a relatively slow release over the first 2 h (acidic environment) followed by a faster release over the next 6 h (neutral environment). The total polyphenol release was 97% after 8 h. This was a similar amount of polyphenols released when compared to the Kolidon SR®

4 formulation but the rate of dissolution increased substantially at the change of the pH of the and started to plateau after 3 h in the neutral pH.

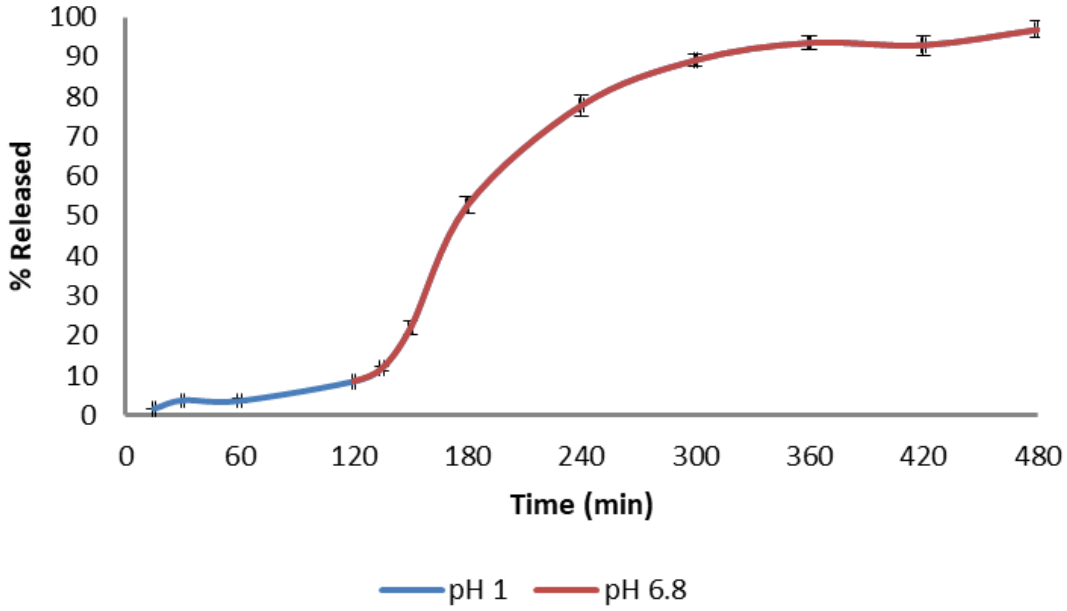


Figure 24: Dissolution profile for Kolidon SR® 5 sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Kolidon SR® 5 mini-tablet formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 38). However, similar R^2 values were obtained for the zero-order and Hixon-Crowell models (Table 38). All the models exhibited a poor fit with low R^2 values. The lowest value for ACI and the highest value for MSC were for the zero order model, indicating that this model has the best fit. The value for n in the Korsmeyer-Peppas fit is 1.04, indicating that the dissolution is a near zero order erosion controlled release. This also explains why the ACI and MSC values indicate a zero order best fit rather than Korsmeyer-Peppas.

Table 38: R^2 values for the mathematical fit of the dissolution data of the Kolidon SR® 5 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.8736	95.1748	1.9017	k0 = 0.2325
First order	0.7927	101.1116	1.4070	k1 = 3.880E-03
Higuchi	0.7007	105.5176	1.0398	kH = 3.9451
Korsmeyer-Peppas	0.8742	97.1188	1.7397	kKP = 0.1878 n = 1.0366
Hixson-Crowell	0.8385	98.4092	1.6322	kHC = 1.130E-03

4.4.14 Retalac® 5

The percentage release of total polyphenols plotted as a function of time from maintenance dose mini-tablets made of 15.25 % (w/w) Retalac® is shown in Figure 25. The dissolution profile in Figure 23 shows a relatively slow release over the first 2 h (acidic environment) followed by a faster release over the next 6 h (neutral environment). The total polyphenol release was 91% after 8 h. This was a similar amount of polyphenols released when compared to the Retalac® 4 formulation this formulation did not exhibit the rapid dissolution rate and plateauing that was seen in the Kolidon SR® 5 formulation.

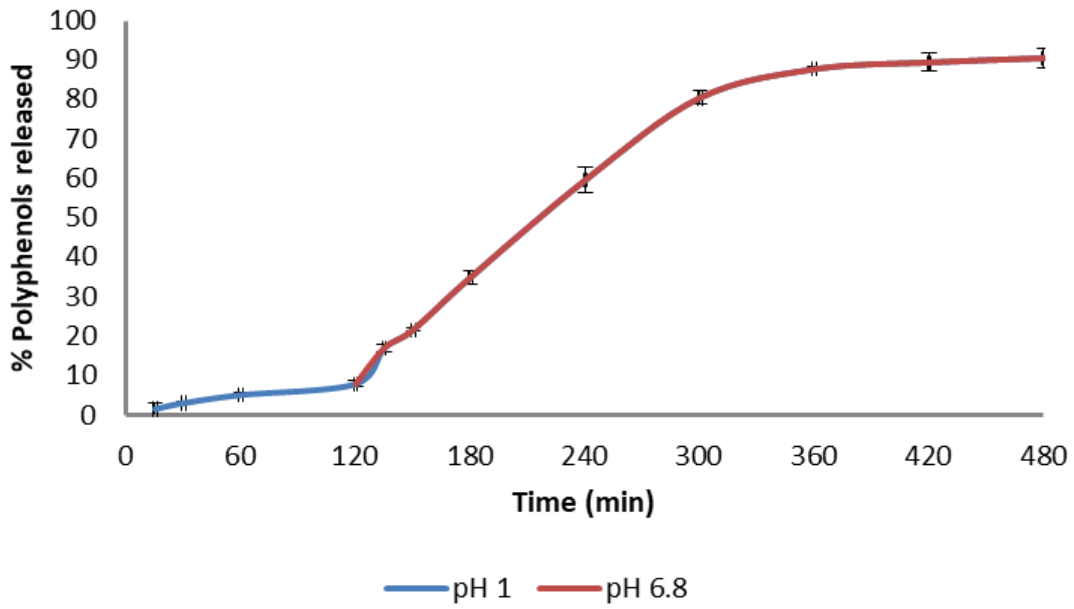


Figure 25: Dissolution profile for Retalac® 5 sustained release mini tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the Retalac® 5 mini-tablet formulation showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 39). However, similar R^2 values were obtained for the zero-order model (Table 39). The lowest value for ACI and the highest value for MSC were for the zero order model, indicating that this model has the best fit. The value for n in the Korsmeyer-Peppas fit is 1.11, indicating erosion controlled release.

Table 39: R^2 values for the mathematical fit of the dissolution data of the Retalac® 5 formulation.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9190	87.3985	2.3466	k0 = 0.2118
First order	0.8312	96.2122	1.6121	k1 = 3.312E-03
Higuchi	0.7176	102.4213	1.0947	kH = 3.5710
Korsmeyer-Peppas	0.9240	88.6362	2.2434	kKP = 0.1104 n = 1.1113
Hixson-Crowell	0.8704	93.0333	1.8770	kHC = 9.752E-04

The dissolution studies showed that the Kolidon SR® (K-5) released 96.9 % of the Rooibos polyphenols (Figure 24), but the dissolution curve had a very steep slope once the pH was adjusted after 2 hr. Within the next 2 hr, 77.9 % of the payload was released and then plateaued over the next 4 hours. The Retalac® (R-5) formulation released only 90.6 % of the Rooibos polyphenols (Figure 25), but the dissolution profile was more linear up to 6 hours and then plateaued over the next 2 hr. This more linear dissolution profile suggests that the Retalac® (R-5) formulation will release the Rooibos polyphenols at a steadier rate than the Kolidon SR® (K-5) formulation. For this reason the Retalac® formulation (R-5) was chosen as the sustained release formulation.

4.4.15 Immediate release mini-tablet production scale formulation

The sodium bicarbonate formulation was chosen as the best formulation that resulted in a more rapid dissolution. This was what was required from the immediate release formulation to act as a loading dose. The percentage of total polyphenols released plotted against time for this formulation is shown in Figure

26. The dissolution profile in Figure 26 shows a relatively rapid release with 80% of the polyphenols being released in the 2 h in acid solution.

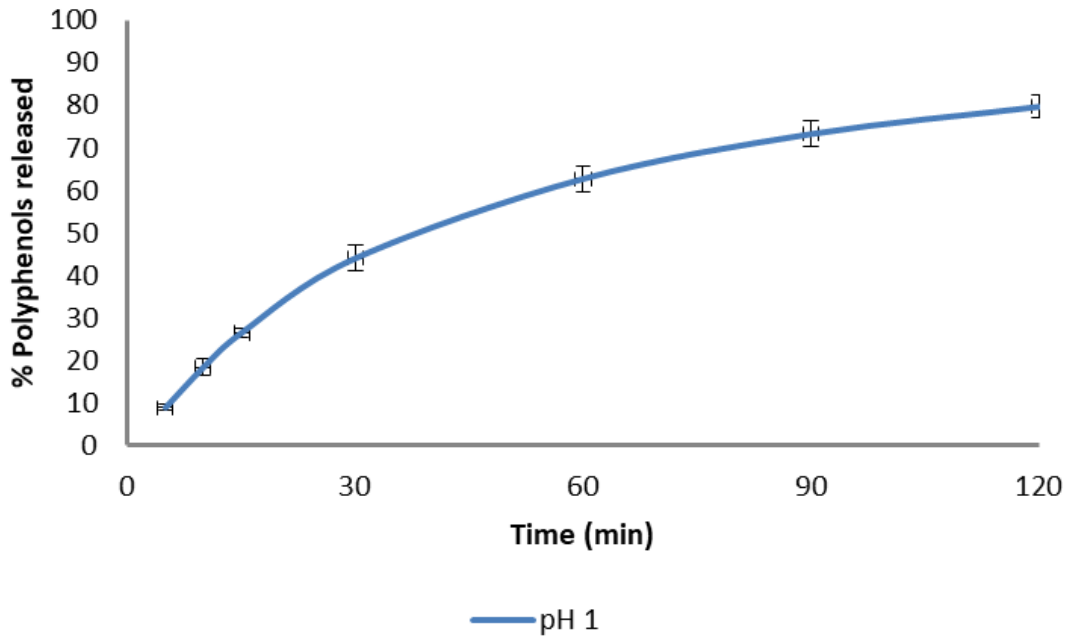


Figure 26: Dissolution profile for production scale immediate release mini-tablets at pH 1 simulating the stomach. The blue line represents the acid phase (simulated stomach conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the immediate release mini-tablet formulation showed the best fit to the first-order model according to the R^2 value (Table 40). However, similar R^2 values were obtained for the Korsmeyer-Peppas model (Table 40). The lowest value for ACI and the highest value for MSC were for the first order model, confirming that this model has the best fit. The value for n in the Korsmeyer-Peppas fit is 0.55, indicating an anomalous release leaning towards a diffusion controlled release.

Table 40: R^2 values for the mathematical fit of the dissolution data of the production scale immediate release mini-tablets.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.7387	51.7526	1.0564	k0 = 0.7988
First order	0.9754	35.2006	3.4210	k1 = 1.635E-02
Higuchi	0.9702	36.5449	3.2289	kH = 7.4942
Korsmeyer-Peppas	0.9753	37.2358	3.1302	kKP = 6.0912 n = 0.5488
Hixson-Crowell	0.9329	42.2380	2.4157	kHC = 4.445E-03

Decreasing magnesium stearate from 1 % (w/w) to 0.5 % (w/w) to increase the dissolution rate as suggested (Rowe, 2012) resulted first order dissolution profile in which 79.6 % of the payload was released in 2 h at pH 1

4.4.16 Sustained release mini-tablet production scale batch

The Retalac® 5 formulation was chosen as the final sustained release formulation as it showed a more steady release when compared to the Kolidon SR® 5 formulation. The percentage of polyphenols released plotted against time for this formulation in pH 6.8 buffer is shown in Figure 27.

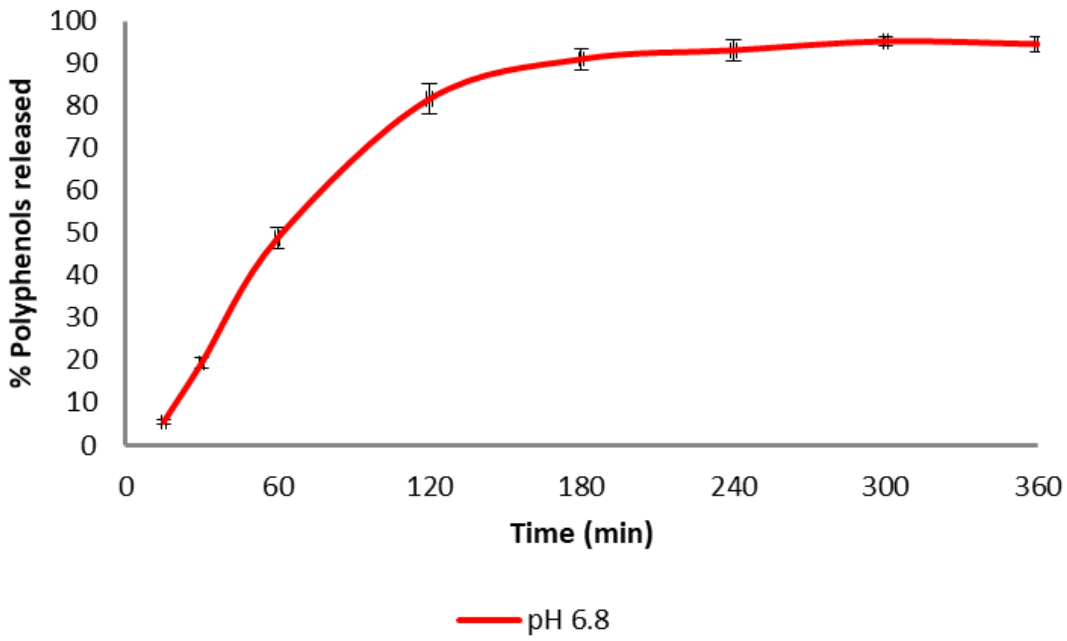


Figure 27: Dissolution profile for production scale sustained release mini tablets. The red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the sustained release mini-tablet formulation showed the best fit to the Hixson-Crowell model according to the R^2 value (Table 41). However, similar R^2 values were obtained for the first-order model (Table 41). The lowest value for ACI and the highest value for MSC were for the Hixson-Crowell model, confirming that this model has the best fit. The value for n in the Korsmeyer-Peppas fit is 0.49, indicating a diffusion controlled release profile.

Table 41: R^2 values for the mathematical fit of the dissolution data of the production scale sustained release mini-tablets.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.5338	69.0846	0.5130	k0 = 0.3492
First order	0.9707	46.9413	3.2810	k1 = 1.117E-02
Higuchi	0.9698	58.9346	1.7818	kH = 5.7556
Korsmeyer-Peppas	0.8694	60.9054	1.5354	kKP = 6.2258 n = 0.4854
Hixson-Crowell	0.9761	45.3060	3.4854	kHC = 3.121E-03

4.4.17 The mini-tablet-in capsule final formulation

Gelatine capsules were filled with two production scale immediate release and eight production scale sustained release mini-tablets. These capsules were used to test the dissolution profile as well as the stability testing.

Figure 28 shows the plot of the percentage polyphenols released against time for the final mini-tablet-in-capsule system. The dissolution test was performed using 6 capsules and an almost linear dissolution curve was obtained with a release of 93.5% (SD = 10.06) of the Rooibos polyphenols (Figure 28).

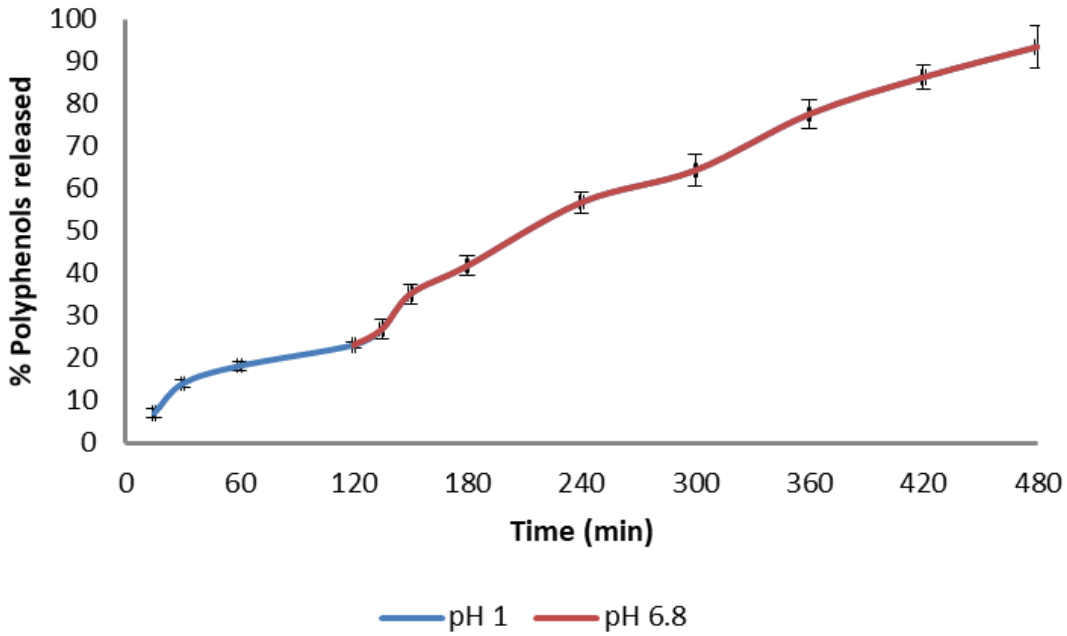


Figure 28: Dissolution profile for final mini-tablet-in-capsule system containing two production scale immediate release mini-tablets and eight production scale sustained release mini-tablets. The blue line represents the acid phase (simulated stomach conditions) and the red line represents the neutral pH buffered phase (simulated small intestine conditions).

The kinetic analysis (i.e. non-linear mathematical fit of the dissolution data) for the production scale mini-tablet-in-capsule system showed the best fit to the Korsmeyer-Peppas model according to the R^2 value (Table 42). However, similar R^2 values were obtained for the zero-order model (Table 42). The lowest value for ACI and the highest value for MSC were for the Korsmeyer-Peppas model, confirming that this model has the best fit. The value for n in the Korsmeyer-Peppas fit is 0.86, indicating an anomalous release profile.

Table 42: R^2 values for the mathematical fit of the dissolution data of the production scale mini-tablet-in-capsule system.

	R^2	ACI	MSC	Dissolution release constants
Zero order	0.9741	68.2401	3.4876	k0 = 0.2098
First order	0.9396	78.4111	2.6400	k1 = 3.488E-03
Higuchi	0.8724	87.3875	1.8920	kH = 3.6511
Korsmeyer-Peppas	0.9865	62.4619	3.9691	kKP = 0.4826 n = 0.8572
Hixson-Crowell	0.9680	70.7901	3.2751	kHC = 1.003E-03

The analysis of the dissolution data showed that the best fit was the Korsmeyer-Peppas model (Figure 29) with only a small deviation at the point where the pH is adjusted at 120 min. The Korsmeyer-Peppas model is used to describe dissolution of formulations where both diffusion of the active compounds and the relaxing of the polymer matrix occur (Jose *et al.*, 2013).

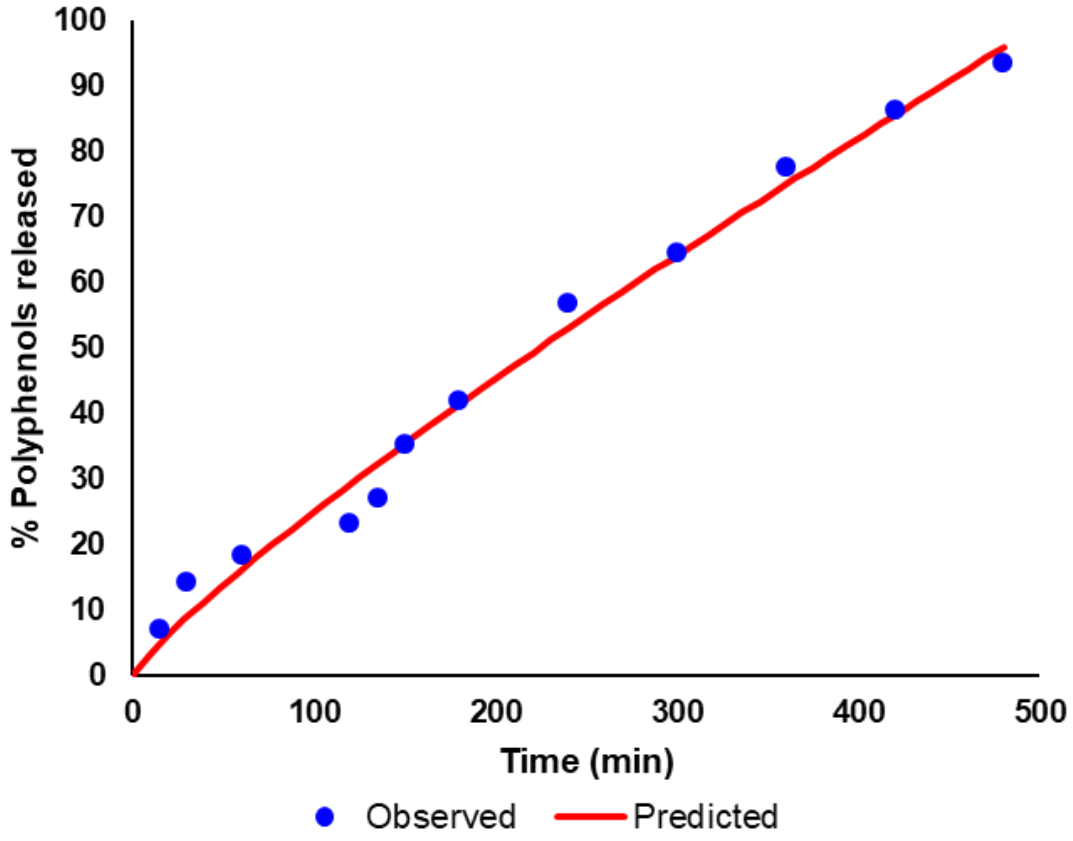


Figure 29: Korsmeyer-Peppas fit, performed by DD Sovler, to the dissolution profile for the final mini-tablet-in-capsule system (R^2 = 0.9865)

4.5 Chemical analysis of the Rooibos content of the mini-tablets

The antioxidant content and capacity assays were performed on the final immediate release- and sustained release mini-tablets. The mini-tablets were crushed and dissolved in hot water (70 °C) water to increase the dissolution rate.

4.5.1 Total polyphenol content

The total polyphenol assay was performed in triplicate on the final formulation of the immediate release as well as sustained release mini-tablets and reported as the average with the standard deviation in brackets (Table 43).

Table 43: Total polyphenol assay results performed on the final mini-tablet formulations

	Total polyphenols mg GAE/tablet
Immediate release mini-tablet	**13.99 (0.0251)**
Sustained release mini-tablet	**12.47 (1.035)**

The immediate release tablets were shown to contain 13.99 mg GAE/tablet (SD= 0.0251) and the sustained release tablets 12.47 mg GAE/tablet (SD=1.035) (Table 43) of polyphenols. Each capsule is packed with two immediate release- and eight sustained release tablets, which would yield a total theoretical polyphenol content of 127.74 mg GAE. When taken twice a day this would deliver Rooibos polyphenols at a dose of 255.5 mg GAE per day. This is lower than the amount of polyphenols taken by participants in the study by Marnewick *et al.* (2011), where a daily intake was took 380 mg GAE/day but the dietary intake of polyphenols were reduced during this study and a balanced diet contains polyphenols and other antioxidants.

4.5.2 Antioxidant capacity of the tablets

Antioxidant capacity assays were performed on both the final formulation immediate release and sustained release mini-tablets. The assays were performed in triplicate and the results reported in Table 44 as the average of triplicate samples assayed (standard deviation is given in brackets).

Table 44: Result of the antioxidant capacity assays performed on the immediate release and sustained release final formulation tablets

	TEAC (µmol TE /tablet)	ORAC (µmol TE /tablet)	FRAP (µmol AAE /tablet)
Immediate release mini-tablets	101.14 (4.47)	212.89 (5.93)	58.51 (2.10)
Sustained release mini-tablets	93.81 (6.23)	191.15 (5.19)	54.34 (4.37)

The TEAC and FRAP assays were performed to measure the ability of the Rooibos extract in the tablets to act as antioxidants in electron transfer reactions. The TEAC assay performed on the immediate release tables resulted in 101.14 µmol Trolox equivalence per tablet and the sustained release tablets in 93.81 µmol Trolox equivalence per tablet (Table 44). The mini-tablet-in-capsule system would have the equivalent activity to 952.76 µmol Trolox. The FRAP assay result showed the immediate release tablets to have an activity equivalent to 58.51 µmol ascorbic acid and the sustained release 54.34 µmol AAE (Table 44). This indicates that the mini-tablet-in-capsule system would result in 551.74 µmol AAE. This is slightly less than the amount reported by Marnewick et al. (2011), but the amount of Rooibos extract used in two capsules is less than the six cups used by the participants in that study.

The ORAC assay was performed to investigate the ability of the Rooibos extract to scavenge oxygen radicals. The results (Table 44) for both the immediate release tablets and the sustained release tablets show that the Rooibos extract has a good ability to partake in the removal of oxygen radicals. The ORAC values for the mini-tablet-in-capsule system would be 1955 µmol TE per capsule. When taking the capsules twice daily this would equal an intake of ± 4000 µmol TE per day compared to the 8400 µmol TE per day in the clinical study of Marnewick *et al* (2011).

The antioxidant content and activity of one mini-tablet-in-capsule system thus equals to about 2 cups of Rooibos.

4.5.3 High performance liquid chromatography analysis of the mini-tablets

The HPLC analysis was performed on both the immediate release and the sustained release mini-tablets to determine the concentrations of the phenolic compounds shown in Table 45. The elution profiles were recorded at 287 nm for aspalathin and 360 nm is depicted in Figure 30, Figure 31, Figure 32 and Figure 33.

Table 45: The concentrations of the different phenolic compounds in the immediate release and sustained release final mini-tablet formulations.

	Aspalathin µg/tablet	Orientin µg/ tablet	Isoorientin µg/ tablet	Isovetixin µg/ tablet	Vetexin µg/ tablet	Hyperoside µg/ tablet	Quercetin µg/ tablet	Luteolin µg/ tablet
Immediate release	58.40	23.12	38.14	5.70	7.22	38.39	2.66	1.26
Sustained release	48.18	23.12	35.00	5.14	8.03	28.90	1.96	0.89

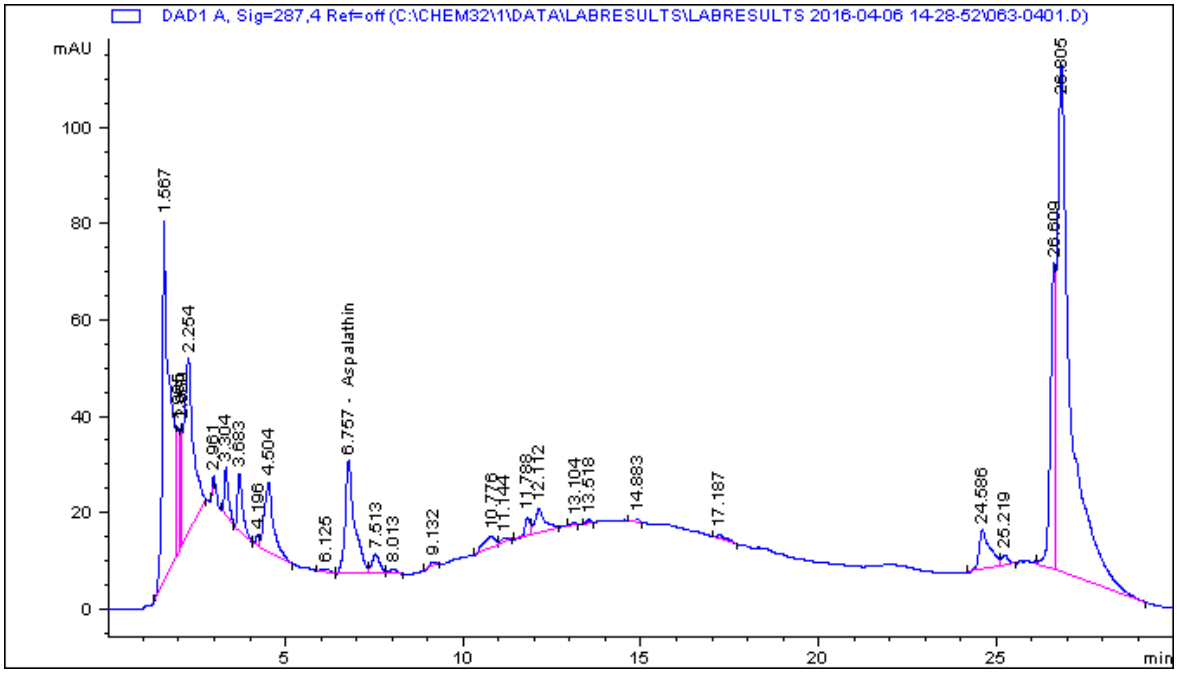


Figure 30: The chromatogram of immediate release mini-tablets monitored at 287 nm

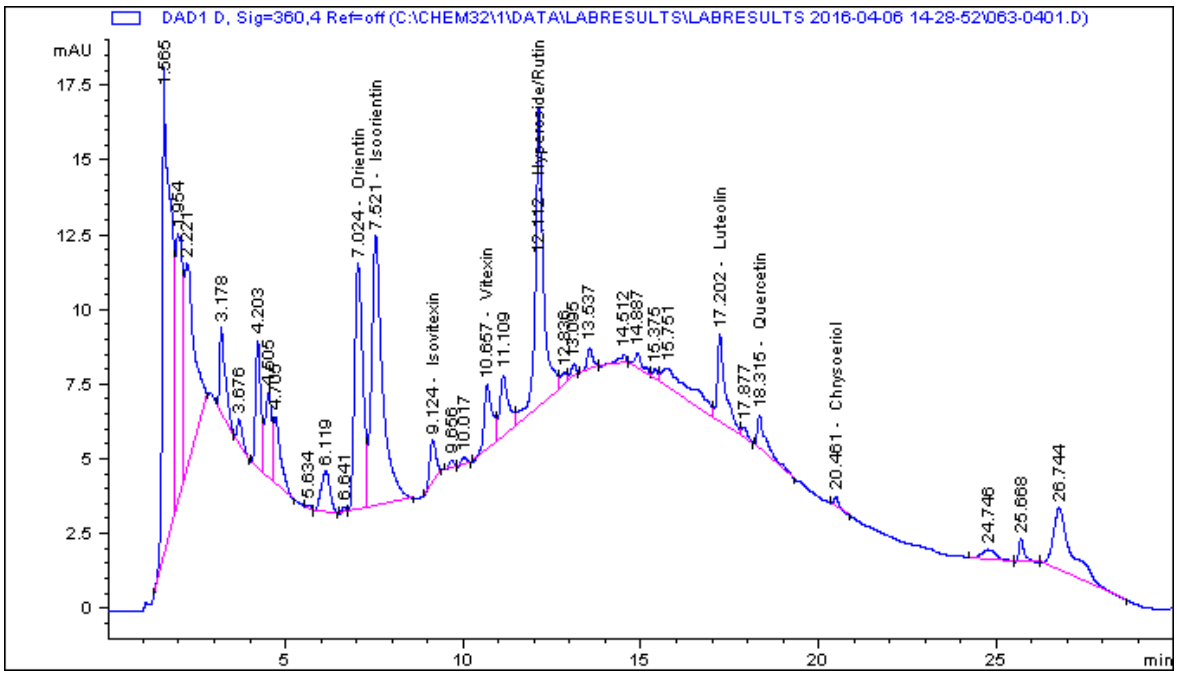


Figure 31: The chromatogram of immediate release mini-tablets monitored at 360 nm

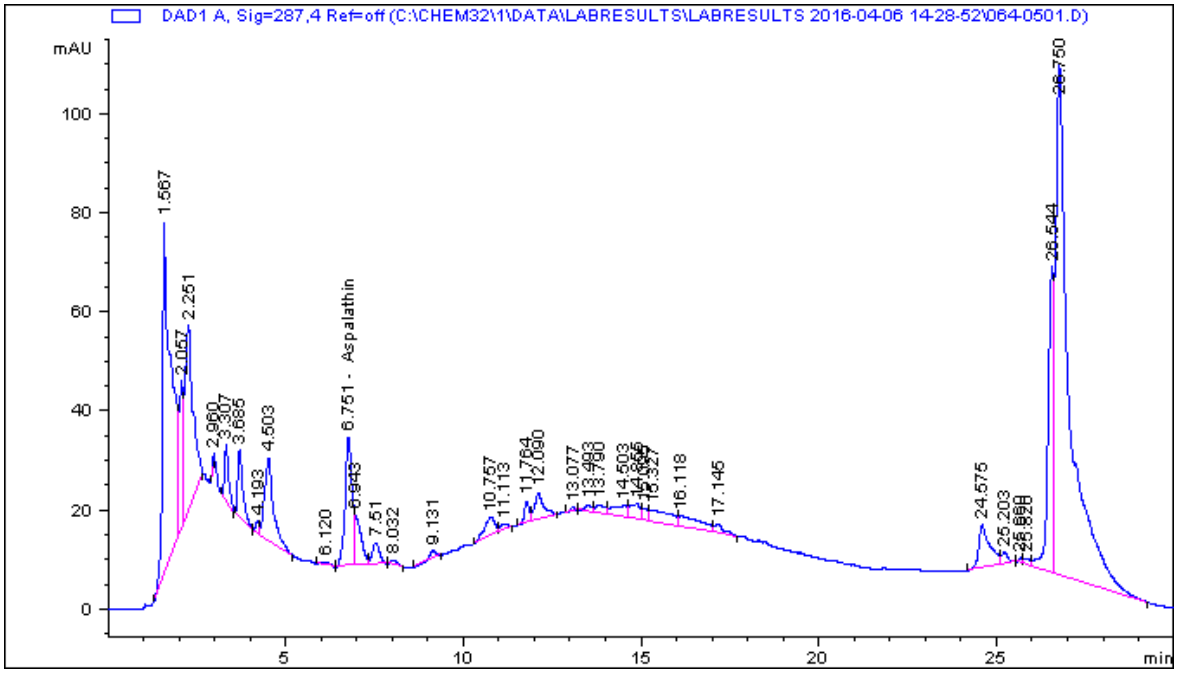


Figure 32: The chromatogram of sustained release mini-tablets monitored at 287 nm

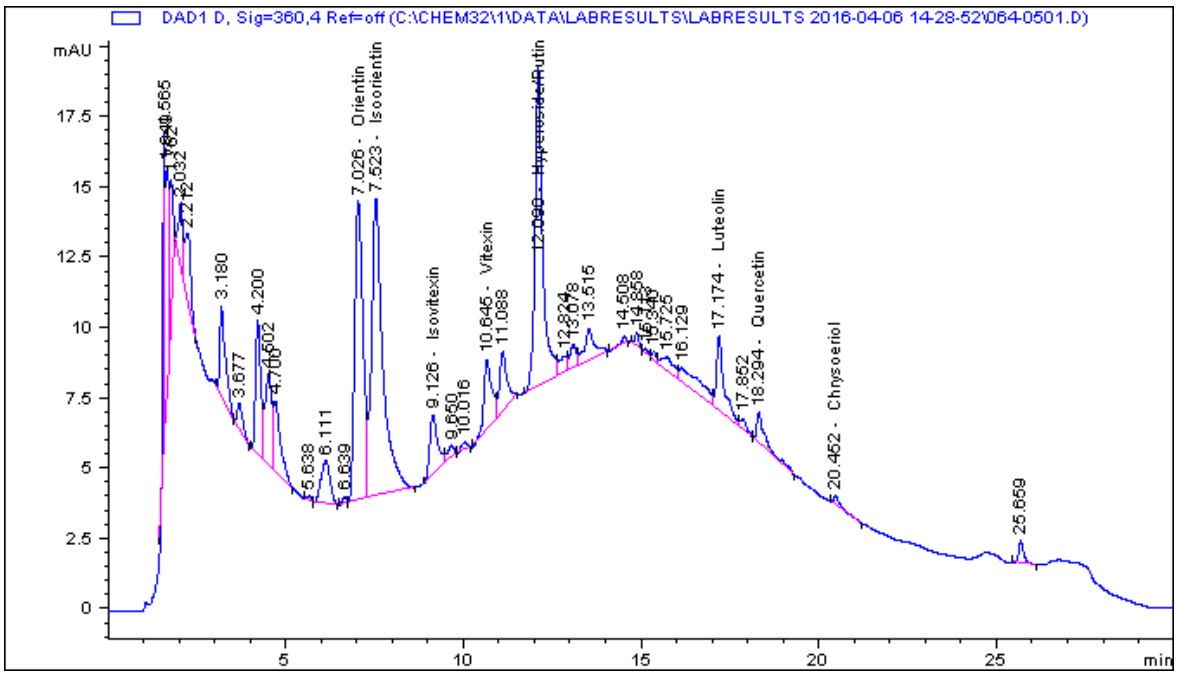


Figure 33: The chromatogram of sustained release mini-tablets monitored at 360 nm

The HPLC analyses of the immediate release- and sustained release tablets to determine the concentrations of the different phenolic compounds were performed. The results are summarised Table 45 and Aspalathin was found to be the most abundant of all the main phenolic compounds at concentrations of 957.4 µg/g for the immediate release tablets and 876.1 µg/g for the sustained release tablets. This compares well with the results obtained in Table 11.

4.6 Stability testing of the final product

Accelerated stability testing was performed at both 25°C and 60% humidity and at 40 °C and 75% humidity over a 203 day period. The total polyphenols assay and TEAC assay was used to evaluate the stability of the samples (see Table 46).

Table 46: The results for the stability testing performed over a 203 day period for Mediterranean conditions (25°C and 60% humidity) and stressed conditions (40°C and 75% humidity). Results are expressed as a percentage of the initial concentration.

Days	Total Polyphenols	Total Polyphenols	TEAC	TEAC
	25°C@60%RH	40°C@75%RH	25°C@60%RH	40°C@75%RH
0	100	100	100	100
7	99.64	97.02	75.95	91.48
14	100.20	95.85	89.40	89.80
21	91.89	100.30	74.86	91.98
49	93.82	102.81	84.19	95.60
84	95.69	100.17	90.93	89.71
112	99.13	97.78	86.19	100.30
140	96.80	100.34	80.60	84.64
175	98.53	103.67	87.04	100.48
203	96.27	98.28	80.93	95.00

The total polyphenols assay would indicate if there was any breakdown of the polyphenols (the active pharmaceutical ingredient in the Rooibos extract) during this time and the TEAC assay was chosen as it would indicate if there was any decrease in the antioxidant capacity (the effectiveness of the active pharmaceutical ingredient in the Rooibos extract) of the tablets. The total polyphenols assay results for the accelerated stability testing was within the 95 % levels (Table 46) indicating that the product is sable and can potentially be given a 24 months expiry date. The TEAC assay results showed a large variation and this would indicate that this assay is not an ideal assay to use for testing the stability of the active components.

CHAPTER 5: CONCLUSIONS

This study aimed to design and prepare a modified release oral delivery system for a commercial Rooibos extract that could deliver the polyphenol payload in a close to zero order delivery over an eight hour period.

A novel mini-tablet-in-capsule delivery system was used in which 10 mini-tablets (6 mm diameter) were packed into size 0 hard gelatin capsules. The 10 mini-tablets would incorporate 400 mg of Rooibos extract. The photograph below (Figure 34) of the mini-tablet-in-capsule system shows what the completed product looks like. This delivery system allows greater flexibility to manipulate the delivery rate of the active ingredient (i.e. Rooibos extract in this study). This system makes provision for an immediate release loading dose, in addition to a sustained release maintenance dose.

Figure 34: A photograph of the mini-tablets and two capsules packed with ten mini-tablets (mini-tablet-in-capsule system). In the capsule on top two mini-tablets were coated white to illustrate the two immediate release mini-tablets and the eight sustained release mini-tablets when packed into the hard gelatine capsule.

The major excipients and the final tablet formulations were evaluated for powder flow by different methodologies before being manufactured. These results showed in general that the powder mixture of both the immediate release and sustained release mini-tablets had acceptable flow properties and that there was no need to add flow enhancers.

Different excipients were evaluated for both the immediate release and sustained release formulations. The final immediate release formulation used sodium bicarbonate together with Explotab® as a disintegrant to increase the disintegration (by increasing the pH of the solution surrounding the mini-tablets) of the mini-tablet and reducing the jelly-like layer around the mini-tablet. Kolidon SR® and Retalac® were used to optimise the sustained release formulation and the 15.24 % (w/w) Retalac® formulation produced a more linear dissolution profile. These formulations were chosen as the final formulations.

The physical properties of the mini-tablet were determined after production. The tablets had acceptable hardness and friability indicating that the tablets should withstand handling during post manufacture storage and packaging. The mass variation was also within an acceptable range indicating that the tablets had a uniform mass and good powder flow during production. This also indicates that the concentration of the active compounds in the individual mini-tablets will have a small variation. There was very little variation in the thickness of the tablets, which goes hand in hand with the mass variation. The disintegration tests for the final formulation of the immediate release mini-tablets was longer than the normally acceptable standard, which states that all 6 tablets must disintegrate within 15 min. This was overcome to a large extent with the use of sodium bicarbonate as an excipient. The sodium bicarbonate in the matrix would potentially form a layer with a slightly increased pH surrounding the tablet that would decrease the formation of the jelly layer around the mini-tablet.

The dissolution studies showed that the immediate release formulation released ±80 % of the Rooibos polyphenols within the first two hours in the acidic (0.1 M HCl) dissolution medium. This shows that the dissolution of the immediate release mini-tablets will take place rapidly in the stomach. The sustained release tablets

released ±95 % of their payload in six hours in the pH 6.8 buffer indicating a steady release in the small intestine. The final product made up of the gelatine capsule filled with two immediate release tablets and eight sustained release tablets delivered ±93 % of the payload in an almost linear fashion over the 8 hours in the dissolution study. A curve fit of the data indicates that the dissolution adhered to the Korsmeyer-Peppas model, with the value of n of 0.85 which indicates a near zero-order release kinetics that are ideal to maintain blood levels over an extended period of time.

The biochemical analysis of the mini-tablet-in-capsule system showed that the total polyphenol content of each capsule to be 127.74 mg GAE per capsule. The available space in a size 0 capsule would allow an increase in the number of tablets or the size of the mini-tablets and this would potentially increase the polyphenol intake from 255.5 mg GAE/day to 335 mg GAE/day. This is still lower, but closer to the 380 mg GAE/day used in the clinical study by Marnewick *et al.* (2011). In the abovementioned clinical study, the participants had to adhere to a polyphenol restricted diet. A normal diet would include polyphenols, thus the decision to produce a formulation with a lower polyphenol content was made. The rapid elimination of the Rooibos polyphenols and the fact that it is released at a constant rate from the mini-tablet-in-capsule system would result in a more stable plasma concentration when compared to drinking the six cups of tea a day. This could potentially have a major effect on the health benefits of Rooibos when compared to drinking Rooibos as an herbal tea. A clinical study would have to be performed to determine if this formulation would deliver an optimum amount of polyphenols if taken with a normal diet.

To take this from a concept into a marketable product some further studies and developments need to be performed.

- The health benefits of the mini-tablet-in-capsule system for the extended delivery of Rooibos should be quantified by a comparative clinical study between drinking the Rooibos as a beverage vs. the mini-tablet-in-capsule delivery system. These results should indicate if the number of tablets should be increased in the gelatine capsule to have a similar effect to the

published clinical data. A study to investigate any harmful side-effects that could possibly exist if the prescribed dosage is exceeded should also be undertaken.

- In order to register this product with the South African Health Products Regulatory Authority three batches of at least 50 000 capsules should be produced and subjected to stability testing over a minimum period of 12 months. These results will indicate the shelf life and this together with the data from a clinical study would be needed for such registration.
- The amount of free space in the gelatine capsule may also be used to add other nutraceuticals or phytochemical compounds that can be beneficial to the consumer. This addition could be for special applications such as sport supplements, maternal health etc.
- Currently the mini-tablets are packed into the gelatine capsules manually, but if it is to be used in full scale production this labour intensive process could prove costly and it could be necessary to invest time and money in designing a machine that can pack the mini-tablets into the gelatine capsules.

This mini-tablet-in-capsule system concept is the first of its kind to be produced for the sustained delivery of Rooibos bio-actives. The mini-tablet-in-capsule system allowed for 400 mg of the Rooibos extract and delivered the payload in an almost linear fashion over an 8 hr period as set out in the aim of this study.

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