Development of vitamin C and E fixed-dose combination, multiple-unit, solid oral delivery systems

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Dissertation submitted in fulfilment of the requirements for the degree Master of Science in Pharmaceutics at the North West University

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Graduation: May 2019

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DECLARATION BY CANDIDATE

“I hereby declare that the dissertation submitted in fulfilment of the requirements for the degree Magister Scientiae in Pharmaceutics at the Potchefstroom Campus of the North-West University, is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references”

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ACKNOWLEDGEMENTS

This truly has been a great adventure, consisting of a lot of ups and downs. I would not have been capable to complete this study without the help, guidance and support of the following:

Firstly to Jessica Röder, you were the inspiration behind this. I never thought I was capable but your belief in me served as all the motivation I needed for this venture. Your love and support carried me through some deep depths. Thank you for being there for me, always. You were the foundation I needed to achieve this. We will someday look back at the last two years and we'll see its worth embedded throughout our lives. I love you.

My parents, thank you for teaching me values that made this possible. My mother, Hilda, your kindness, love and support kept me going. Thank you for honestly trying to understand what my study was about and always asking me how it was getting along. My father, Noeks, you taught me that there is no such thing as failure, only learning experiences. When I failed you encouraged me to get back up, and now look how far I've come.

Marco Swart, you've been a brother to me for most of my time on varsity. Your unconditional support and kindness I will forever cherish, but during the last two years you've been incredible. We've had some great times. Thank you Boeta.

Werner Gerber, firstly your keen assistance throughout this study is greatly appreciated. Your friendship I appreciate more. Thank you for the fun times. Your banter was always a timely distraction from the chaos.

My brothers, Martin and Barnie, you have always been great friends for me throughout our lives, but the last two years you were exceptional. I'm so proud to have you both in my life.

My supervisor, Prof Sias Hamman, thank you for believing in me and for the opportunity to complete this study. Your academic advice and guidance is greatly appreciated. I've learned so much from you, some lessons I'll carry with me throughout the entirety of my life.

My co-supervisors, Prof Jan Steenekamp and Dr Liezl Badenhorst, thank you for your input to make this study possible. Your kind words of encouragement throughout this time is truly appreciated.

Prof Jan du Preez, without your assistance the greatest part of my study would not have been possible. Thank you for your patience and advice during the HPLC validation. I've learned a lot of new skills from you and for that I'm am forever grateful.

Mr Francois Viljoen, thanks for the advice and support during this study, especially in regards to the HPLC validation.

The North-West University for the opportunity granted to me and for the master's scholarship and institutional bursary.

And lastly, the National Research Foundation of South-Africa, for the DST-NRF Innovation Master's Scholarship (grant number: 113602). Without this grant this study would truly not have been possible. Thank you for continuously supporting students in their pursuit of achieving greatness.

This work is based on the research supported wholly or in part by the National Research Foundation of South Africa (Grant number: 113602)

ABSTRACT

Oral drug delivery is one of the most preferred and user-friendly routes of drug administration, however, vitamin C and vitamin E have poor and unreliable bioavailability at doses intended for anti-oxidant effects. This may be due to the instability (vitamin C), poor solubility (vitamin E) or the poor permeability in the intestinal tract. Functional excipients such as *Aloe vera* gel (AVG) and sodium lauryl sulphate (SLS) may be incorporated into drug delivery systems to overcome the poor bioavailability. AVG and SLS act as absorption enhancers and have been proven to increase membrane permeability and bioavailability of both vitamins C and E.

The general aim of this study was to formulate multiple unit pellet systems (MUPS) with AVG and SLS to enhance the bioavailability and permeability of vitamins C and E. MUPS are considered to be an interesting alternative to conventional tablets and capsules. MUPS provide several advantages over single-unit dosage forms, which include a relatively high degree of homogeneous dispersion of the sub-units (e.g. pellets) in the gastro-intestinal tract, less local irritation effects, less variation in transit time, and lower fluctuations in peak plasma levels.

Beads containing both vitamin C and E with AVG and SLS as functional excipients were produced by means of extrusion spheronisation. The beads were characterised in terms of uniformity of mass, friability, assay and bead size distribution. Drug release studies were performed by means of dissolution tests. *Ex vivo* transport studies were done using a Sweetana-Grass diffusion apparatus to determine the transport of vitamin C and E for a 2 hour period. The samples obtained were analysed by means of high-performance liquid chromatography (HPLC) using validated methods.

Ex vivo transport studies showed that the tested absorption enhancers (AVG and SLS) formulated into MUPS capsule formulations succeeded in enhancing the permeability of vitamin C across excised pig intestinal tissue. No transport of vitamin E across the excised intestinal tissues was observed for the vitamin E MUPS capsule formulations and retention of vitamin E in the tissues was therefore assessed. These retention studies showed that AVG and SLS improved the ability of vitamin E to be delivered into the epithelial tissues. Promising results were obtained, but *in vivo* studies are needed to prove that the bioavailability enhancement effects can lead to clinically significant blood plasma levels.

Key words: *Absorption enhancer, Aloe vera gel, ex vivo transport, MUPS capsule formulations, sodium lauryl sulphate, Vitamin C, Vitamin E*.

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LIST OF ABBREVIATIONS:

AVG	*Aloe vera* gel
BP	British Pharmacopoeia
D[4;3]	Mean particle diameter
d(0.5)	Median of the size distribution
FeSSIF	Fed state simulated intestinal fluid
HPLC	High performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MCC	Microcrystalline cellulose
MUPS	Multiple-unit pellet systems
P_{app}	Apparent permeability coefficient
P-gp	P-glycoprotein
PPBS	Potassium phosphate buffer solution
R^2	Relevant correlation coefficient
RSD	Relative standard deviation
SMBS	Sodium metabisulphite
SLS	Sodium lauryl sulphate
TEER	Trans-epithelial electrical resistance

LIST OF EQUATIONS:

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CHAPTER 1: INTRODUCTION, AIM AND OBJECTIVES

1.1. Vitamin C and E supplementation

Vitamin supplementation is annually growing because of the higher consumer awareness of preventative healthcare. While some vitamins are considered to be well absorbed with good bioavailability when taken orally as part of a meal, the absorption of certain vitamins still varies quite considerably inter- and intra-individually (bioavailability of some vitamins may range between 20 and 98%) (Bates & Heseker, 1994:95).

Vitamin C (ascorbic acid) is a water soluble vitamin and a potent anti-oxidant. The bioavailability of vitamin C, when taken at the daily recommended dose, ranges between 80 and 95%. For higher doses, its bioavailability drops to below 50% (Rivers, 1987:445). Vitamin E (tocopherol) is very lipid soluble and poorly soluble in water. This physico-chemical property causes a challenge for efficient oral delivery. The absorption efficiency of vitamin E is highly variable and estimated to be between 10 and 79% depending on the amount of fat ingested with the vitamin (Borel *et al.*, 2013:320).

1.2. Multiple-unit pellet systems

A multiple-unit pellet system (MUPS) is a dosage form that consists of a compilation of pellets either compacted into tablets or loaded into hard gelatine capsules. MUPS dosage forms are known to exhibit a high degree of homogeneous dispersion of the units (i.e. the pellets) in the gastro-intestinal tract after oral administration, which provides more consistent drug absorption. Furthermore, MUPS have less local irritation effects, less variation in transit time and lower fluctuations in peak plasma levels. MUPS have become one of the main dosage forms of providing modified drug release. This offers the advantage of achieving an optimum therapeutic response, while prolonging therapeutic action and decreasing toxicity. Another important advantage is the improved patient compliancy achieved by reducing the dosing frequency. In addition, the avoidance of high concentrations of irritable active agents, a decreased chance of dose dumping, better flow properties and a relatively narrow particle size distribution are other advantages related to this dosage form (Hamman *et al.*, 2017:201).

The pellets to be included in the MUPS formulations can be prepared by using the extrusion-spheronisation technique. Advantages of extrusion-spheronisation as pellet manufacturing technique include the formulation of pellets with uniform sizes, a high drug loading capacity, easy operation, low wastage compared with high output, narrow particle size distribution, lower friability of pellets, pellets better suitable for film coating and a better controlled drug release profile. Another advantage of this technique is the relative short processing time, which consequently saves on production costs (Gandhi *et al.*, 1999:161-163).

1.3. Selected models that are used to evaluate drug delivery

1.3.1. *In vitro* models

In vitro models play an important role during drug development since they usually eliminate compounds that don't adhere to permeation characteristics (i.e. required drug like properties with acceptable biopharmaceutical properties) needed to succeed during clinical trials. Even though *in vitro* models can't take complex physiological processes into consideration, these models are still useful screening tools during early drug or compound development (Joubert *et al.*, 2017:184).

The Caco-2 cell line remains a customary *in vitro* model to test drug permeation across the intestinal epithelium. Caco-2 cells are derived from human colon adenocarcinoma, but form polarized monolayers that closely resemble the functional characteristics of human intestinal enterocytes. Caco-2 cells form tight junctions between each other and additionally express several enzymes and active transporter systems (e.g. efflux transporters such as p-glycoprotein). One disadvantage is that Caco-2 cell monolayers need to be cultured for 21 days in order to form tight junctions and express efflux transporters. Other disadvantages include underestimation of paracellular transport of compounds (because Caco-2 cells have tighter junctions compared to human intestinal enterocytes), the underestimation of absorption of drugs with an affinity for P-gp, variability between different laboratories and wide variations on the passage number from different Caco-2 cell studies (Alqahtani *et al.*, 2013:2-3).

1.3.2. *Ex vivo* models

Ex vivo models consist of excised tissue pieces mounted between two diffusion cell compartments, usually in Ussing type diffusion apparatuses. This model can be used to determine both absorptive and secretory transport of compounds across mucosal surfaces. Drug efflux can be studied when the drug is placed in the basolateral compartment and samples are withdrawn from the apical compartment, which can then be compared to the drug transport in the absorptive direction. The main disadvantage of this model is the viability of the excised tissue as a function of time after removal from the organism. The integrity of the intestinal tissue decreases after removal from the animal and can only be used for limited periods of time before drug permeability is affected. Trans-epithelial electrical resistance (TEER) measurement can be used as one technique to monitor the viability of the excised tissue (Alqahtani *et al.*, 2013:3-5).

Advantages of the excised tissue model over the Caco-2 cell culture model include the shorter time needed to prepare tissue for absorption studies, the good correlation between membrane permeability and *in vivo* absorption percentage, better correlation for drugs with affinity for P-gp

and a better gradual slope for absorption percentage, allowing a better prediction of the absorption percentage that will occur *in vivo* (Gotoh *et al.*, 2005:520).

1.4. Problem statement

The oral route of administration is generally the most used route for taking medicines and supplements, but it is not always optimal in terms of bioavailability. For example, oral delivery of vitamin E poses challenges such as high variability in bioavailability (ranging roughly between 10 and 79%) as well as low solubility in the aqueous environment of the gastro-intestinal tract (Borel *et al.*, 2013:319). Vitamin C also experiences bioavailability challenges when administered orally in high doses such as those intended for anti-oxidant activity (Hornig *et al.*, 1980:309).

The variability of vitamin absorption can be reduced by formulation approaches such as the design of multiple-unit drug delivery systems, while poor solubility can be improved by inclusion of surface active agents in the formulation. In addition, poor membrane permeation can be overcome by inclusion of absorption enhancers in the formulation.

1.5. General aim

The general aim of this study is to develop MUPS capsule formulations containing vitamin C (ascorbic acid) and E (in the form of D-α-tocopherol succinate), while incorporating *Aloe vera* gel (AVG) and sodium lauryl sulphate (SLS) as functional excipients to enhance the solubility and permeation of these vitamins across the intestinal epithelium.

1.6. Specific objectives

- To formulate vitamin C and E containing spherical beads by means of extrusion-spheronisation with different compositions,
- To prepare MUPS capsule formulations (i.e. beads encapsulated in hard gelatine capsules) containing the different bead formulations,
- To validate an analytical method for detection of both vitamin C and E by means of high-performance liquid chromatography (HPLC),
- To evaluate the quality of the different MUPS capsule formulations by means of mass variation, friability, disintegration, dissolution and assay testing,
- To evaluate the intestinal delivery of vitamins C and E from the formulated MUPS capsule formulations by means of an *ex vivo* technique, i.e. across excised pig jejunum tissues mounted in a Sweetana-Grass diffusion apparatus.

CHAPTER 2: LITERATURE REVIEW

2.1. Vitamin supplementation

2.1.1. The necessity of vitamin C and E

Vitamin C, also referred to as ascorbic acid, is a water soluble vitamin and a very potent anti-oxidant. Vitamin C is widely found in fruits and vegetables. Anti-oxidants offer protection to the body against free radicals that cause oxidative stress and cell damage (Varatharajan *et al.*, 2015:54). The anti-oxidant ability of vitamin C leads to removal of reactive oxygen species such as free radicals in the body thereby reducing oxidative damage to cells (Sorice *et al.*, 2014:445). Scurvy is a clinical syndrome that occurs as a result of deficiency in vitamin C supplementation (Padayatty *et al.*, 2003:19). Without vitamin C intake, scurvy may progress to the stage that it can cause poor wound healing, bleeding gums, anaemia and may also impair bone growth (Sorice *et al.*, 2014:445). A reduction in supplementation of vitamin C has also been proven to cause an impaired immune system. This was shown by means of a delayed skin reaction test, which was used as an appropriate way for measuring immune response (Jacob *et al.*, 1991:1302). Oxidative stress contributes to the pathology of hypertension and vascular endothelial dysfunction and therefore vitamin C can have beneficial effects on vascular endothelial diseases by way of its anti-oxidant effects (Brown & Hu, 2001:679). Vitamin C improves the absorption of iron from the gastro-intestinal tract and therefore aids in preventing an iron deficiency (Vinson *et al.*, 2005:761). Vitamin C also plays a role in metabolic reactions that are crucial to physiological systems and biochemical processes including cell division, gene expression and biological defence mechanism activation (Arrigoni & De Tullio, 2002:2). Another important role of vitamin C is the utilisation thereof by enzymes located in the endoplasmic reticulum. These enzymes are responsible for catalysing the production and regulation of cross linked collagen and elastin in vascular smooth muscle cells, which is done via post-translational modification and folding of proteins (Griffiths & Lunec, 2001:174).

Vitamin E is the generic term for eight natural isoforms of α-tocopherol and consists of four tocopherols (including α, β, γ, and δ) and four tocotrienols (including α, β, γ, and δ). Vitamin E is essential for human health. However, three out of four American citizens (19 – 30 years of age) consume less than 10 mg/day and in Europe, 8% of men and 15% of women fail to meet 67% of the recommended dietary allowance for α-tocopherol, which is 15 mg/day for persons older than 14 years of age (Borel & Desmarchelier, 2016:2094). α-Tocopherol is the isoform that is most abundantly found in nature and is also the form of vitamin E with the highest biological activity (Brigelius-Flohe & Traber, 1999:1145).

Vitamin E plays a role in the prevention of degenerative diseases, such as Alzheimer's disease and other dementias, because of its protective anti-oxidant properties. Oxidative stress is

continuously present in the human body, which may originate from external or internal sources. The major internal source of reactive oxygen species is the mitochondrion (Khan *et al.*, 2011:789). Vitamin E protects against lipid peroxidation by acting as a scavenger for lipid peroxyl radicals (Morrissey *et al.*, 1994:571). Furthermore, vitamin E is of great importance because of its function to protect the integrity of the lipid structures and is the major lipid-soluble anti-oxidant found throughout the body (Burton & Traber, 1990:360).

Non-anti-oxidant activities of vitamin E include gene expression modulation, inhibition of cell proliferation, platelet aggregation, monocyte adhesion and bone mass regulation (Borel *et al.*, 2013:319). Vitamin E in the form of α-tocopherol succinate has also been proven to inhibit the growth of different types of cancer cells, including pancreas, breast, gastric and prostate (Rose & McFadden, 2001:19). It has been proven that a regression in small intestinal metaplasia, a predecessor of gastric carcinoma, can be obtained with high doses of vitamin E (Bukin *et al.*, 1997:543). Vitamin E intake in the form of supplements and/or general food sources has been associated with reduction in age related cognitive declination (Morris *et al.*, 2002:1125). Extreme deficiency of vitamin E may lead to peripheral neuropathological disorders including spinocerebellar ataxia and myopathy (Brigelius-Flohe & Traber, 1999:1148). As stated by Borel et al. (2013:319), the main dietary sources of vitamin E are vegetable oils and nuts, but the average intake of this vitamin is still below the recommended dietary allowance in the United States of America. This may be owing to a variety of reasons, but also because of its poor bioavailability. The main reasons for vitamin E's low bioavailability is the poor dissolution and absorption rate (Parthasarathi & Anandharamakrishnan, 2016:469).

Combined use of vitamin C and E, especially at doses intended for anti-oxidant effects (from 280 mg for vitamin E and from 500 mg for vitamin C), have been proven to lower the prevalence and incidence of Alzheimer's disease (Zandi *et al.*, 2004:82). Treatment with a combination of vitamin C and E also has the ability to inhibit atherosclerotic progression in people with high cholesterol levels (Salonen *et al.*, 2003:947). Vitamin C also acts as a co-anti-oxidant by means of rejuvenating α-tocopherol (Vitamin E) from its radical, which is formed from scavenging lipid-soluble radicals. This is also of importance because α-tocopherol may serve as a pro-oxidant in the absence of Vitamin C, further demonstrating the co-functioning of both these vitamins (Carr *et al.*, 2012:1087).

2.2. Challenges in oral delivery of Vitamin C and E

2.2.1. Vitamin C

Vitamin C is a water-soluble compound that is arguably the most commonly used vitamin supplement because of its well-known anti-oxidant effects and health benefits. A study on the pharmacokinetics of vitamin C in humans concluded that the maximum bioavailability occurs at

200 mg/day, while at levels of more than 1000 mg/day, complete plasma saturation takes place resulting in a decrease of bioavailability with an increase in urinary excretion (Levine *et al.*, 1996:3708-3709). Another study also demonstrated a decrease in bioavailability of vitamin C with increase in dose. The bioavailability decreased from 100% (at a 200 mg dose) to 75% (at a 1 g dose) and this decrease continued with the bioavailability falling to 44% (at a dose of 2 g), 39% (at a dose of 3 g), 28% (at a dose of 4 g) and to only 20% (at a dose of 5 g) (Hornig *et al.*, 1980:309). *Hylicobacter pylori* infections, which are extremely common in people affected with peptic ulcers, have been proven to impair the bioavailability of vitamin C. The study showed a decrease in plasma vitamin C concentration of 20% in persons infected with *H. pylori* compared to those whom tested negative (Woodward *et al.*, 2001:233).

2.2.2. Vitamin E

Vitamin E is a lipophilic bioactive compound with many health benefits, but it exhibits insufficient bioavailability because of its poor dissolution and rapid degradation in the presence of oxygen (Parthasarathi & Anandharamakrishnan, 2016:469). Noticeable degradation of α-tocopherol in the gastro-intestinal tract has also been indicated (Borel *et al.*, 2001:102). The oral-delivery of vitamin E exhibits relatively large inter-individual variation with many factors that can contribute to both poor and variable bioavailability (Dimitrov *et al.*, 1991:726).

Vitamin E is practically insoluble in water, but easily soluble in different oils and organic solvents such as ethanol, methanol and ether (Rowe *et al.*, 2006:33). Vitamin E should be taken with meals because the secretion of bile salts aids its solubility in the gastro-intestinal tract. The vitamin E gets incorporated into bile salt micelles in the small intestine, which form a fine emulsion that facilitates moving of vitamin E molecules across the epithelial cell membrane (Julianto *et al.*, 2000:54).

Because vitamin E is a lipophilic compound, the amount of fat in the gastro-intestinal tract influences the efficiency of vitamin E absorption. A study was done where apples were fortified with α-tocopherol to determine the effect of fat in the delivery of vitamin E. This study concluded that the absorption of vitamin E was between 10 and 33%. Combining vitamin E with low fat meals showed vitamin E absorption closer to the 10% spectrum and with higher fatty meals it was more towards the higher end of the spectrum of 33% (Bruno *et al.*, 2006:299). Another study also compared the absorption of vitamin E when given with meals containing higher fat amounts to meals with a lower fat amounts. It was proven that the absorption of vitamin E improved when given with the higher fatty meals than with the lower fatty meals (Jeanes *et al.*, 2004:575).

The type of fat with which vitamin E is consumed may also have an influence on its absorption. It was found that long-chain triacylglycerols have a diminished absorption efficiency compared

to medium-chain triacylglycerols(Gallo-Torres *et al.*, 1978:240-241). Vitamin E absorption may also be negatively influenced by the presence of dietary fiber. The reason for this is that dietary fiber inhibits lipases and therefore affects the formation of micelles. Consequently, this decreases the formation of micelles that contain the vitamin E at the site of absorption (Borel *et al.*, 2013:326).

2.3. Multiple-unit dosage forms

Single-unit dosage forms contain the complete dose of active ingredient within a single dose unit that is intended to be administered singularly. Advantages of single-unit dosage forms include high drug loading, simple and cost effective manufacturing and the ability to use different mechanisms for drug release. Multiple-unit dosage forms differ from single-unit dosage forms because they consist of a number of sub-units, each containing a portion of the dose, formulated into a dosage unit. There are several advantages attributed to the use of multiple-unit dosage forms over the use of single-unit dosage forms, which include (Gandhi *et al.*, 1999:161):

- A relatively high degree of homogeneous dispersion of the sub-units (e.g. pellets) in the gastro-intestinal tract,
- Less local irritation effects,
- Less variation in transit time, and
- Lower fluctuations in peak plasma levels.

In addition to the aforementioned advantages, multiple-unit dosage forms have superior pharmacokinetic and pharmacodynamic properties compared to solid oral single-unit dosage forms. Furthermore, multiple-unit dosage forms have better transit from the stomach into the duodenum, give an even distribution of the sub-units (e.g. pellets) upon reaching the small intestine and consequently provide more consistent drug absorption (Hamman *et al.*, 2017:201)

2.3.1. Multiple-unit pellet system

A multiple-unit pellet system (MUPS) is a dosage form that consists of a compilation of pellets compacted into tablets or loaded into hard gelatine capsules. Pellets are sphere shaped particles varying in diameter and size depending on the application thereof. Pellets are not only restricted to the pharmaceutical industry, but are also commonly used in agriculture (e.g. fertiliser) and in the polymer industries. MUPS as a drug delivery system offers similar advantages as mentioned for multiple-unit dosage forms in general such as a lowered risk of side effects caused by dose dumping and less irritation of the gastro intestinal tract (Vervaet *et al.*, 1995:131). Other important advantages include the improved patient compliance achieved by reducing the dosing frequency, avoidance of high concentrations of irritable bioactive agents, a decreased chance of dose dumping, better flow properties and a relatively narrow particle size

distribution (Gandhi *et al.*, 1999:161-163). MUPS have become one of the key drug delivery systems for controlled drug release. This offers the advantage of achieving an optimum therapeutic response while prolonging efficacy and decreasing toxicity.

2.3.2. Pharmaceutical pelletisation techniques

Pellets for use in MUPS can be produced in a number of ways including the building of pellets layer by layer; spray-congealing; spray-drying and evaporation of the fluid phase and the most popular and commonly used method is extrusion-spheronisation (Vervaet *et al.*, 1995:132). Advantages of the extrusion-spheronisation technique include the production of relatively dense and homogenous beads with low surface porosity at short processing times (Mallipeddi *et al.*, 2010:54).

The spray-drying process forms pellets by means of evaporating water from the core material mixture. Pellets are formed as a result of the dry solids being separated. Negatives of the spray-drying technique is the possibility of producing hollow pellets, due to the liquid mixture evaporating faster than the diffusion rate of the dissolved solids back into the droplet interior (Gandhi *et al.*, 1999:161).

Spray congealing is the formation of pellets from a fluid mixture to a solid state by means of coagulation. The material mixture (insoluble in a molten mass) is spray-congealed to form small particles (Gandhi *et al.*, 1999:161).

Fluidized bed technology uses the process of suspending the material mixture in a stream of hot air. Binder or granulating liquid can be sprayed onto the suspended particles causing a reaction prior to vaporization. This causes agglomeration of the ingredient particles to form solid pellets (Govender & Dangor, 1997:456-457).

Powder layering involves the process of forming pellets by successively adding layers of dry powder of the material mixture and binding liquid to neutral starter seed cores. The successive layering will subsequently form pellets until the desired pellet sizes are achieved (Kumar *et al.*, 2011:122). Conventional coating pans can be used to manufacture pellets by this method (Panda *et al.*, 2013:54). Layering methods do have limitations such as non-uniformity in pellet size and a relatively low drug loading capacity (Rahman *et al.*, 2009:122).

The pelletisation process known as balling consists of converting finely divided particles to spherical particles by the constant addition of certain predetermined quantities of liquid during a continuous rolling and tumbling motion (Kumar *et al.*, 2011:123).

2.3.3. Extrusion-spheronisation as manufacturing method

As mentioned before, extrusion-spheronisation remains the most used formulation process in producing pellets, because it results in dense spherical pellets of uniform size and shape. Extrusion-spheronisation involves the following manufacturing steps (Rahman *et al.*, 2009:121-122):

- Dry mixing: Mixing of dry powder ingredients to obtain a homogeneous mixture of active ingredients and excipients. Mixing can be done using any acceptable powder mixer such as a planetary mixer, a twin shell blender, a high speed mixer, a shaker mixer or a tumbler mixer.
- Wet massing: The powder mixture is then wet massed by adding an appropriate liquid to the powder mixture during continuous stirring or mixing to produce a mass with the correct consistency for extrusion (this liquid is known as the granulation liquid).
- Extrusion: The third step of the process is extrusion of the wet mass to form spaghetti like cylinders of uniform diameter. The wet powder mass is forced through openings of particular diameter to form the cylinders. A certain amount of plasticity has to be exhibited by the wet powder mass to allow deformation, but not too much otherwise spherical particles will not form during the spheronisation process. There are three different types of extruders that can be used for this step in the process including the screw feed, gravity feed and piston feed extruders.
- Spheronisation: The spheroniser consists of a static cylinder and a rotating friction plate in which the extrudate is placed for spherical pellet formation. These cylindrical extrudates are broken up into smaller particle sizes of roughly the same length as diameter, these particles are then rounded to create pellets due to the frictional forces created by the spheroniser. The rotational speeds of the spheroniser vary from 200 to 2000 rpm. A speed of 200 rpm is sufficient to create highly spherical pellets. The time needed to form pellets usually takes 2-10 min. Dusty beads may be formed if the extrudate mass is too dry, and consequently this will not produce pellets of consistently the same size and diameter. On the other hand, agglomeration of the pellets and dumbbell formation may take place if the extrudate is too wet.
- Drying: Drying of the pellets is required to obtain the desired moisture content. Drying can be done at room temperature, at an elevated temperature such as oven drying or at lower temperatures during freeze drying. Fluidized bed driers can also be used.
- Screening: The desired size distribution of the pellets can be achieved by screening the formed pellets through various size sieves.

2.3.4. Factors affecting pellet quality (pellets prepared by extrusion-spheronisation)

A number of factors determine the quality of the pellets produced by means of extrusion-spheronisation such as the moisture content in the powder mass, solubility of the active ingredient in the wetting agent, the type of wetting agent and physical properties of the excipients. Moisture content of the wet mass is of great importance in terms of the quality of the pellets that will be obtained by means of extrusion-spheronisation. A moisture content above the threshold value will lead to formation of big spheres because of agglomeration of the pellets during spheronisation. A moisture content below a certain threshold value will lead to dust formation and brittle pellets as a result of some of the powder particles not being incorporated into the pellets (Vervaet *et al.*, 1995:137).

The solubility of the active ingredient is the determining factor in the amount of wetting agent to be used during the wet massing step. A very soluble drug, e.g. vitamin C, requires less wetting agent (or granulation liquid) than an insoluble or poorly soluble drug, e.g. vitamin E. The solubility of the active ingredient may cause a problem because it easily results in either over-wetting or under-wetting of the powder mixture (Vervaet *et al.*, 1995:137). The type of granulation liquid used also plays a role in the quality of the pellets. A mixture of water and alcohol is usually used to ensure formation of pellets with acceptable physical properties. An increase in water content of the granulation liquid results in an increase in pellet hardness. On the other hand, an increase in alcohol content of the granulation liquid results in a softer pellet with a faster *in vitro* drug release rate. The compressibility of pellets into MUPS tablets also varies with composition of the granulation liquid, e.g. pellets formed with wetting agent of higher water content usually exhibit less compressibility than pellets formed by wetting agent with higher alcohol content (Millili & Schwartz, 1990:1415).

The physical properties of the excipients have an effect on the quality of the pellets produced by means of extrusion-spheronisation. For example, the bulking agents may influence the release rate of the drug from the pellets. The pellet size, hardness and sphericity may also be affected (Gandhi *et al.*, 1999:164). The particle size of the powder mass affects the bead size and sphericity, e.g. a finer grade microcrystalline cellulose (MCC) produced smaller beads than a coarser grade of MCC (Vervaet *et al.*, 1995:138).

Pellet quality is affected by the type of extrusion utilised as well as variables in the spheronisation process. An axial screw extruder produces more dense pellets compared to a radial screw extruder (Gandhi *et al.*, 1999:164). The thickness and pore size of the extruder screen will also have an effect on the quality of the extrudate and consequently also the pellets. Pellet size is determined by the diameter of the screen pores, whereas the screen thickness determines the length-to-radius ratio of the produced extrudate (Baert *et al.*, 1993:12). The rotation speed used during spheronisation also plays a role in the quality of the pellets that are

produced by the extrusion-spheronisation technique. A slow rotation speed barely changes the shape of the extrudate and results in non-spherical pellets, while a higher rotation speed results in reducing the size of the pellets (Newton *et al.*, 1995:101). The load size of the extrudate fed into the spheroniser determines certain characteristics of the pellets. Small loads (i.e. between 50 and 100 g) produced pellets of greater diameter, but with lower density compared to larger loads (i.e. between 700 and 1000 g) (Newton *et al.*, 1995:106).

2.4. Functional excipients

Functional excipients are defined as additives in dosage forms responsible for an increase in the bioavailability of the active ingredient, assistance in the stability of the active ingredient and improvement of the membrane permeability of orally administrated drugs. Incorporation of functional excipients can also optimize manufacturability of the dosage form and to facilitate the drug release and drug delivery process (Hamman & Steenekamp, 2012:219). The following are examples of functional excipients:

- fillers or bulking agents,
- binders used during compacting of tablets,
- disintegrants,
- permeation enhancers,
- lubricants,
- propellants used in the delivery of inhalants,
- emulsifying and solubilizing agents,
- colorants and flavourants, and
- coating agents.

The need for a multi-functional excipient has risen in the last couple of years that can possibly fulfil more than one role in the dosage form, e.g. to enhance drug permeation and to increase the overall stability in the gastro-intestinal tract. Excipients that can be used in combination with microcrystalline cellulose to improve the delivery of the drug from pellets include inclusion complex formers (e.g. β-cyclodextrin), super-disintegrants (e.g. croscarmellose sodium) and surface active agents to improve wettability and dissolution rate (e.g. sodium lauryl sulphate) (Hamman *et al.*, 2017:203).

Permeation enhancers are functional excipients that may be included in formulations to improve the absorption of an active pharmaceutical ingredient. Mechanisms used for permeation enhancement include membrane perturbation and disruption to increase transcellular movement of molecules, opening of tight junctions to increase paracellular movement of molecules, stimulation of active transporters in the absorptive direction and efflux inhibition in the secretory direction (Aungst, 2012:13). Permeation enhancement may be achieved by

means of incorporating a chemical permeation enhancer in the dosage form together with the active ingredient, but the downside is that these chemical excipients may be associated with toxicity (Hamman & Steenekamp, 2012:220). Therefore, the selection of a functional excipient must be carefully considered, taking into account its toxicity profile as well as its compatibility with the active pharmaceutical ingredient (Hamman & Steenekamp, 2012:221).

2.4.1. Sodium lauryl sulphate as a functional excipient

Sodium lauryl sulphate (SLS), also referred to as sodium dodecyl sulphate, is an anionic surfactant and has a number of functional uses in oral preparations, which include its use as an emulsifying agent, use as a solubilising agent, to modify drug release, to enhance permeation, and use as a tablet and capsule lubricant. SLS is freely soluble in water, partly soluble in ethanol and practically insoluble in ether or chloroform. One of the advantages of SLS is that it is effective in both alkaline and acidic conditions (Rowe *et al.*, 2006:687). SLS is the most commonly used wetting agent in solid oral dosage forms and has been proven to increase permeation via the reversible opening of tight junctions at concentrations higher than 0.4 mM (Anderberg & Artursson, 1993:392). SLS has the ability to improve solubility of poorly water-soluble drugs via micelle formation (Desai & Park, 2004:46). Desai & Park (2004:47) concluded that SLS has a greater solubility enhancement ability compared to Tween-20 and Tween-80.

2.4.2. *Aloe vera* gel as a functional excipient

Aloes belong to the succulent plant family known as *Asphodelaceae*. The Aloe plant group is very unique and is mostly found in Africa, especially in the southern and eastern parts. Its name is derived from the Arabic term *alloeh,* meaning shining bitter substance. The genus Aloe consists of nearly 420 species and are source to more than 130 active compounds including: polysaccharides, anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes and flavonoids (Dagne *et al.*, 2000:1058). The synergistic action of these active compounds are responsible for the biological and pharmaceutical activity of *A. vera* (Dagne *et al.*, 2000:1075). *Aloes* are classified as xerophytes because of their ability to store water in their specialised leaves, making them adaptable in dry and harsh environmental conditions (Rodríguez *et al.*, 2010:306). Aloes are also characterised by their stemless fleshy leaves, arranged in rosettes, which usually contain jagged thorns along the edges (Cousins & Witkowski, 2012:1). Their leaves consist of three parts, each known for different applications. The two outermost layers are the green cuticle rind and the yellow bitter latex exudate. These layers are known for their laxative effects. The innermost pulp is externally used to treat burns, wounds, skin irritations, infections and parasite infestations. Orally it can also be administered to treat coughs, constipation and ulcers (Lebitsa *et al.*, 2012:297). *Aloe vera* and *Aloe ferox* are the only *Aloe* species in South-Africa of commercial significance in international trade (Dagne *et al.*, 2000:1055).

Aloe vera, a perennial succulent xerophyte, is the most commercialised aloe species. It plays a part in the food, cosmetic and food industry, but also in the pharmaceutical industry. It is incorporated in many household products including toiletries and cosmetic products. The most important contribution in the pharmaceutical industry made by *A. vera* is the latex that is used as laxative, but its properties to improve bioavailability of certain poorly absorbable substances have shown a lot of potential for future applications (Hamman, 2008:1600). *A. vera* gel has been previously shown to increase the bioavailability of vitamins C and E. A bioavailability study on human subjects was conducted where *A. vera* liquid preparations were conjointly administered with vitamins C and E, which was measured against that of the control (vitamins E and C alone). *A. vera* gel excelled in its ability to improve absorption of both vitamins C and E and it was concluded that *A. vera* juice should be taken with vitamin C and E to maximise its absorption (Vinson *et al.*, 2005:760). *A.vera* gel has also shown the ability to enhance the permeation of macromolecular drugs across intestinal epithelial membranes (Lebitsa *et al.*, 2012:297). *A. vera* has been proven to decrease the trans-epithelial electric resistance (TEER) of Caco-2 cell monolayers. This indicated the opening of tight junctions between adjacent epithelial cells, resulting in permeation enhancement (Chen *et al.*, 2009:589). The study done by Chen *et al.* also showed that *A. vera* gel reduces the TEER values to a much higher extend as compared to the *A.vera* whole leaf extract. Muco-adhesion of formulations can also be enhanced by means of incorporating *A. vera* gel. It has been shown to have better muco-adhesive properties than that of Carbopol®, which is considered to have good muco-adhesive properties (Jambwa *et al.*, 2011:52). Another study also showed this muco-adhesive increase of *A. vera* gel and concluded that this may be because of the large number of large polysaccharide molecules present in the *A. vera* gel (De Bruyn *et al.*, 2018:57).

The dry powder form of *A. vera* gel can also be used to successfully manufacture compressible matrix type tablets. A study showed that it was possible to formulate dosage forms that slowly released the model compound over an extended time period. Therefore *A. vera* gel can be used as an excipient in modified release dosage forms (Jani *et al.*, 2007:90). Additional to the above mentioned excipient properties, *A. vera* gel also has significant therapeutic and biological properties, which include wound healing, anti-microbic, radiation damage repair, anti-inflammatory, immune stimulation and anti-oxidant effects (Hamman, 2008:1608).

CHAPTER 3: MATERIALS AND METHODS

3.1. Introduction

A number of different MUPS capsule formulations were prepared by filling size 00 hard gelatine capsules with bead formulations containing different vitamin and functional excipient combinations. The different bead formulations were manufactured by means of extrusion-spheronisation containing either vitamin C or E with different combinations of functional excipients including *Aloe vera* gel and sodium lauryl sulphate. Seven different MUPS capsules containing500 mg of vitamin C and seven different MUPS capsules containing 300 mg of vitamin E were prepared in addition to different concentrations of each functional excipient.

An *ex vivo* drug delivery model in the form of excised pig jejunum tissue mounted in a Sweetana-Grass diffusion apparatus was used to determine the effect of AVG and SLS on the permeability of both vitamin C and E across the intestinal epithelium. The transport studies were performed in the apical-to-basolateral direction to simulate absorption from the intestinal tract into the blood circulation. Two different transport media were used in the permeations studies namely potassium phosphate buffer solution (PPBS) for the vitamin C formulations and fed state simulated intestinal fluid (FeSSIF) was used for the poorly water-soluble vitamin E formulations.

3.2. Materials

Aloe vera gel (AVG) (batch number: 700AQ11PK01) was obtained from Improve USA, INC (USA, Texas, De Soto) and Pharmacel® (microcrystalline cellulose, MCC) was obtained from Warren Chem Specialities (SA, Johannesburg). The vitamins C and E (ascorbic acid and D-α-tocopherol succinate) was acquired from Sigma Aldrich (SA, Johannesburg). Sodium lauryl sulphate (SLS) (batch number: SAAR5823610EM) was obtained from Merck (SA, Johannesburg).

The potassium phosphate buffer solution (PPBS) consisted of sodium hydroxide pellets (NaOH) (batch number: MH6M562064) acquired from Merck, (SA, Johannesburg) and potassium phosphate(KH_2PO_4) (lot number: 20K0229) acquired from Sigma Aldrich (SA, Johannesburg).FeSSIF ingredients included sodium taurocholate hydrate (product number: 86339) purchased from Sigma Aldrich (SA, Johannesburg), lecithin (product number: J61675) obtained from Alfa Aesar (SA, Kyalami), sodium chloride (lot number: D00130978) obtained from Sigma Aldrich (SA, Johannesburg) and glacial acetic acid (100%) (Batch number: SAAR1021020LC) acquired from Merck (SA, Johannesburg). Sodium metabisulphite (SMBS) (Batch number: 25956) was used to stabilise the vitamin C in solution, which was obtained from SAARCHEM (SA, Krugersdorp).

Acetonitrile, phosphoric acid, and methanol (100%) (HPLC grade) were acquired from Sigma Aldrich (SA, Johannesburg). The excised pig jejunum tissue was collected at Potchefstroom Abattoir (SA, Potchefstroom).

3.3. Manufacturing of multiple-unit-pellet systems (MUPS)

3.3.1. Extrusion spheronisation method

Different bead formulations were manufactured by means of the extrusion-spheronisation technique as listed in Table 3.1.Firstly, 100 g batches of the dry materials that included the Pharmacel® (MCC), vitamin C or E, SLS and/or AVG, depending on the formulation, were mixed using a Turbula®T2B mixer (Switzerland, Willy, A. Bachofen) for 10 min. For vitamin C, 25g of vitamin powder were incorporated per 100g of powder mass and for vitamin E, 15g vitamin powder per 100g of powder mass were incorporated. The wetting agent, which consisted of a mixture of deionised water and alcohol (80:20), was slowly added to the dry powder mixture while mixing with a Kenwood® (SA, Maraisburg) planetary mixer. For the vitamin C containing bead formulations, the volume of wetting agent added per 100 g of powder mass was determined to be 80ml and for the bead formulations containing vitamin E, it was determined to be 110ml. The resulted wetted powder mass was then passed through the screen of the extrusion apparatus (Caleva® Extruder 20, England, Dorset). The screen size fitted to the extruder had a 1mm aperture size and the rotation speed of the extruder was set at 35 rpm. The resulted spaghetti-like extrudates were added to the spheroniser (Caleva® Multibowl Spheronizer, England, Dorset). This final step formed the spherical beads that were used in the MUPS formulations. The spheroniser was operated at a speed of 1700 rpm for 5 min. The resulting beads were added to a glass container intended to be used on a freeze dryer and placed for at least 24 h in a-80 ˚C freezer (Forma™ scientific lab freezer, Thermo Fisher Scientific, USA, Massachusetts, Waltham). The resulted frozen beads were lyophilised for at least 48 hours (Virtis™ bench-top freeze dryer, SP Scientific, USA, New-York, Gardiner). Afterwards, the dry spherical beads were manually filled into size 00 hard gelatine capsules (500 mg for each of the vitamin C and E formulations).

Table 3.1: Composition of different bead formulations (C1 - C7 for vitamin C containing beads and E1 – E7 for vitamin E containing beads)

Functional excipient	Vitamin C (25% w/w)	Vitamin E (15% w/w)
Aloe vera gel (AVG) 5% w/w	C1	E1
Aloe vera gel (AVG) 10% w/w	C2	E2
Sodium lauryl sulphate (SLS) 0.1% w/w	C3	E3
Sodium lauryl sulphate (SLS) 0.5% w/w	C4	E4
AVG 5% w/w + SLS 0.1% w/w	C5	E5
AVG 10% w/w + SLS 0.5% w/w	C6	E6
Control (without functional excipients)	C7	E7

3.3.2. Bead characterization

3.3.2.1. Uniformity of mass

A total of 20 MUPS capsules were randomly taken from each formulation. Each complete capsule was weighed individually. Each capsule was emptied and each capsule shell was weighed. The weight of the beads was determined by the difference in weight of the complete capsule and its shell. This process was repeated for the 20 MUPS capsules from each of the different bead formulations as outlined in Table 3.1. The deviation in mass of the contents of 20 capsules should not be more than 7.5%(BP, 2017:XII).

3.3.2.2. Friability

According to the British Pharmacopoeia (2017:XVII), friability is the reduction in the mass of solid dosage forms when they are subjected to mechanical strains. Such strains may be caused by tumbling produced by a friabilator. Abrasion, deformation and breakage is caused by the tumbling of the beads, this may serve as parameters to determine the ability to withstand physical strain caused by handling and packaging.

Bead samples of approximately 3 g from each formulation were individually added to a Parvalux® friability tester (Parvalux Electric motors, England, Bournemouth) along with 25 glass beads (diameter of 5 mm). A total of 100 revolutions of the friabilator drum were then applied to the test sample, which was achieved by operating the friabilator at 25 rpm for 4 min. The beads were removed from the drum and the glass beads were separated from the test sample. The beads were placed on a 500µm sieve to remove dust and to allow for the smaller particles to pass through. After this, the beads were weighed. Friability (represented by F) was determined by means of calculating the percentage loss using the following equation:

$$F = \frac{W1-W2}{W1} \times 100 \qquad \text{(Equation 3.1)}$$

W_1 represents the initial weight and W_2 represents the weight of the beads after undergoing the friability test. Friability was assessed in triplicate for each of the different formulations. A maximum mean weight loss not more than 1.0% was considered acceptable for the bead formulations (BP, 2017:XVII).

3.3.2.3. Assay

The content of each bead formulation was determined by crushing 32 mg of beads containing vitamin C (to give a concentration of 80µg/ml) and 33 mg of beads containing vitamin E (to give a concentration of 200 µg/ml), using a pestle and mortar. The crushed bead masses of the vitamin C bead formulations were each transferred to a 100 ml volumetric flask and made up to

volume using deionised water, while the crushed bead masses of the vitamin E bead formulations were each transferred to a 25 ml volumetric flask and made up to volume using high-performance liquid chromatography (HPLC) grade methanol. The different masses of the bead formulations and volumes to which they were made up were determined by the difference in limit of detection and limit of quantification values of the two vitamins. The flasks were placed in an ultra-sonic bath for 5 min to ensure total dissolution of each vitamin in the crushed bead masses. The vitamin concentration in each solution was determined using an HPLC method as described in section 3.4.1. The concentration of the vitamin in the prepared solution was compared to the theoretical content of each bead formulation. The real vitamin content was expressed as a percentage of the intended content which was calculated using the following equation:

$$\% \text{ content} = \frac{\text{experimental vitamin content}}{\text{theoretical vitamin content}} \times 100 \qquad \text{(Equation 3.2)}$$

From the % content, the actual vitamin concentration was determined using the following equation:

$$\text{vitamin concentration} = \text{theoretical vitamin concentration} \times \% \text{ content} \qquad \text{(Equation 3.3)}$$

3.3.2.4. Bead size and size distribution

Laser diffraction was used to determine the particle size and size distribution of the beads. This method entails exposing the particles, in this case the beads, to a beam of monochromatic light to produce a diffraction pattern. The particles must be dispersed at an adequate amount (as measured by obscuration) in a suitable liquid. Thereafter a multi-element detector measures the scattered pattern of light. Numerical values are assigned to the diffraction patterns and analysed. A mathematical algorithm is used to assign the particles to different size classes, which makes up the volumetric particle-size distribution (BP, 2017:XVII).

A Malvern® Mastersizer 2000 (Malvern Instruments Ltd. Worcestershire, UK) fitted with a Hydro 2000SM sample dispersion unit was used for determination of particle size of each bead formulation. A small sample of beads from each formulation was added to the sample dispersion unit and the particle size was measured, while the Mastersizer software was used to obtain and capture the data. Ethanol was used as the liquid dispersant in the system and also to flush and align the lasers within the apparatus. The d(0.5) (median of the size distribution) and D[4;3] (mean particle diameter) were obtained.

3.4. HPLC analysis method validation

3.4.1. Analytical instrument and chromatographic conditions

Different conditions for HPLC analysis was applied on each of the two vitamins, because of differences in solubility. The HPLC analytical parameters used in this study for vitamin C and E are summarized in Table 3.2 and Table 3.3 respectively.

Table 3.2: HPLC analytical parameters for vitamin C

Analytical conditions	Description
Analytical instrument	Agilent HP1100 series equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.10.03 data acquisition and analysis software
Column	VenusilC18 250x 4.6 mm
Mobile phase	Acetronitrile/deionised water (5:95) with 0.1% (v/v) H_3PO_4
Flow rate	1.0 ml/min
Injection volume	10 µl
Detection wavelength	243 nm
Retention time	3.8 min
Stop time	6 min
Solvent	PPBS stabilised with 0.2% (m/v) sodium metabisulphite

Table 3.3: HPLC analytical parameters for vitamin E

Analytical conditions	Description
Analytical instrument	Agilent HP1100 series equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.10.03 data acquisition and analysis software
Column	Venusil C18 150 x 4.6 mm
Mobile phase	100% HPLC grade methanol; pH adjusted to 3.5
Flow rate	1.5 ml/min
Injection volume	10 µl
Detection wavelength	208 nm
Retention time	6.0 min
Stop time	8 min
Solvent	PPBS stabilised with 0.2% (m/v) sodium metabisulphite, FeSSIF and 100% methanol

3.4.2. Determination of vitamin concentration in the samples using the standard curves

To calculate the concentrations in the test samples of vitamin C and E, a standard curve was used that was freshly prepared for each vitamin before each analysis. This was done by dissolving 10 mg of vitamin C in 100 ml of PPBS or dissolving 20 mg of vitamin E in 100ml methanol. A series of dilutions of the stock solution of each vitamin were injected into the HPLC by varying the injection volume as follows: 2, 4, 6, 8 and 10 µl. Standard curves were obtained by plotting the HPLC chromatogram peak area (y-axis)as a function of the concentration (x-axis) for each vitamin.

The concentrations of each vitamin in the test sample were determined from the standard curves by using the following equation:

$$\text{concentration in sample} = \frac{(\text{peak area} - \text{y-intercept})}{\text{slope}} \qquad \text{(Equation 3.3)}$$

3.4.3. Specificity

Specificity is defined as the ability to analyse the analyte in the presence of other components that may be present in the analytical process. These usually include impurities and degradation products, but may be any other component of a formulation such as excipients(BP, 2017:SC III).

A quantity of 17.61 mg of Vitamin C was dissolved in 100 ml PPBS stabilised with SMBS. A quantity of11.42 mg of Vitamin E was dissolved in 20 ml methanol and made up to 100 ml with FeSSIF. Both these solutions were analysed (in triplicate) in the presence of SLS and AVG by means of the HPLC methods described for each vitamin. The resulting chromatograms were then inspected to establish that the vitamin (C and E) peaks were clearly separated from the peaks caused by the other components and the percentage recovery of the vitamins was determined by the following equation:

$$\%\ \text{recovery} = \frac{\text{Actual peak}}{\text{Expected peak}} \times 100 \qquad \text{(Equation 3.4)}$$

3.4.4. Linearity

Linearity of an analytical method is defined as the ability to obtain responses (i.e. chromatogram peak areas) that are directly proportional to the concentration of the analyte within the given analytical range (BP, 2017:SC III). For validation of the HPLC method in terms of linearity, a regression was done on the curve obtained when the peak area (y-axis) was plotted as a function of concentration (x-axis) for each vitamin.

Vitamin C solutions with concentrations ranging between 25 and 500 µg/ml and vitamin E solutions ranging between 15 and 300 µg/ml were injected in duplicate into the HPLC. The resulting peak areas were then plotted as a function of the concentration of the specific vitamin in solution. A linear regression of the curve was performed using Microsoft Excel® software from which the relevant correlation coefficient (R^2) values were obtained. The R^2value should be above 0.99 for the analytical method to be acceptable in terms of linearity (BP, 2017:SC III).

3.4.5. Accuracy

The accuracy of an analytical method describes the closeness of the test results obtained by the analytical method to the true concentration of the analyte (Shabir, 2003:61). Three different samples of each vitamin were accurately weighed and dissolved in the required solvent (PPBS

for vitamin C and methanol for vitamin E). For vitamin C, the three concentrations were 25, 50 and 100 µg/ml and for vitamin E the concentrations were 25, 50 and 200 µg/ml. Both were done in duplicate. The samples were analysed and the amount of vitamin was determined from the standard curves by using Equation 3.3 as previously described.

The concentration, determined by the HPLC in terms of peak areas, was compared to the weighed concentrations (i.e. the real concentrations) to determine the percentage recovery. This recovery may not be less than 90% and not be higher than 110% for the method to be considered acceptable in terms of accuracy (Johnson & Van Buskirk, 1998:99).

3.4.6. Limit of detection and limit of quantification

Detection limit is the lowest concentration of an analyte in a sample that may be detected, this does not mean it can be quantified as an exact value (BP, 2017:SC III). The limit of detection (LOD) was determined at a peak area three times greater than the baseline noise. LOD can be calculated using the following equation (Shabir, 2003:63):

$$LOD = 3.3 \times \left(\frac{SD}{S}\right) \qquad \text{(Equation 3.5)}$$

Quantification limit is the lowest concentration of analyte in the sample that can be determined quantitatively in terms of acceptable precision and accuracy (BP, 2017SC III). The limit of quantification (LOQ) was determined by means of injecting three replicate samples of each vitamin at a low concentration, into the chromatograph. The limit of quantification was determined at a peak area 10 times greater than the baseline noise. LOQ can be calculated using the following equation (Shabir, 2003:63):

$$LOQ = 10 \times \left(\frac{SD}{S}\right) \qquad \text{(Equation 3.6)}$$

Whereas (for both Equations 3.5 and 3.6 respectively) SD represents the standard deviation of the residual regression line and S represents the slope of the calibration curve.

3.4.7. Precision

Precision can be defined as the closeness between a series of measurements obtained from numerous tests from the same homogenous sample under previous determined conditions. The results are expressed as the standard deviation or percentage relative standard deviation(% RSD) for the series of measurements (BP, 2017:SC III).

Intra-day precision (repeatability)

Intra-day precision can also be defined as intra-assay precision and it expresses the precision under the exact same conditions of operation over a short time interval (typically on the same day). The repeatability was determined using the same concentration range that was used for the accuracy test for both vitamins on three different times during the same day. For vitamin C, the acceptable %RSD for intra-day precision was considered to be less than 10% because of the relatively poor stability of this vitamin in solution. For vitamin E, the acceptable %RSD was considered to be below 5%.

Inter-day precision

For inter-day repeatability, solutions with concentrations of 50 µg/ml for both vitamins were injected into the HPLC at the same time on three consecutive days. The same solutions were used for vitamin E and it was kept away from light at a temperature of 6 °C. For vitamin C new solutions were prepared each day because of its instability in solution. Acceptable %RSD values for inter-day precision were considered to be below 10% for vitamin C and for vitamin E it should be less than 5%.

3.4.8. Ruggedness

Sample stability

Standard solutions of both vitamins were prepared at concentrations of 100 µg/ml. Vitamin E was firstly dissolved in 10 ml methanol then made up to volume with FeSSIF, whereas vitamin C was directly dissolved in PPBS stabilised with sodium metabisulphite. These samples were then injected into the chromatograph to determine the stability of the vitamins in the specific solvents. The vitamin samples were left in the auto sampler tray and analysed at hourly intervals for 24 hours. The samples only needed to be stable for 6 hours because the experiments combined with HPLC analysis didn't exceed this amount of time. Acceptable %RSD for vitamin C was considered to be less than 5% and for vitamin E, it was considered acceptable when less than 2%.

Repeatability

The repeatability of peak area and retention time of each sample was also tested. This was done by means of consecutively injecting a standard solution of the vitamin six times into the chromatograph. For both vitamins the acceptable %RSD for the six replicates for both the peak area and retention time should be less than 2%.

3.5. Drug release

3.5.1. Preparation of potassium phosphate buffer solution (PPBS)

For all the dissolution and transport studies done on the vitamin C formulations, a potassium phosphate buffer solution (PPBS) was used as liquid medium. To prepare 1000 ml PPBS, 1.5 g of NaOH were dissolved in 400 ml of deionised water and 6.5 g of KH_2PO_4 were dissolved in 250 ml of deionised water. The NaOH-solution was then added to the KH_2PO_4-solution, while stirring. Deionised water was added to make the volume up to 1000 ml. The pH of the buffer solution was adjusted to 7.4 by adding sufficient volumes of 0.1 M hydrochloric acid (HCl) or 0.1 M NaOH. A quantity of 2 g of sodium metabisulphite (SMBS) was added afterwards to stabilise the vitamin C in solution.

3.5.2. Preparation of fed state simulated intestinal fluid (FeSSIF)

The vitamin E solubility and transport studies were done using FeSSIF (Fed state simulated intestinal fluid) as liquid medium. The quantities of each compound required for preparing the solution is indicated in Table 3.4.

Table 3.4: Composition of fed state simulated intestinal fluid (FeSSIF)

Component	**Quantity**
Sodium taurocholate	8.09 g
Phosphatidylcholine as lechitin	2.41 g
Acetic acid (glacial)	9.1 ml
NaCl	10.11 g
Deionised water	To 1000 ml

Each of the abovementioned dry compounds (Table 3.4) was accurately weighed and transferred to a 1000 ml volumetric flask. A volume of 500 ml of the deionised water was added while the content was magnetically stirred. Thereafter the glacial acetic acid was added and another 400 ml of water was added. The mixture was heated to 40 °C, while continuously being stirred. This was done until everything was clearly in solution. The pH was adjusted to 7.4 with the use of 0.1 M NaOH solution. Thereafter the magnetic stirrer was removed and the solution was made up to 1000 ml with deionised water (Marques, 2004:16).

3.5.3. Dissolution of vitamin C MUPS capsule formulations (C1 – C7)

Dissolution is defined as the process during which a drug is dissolved in a pre-determined dissolution medium. Dissolution studies on the vitamin C MUPS capsule formulations (C1 – C7) was carried out using the basket method (BP, 2017:SC I) with PPBS as dissolution medium in a six vessel Distek 2500 dissolution apparatus (US, NJ, North Brunswick). Two MUPS capsules

containing 500 mg beads each were placed in each basket to make up the total dosage of 1000 mg. The dissolution of the different bead formulations were tested in triplicate. The stirring rate was set at 150 rpm with each vessel filled with 900ml of the PPBS dissolution medium. The dissolution medium temperature was maintained at 37 ± 0.5°C. Samples of 4 ml were drawn from each individual vessel using a Distek evolution 4300 auto sampler (US, NJ, North Brunswick) at established time intervals (i.e. 15, 30, 60, 90, 120, 150, and 180 min).The sample volume was replaced by the same amount of fresh dissolution medium in each of the six vessels after each sample withdrawal.

The samples obtained were analysed with the aforementioned HPLC analytical method to determine the concentration of vitamin C in the samples withdrawn at each time point and to calculate the % dissolution as a function of time. This was done using the following equation:

$$\% \text{ dissolution} = \frac{\text{vitamin amount released at set time interval}}{\text{total vitamin amount}} \times 100 \qquad \text{(Equation 3.7)}$$

The total amount of the vitamin C in each MUPS capsule formulation was determined by means of the assay testing as described before.

3.5.4. Dissolution of vitamin E MUPS capsule formulations (E1 – E7)

The same dissolution method as described for vitamin C MUPS capsule formulations was also performed on the vitamin E formulations. However, very little vitamin E could be detected in the samples withdrawn from the dissolution vessels for all of the vitamin E formulations. Since this result was most probably a consequence of the low solubility of vitamin E in an aqueous environment (i.e. the PPBS dissolution medium), it was decided to conduct a solubility study in an alternative dissolution medium in order to find a medium in which the *ex vivo* transport studies could be conducted.

3.5.5. Dissolution studies of vitamin EMUPS capsule formulations (E1–E7) in FeSSIF

A quantity of 1400 mg beads (to replicate the same concentration, 21 mg/ml, which was set to be used for the transport studies) was added to 10 ml of FeSSIF in a 50 ml Corning Centristar® tube. The tubes were sealed using Parafilm® and attached to the rotating axis inside the water bath of a customised dissolution apparatus at 37°C. Test tubes were prepared in triplicate for each different vitamin E bead formulation (E1 – E7). Samples (1 ml) were drawn at time intervals of 60, 120 and 180 min and filtered through 0.45 µm membrane filters.

The samples obtained were analysed with the HPLC analytical method as described before to determine the % dissolved at each time point. This was done using the same equation that was used for the dissolution studies of vitamin C (i.e. Equation 3.7):

$$\% \text{ dissolution} = \frac{\text{vitamin amount released at set time interval}}{\text{total vitamin amount}} \times 100$$

The total amount of vitamin E that dissolved from each MUPS capsule formulation was determined by means of the assay testing as described before. This dissolution test was also done using PPBS as dissolution medium to compare the dissolution within the different mediums.

3.6. *Ex vivo* transport studies

Ex vivo refers to experiments where excised animal/human tissues are being used outside the body. Excised pig intestinal tissues were mounted in a Sweetana-Grass diffusion apparatus for the *ex vivo* vitamin transport experiments conducted in this study. The transport media consisted of PPBS stabilised with SMBS for the vitamin C MUPS capsule formulations, while FeSSIF was used for the transport studies with the vitamin E MUPS capsule formulations. For each vitamin bead formulation, a batch of MUPS capsule formulations were prepared by loading 500 mg of the specific bead formulation per hard gelatine capsule. Two MUPS capsules were applied per donor chamber in the assembled Sweetana-Grass diffusion apparatus, which means that 1000 mg of beads were applied into each donor chamber. Two MUPS capsules contained 250 mg vitamin C and 150 mg vitamin E in total.

3.6.1. Preparation of the excised pig jejunum tissue

Directly after a pig was slaughtered for meat production purposes, a piece of jejunum, approximately 30 cm, was collected from Potchefstroom Abattoir. Before transporting the excised tissue to the laboratory, the tissue was rinsed with ice cold PPBS. The tissue was placed in a Schott® bottle with the remainder of the ice cold PPBS and added to a cold box to adhere to the cold chain. The excised pig jejunum tissue was transported and processed within 30 min from the time that the pig was slaughtered.

In the laboratory, the excised pig jejunum tissue was pulled onto a glass tube (Figure 3.1 A). Blunt dissection was used to remove the serosa from the tissue (Figure 3.1 B) and a scalpel was used to cut the excised jejunum along the mesenteric border (Figure 3.1 C). The resulting tissue sheet was washed onto a piece of filter paper and the mucus was rinsed off using PPBS, which resulted in the apical side of tissue being faced upwards (Figure 3.1 D). All of the transport studies were done in the apical to basolateral direction.

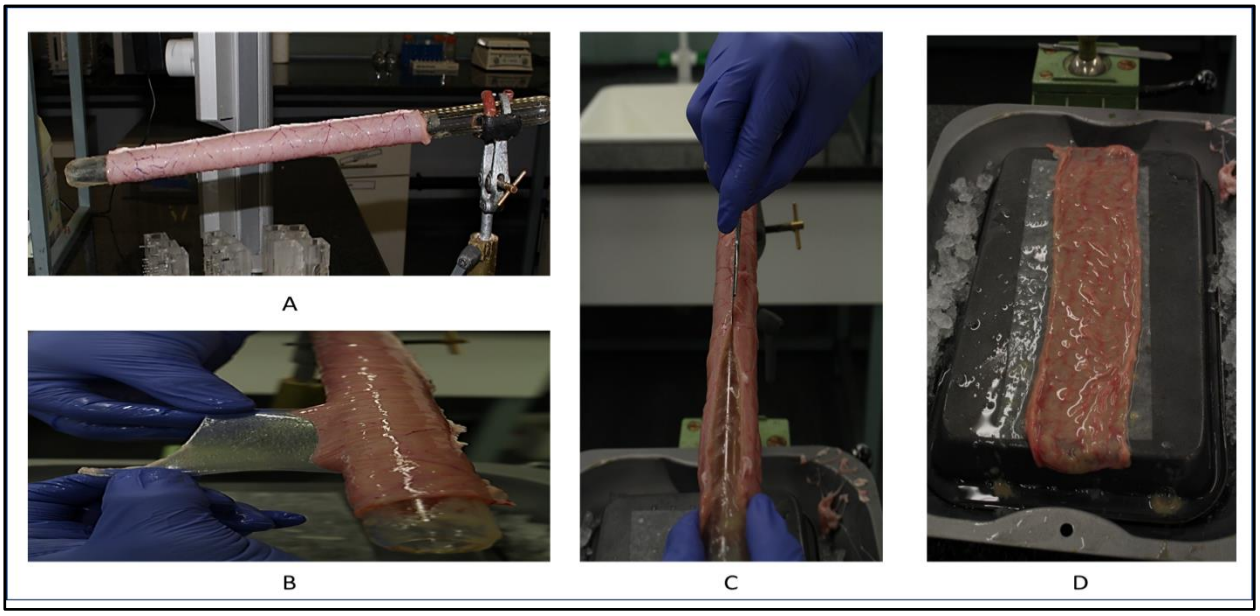

Figure 3.1: Images A-B illustrate the process of removing the serosa, image C illustrates the cutting of the tissue along the mesenteric border and image D illustrates the sheet of excised jejunum tissue on filter paper

The resulting jejunum sheet was cut into smaller pieces to be mounted on the Sweetana-Grass diffusion chamber half-cells (Figure 3.2 A). While cutting the jejunum, Peyer's patches were avoided because of their potential effect on the drug transport (Figure 3.2 B). The half-cells are designed to expose a surface area of 1.72 cm^2 of the excised tissue to the transport media. The small pieces of tissue were fitted to the chambers, the filter paper was removed and the chambers were assembled (Figure 3.2 C). Metal rings were used to clamp the two chamber half-cells together (Figure 3.2 D).

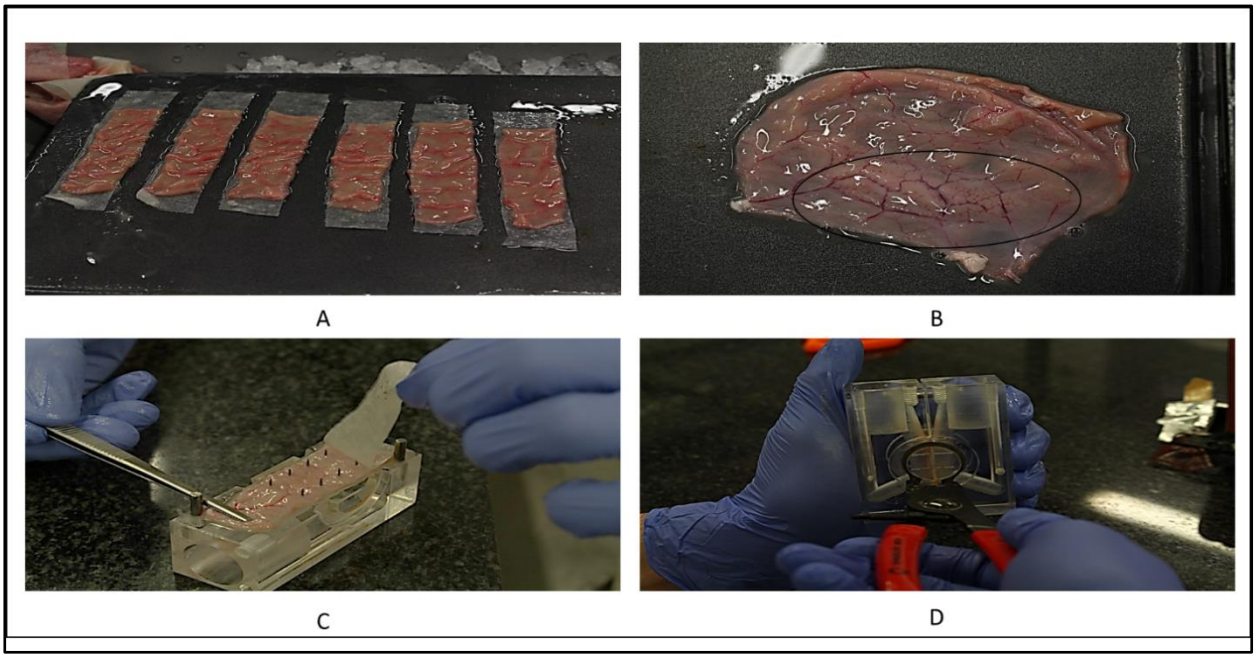

Figure 3.2: Image A illustrates small jejunum pieces, image B indicates a Peyer's patch and images C - D illustrate the removal of the filter paper and the assembly of the diffusion chamber

The assembled diffusion chambers were placed in the manifold, which is linked to a heating block (set at 37 °C), and supplied with carbogen gas (95% O_2:5% CO_2) (Figure 3.3 A). The chambers were filled with 7 ml of PPBS for vitamin C formulations and 7 ml FeSSIF for vitamin E formulations to acclimatise the tissue to the particular diffusion medium. Trans-epithelial electrical resistance (TEER) was measured in the beginning and end of the transport experiment, to measure if the membrane integrity was maintained during the transport study (Figure 3.3 B).

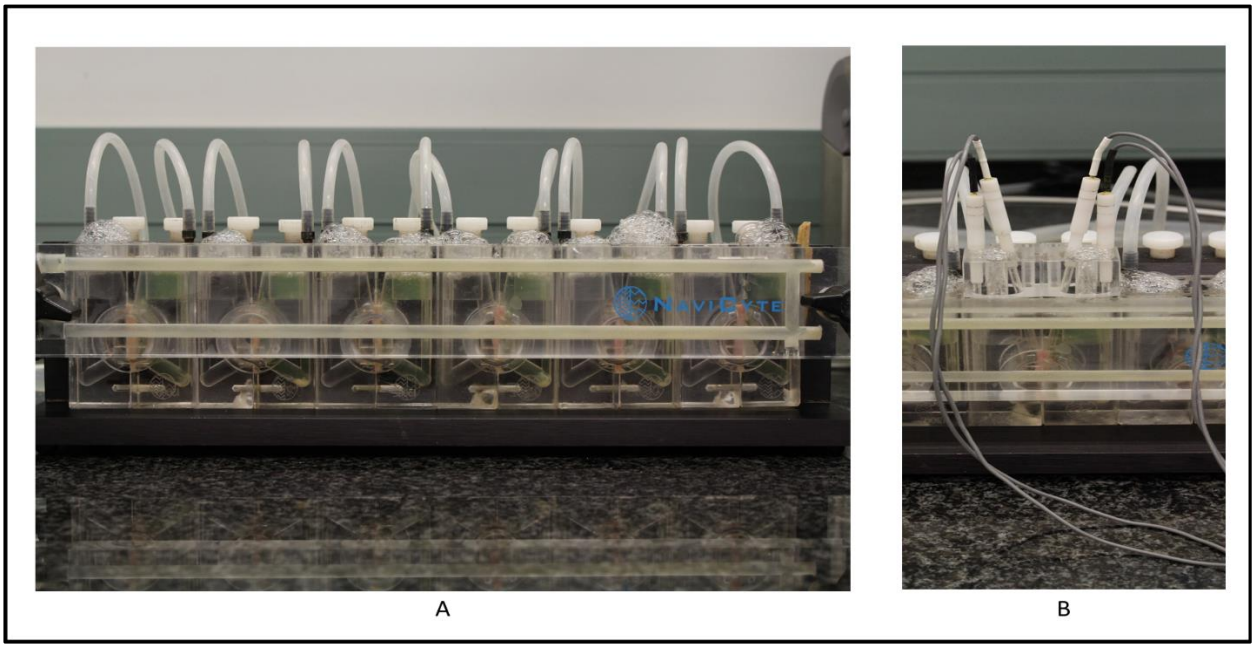

Figure 3.3: Image A illustrates the assembled Sweetana-Grass diffusion apparatus with diffusion chambers inserted into the manifold connected with carbogen gas supply and image B illustrates the electrodes with which trans-epithelial electrical resistance (TEER) was measured

3.6.2. Transport studies

Transport of vitamin C and E across excised intestinal tissue was done by suspending the different bead formulations in the specific diffusion medium in the Sweetana-Grass diffusion chamber apparatus. The apical side of the chambers were emptied after acclimation period (15 min) and filled with the appropriate diffusion medium. Thereafter, 2 MUPS capsule formulations (containing 1000 mg of beads) were emptied in the apical chambers, which were done separately for each formulation. Samples (500 µl) were drawn from the basolateral chamber at the specified time intervals (15, 30, 60, 90, and 120 min) and transferred to HPLC vials. The withdrawn samples were replaced with equal volumes of the diffusion medium. The samples were analysed to determine the vitamin concentration by means of the specific validated HPLC method. Transport studies were done in triplicate. After the transport studies were completed, the excised tissues used in the transport of vitamin C were safely discarded as bio-material (according to SOP for waste removal of bio hazardous material). The excised tissues used in the vitamin E transport was further processed and tested for vitamin E retained in the tissue (to be explained in 3.6.3).

3.6.3. Vitamin E retained in the excised intestinal tissue

Because there was no transport for vitamin E observed across the excised tissues, the vitamin E retained inside the tissues was analysed. Each separate tissue sample was taken, rinsed with deionised water and added to a 15 ml glass vial. A volume of 5 ml of 100 % HPLC grade methanol was added to each vial and the contents mixed using a Vortex® mixer. After mixing,

the vials were placed in an ultra-sonic bath for 10 min to cause lysis of the tissue. This was done to extract any vitamin E that accumulated in the tissue. Because of the lipophilic nature of vitamin E, the vitamin E molecules probably stayed inside of the intestinal tissue and did not partition into the aqueous environment of the basolateral chamber. After the lysis of the tissues, the vials were centrifuged (for 5 min at 3000 rpm) to separate the supernatant from the solid materials. Samples of 500 µl each were withdrawn from the supernatant and analysed using the HPLC apparatus. The percentage vitamin E retained in the tissue was calculated with the following equation:

$$\% \text{ retention} = \frac{\text{Amount extracted from tissue}}{\text{Amount in apical chamber at end}} \times 100 \qquad \text{(Equation 3.8)}$$

The amount extracted from the tissue during the lysis process was compared to the amount of vitamin E released in the apical chamber at the 120 min time interval, this ratio was used to determine the percentage retention.

3.6.4 Analysis and processing of transport data of vitamin C

The percentage transport for vitamin C was calculated using the following equation:

$$\% \text{ transport} = \frac{\text{vitamin amount at set time interval}}{\text{total vitamin amount}} \times 100 \qquad \text{(Equation 3.9)}$$

The percentage transport was plotted as a function of time. Apparent permeability coefficient (Papp) values were calculated using the following equation:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A \times Co \times 60} \qquad \text{(Equation 3.10)}$$

Where P_{app} is the apparent permeability coefficient (cm/s), dQ/dt (µg.s^{-1}) represents the increase in the amount of vitamin in the receiver chamber within a given time period, which is equivalent to the slope of the plotted drug concentration transported versus time. A (cm^2) is the effective surface area of the excised pig intestinal cell layer between the apical and basolateral chambers and C_o is the initial vitamin concentration in the apical chamber (µg.cm^{-3}).

3.7. Muco-adhesion studies

The method used for evaluating the muco-adhesive properties of the different bead formulations was adapted from a previously published method based on two different methods namely the “falling liquid film method” and the “adhesion number method” (De Bruyn *et al.*, 2018:55). A number of 100 beads from each formulation were filled into hard gelatine capsules. The content of one capsule was emptied at a height of 1 cm on to a marked area (approximately 1 cm^2) of the excised pig jejunum tissue sheet, which was mounted to a concave surface at an angle of 15°. The tissue was acquired in the same manner as for the *ex vivo* transport studies, except

the mucus was not rinsed from the tissue. Deionised water (50 ml) was filled into a burette. The water in the burette was emptied onto the beads on the tissue, which rinsed down the slope into a beaker. The result of this was that a number of beads were washed away and some beads stayed behind on the tissue. The beads that were washed away ended in the glass beaker, from which the beads were removed and counted. The counted beads were subtracted from the initial number to determine the number of beads retained on the tissue. The percentage muco-adhesion was then calculated using the following equation:

$$\% \text{ muco-adhesion} = \frac{\text{number of beads retained on tissue}}{\text{initial number of beads}} \times 100 \qquad \text{(Equation 3.11)}$$

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Introduction

Different MUPS capsule formulations containing vitamin C and E were manufactured from beads that were produced by the extrusion spheronisation technique as described in section 3.3.1 to create the different bead formulations as listed in Table 3.1. Bead characterisation including uniformity of mass, friability, assay and particle size was done on each formulation. Validation results obtained from the HPLC analytical method for vitamin C and E served as proof that the analytical method produced accurate, repeatable and reliable results. Vitamin release studies were done on all the different MUPS formulations. Transport of each vitamin from each formulation was measured across excised pig intestinal tissues using a Sweetana-Grass diffusion apparatus to determine the vitamin delivery ability of each formulation. P_{app} and percentage transport across the excised pig intestinal tissues were calculated for vitamin C and the percentage retention in the excised pig intestinal tissues was calculated for vitamin E. The muco-adhesive properties of the beads were also tested.

4.2. Bead characterisation

4.2.1. Uniformity of mass

The average masses of the beads in each MUPS capsule bead formulation, the percentage relative standard deviation (%RSD) and the standard deviation are given in Table 4.1.

Table 4.1: The average masses, the standard deviation and percentage relative standard deviation (%RSD) for each MUPS capsule bead formulation

MUPS capsule formulation	Average mass (mg)	Standard deviation (n = 20)	%RSD (n = 20)
C1	506.23	4.71	0.93
C2	509.76	2.70	0.53
C3	507.80	1.90	0.37
C4	505.81	1.82	0.36
C5	511.38	2.18	0.43
C6	510.65	3.63	0.71
C7	501.28	2.58	0.51
E1	511.30	2.48	0.48
E2	508.37	2.25	0.44
E3	510.61	2.64	0.52
E4	510.45	2.90	0.57
E5	509.93	2.09	0.41
E6	510.52	2.90	0.57
E7	510.07	3.68	0.72

The criterion for acceptable uniformity of mass for capsules with a content of greater than 300 mg is a percentage deviation equal to or lower than 7.5% (BP, 2017:XII). Table 4.1 clearly indicates that each MUPS capsule bead formulation complied with the prescribed standards of the BP.

4.2.2. Friability

Friability can be defined as the percentage loss in mass when solid dosage forms are subjected to mechanical strain (BP, 2017:XVII). The average initial mass, the average mass after friability testing, the average percentage friability and the standard deviation for each formulation is given in Table 4.2. The average percentage friability of all the formulations adhered to the BP standard for friability (which is less than 1% mass loss), except for formulation C1 (1.02%) that marginally exceeded the limit. The friability of the bead formulations was considered acceptable in terms of percentage friability as seen in Table 4.2.

Table 4.2: The initial mass (mg), mass after friability test (mg), friability (%) and standard deviation for each MUPS capsule bead formulation

Bead formulation	Average initial mass (mg)	Average mass after test (mg)	Average Friability (%)	Standard deviation (n = 3)
C1	3047.13	3016.00	1.02	0.31
C2	3041.33	3011.80	0.97	0.93
C3	3031.10	3012.40	0.62	0.29
C4	3036.67	3021.00	0.52	0.10
C5	3012.43	2989.93	0.75	0.24
C6	3022.70	2993.17	0.98	0.34
C7	3000.73	2973.83	0.90	0.26
E1	3031.87	3007.47	0.80	0.22
E2	3004.37	2978.43	0.86	0.21
E3	3030.43	3002.20	0.93	0.30
E4	3011.07	2982.53	0.95	0.23
E5	3029.13	3002.97	0.86	0.35
E6	3002.93	2980.40	0.75	0.54
E7	2997.10	2967.23	0.99	0.39

4.2.3. Assay

The content of vitamin C in the MUPS capsule bead formulations was aimed to be 25% w/w and for vitamin E, it was 15% w/w. The measured (or actual) concentration of each vitamin was compared with the theoretical concentration to determine the percentage (%) content (assay). A summary of the assay results is given in Table 4.3.

Table 4.3: The average experimental peak area, the actual vitamin concentration (% m/m) and the average % content of each different MUPS bead formulation

Bead formulation	Average experimental peak area	Actual vitamin concentration (%w/w)	Average % Content
*C1	2580.92	25.91	103.65
C2	2304.27	22.72	90.89
C3	2607.42	25.19	100.74
C4	2614.77	25.44	101.75
C5	2474.23	24.30	97.19
C6	2503.12	24.77	99.07
C7	2597.87	25.77	103.08
**E1	4027.23	13.20	88.00
E2	4443.17	14.52	96.77
E3	3570.63	11.50	76.67
E4	3648.80	11.84	78.93
E5	3725.82	13.11	87.43
E6	4806.08	15.97	106.48
E7	3701.42	12.42	82.80

Theoretical vitamin concentration (%w/w) = *25 and **15.

4.2.4. Bead size and size distribution

The d(0.5) (median of the size distribution), the D[4;3] (mean particle diameter) and the span obtained during the size analysis of the different bead formulations are given in Table 4.4. The Span is the measurement of the width of the distribution. Small span values are a representative of relatively narrow size distribution.

Table 4.4: The d(0.5) (median of the size distribution), the D[4;3] (mean particle diameter) and the span of the different bead formulations

Bead formulation	d(0.5) µm	D[4;] µm	Span
C1	1043.75	1071.54	0.630
C2	996.48	1029.75	0.684
C3	1017.57	1055.39	0.841
C4	946.901	989.09	0.813
C5	912.55	945.15	0.668
C6	985.71	1018.02	0.664
C7	994.29	1024.35	0.198
E1	934.80	974.80	0.761
E2	903.88	941.60	0.740
E3	993.81	1026.59	0.681
E4	1028.94	1071.94	0.884
E5	928.89	967.42	0.741
E6	1007.99	1039.66	0.667
E7	975.26	1006.31	0.644

From Table 4.4, it is evident that narrow particle size distributions were obtained, because of the small span values. Furthermore, the similarities between the values of d(0.5) µm and D[4;] µm

for each bead formulation indicates that the distributions are close to symmetrical. The difference in these values was found to be small for each different bead formulation. The d(0.5) (median of the size distribution) and the D[4;3] (mean particle diameter) of each bead formulation was determined to be close to the aperture size of 1000 µm. This is expected for beads prepared by means of extrusion spheronisation. Figure 4.1 illustrates an example of a size distribution plot of formulation C1. The size distribution plots obtained for each different bead formulation are shown in Addendum E.

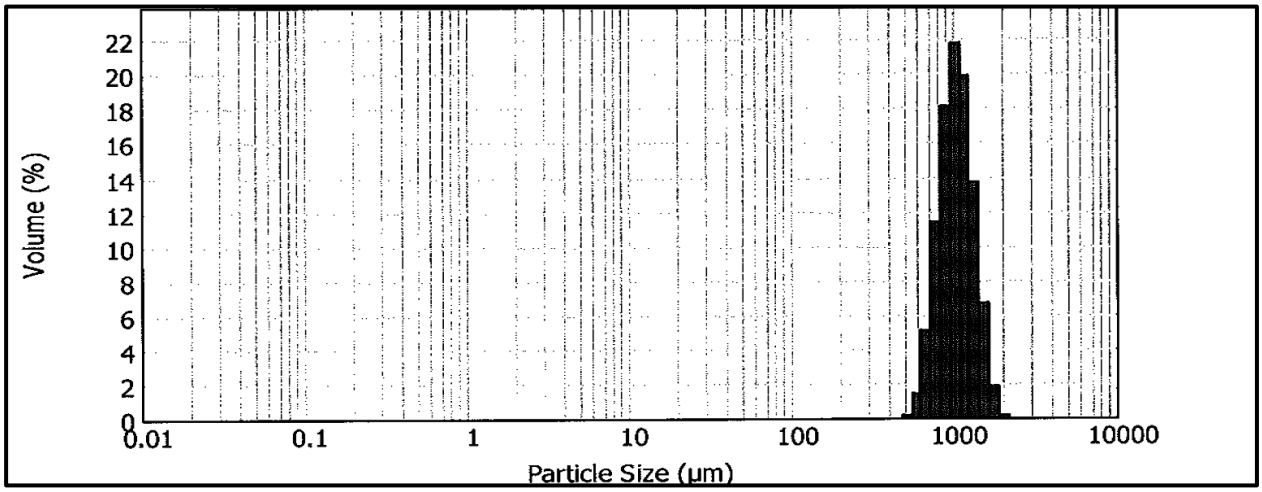

Figure 4.1: Size distribution plot for bead formulation C1

4.3. HPLC analysis method validation

4.3.1. Specificity

4.3.1.1. Vitamin C specificity

Figure 4.2 illustrates the chromatograph and the peak areas of all the peaks for vitamin C specificity validation. From the chromatograph it is clear that no other components interfered with the vitamin C peak at the retention time of 4.129 min. A narrow symmetrical peak was achieved. The % recovery for vitamin C (in the presence of SMBS, PPBS, AVG and SLS) was found to be 99.47 %. Because no interference between components was found, the specificity validation is acceptable according to the criteria for specificity (Johnson & Van Buskirk, 1998:90).

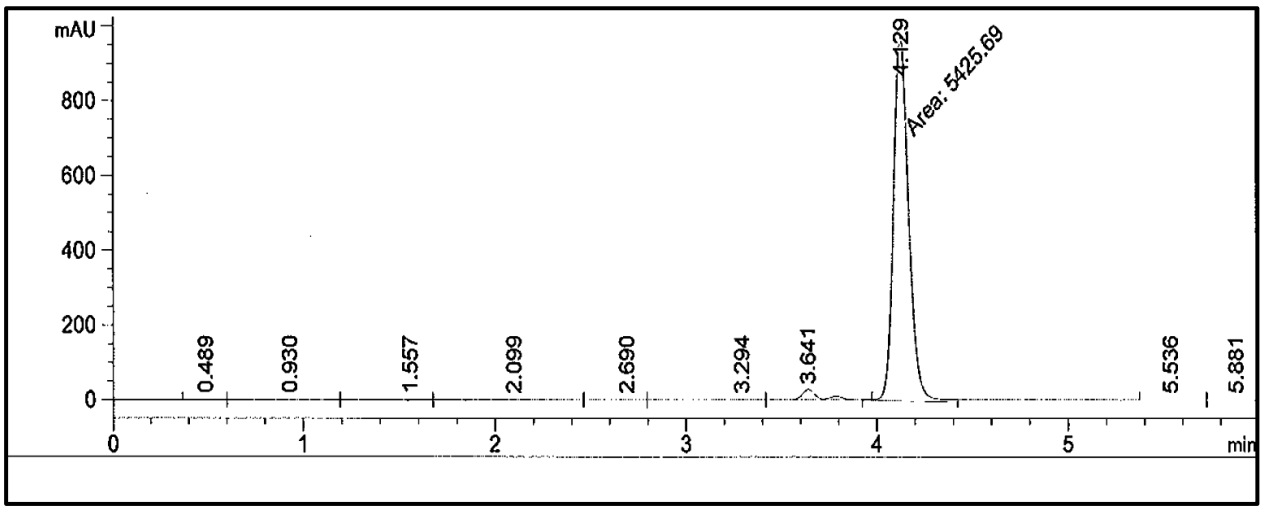

Figure 4.2: The HPLC chromatograph of vitamin C test sample

4.3.1.2. Vitamin E specificity

The % recovery for vitamin E was determined to be at 98.92 % using Equation 3.4. Figure 4.3 illustrates the chromatograph and the peak areas for vitamin E specificity validation. From the chromatograph it is clear that no other components interfered with the vitamin E peak at the retention time of 6.605 min and that the vitamin E peak was clearly separated. A narrow symmetrical peak was also achieved.

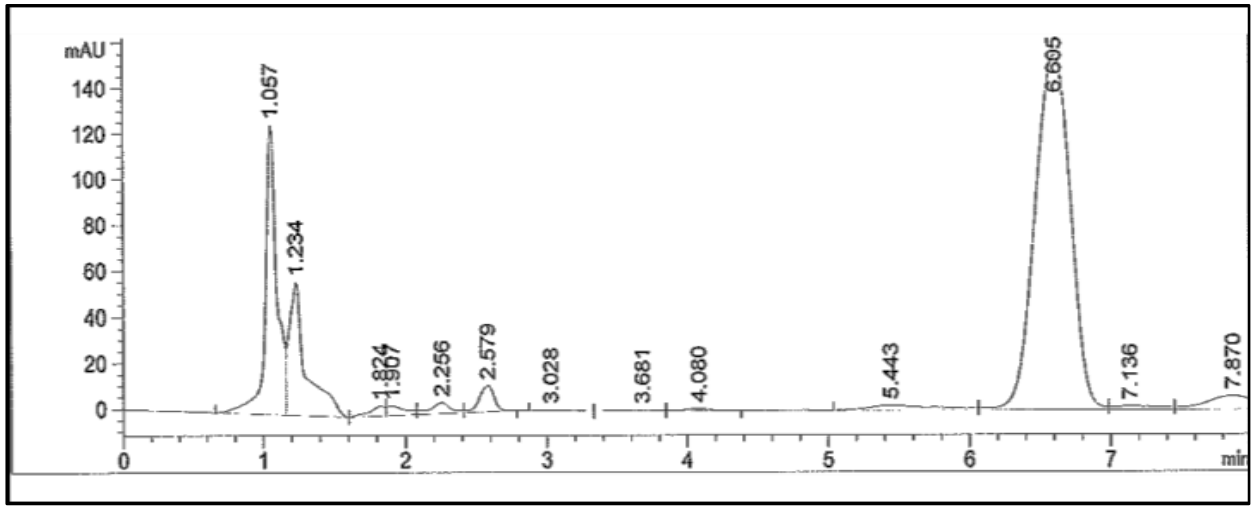

Figure 4.3: The HPLC chromatograph of vitamin E test sample

4.3.2. Linearity

4.3.2.1. Vitamin C linearity

The peak areas obtained for vitamin C for all the concentrations within the pre-determined concentration range for the linear regression curve are given in Table 4.5.

Table 4.5: HPLC peak areas obtained for different vitamin C concentrations

Concentration (µg/ml)	Peak area 1	Peak area 2	Mean peak area
24.80	793.70	798.40	796.05
49.60	1550.60	1583.70	1567.15
74.40	2381.70	2122.30	2252.00
99.20	3134.90	2932.30	3033.60
124.00	4008.30	4065.30	4036.80
248.00	7945.40	7587.50	7766.45
372.00	11770.00	11269.50	11519.75
496.00	15200.60	15075.30	15137.95

Figure 4.4 illustrates the linear regression curve where peak area was plotted as a function of vitamin C concentration. The slope was determined to be 30.822 and the R^2 value was 0.9995. Therefore, the linearity of the HPLC method for vitamin C is acceptable (i.e. it is > 0.99) over the concentration range of 24.8 µg/ml to 496.0 µg/ml.

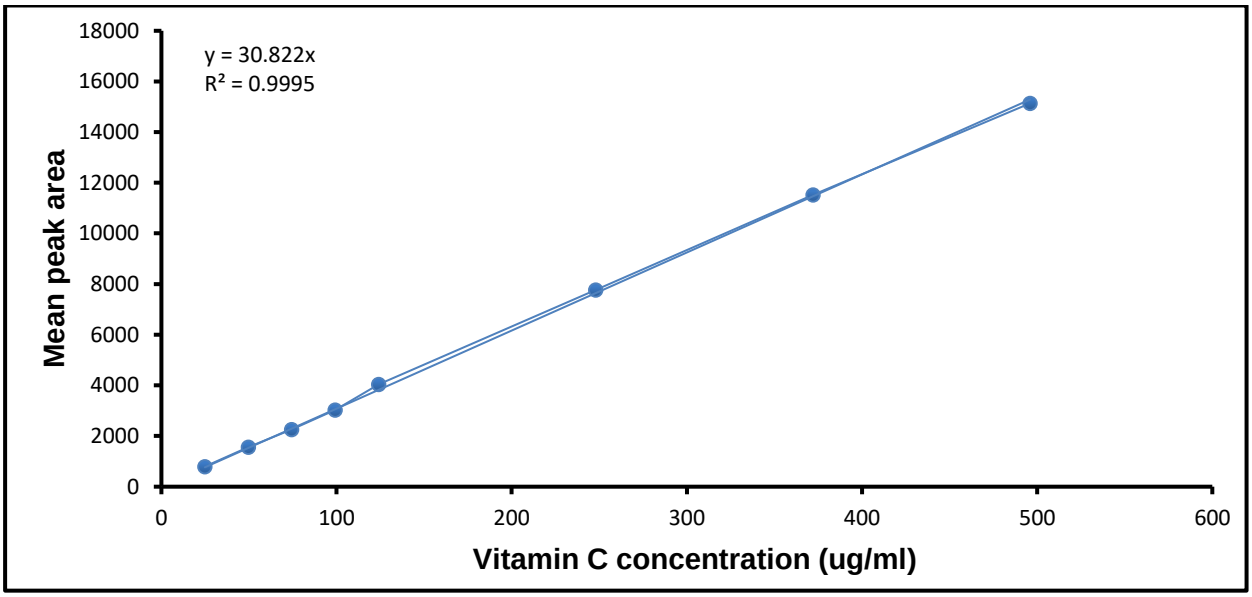

Figure 4.4. Linear regression curve for vitamin C

4.3.2.2. Vitamin E linearity

Vitamin E solutions with a concentration range between 15.6 µg/ml and 312.4 µg/ml were injected in duplicate into the HPLC. Linear regression was performed to determine the relevant correlation coefficient (R^2). Figure 4.5 illustrates the linear regression curve where the mean peak area was plotted as a function of vitamin E concentration. The peak areas obtained for vitamin E over the set concentration range are given in Table 4.6. The slope was determined to be 23.931 and the R^2 value was 0.9997. Therefore the instrument response was also linear over the concentration range between 15.6 µg/ml and 312.4 µg/ml for vitamin E.

Table 4.6: Peak areas obtained over the vitamin concentration range to determine linearity

Concentration (µg/ml)	Peak area 1	Peak area 2	Mean peak area
15.62	370.70	398.70	384.70
31.24	790.00	782.50	786.25
46.86	1140.10	1129.50	1134.80
62.48	1515.80	1541.10	1528.45
78.10	1937.70	1895.10	1916.40
156.20	3787.80	3757.90	3772.85
234.30	5647.10	5630.40	5638.75
312.40	7399.40	7420.80	7410.10

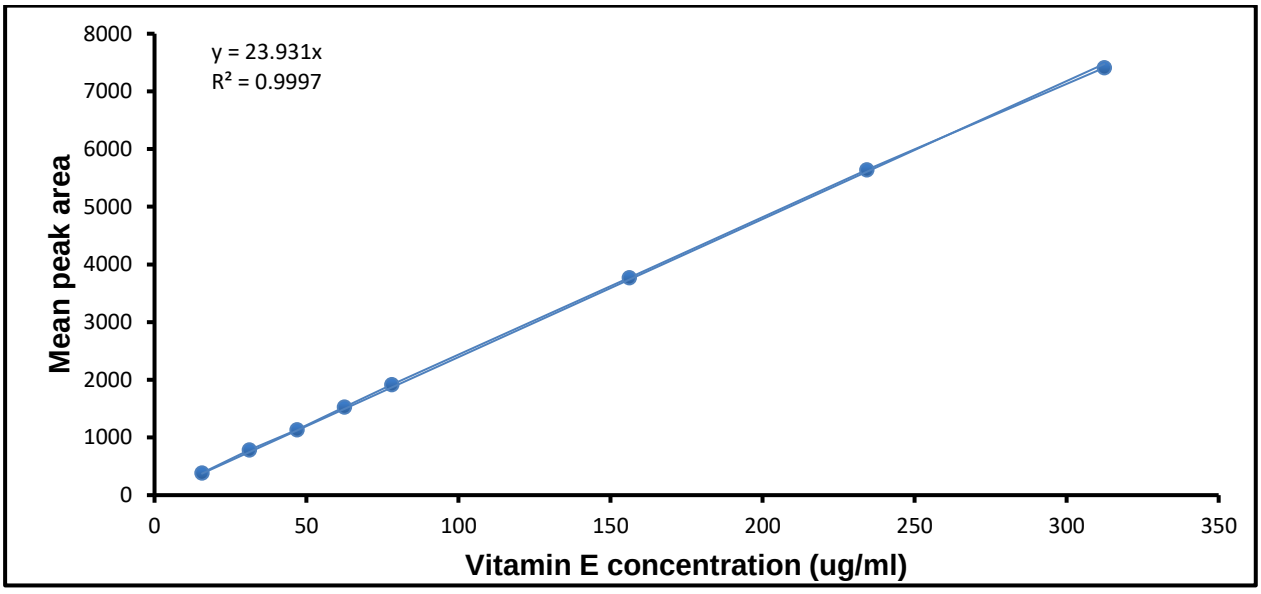

Figure 4.5. Linear regression curve for vitamin E

4.3.3. Accuracy

4.3.3.1. Vitamin C accuracy

Illustrated in Table 4.7 are the recovery values (concentration and percentage) obtained from vitamin C solutions with different concentrations. Table 4.8 illustrates the statistical analysis results of the recovery data of vitamin C. The mean % recovery (105.03%) for vitamin C was acceptable and the method was considered accurate.

Table 4.7: Recovery data for vitamin C accuracy determination

Concentration (µg/ml)	Peak area 1	Peak area 2	Mean peak area	Recovery concentration (µg/ml)	Recovery (%)
30.65	911.60	908.30	909.95	35.01	114.21
28.03	698.30	681.00	689.65	26.53	94.65
24.71	674.20	667.50	670.85	25.81	104.46
61.30	1788.30	1779.00	1783.65	68.62	111.94
56.06	1588.70	1573.10	1580.90	60.82	108.49
49.41	1133.30	1114.00	1123.65	43.23	87.49
122.60	3520.10	3507.80	3513.95	135.18	110.26
112.12	3397.20	3381.60	3389.40	130.39	116.29
98.20	2495.30	2481.00	2488.15	95.72	97.47

Table 4.8: Statistical analysis results of vitamin C recovery data

Mean recovery	105.03%
Standard deviation	9.26
%RSD	8.81%
Estimated median	97.93

4.3.3.2. Vitamin E accuracy

Table 4.9 illustrates the recovery values (concentration and percentage) obtained from three vitamin E solutions. Table 4.10 illustrates the statistical analysis results for the recovery data of vitamin E. The mean % recovery for vitamin E (99.24%) was acceptable and the method was considered accurate.

Table 4.9: Recovery data for vitamin E accuracy determination

Concentration (µg/ml)	Peak area 1	Peak area 2	Mean peak area	Recovery concentration (µg/ml)	Recovery percentage
25.59	569.50	560.00	564.75	26.80	104.75
26.30	580.60	569.50	575.05	27.21	103.47
27.58	579.10	561.00	570.05	27.01	97.96
51.18	1169.30	1154.90	1162.10	50.46	98.61
52.60	1219.60	1231.00	1225.30	52.97	100.70
55.15	1157.40	1168.60	1163.00	50.50	91.57
204.70	4910.60	4900.70	4905.65	198.73	97.09
210.40	5296.80	5303.70	5300.25	214.36	101.88
220.60	5291.60	5301.10	5296.35	214.21	97.10

Table 4.10: Statistical analysis results of vitamin E recovery data

Mean recovery	99.24%
Standard deviation	3.76
%RSD	3.79%
Estimated median	97.93

4.3.4. Limit of detection (LOD) and limit of quantification (LOQ)

4.3.4.1. LOD and LOQ for vitamin C

Table 4.11 illustrates the statistical data obtained during the limit of detection (LOD) and limit of quantification (LOQ) analysis for vitamin C. The LOD for the HPLC analysis of vitamin C was determined to be 0.026 µg/ml, while the LOQ was determined to be 0.079 µg/ml.

Table 4.11: Statistical data and peak areas for vitamin C obtained during LOD and LOQ determination

Inj. Vol (µl)	**1**	**2**	**4**
Concentration (µg/ml)	0.1082	0.2164	0.4328
Area	4.01	8.29	15.42
	4.68	8.4	16.37
	4.72	8.34	15.93
	4.27	8.68	16.24
	4.53	8.39	15.87
	4.09	8.03	15.97
Mean	4.38	8.36	15.97
SD	0.28	0.19	0.30
% RSD	6.32	2.29	1.89

4.3.4.2. LOD and LOQ for vitamin E

Table 4.12 illustrates the statistical data obtained during the limit of detection (LOD) and limit of quantification (LOQ) analysis for vitamin E. The LOD for vitamin E was determined to be 0.543 µg/ml, while the LOQ was determined to be 1.646 µg/ml.

Table 4.12: Statistical data and peak areas for vitamin E obtained during LOD and LOQ determination

Inj. Vol (µl)	**6**	**8**	**10**
Concentration (µg/ml)	1.593	2.124	2.655
Area	29.3	30.9	30.7
	29.3	30.3	33.5
	28.3	30.5	32.2
	28.1	30.2	32.6
	29.4	30.7	30.8
	28.9	30.6	33.3
Mean	28.89	30.53	32.18
SD	0.51	0.24	1.10
% RSD	1.76	0.77	3.41

4.3.5. Precision

4.3.5.1. Vitamin C precision

Intra-day precision (repeatability)

The peak areas and recovery data obtained during intra-day precision determination of vitamin C are given in Table 4.13. The statistical data are given in Table 4.14. The %RSD was 8.87%, which indicated an acceptable intra-day precision.

Table 4.13: Intra-day precision results for vitamin C

Concentration (µg/ml)	Peak area 1	Peak area 2	Mean peak area	Recovery concentration (µg/ml)	Recovery %
30.65	911.60	908.30	909.95	35.01	114.21
28.03	698.30	681.00	689.65	26.53	94.65
24.71	674.20	667.50	670.85	25.81	104.46
61.30	1788.30	1779.00	1783.65	68.62	111.94
56.06	1588.70	1573.10	1580.90	60.82	108.49
49.41	1133.30	1114.00	1123.65	43.23	87.49
122.60	3520.10	3507.80	3513.95	135.18	110.26
112.12	3397.20	3381.60	3389.40	130.39	116.29
98.20	2495.30	2481.00	2488.15	95.72	97.47

Table 4.14: Statistical results obtained for the vitamin C intra-day precision data

Mean	105.97
SD	9.40
% RSD	8.87

Inter-day precision

The % recovery, standard deviation and %RSD for vitamin C over a three day period are given in Table 4.15. The average % recovery for the three day period was determined to be 106.77% and %RSD was 7.54%. Inter-day precision for vitamin C was within acceptable limits for %RSD of less than 10%.

Table 4.15: Inter-day precision results for Vitamin C

	Day 1 Peak areas	Day 2 Peak areas	Day 3 Peak areas	Average
	111.9	118.6	108.1	
	108.5	111.6	104.6	
	87.5	106.9	103.3	
Mean % recovery	102.64	112.36	105.32	106.77
SD	10.80	4.80	2.01	8.05
% RSD	10.53	4.28	1.91	7.54

4.3.5.2. Vitamin E precision

Intra-day precision (repeatability)

The peak areas and recovery data of vitamin E for intra-day precision are given in Table 4.16. The statistical data are given in Table 4.17. The %RSD was 3.93%, which is acceptable for intra-day precision.

Table 4.16: Intra-day precision results for vitamin E

Concentration (µg/ml)	Peak area 1	Peak area 2	Mean peak area	Recovery concentration (µg/ml)	Recovery percentage
25.59	569.50	560.00	564.75	26.80	104.75
26.30	580.60	569.50	575.05	27.21	103.47
27.58	579.10	561.00	570.05	27.01	97.96
51.18	1169.30	1154.90	1162.10	50.46	98.61
52.60	1219.60	1231.00	1225.30	52.97	100.70
55.15	1157.40	1168.60	1163.00	50.50	91.57
204.70	4910.60	4900.70	4905.65	198.73	97.09
210.40	5296.80	5303.70	5300.25	214.36	101.88
220.60	5291.60	5301.10	5296.35	214.21	97.10

Table 4.17: Statistical data results for vitamin E intra-day precision data

Mean	99.50
SD	3.91
% RSD	3.93

Inter-day precision

The % recovery, standard deviation and %RSD for vitamin E over a three day period are given in Table 4.18. The % recovery was determined to be 98.66%, while the %RSD was 3.53%.

Inter-day precision for vitamin E was within acceptable limits since the %RSD was less than 10%.

Table 4.18: Inter-day precision results for Vitamin E

	Day 1 Peak areas	Day 2 Peak areas	Day 3 Peak areas	Average
	98.61	98.02	102.50	
	100.70	97.54	102.41	
	91.57	94.85	101.78	
Mean % recovery	96.96	96.80	102.23	98.66
SD	3.91	1.40	0.32	3.48
% RSD	4.03	1.44	0.31	3.53

4.3.6. Ruggedness

4.3.6.1. Vitamin C ruggedness

Stability in solution

The vitamin C needed to be stable in solution for a 6 hour period because the HPLC analysis done on vitamin C samples in all experiments didn't exceed this amount of time. The peak area and vitamin C stability in solution over a 6 hour time period are given in Table 4.19. After 6 hours, 90.07% vitamin C remained in the solution when it was stabilised with sodium metabisulphite (SMBS).

Table 4.19: Stability results for vitamin C in solution when stabilised with SMBS over a 6 h period

Time (hours)	Peak Area	Sample stability (%)
0	4003.70	100.00
1	3950.00	98.66
2	3894.20	97.27
3	3835.00	95.79
4	3766.50	94.08
5	3681.80	91.96
6	3606.20	90.07
Mean peak area	3819.63	95.40
Standard deviation	133.12	3.32

Table 4.20 shows the data obtained when vitamin C was not stabilised with SMBS in solution. At the 6 h interval, only 1.28% vitamin C remained intact in solution. Therefore it is of great importance to stabilise vitamin C by addition of a preservative (anti-oxidant) when in solution.

Table 4.20: Stability results for vitamin C without stabilising with SMBS over a 6 h period

Time (hours)	Peak area	Sample stability (%)
0	4013.20	100.00
1	3278.80	81.71
2	2243.40	55.90
3	1129.30	28.14
4	367.60	9.16
5	130.40	3.25
6	51.40	1.28
Mean peak area	1602.01	39.92
Standard deviation	1481.03	36.91

System repeatability

Table 4.21 shows results when a vitamin C sample was consecutively injected into the chromatograph. The %RSD for the peak areas was determined at 0.370% and the %RSD for the retention times was found to be 0.324%. The results obtained were acceptable and the method proved to be repeatable.

Table 4.21: System repeatability results for Vitamin C analysis

Sample	Peak area	Retention time (min)
1	3437.30	3.70
2	3428.60	3.67
3	3423.00	3.66
4	3409.30	3.67
5	3410.50	3.67
6	3400.60	3.67
Mean	3381.6	3.665
Standard deviation	12.55	0.012
RSD %	0.370	0.324

4.3.6.2. Vitamin E

Stability in solution

Vitamin E proved to be very stable in solution over a period of 24 hours, while only stability over 6 h is required for the analytical method. The peak area and stability for vitamin E are given in Table 4.22. The vitamin E was highly stable with 99.41% still intact at the 6 h interval.

Table 4.22: Stability results for vitamin E over a 6 h period

Time (hours)	Peak Area	Sample stability (%)
0	2318.10	100.00
1	2304.40	99.41
2	2296.70	99.08
3	2298.10	99.14
4	2313.80	99.81
5	2306.90	99.52
6	2304.50	99.41
Mean	2303.232	99.359
Standard deviation	9.635	0.416

System repeatability

Table 4.23 shows results when a vitamin E sample was consecutively injected into the chromatograph. The %RSD for the peak areas was determined at 0.556% and the %RSD for the retention times was found to be 0.313%. The results obtained were acceptable and the method proved to be repeatable.

Table 4.23: System repeatability results for Vitamin E analysis

Sample	Peak area	Retention time (min)
1	5520.80	6.42
2	5534.50	6.46
3	5481.90	6.42
4	5558.80	6.45
5	5479.50	6.40
6	5481.30	6.40
Mean peak area	5509.467	6.425
Standard deviation	30.660	0.020
RSD %	0.556	0.313

4.3.7. Conclusion

The validation requirements were met and the HPLC analytical methods for both vitamin E and vitamin C were found to be sensitive, repeatable, selective and accurate.

4.4. Drug release studies

4.4.1. Drug release for vitamin C

Figure 4.6 illustrates the % dissolution plotted as a function of time for the different vitamin C MUPS formulations. All the vitamin C MUPS formulations showed immediate drug release. Almost all the formulations reached maximum dissolution at 30 min, except for formulations C2 and C4 where the % dissolution already peaked at 15 min. The gradual decrease in dissolution percentage was as a result of the degradation of vitamin C in solution. With the dissolution temperature set at 37°C this may result in a faster degradation of the vitamin C compared to previous degradation results seen with the stability tests at room temperature in section 4.3.6.1. The control formulation (C7), containing no functional excipients, exhibited the same type of drug release rate. With the % dissolution also peaking at 30 min. Therefore it may be concluded that the functional excipients AVG and SLS had no expressive impact on the vitamin C release rate.

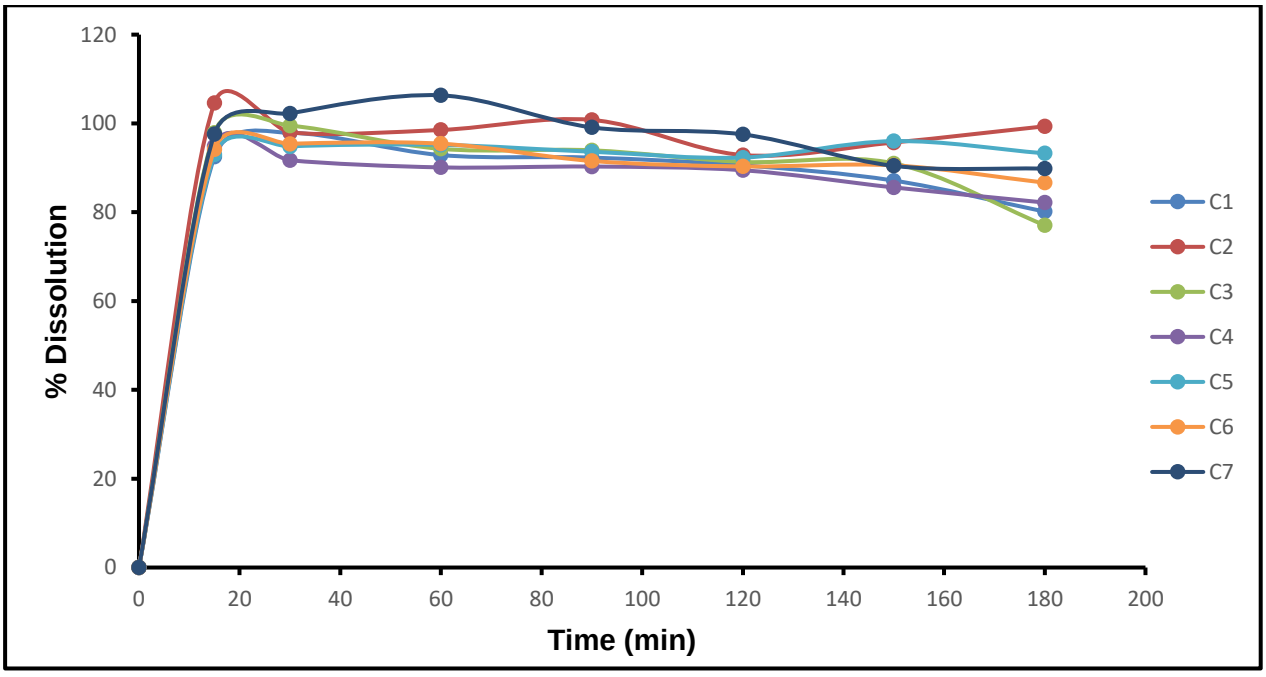

Figure 4.6. Percentage dissolution of the vitamin C MUPS capsule formulations plotted as a function of time

4.4.2. Drug release for vitamin E

PPBS was initially used as the dissolution medium for the dissolution testing of vitamin E MUPS capsule formulations, but the results showed very low vitamin E release (below 6%). Figure 4.7 illustrates the % dissolution of the vitamin E MUPS capsule formulations plotted as a function of time with PPBS as dissolution medium. The extremely low vitamin E release can be explained by its poor solubility in an aqueous environment.

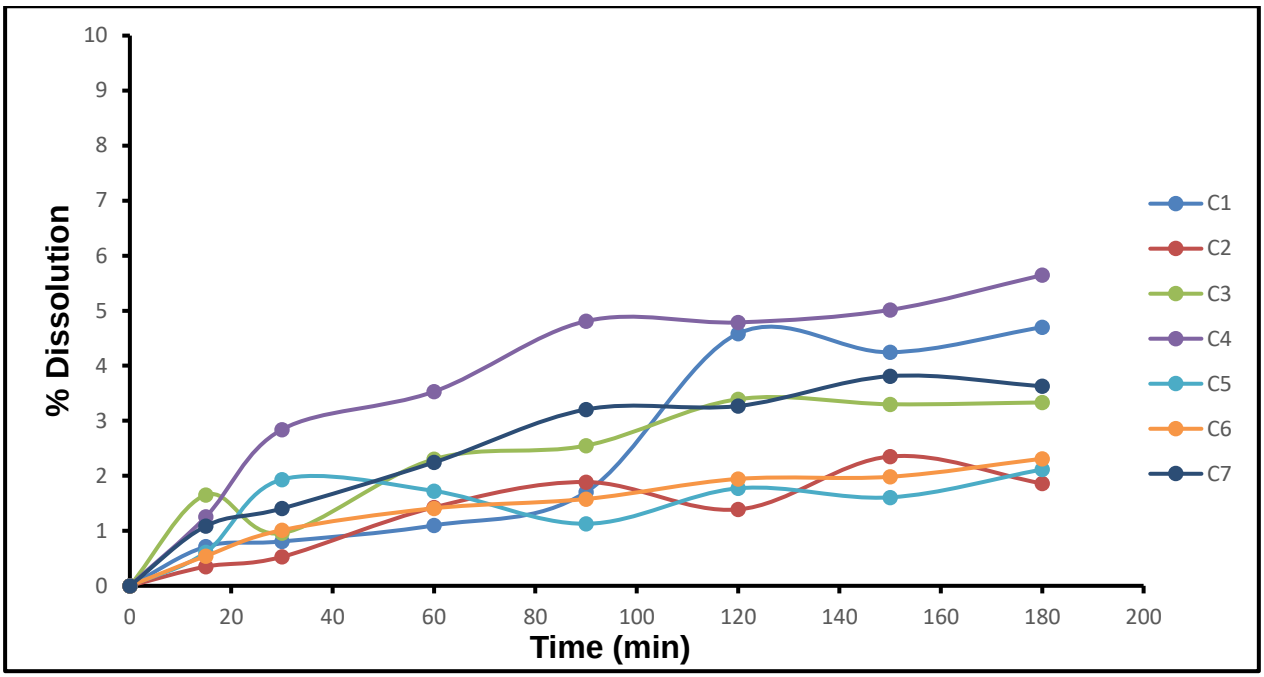

Figure 4.7. Percentage dissolution of the vitamin E MUPS capsule formulations plotted as a function of time with PPBS as dissolution medium

Fed state simulated intestinal fluid (FeSSIF) was used as an alternative dissolution medium in order to find a medium in which the *ex vivo* transport studies could be done. Figure 4.8 illustrates the % dissolution of the vitamin E MUPS capsule formulations plotted as a function of time with FeSSIF as the dissolution medium. In this dissolution medium, the vitamin E MUPS capsule formulations achieved a higher % dissolution at the respective time intervals of 60, 120 and 180 minutes. Table 4.24 compares the % dissolution of vitamin E MUPS formulations in both dissolution mediums at the 180 min time interval. From the table it is clear that the vitamin E formulations rendered better drug release in FeSSIF compared to PPBS. Therefore it was decided that FeSSIF will be used as transport medium during the transport studies to evaluate the delivery performance of the vitamin E MUPS capsule formulations.

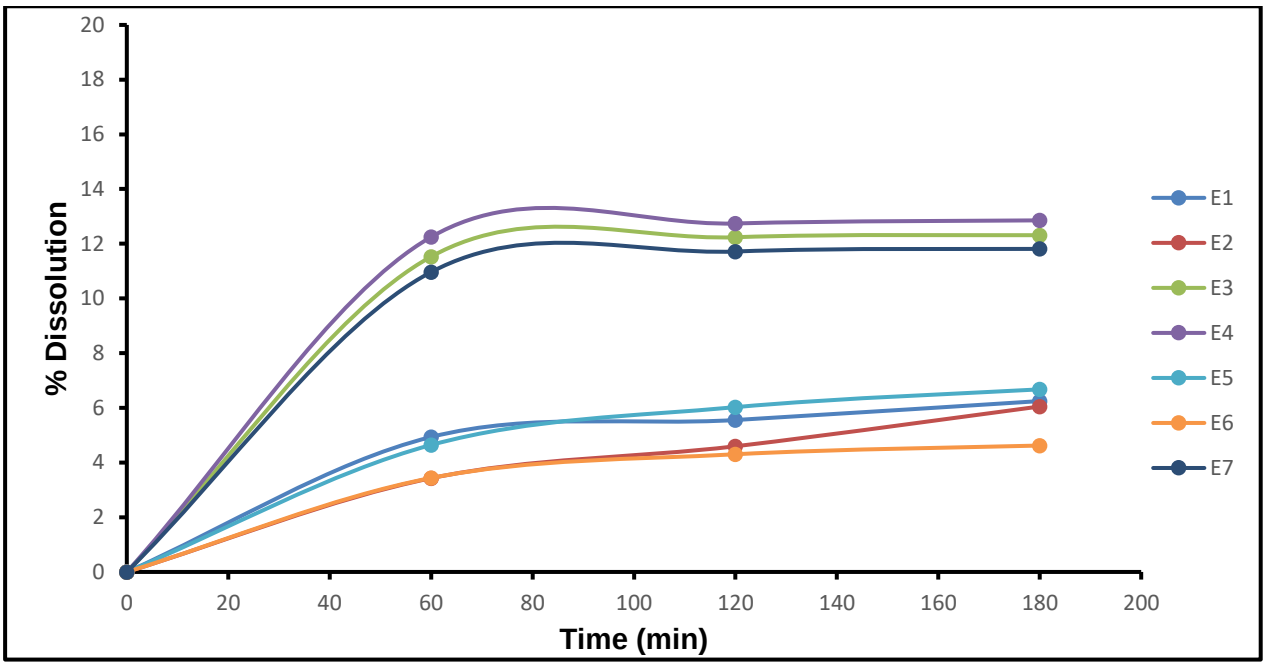

Figure 4.8. Percentage dissolution of the vitamin E MUPS capsule formulations plotted as a function of time with FeSSIF as dissolution medium

Table 4.24: Dissolution % of the vitamin E MUPS capsule formulations for PPBS compared to FeSSIF at the 180 min time interval

Formulation	% Dissolution PPBS	% Dissolution FeSSIF
E1	4.70	6.25
E2	1.86	6.05
E3	3.33	12.31
E4	5.65	12.85
E5	2.11	6.68
E6	2.31	4.62
E7	3.63	11.81

4.5. *Ex vivo* transport studies

4.5.1. Transport studies for vitamin C

Figure 4.9 illustrates the percentage *ex vivo* transport for the vitamin C from the MUPS capsule formulations plotted as a function of time. From Figure 4.9, it is evident that the vitamin C MUPS capsule formulations containing AVG and/or SLS as functional excipients improved the % cumulative transport compared to the control vitamin C MUPS capsule formulation (C7). As expected, the formulations (C5 and C6) that consisted of a combination of AVG and SLS, exhibited the best cumulative transport over the 120 min time period. With AVG as functional excipient, formulation C2 (10% AVG) resulted in higher transport compared to C1 (5% AVG). For the formulations containing SLS as excipient, formulation C4 (0.5% SLS) resulted in higher transport than C3 (0.1% SLS). Formulation C6 (10% AVG and 0.5% SLS) exhibited the best %

cumulative transport over the 120 min period and therefore performed the best in terms of vitamin C delivery across excised intestinal tissue. The combination of the two functional excipients in the vitamin C MUPS capsule formulation therefore resulted in an increased vitamin C transport enhancement effect when compared to the two components each on their own.

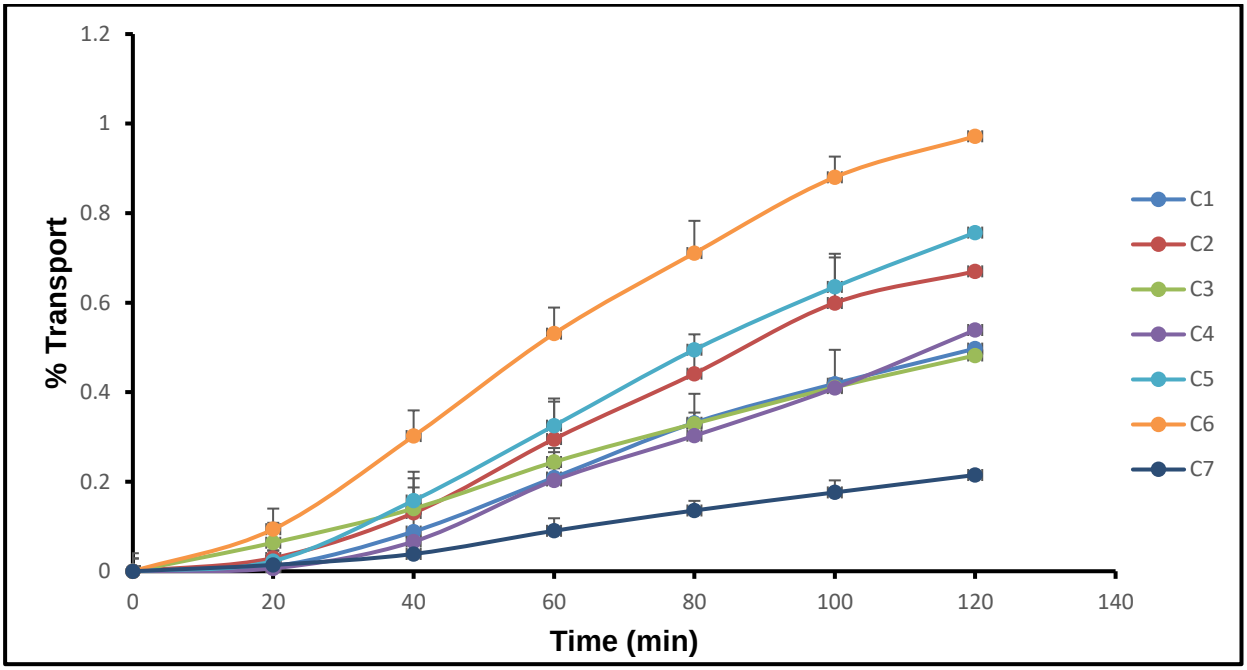

Figure 4.9. Percentage *ex vivo* transport of the vitamin C MUPS capsule formulations plotted as a function of time

The apparent permeability coefficient (P_{app}) values for each of the vitamin C MUPS capsule formulations are shown in Figure 4.10. From Figure 4.10, it is evident that that the highest amount of vitamin C transport occurred from formulation C6 (10% AVG and 0.5% SLS). Furthermore, formulations with AVG as functional excipient (C1 and C2) exhibited greater transport compared to the use of SLS as functional excipient (C3 and C4). To summarize, it is clear that the vitamin C formulations containing AVG and/or SLS as functional excipients (C1 – C6) exhibited greater transport compared to the control formulation containing only vitamin C (C7). Formulations C2, C5 and C6 showed statistically significant differences compared to the control, $p \leq 0.05$, according to statistical analysis done by means of a Kruskal-Wallis test.

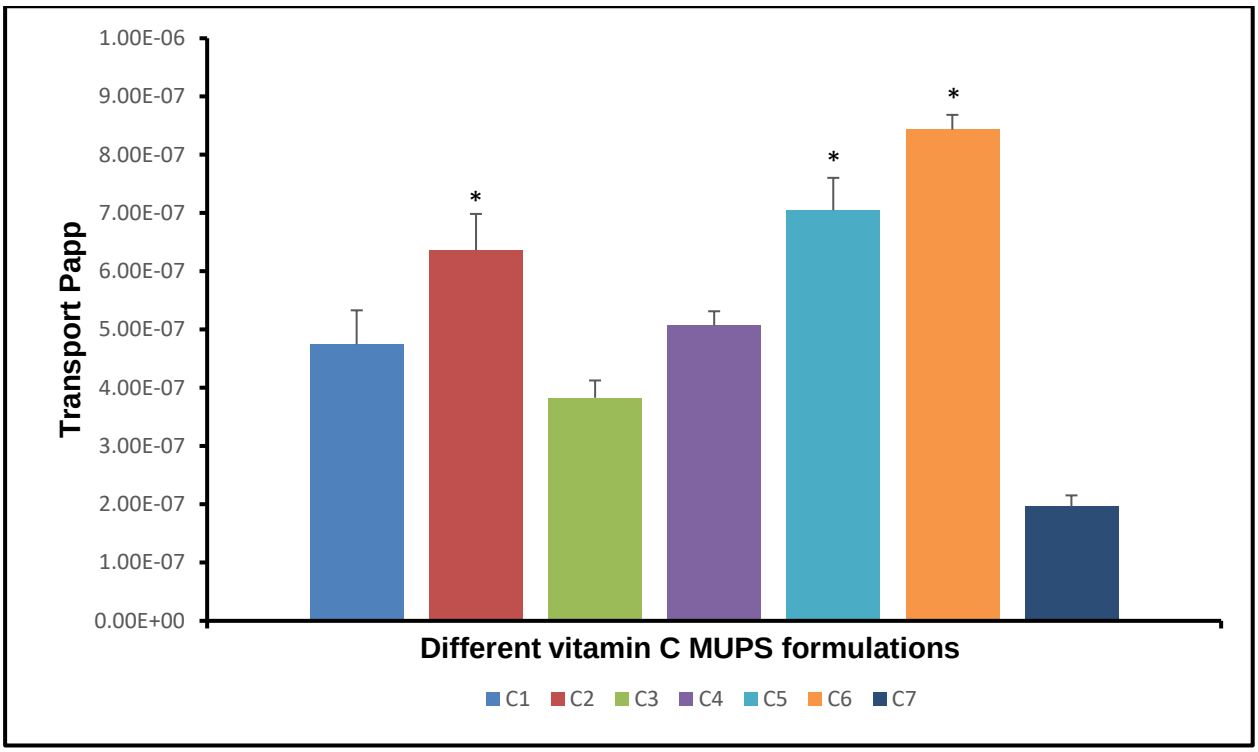

Figure 4.10. Average P_{app} values for *ex vivo* transport of vitamin C from MUPS capsule formulations (*statistically significant differences compared to the control, $p \leq 0.05$)

4.5.2. Vitamin E retained in the excised pig jejunum tissue

No transport was observed for vitamin E across the excised intestinal pig tissue during the *ex vivo* transport studies, therefore retention studies were done to determine the quantity of vitamin E that retained inside the excised intestinal tissue. Figure 4.11 illustrates the percentage retention of vitamin E in the excised pig intestinal tissues at the end of a 120 min transport period. From Figure 4.11, it is clearly evident that the vitamin E MUPS capsule formulations that contained a combination of AVG and SLS as functional excipients (E5 and E6) had the highest ability to deliver vitamin E into the intestinal tissue compared to the control vitamin E MUPS capsule formulation (E7). Formulations containing only AVG as functional excipient (E1 and E2) exhibited greater vitamin E retention inside the excised tissues than formulations containing only SLS as functional excipient (E3 and E4). According to statistical analysis done by means of a Kruskal-Wallis test, the data showed not to be statistically significant.

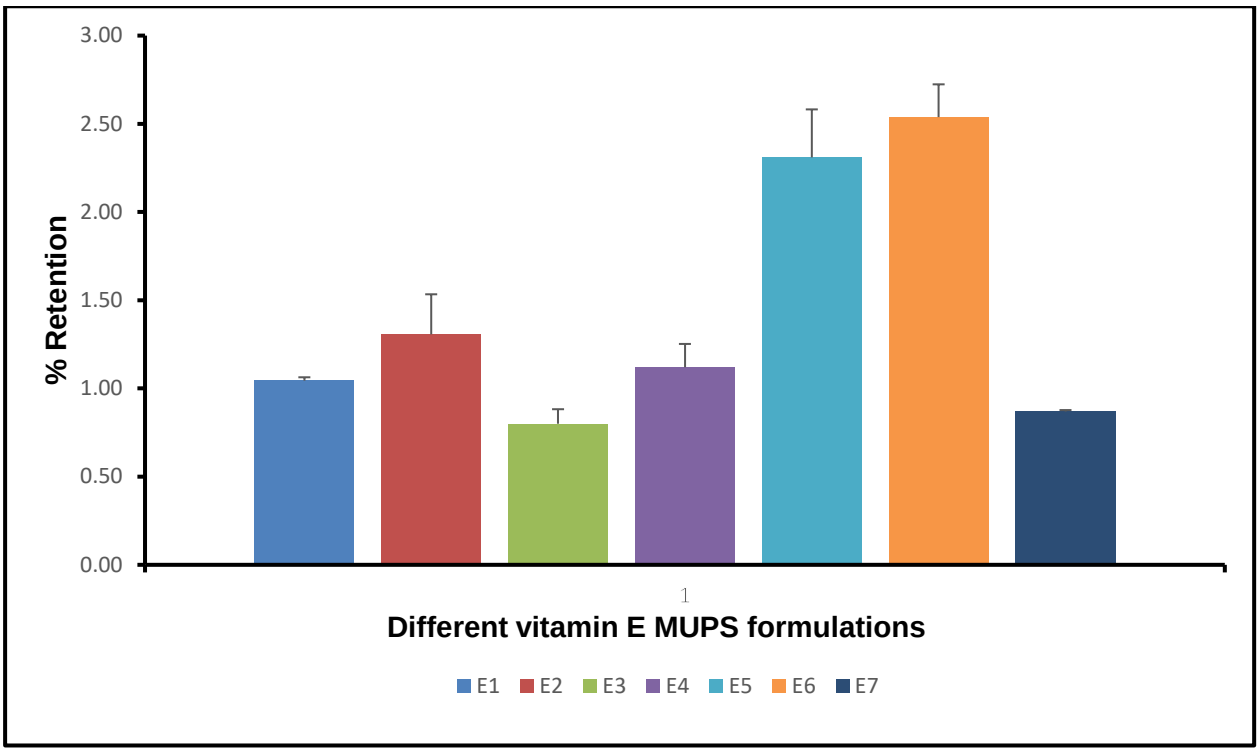

Figure 4.11. Percentatage vitamin E retained in the excised intestinal tissue at 120 min

4.6. Muco-adhesion studies

4.6.1. Muco-adhesion of vitamin C bead formulations

Data obtained from muco-adhesion studies done on the vitamin C bead formulations are given in Table 4.25. The percentage muco-adhesion of the different vitamin C bead formulations are graphically illustrated in Figure 4.12. The control formulation (C7) exhibited an average % muco-adhesion of 50%. From Figure 4.12, it is evident that the formulations containing AVG as a functional excipient (C1, C2, C5 and C6) are more muco-adhesive compared to the control and compared to formulations containing only SLS as excipient (C3 and C4). Formulation C5 (5% AVG and 0.1% SLS) proved to be the most muco-adhesive, at 70%.

Table 4.25: Data obtained from muco-adhesion studies for vitamin C formulations

Formulation		Total beads			Average total beads	Standard deviation	% Muco-adhesion
C1	**beads on**	67	65	59	63	3.40	63
	beads off	33	38	41	37	3.30	
C2	**beads on**	61	68	63	64	2.94	64
	beads off	39	32	37	36	2.94	
C3	**beads on**	58	45	32	45	10.61	45
	beads off	42	55	68	55	10.61	
C4	**beads on**	63	53	50	55	5.56	55
	beads off	37	47	50	45	5.56	
C5	**beads on**	73	76	60	70	6.94	70
	beads off	27	24	40	30	6.94	
C6	**beads on**	58	60	81	66	10.40	66
	beads off	42	40	19	34	10.40	
C7	**beads on**	69	46	36	50	13.82	50
	beads off	31	54	64	50	13.82	

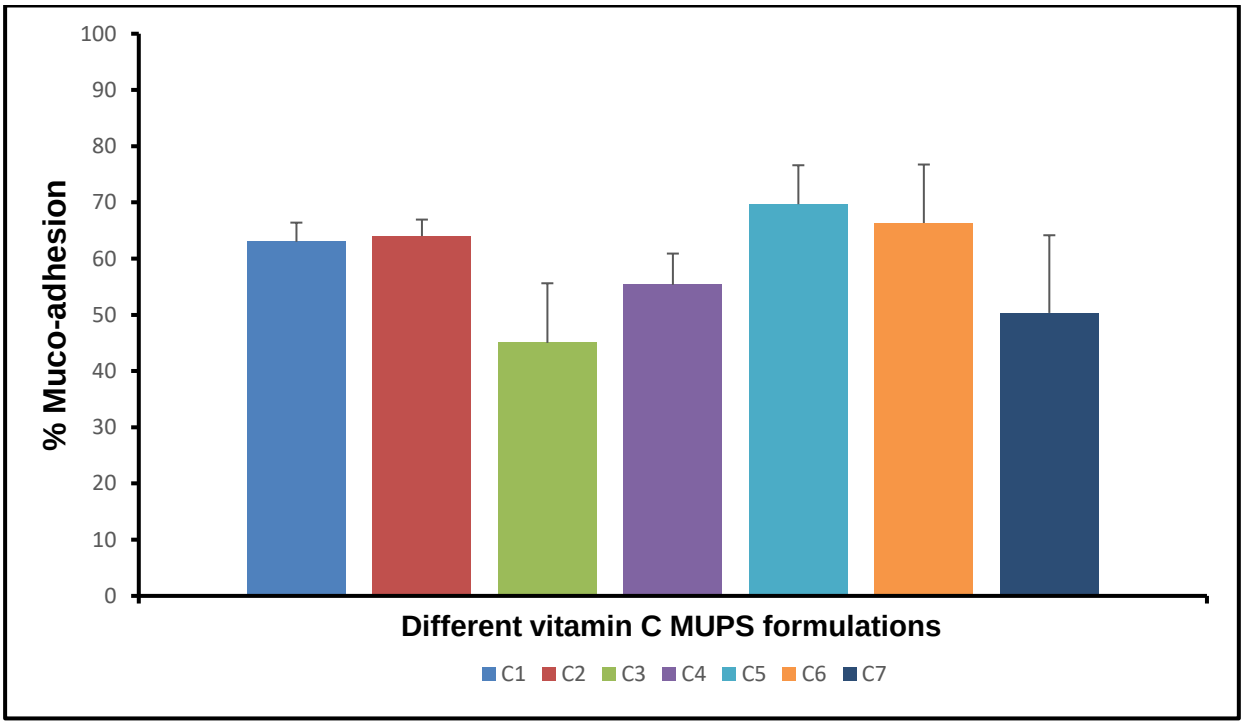

Figure 4.12. Percentage muco-adhesion for vitamin C bead formulations

4.6.2. Muco-adhesion of vitamin E bead formulations

Data obtained from muco-adhesion studies done on the vitamin E bead formulations are given in Table 4.26. The percentage muco-adhesion of the different vitamin E bead formulations are graphically illustrated in Figure 4.13. From Figure 4.13 the control vitamin E bead formulation (E7) is shown to be 45% muco-adhesive. The formulations containing AVG as a functional excipient (E1, E2, E5 and E6) proved to be more muco-adhesive compared to the formulations containing only SLS (C3 and C4) and compared to the control. The formulation with the highest % muco-adhesion proved to be E5 (5% AVG and 0.1% SLS). The concentrations of functional excipients (5% AVG and 0.1% SLS) used in formulations C5 and E5 proved to improve the % muco-adhesion the most for both vitamin C and vitamin E formulations.

Table 4.26: Data obtained from muco-adhesion studies for vitamin E bead formulations

Vitamin E		Total beads			Average total beads	Standard deviation	% Muco-adhesion
E1	**beads on**	44	71	73	63	13.22	63
	beads off	56	29	27	37	13.22	
E2	**beads on**	67	61	65	64	2.49	64
	beads off	33	39	35	36	2.49	
E3	**beads on**	63	60	55	59	3.30	59
	beads off	37	40	45	41	3.30	
E4	**beads on**	66	37	46	50	12.12	50
	beads off	34	63	54	50	12.12	
E5	**beads on**	73	76	60	70	6.94	70
	beads off	27	24	40	30	6.94	
E6	**beads on**	58	60	81	66	10.40	66
	beads off	42	40	19	34	10.40	
E7	**beads on**	52	50	33	45	8.52	45
	beads off	48	50	67	55	8.52	

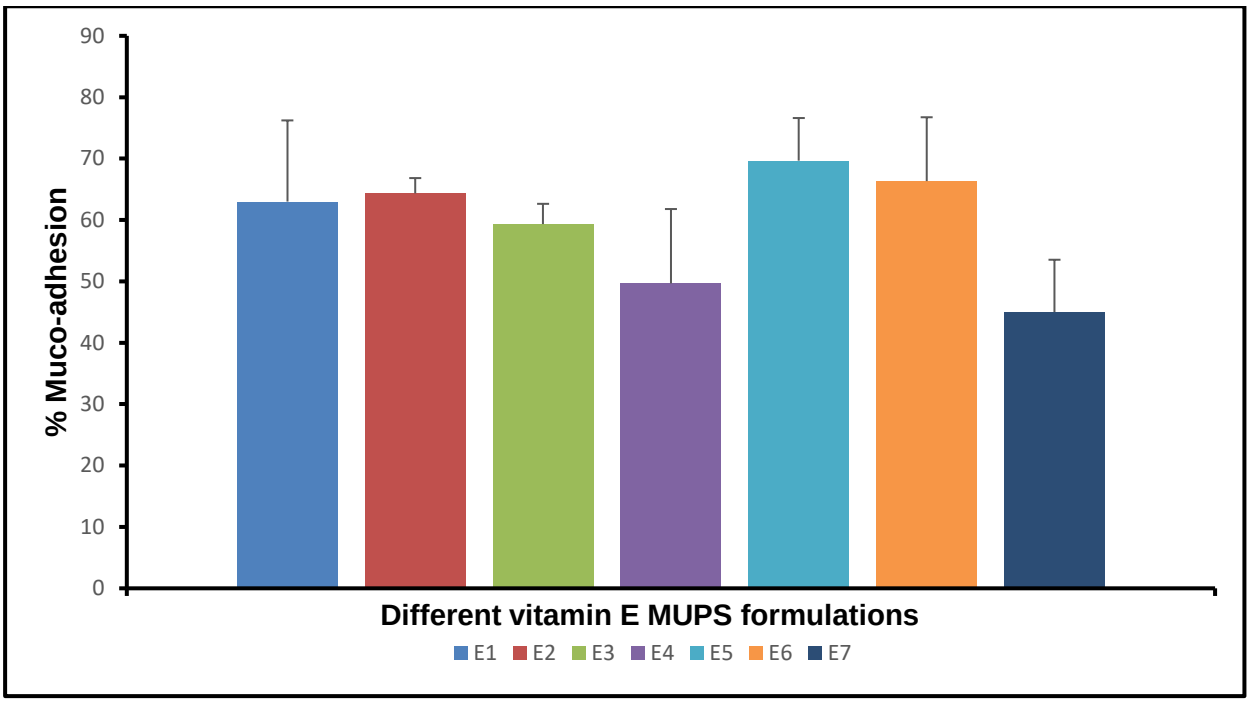

Figure 4.13. Percentage muco-adhesion for vitamin E bead formulations

4.7. Conclusion

The analytical method, used to determine the concentrations of vitamin C and E in the dissolution and transport samples, complied with all the validation specifications of specificity, linearity, accuracy, limit of detection, limit of quantification, precision and robustness. Formulated beads were characterised in terms of uniformity of mass, friability, assay and particle size. The beads complied with specifications for acceptable bead formulations as set by the British Pharmacopoeia. Dissolution studies done on the vitamin C MUPS capsule formulations showed immediate and complete drug release profiles. For the dissolution studies of vitamin E MUPS capsule formulations, FeSSIF was used as an alternative to PPBS. FeSSIF as a dissolution medium achieved a higher % dissolution for the vitamin E MUPS capsule formulations. Therefore it was decided that FeSSIF will be used as transport medium during the transport studies to evaluate the delivery performance of the vitamin E MUPS capsule formulations. The vitamin C MUPS capsule formulations, with AVG and SLS as functional excipients, showed an improvement in transport across the intestinal epithelium compared to the control vitamin C MUPS formulation containing only vitamin C. With formulation C6 (10% AVG and 0.5% SLS) exhibiting the best % cumulative transport over the 120 min period. Formulations C2, C5 and C6 proved statistically significant differences compared to the control (C7), $p \leq 0.05$.No transport was observed for vitamin E across the excised intestinal pig tissue during the *ex vivo* transport studies. Retention studies were therefore done to determine the quantity of vitamin E retained in the excised intestinal tissue. AVG and SLS as functional excipients proved to enhance the vitamin E retention in the excised intestinal tissue. Vitamin E MUPS capsule formulation E6 (10% AVG and 0.5% SLS) had the highest ability to deliver

vitamin E into the intestinal tissue compared to the control vitamin E MUPS capsule formulation (E7). Although, this was not proven to be statistically significant. The % muco-adhesion showed to be the highest for formulations containing AVG as a functional excipient compared to the control vitamin bead formulations for both vitamin C and vitamin E bead formulations.

CHAPTER 5: FINAL CONCLUSIONS AND FUTURE RECOMMENDATIONS

5.1. Final conclusions

The main aim of this study was to develop MUPS capsule formulations to effectively deliver vitamin C and E across the gastrointestinal tract, therefore improving the solubility and permeation of vitamin C and E. The evaluation of the solubility and absorption enhancing abilities of *Aloe vera* gel (AVG) and sodium lauryl sulphate (SLS) when included as functional excipients in MUPS capsule formulations of vitamin C and E was done across excised pig intestinal-tissue.

Different vitamin MUPS capsule formulations were manufactured by filling hard gelatine capsule with beads formulated by means of extrusion spheronisation. The beads were successfully evaluated in terms of uniformity of mass, friability, assay and size distribution. The beads complied with the standards of all the physical tests and exhibited narrow particle size distributions, which was expected for beads produced with extrusion spheronisation. The analytical method, used to determine the concentrations of vitamin C and E in the dissolution and transport samples complied with all the validation specifications of specificity, linearity, accuracy, limit of detection, limit of quantification, precision and robustness.

The vitamin C MUPS capsule formulations showed excellent and complete drug release profiles, whereas the vitamin E MUPS capsule formulations only released about 12% of the vitamin within the 180 min period. The extremely low vitamin E release can be explained by its poor solubility in an aqueous environment, even when FeSSIF was used as the dissolution medium.

Ex vivo transport studies were completed for both vitamin C and E MUPS capsule formulations. This drug delivery model proved to be a successful model to determine the delivery of the MUPS formulations containing vitamin C. For vitamin E the formulation design was inadequate because of the poor aqueous solubility of vitamin E. From the *ex vivo* transport studies it is clear that AVG and SLS had an increased effect on the transport of vitamin C across the excised intestinal tissues compared to the control formulation C7 (vitamin C alone). However, vitamin C formulations containing both AVG and SLS as functional excipients exhibited the highest % cumulative transport over the 120 min period as well as the highest apparent permeability coefficient (P_{app}) values, with formulations C2, C5 and C6 showing statistically significant differences compared to the control, $p \leq 0.05$. This clearly indicates that the combination of both AVG and SLS as functional excipients offers the highest amount of vitamin C transport across the excised intestinal tissue. The absorption enhancement of AVG was found to be greater than SLS at the tested concentrations.

No transport across the excised intestinal tissue was found for vitamin E. This may be a result of the poor solubility of vitamin E in an aqueous environment and the lipophilic nature of vitamin E. It was speculated that the vitamin E was retained in the gastrointestinal epithelium. Therefore, retention studies were done to determine the amount of vitamin E retained in the excised tissue. From these studies it was clear that the combination of AVG and SLS as functional excipients resulted in a greater vitamin E retention inside the excised tissue compared to the control formulation E7 (vitamin E alone), albeit not statistically significant.

Muco-adhesive properties of the formulated beads were also examined. From the results obtained it is clear that the beads containing AVG as a functional excipient exhibited higher muco-adhesive properties compared to the formulations containing no AVG. This was evident for both vitamin C and E bead formulations.

5.2. Future recommendations

This study showed promising results for the delivery of vitamin C MUPS capsule formulations. Delivery of vitamin E MUPS capsule formulations, however proved more challenging and several changes can be made in similar future studies. Further recommendations follow:

- *In vivo* testing on the vitamin C MUPS capsule formulations would further prove the delivery efficacy in terms of bioavailability and whether the MUPS capsule formulations can provide clinically effective blood plasma levels.
- Both vitamin C and vitamin E can be formulated into MUPS tablet formulations to further test the *ex vivo* delivery of both vitamins.
- Formulation of a solid emulsion consisting of lipophilic MUPS to improve the *ex vivo* delivery of vitamin E.
- Enteric coating with a muco-adhesive polymer could be applied to further increase the retention time in the gastro-intestinal tract of the formulated beads.
- Different transport models could also be investigated, including human intestinal epithelial cell culture monolayers (Caco-2) as well as tissue from different species of animals.
- Different types of transport medium can be tested to effectively deliver lipophilic compounds such as vitamin E.
- Investigating different preservatives to stabilise vitamin C in solution may prove to be significant.
- Solubility enhancers could also be added especially with the aim to improve the solubility of lipophilic compounds during the *ex vivo* transport studies.
- Different excipients (disintegrants, surfactants and bile salts) in combination with the tested excipients AVG and SLS should also be investigated.

- Micro-tablets could also be formulated containing the studied compounds and filled into capsules to investigate the effects of a different dosage form.

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ADDENDUM A

ETHICS APPROVAL

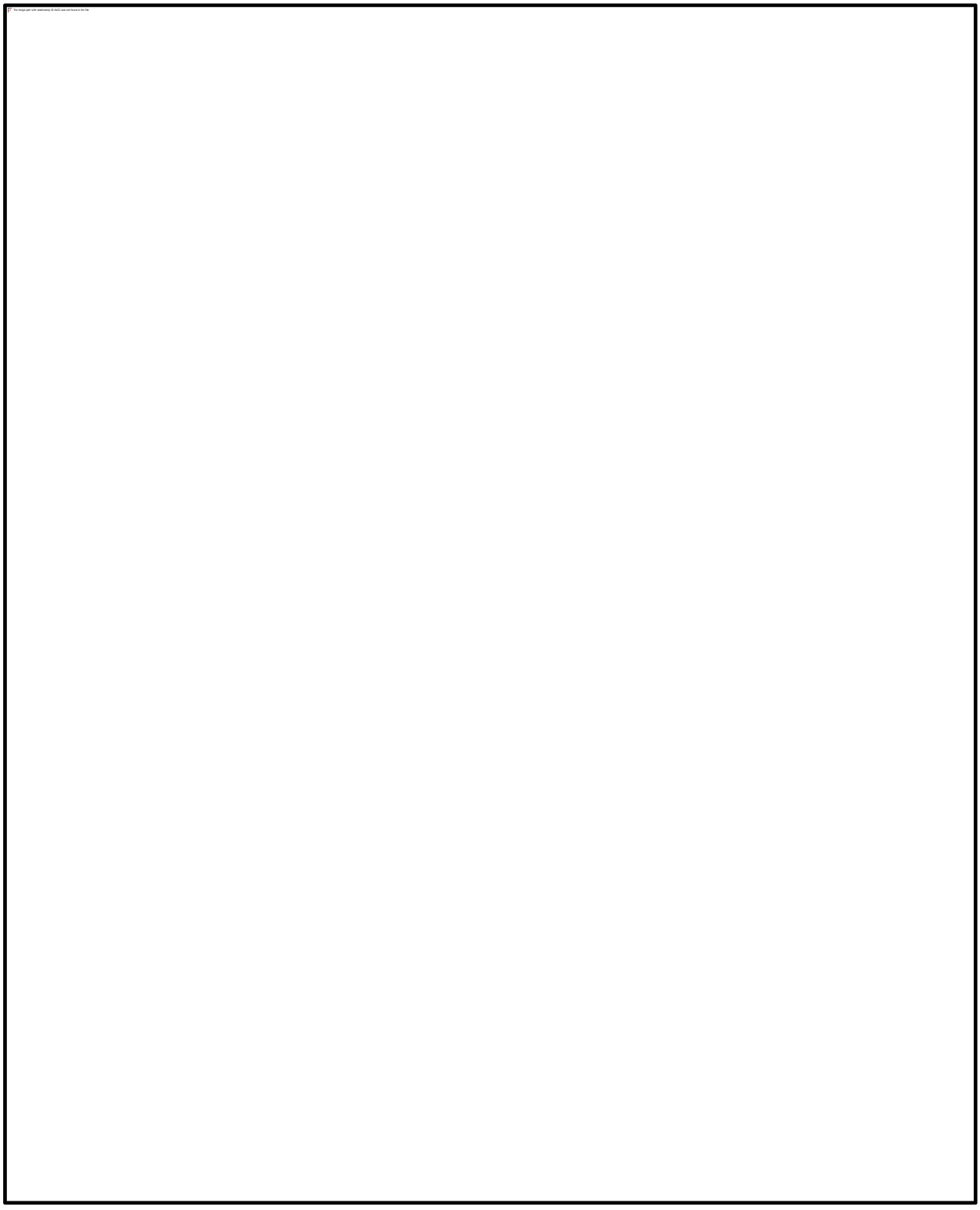

Figure A.1: Ethics approval

ADDENDUM B

DISSOLUTION DATA

Table B.1.1: Dissolution data for Vitamin C MUPS capsule formulation C1

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	97.85	86.30	93.37	92.51	4.75
30	100.94	95.26	97.44	97.88	2.34
60	90.48	94.35	93.81	92.88	1.71
90	90.48	92.62	93.83	92.31	1.39
120	89.64	92.06	90.02	90.57	1.06
150	88.19	85.93	87.27	87.13	0.93
180	78.49	83.73	78.21	80.14	2.54

Table B.1.2: Dissolution data for Vitamin C MUPS capsule formulation C2

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	97.14	110.40	106.27	104.60	5.54
30	99.91	97.57	96.60	98.02	1.39
60	99.40	100.03	96.17	98.54	1.69
90	106.05	100.34	96.00	100.80	4.12
120	94.95	93.56	90.15	92.89	2.02
150	98.47	95.57	93.10	95.71	2.19
180	99.21	97.33	106.27	99.36	1.72

Table B.1.3: Dissolution data for Vitamin C MUPS capsule formulation C3

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	100.46	95.73	97.54	97.91	1.95
30	95.24	103.22	100.00	99.48	3.28
60	96.01	95.32	91.50	94.28	1.99
90	95.76	93.84	92.25	93.95	1.44
120	92.82	89.72	91.47	91.34	1.27
150	93.17	89.53	90.33	91.01	1.56
180	77.36	76.50	77.34	77.07	0.40

Table B.1.4: Dissolution data for Vitamin C MUPS capsule formulation C4

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	90.35	98.93	95.41	94.90	3.52
30	92.36	88.99	93.79	91.71	2.01
60	89.83	91.17	89.31	90.10	0.78
90	90.83	90.20	89.86	90.30	0.40
120	94.56	87.15	86.67	89.46	3.61
150	86.74	85.61	84.42	85.59	0.95
180	83.41	83.47	79.68	82.19	1.77

Table B.1.5: Dissolution data for Vitamin C MUPS capsule formulation C5

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	92.26	93.79	92.95	93.00	0.63
30	95.73	90.73	98.06	94.84	3.06
60	95.06	93.02	97.43	95.17	1.80
90	92.67	93.67	94.55	93.63	0.77
120	92.84	91.81	92.32	92.32	0.42
150	92.65	97.70	97.73	96.03	2.39
180	91.29	92.00	96.52	93.27	2.32

Table B.1.6: Dissolution data for Vitamin C MUPS capsule formulation C6

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	97.04	93.71	91.75	94.16	2.18
30	99.50	92.38	94.39	95.42	2.99
60	98.26	95.36	92.71	95.44	2.26
90	94.17	89.25	91.24	91.55	2.02
120	91.05	90.19	89.71	90.32	0.56
150	93.64	89.48	88.55	90.56	2.21
180	85.94	85.98	88.07	86.66	0.99

Table B.1.7: Dissolution data for Vitamin C MUPS capsule formulation C7

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	98.20	98.42	96.30	97.64	1.49
30	103.65	102.95	100.19	102.26	6.10
60	114.96	101.95	102.09	106.33	2.35
90	98.16	102.36	96.83	99.12	5.05
120	98.35	90.97	103.26	97.53	1.65
150	89.61	92.76	88.98	90.45	2.98
180	93.61	89.56	86.33	89.83	0.95

Table B.2.1: Dissolution data for Vitamin E MUPS capsule formulation E1 using PPBS as dissolution medium

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	0.54	0.74	0.85	0.71	0.13
30	0.89	0.86	0.66	0.81	0.10
60	0.61	0.88	1.81	1.10	0.51
90	1.46	1.62	2.03	1.70	0.24
120	5.68	6.55	1.52	4.58	2.20
150	5.54	5.54	1.65	4.24	1.83
180	5.84	6.48	1.78	4.70	2.08

Table B.2.2: Dissolution data for Vitamin E MUPS capsule formulation E2 using PPBS as dissolution medium

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	0.34	0.56	0.15	0.35	0.17
30	0.58	0.72	0.27	0.53	0.19
60	1.29	1.54	1.45	1.43	0.10
90	2.66	1.59	1.41	1.89	0.55
120	1.11	1.47	1.58	1.39	0.20
150	2.64	2.23	2.18	2.35	0.20
180	1.57	2.00	2.02	1.86	0.21

Table B.2.3: Dissolution data for Vitamin E MUPS capsule formulation E3 using PPBS as dissolution medium

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	1.52	1.99	1.44	1.65	0.24
30	0.31	0.99	1.56	0.96	0.51
60	2.18	2.75	1.97	2.30	0.33
90	2.79	2.47	2.38	2.55	0.18
120	4.75	3.05	2.38	3.39	1.00
150	2.83	4.53	2.54	3.30	0.88
180	2.93	3.93	3.14	3.33	0.43

Table B.2.4: Dissolution data for Vitamin E MUPS capsule formulation E4 using PPBS as dissolution medium

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	0.86	1.86	1.05	1.26	0.44
30	2.69	2.93	2.90	2.84	0.11
60	3.01	3.93	3.64	3.53	0.38
90	5.18	4.60	4.65	4.81	0.26
120	4.58	5.59	4.20	4.79	0.59
150	4.07	5.24	5.73	5.02	0.70
180	7.04	5.09	4.81	5.65	0.99

Table B.2.5: Dissolution data for Vitamin E MUPS capsule formulation E5 using PPBS as dissolution medium

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	0.47	0.51	0.85	0.61	0.17
30	1.95	1.93	1.92	1.93	0.01
60	2.06	1.15	1.97	1.72	0.41
90	1.79	0.78	0.81	1.13	0.47
120	2.27	1.88	1.16	1.77	0.46
150	1.84	1.13	1.86	1.61	0.34
180	2.59	2.11	1.64	2.11	0.39

Table B.2.6: Dissolution data for Vitamin E MUPS capsule formulation E6 using PPBS as dissolution medium

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	0.69	0.37	0.57	0.54	0.13
30	0.12	1.53	1.38	1.01	0.63
60	2.96	1.02	0.24	1.41	1.14
90	2.34	2.03	0.36	1.58	0.87
120	1.75	2.59	1.48	1.94	0.47
150	1.21	2.06	2.68	1.98	0.60
180	1.36	2.78	2.79	2.31	0.67

Table B.2.7: Dissolution data for Vitamin E MUPS capsule formulation E7 using PPBS as dissolution medium

Time (min)	% dissolution vessel 1	% dissolution vessel 2	% dissolution vessel 3	Average % dissolution	Standard deviation
0	0	0	0	0	
15	1.02	0.64	1.60	1.09	0.39
30	1.60	1.64	0.97	1.40	0.30
60	2.30	2.12	2.31	2.24	0.09
90	3.43	1.94	4.25	3.20	0.96
120	4.26	2.93	2.62	3.27	0.71
150	4.60	3.63	3.20	3.81	0.58
180	3.56	3.18	4.14	3.63	0.39

Table B.3.1: Dissolution data for Vitamin E MUPS capsule formulations using FeSSIF as dissolution medium

Time (min)	**60**	**120**	**180**	
Formulation	**% dissolution**	**% dissolution**	**% dissolution**	**Standard deviation**
E1	4.93	5.55	6.25	0.54
E2	3.43	4.59	6.05	1.07
E3	11.53	12.24	12.31	0.35
E4	12.25	12.74	12.85	0.26
E5	4.64	6.02	6.68	0.85
E6	3.44	4.30	4.62	0.50
E7	10.96	11.72	11.81	0.38

ADDENDUM C

TRANSPORT DATA

Table C.1: Transport data for Vitamin C MUPS capsule formulation C1

Time (min)	% Transport vessel 1	% Transport vessel 2	% Transport vessel 3	Average % Transport	Standard deviation	Average TEER
0	0	0	0	0		
20	0.02	0.00	0.01	0.01	0.01	48
40	0.14	0.06	0.07	0.09	0.04	
60	0.27	0.19	0.17	0.21	0.04	
80	0.38	0.36	0.25	0.33	0.06	
100	0.51	0.40	0.35	0.42	0.06	
120	0.60	0.44	0.44	0.50	0.08	42

Table C.2: Transport data for Vitamin C MUPS capsule formulation C2

Time (min)	% Transport vessel 1	% Transport vessel 2	% Transport vessel 3	Average % Transport	Standard deviation	Average TEER
0	0	0	0	0		
20	0.05	0.03	0.02	0.03	0.03	59
40	0.16	0.13	0.09	0.13	0.07	
60	0.32	0.27	0.30	0.30	0.09	
80	0.48	0.37	0.47	0.44	0.09	
100	0.60	0.57	0.63	0.60	0.06	
120	0.67	0.61	0.73	0.67	0.10	51

Table C.3: Transport data for Vitamin C MUPS capsule formulation C3

Time (min)	% Transport vessel 1	% Transport vessel 2	% Transport vessel 3	Average % Transport	Standard deviation	Average TEER
0	0	0	0	0		
20	0.01	0.07	0.11	0.06	0.04	52
40	0.08	0.15	0.19	0.14	0.04	
60	0.19	0.24	0.30	0.24	0.05	
80	0.29	0.33	0.37	0.33	0.03	
100	0.38	0.41	0.44	0.41	0.02	
120	0.46	0.50	0.49	0.48	0.01	46

Table C.4: Transport data for Vitamin C MUPS capsule formulation C4

Time (min)	% Transport vessel 1	% Transport vessel 2	% Transport vessel 3	Average % Transport	Standard deviation	Average TEER
0	0	0	0	0		
20	0.00	0.01	0.01	0.01	0.00	38
40	0.06	0.09	0.06	0.07	0.01	
60	0.19	0.23	0.19	0.20	0.02	
80	0.30	0.34	0.27	0.30	0.03	
100	0.38	0.40	0.45	0.41	0.03	
120	0.51	0.54	0.56	0.54	0.02	33

Table C.5: Transport data for Vitamin C MUPS capsule formulation C5

Time (min)	% Transport vessel 1	% Transport vessel 2	% Transport vessel 3	Average % Transport	Standard deviation	Average TEER
0	0	0	0	0		
20	0.02	0.03	0.02	0.02	0.00	41
40	0.15	0.19	0.14	0.16	0.02	
60	0.33	0.38	0.26	0.33	0.05	
80	0.52	0.54	0.42	0.49	0.05	
100	0.68	0.63	0.59	0.64	0.03	
120	0.80	0.82	0.65	0.76	0.07	37

Table C.6: Transport data for Vitamin C MUPS capsule formulation C6

Time (min)	% Transport vessel 1	% Transport vessel 2	% Transport vessel 3	Average % Transport	Standard deviation	Average TEER
0	0	0	0	0		
20	0.07	0.08	0.13	0.09	0.03	32
40	0.26	0.29	0.36	0.30	0.05	
60	0.47	0.52	0.61	0.53	0.06	
80	0.65	0.69	0.79	0.71	0.06	
100	0.80	0.86	0.98	0.88	0.07	
120	0.91	0.97	1.03	0.97	0.05	30

Table C.7: Transport data for Vitamin C MUPS capsule formulation C7

Time (min)	% Transport vessel 1	% Transport vessel 2	% Transport vessel 3	Average % Transport	Standard deviation	Average TEER
0	0	0	0	0		
20	0.01	0.00	0.03	0.01	0.01	35
40	0.03	0.03	0.06	0.04	0.01	
60	0.06	0.09	0.12	0.09	0.02	
80	0.10	0.14	0.17	0.14	0.03	
100	0.15	0.18	0.20	0.18	0.02	
120	0.18	0.22	0.25	0.21	0.03	29

ADDENDUM D

RETENTION DATA

Table D.1: Retention data for Vitamin E MUPS capsules

Formulation	% Retention vessel 1	% Retention vessel 2	% Retention vessel 3	Average % Retention	Standard deviation
E1	1.04	1.07	1.03	1.04	0.02
E2	1.63	1.11	1.18	1.31	0.23
E3	0.46	0.51	0.65	0.80	0.08
E4	1.29	0.97	1.09	1.12	0.13
E5	2.00	2.27	2.66	2.31	0.27
E6	2.79	2.46	2.36	2.54	0.19
E7	0.86	0.88	0.87	0.87	0.01

ADDENDUM E

SIZE DISTRIBUTION PLOTS FOR VITAMIN C AND E BEAD FORMULATIONS

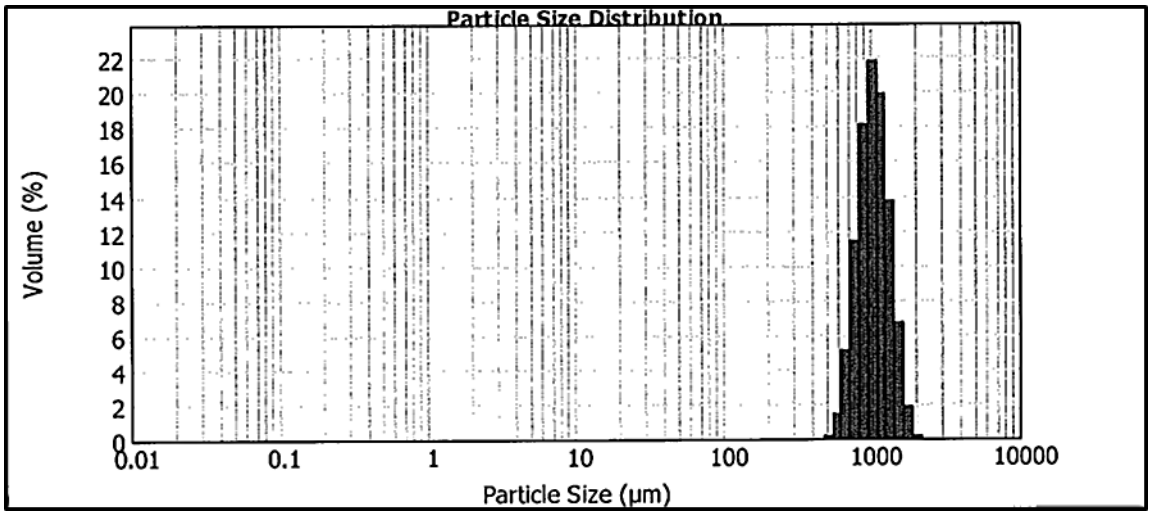

Figure E.1.1: Size distribution plot for bead formulation C1

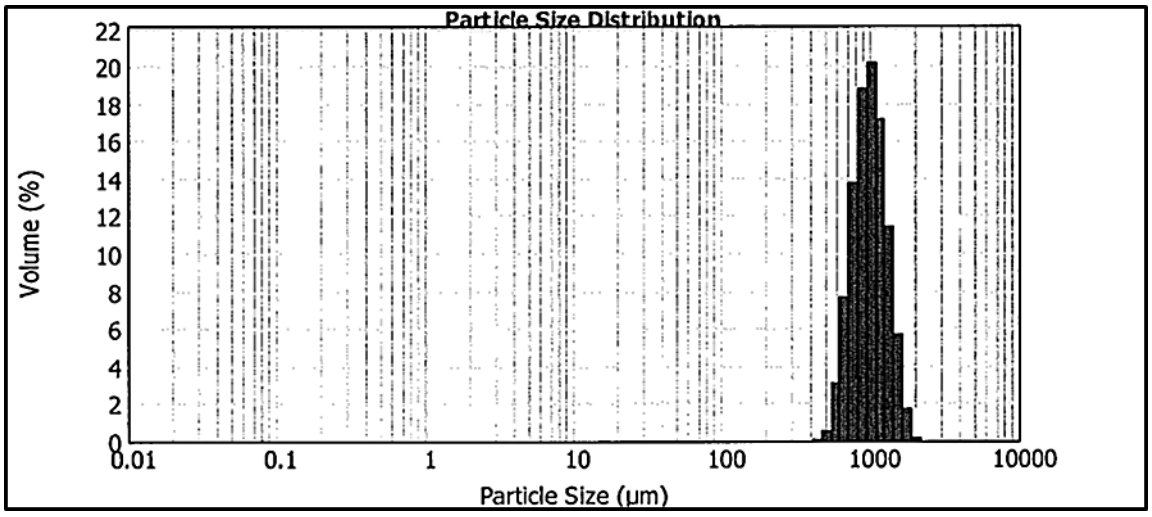

Figure E.1.2: Size distribution plot for bead formulation C2

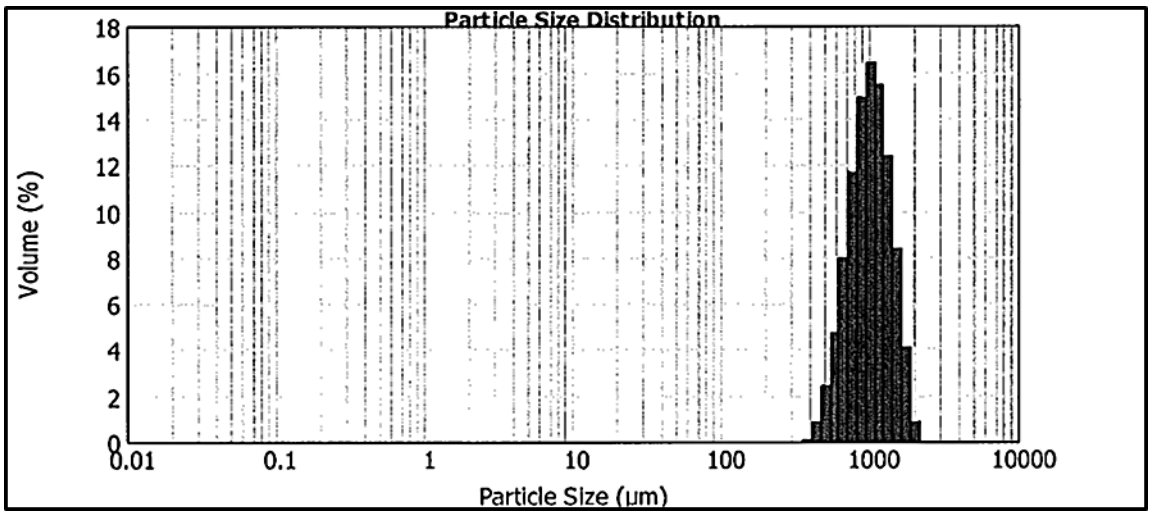

Figure E.1.3: Size distribution plot for bead formulation C3

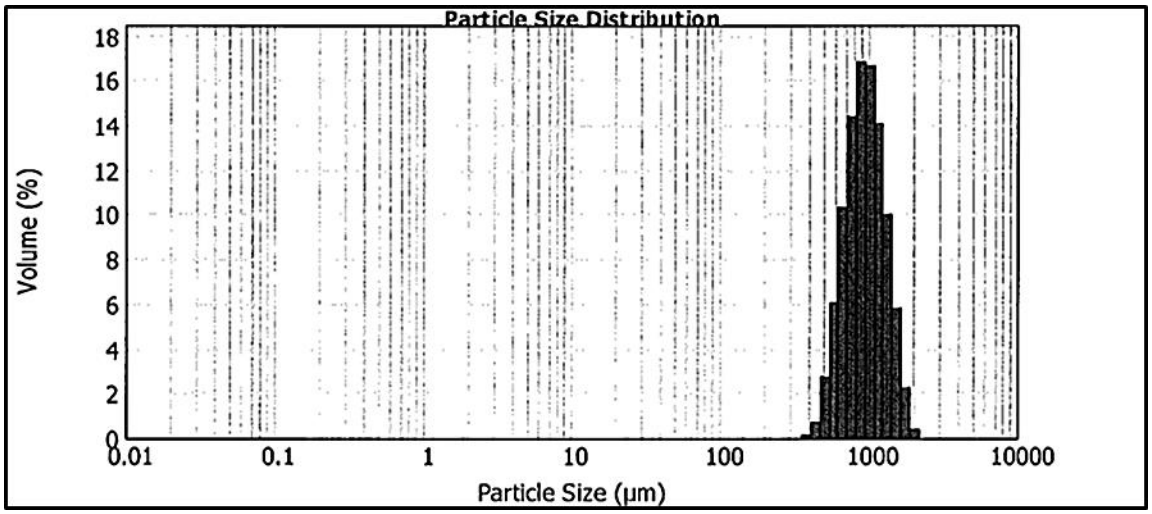

Figure E.1.4: Size distribution plot for bead formulation C4

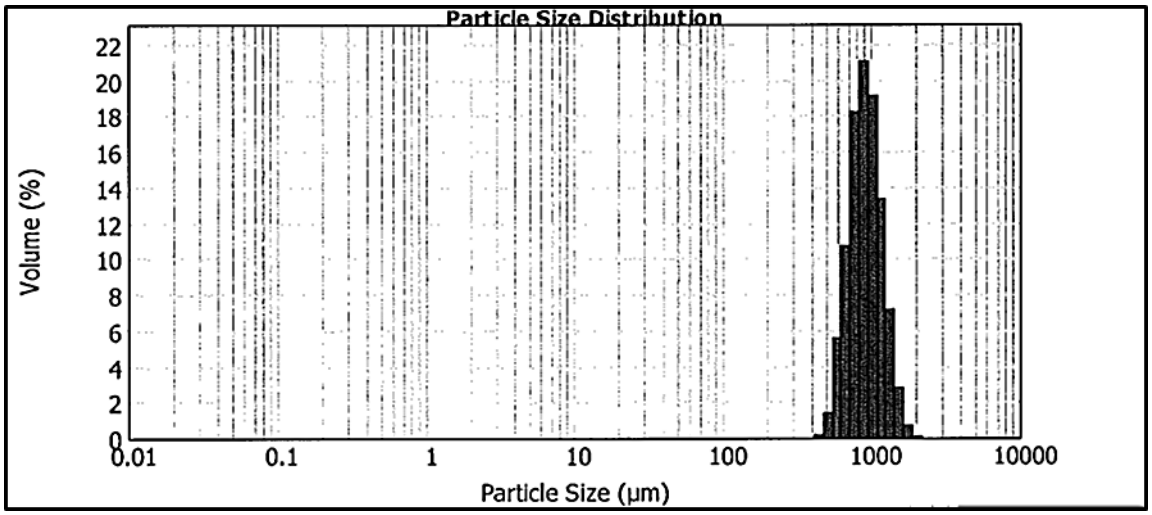

Figure E.1.5: Size distribution plot for bead formulation C5

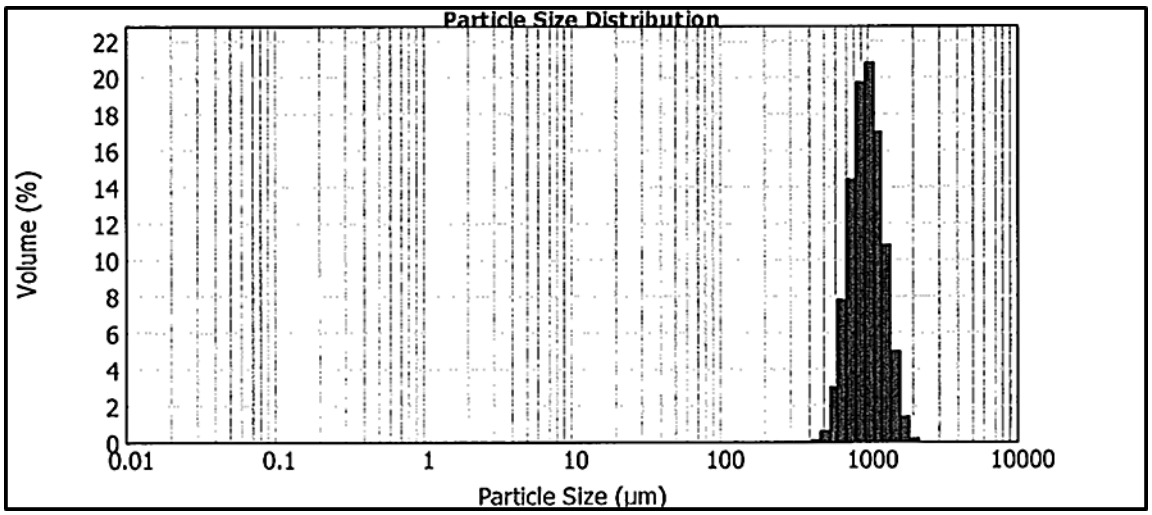

Figure E.1.6: Size distribution plot for bead formulation C6

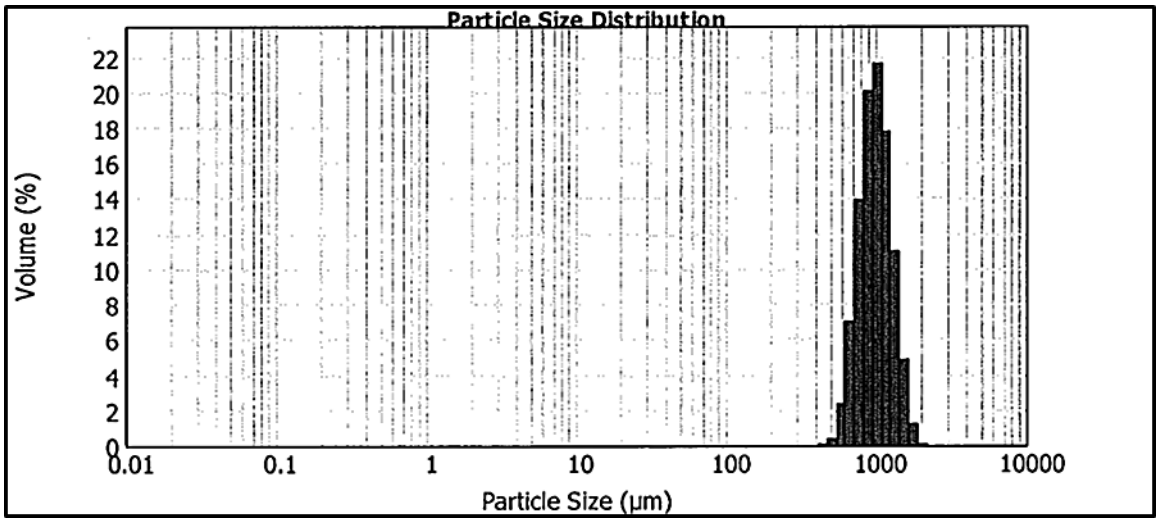

Figure E.1.7: Size distribution plot for bead formulation C7

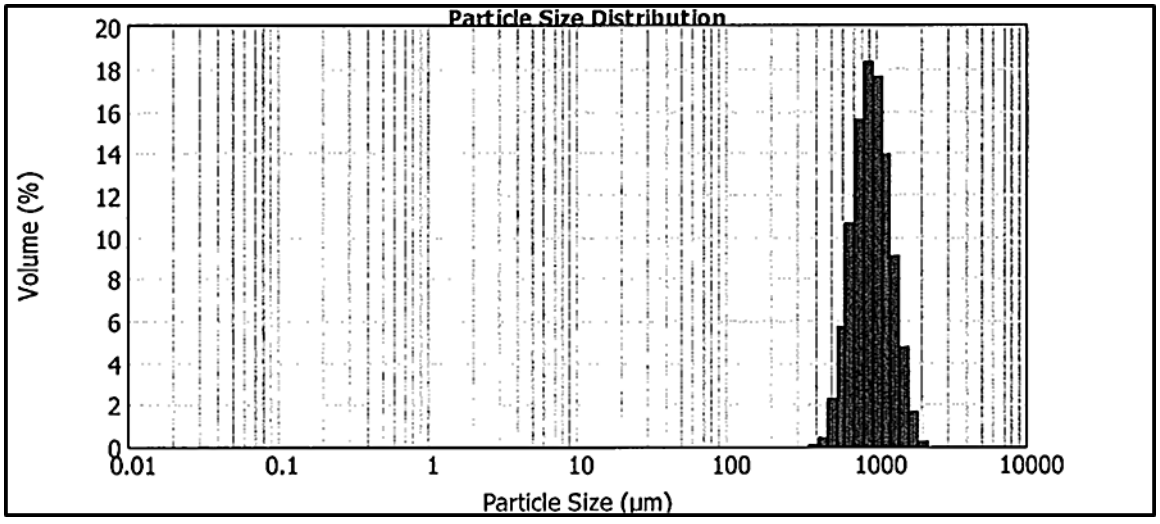

Figure E.2.1: Size distribution plot for bead formulation E1

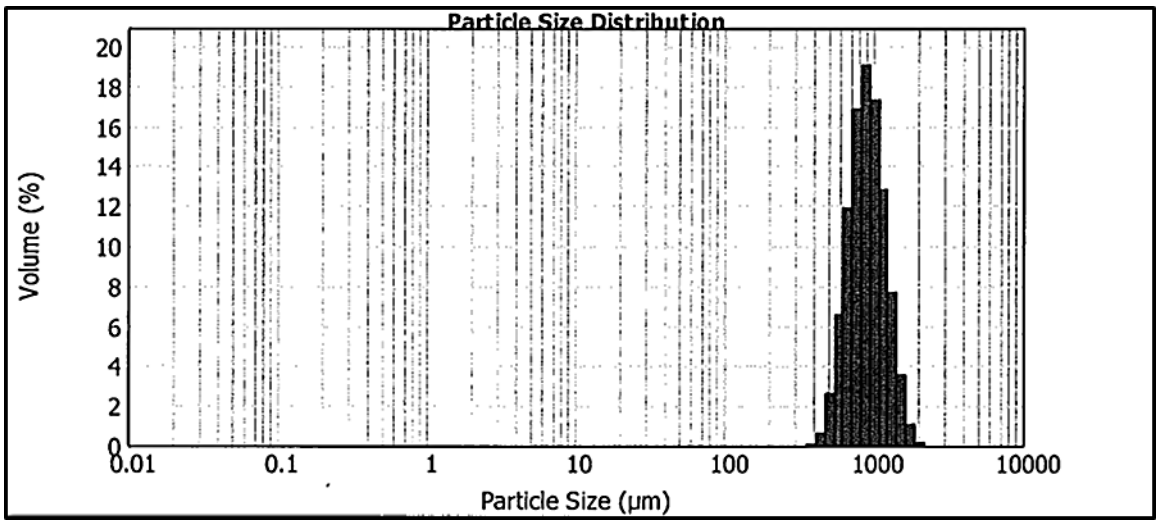

Figure E.2.2: Size distribution plot for bead formulation E2

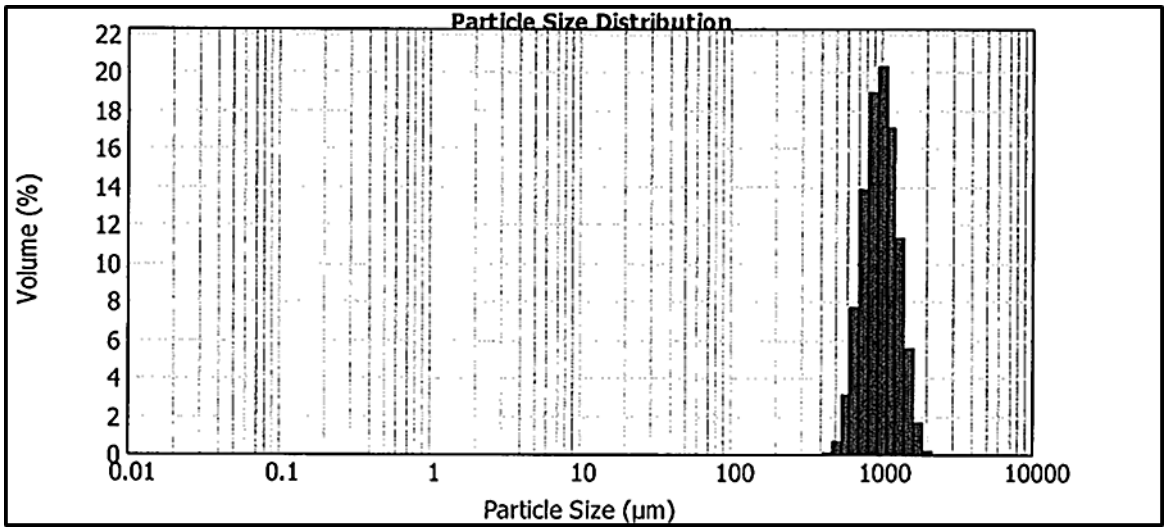

Figure E.2.3: Size distribution plot for bead formulation E3

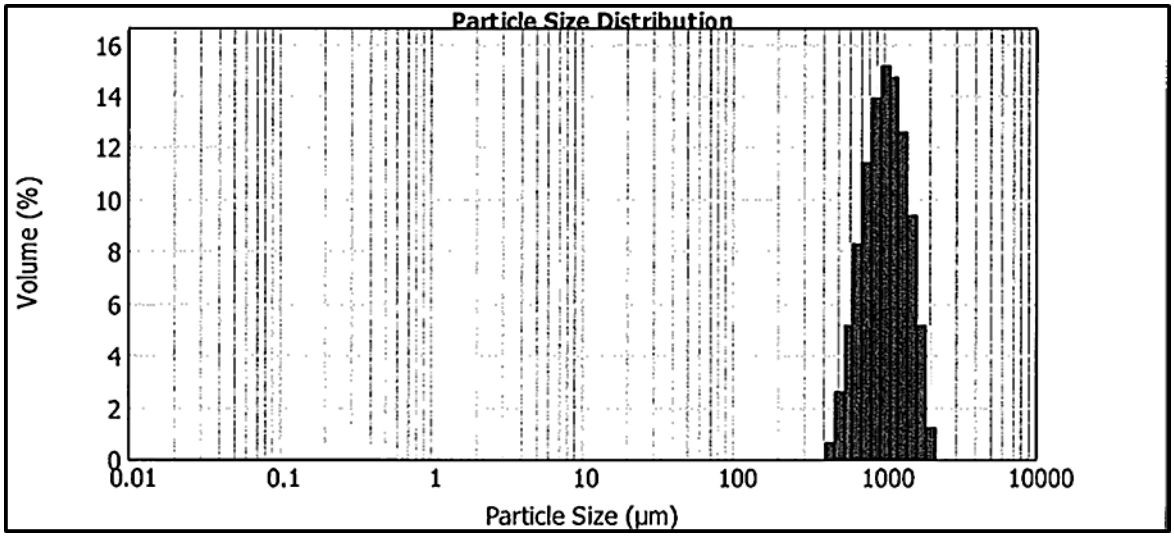

Figure E.2.4: Size distribution plot for bead formulation E4

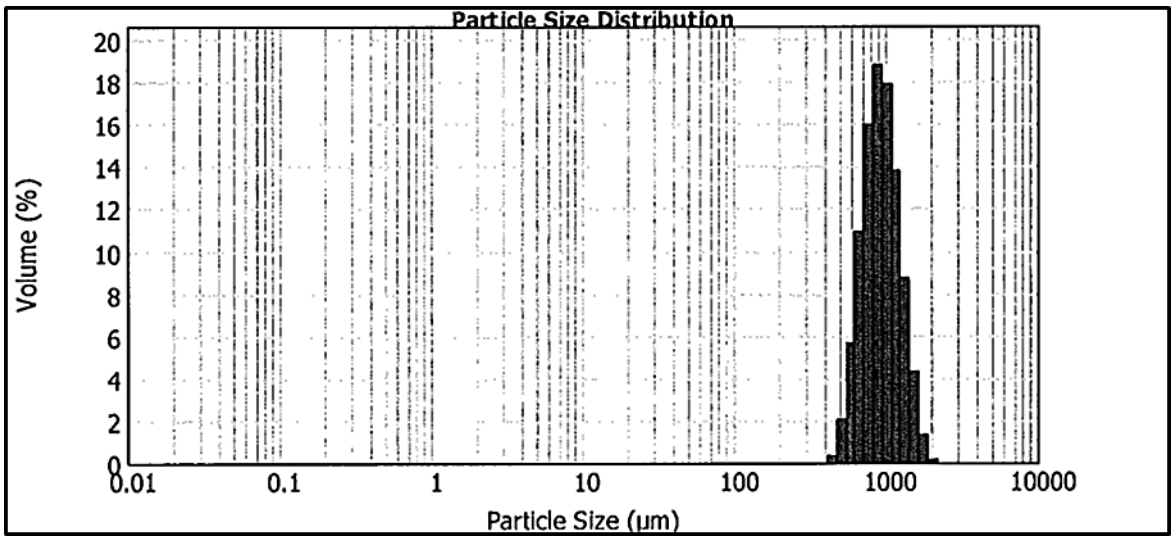

Figure E.2.5: Size distribution plot for bead formulation E5

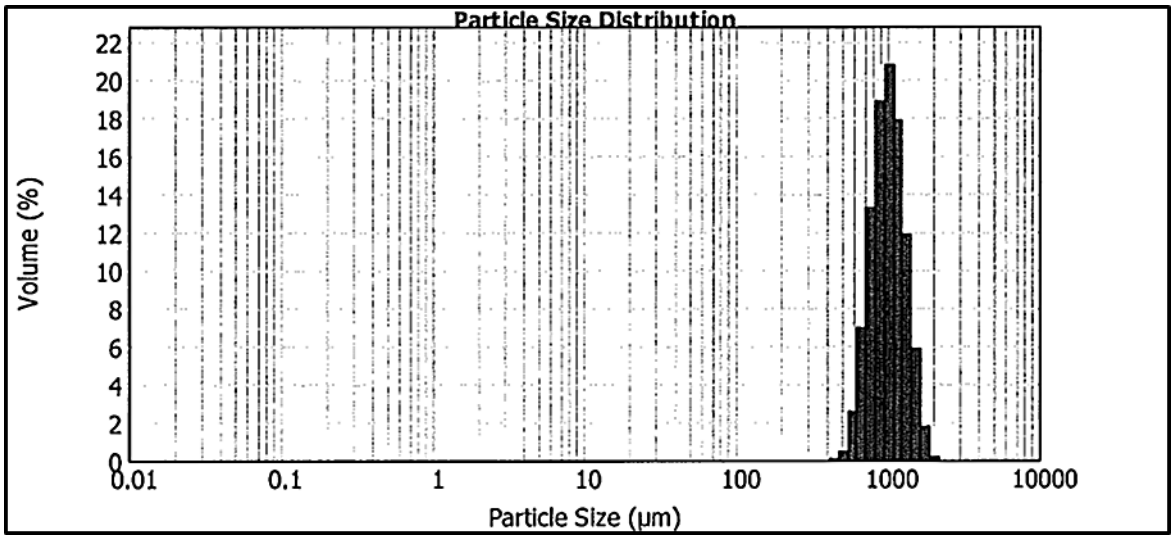

Figure E.2.6: Size distribution plot for bead formulation E6

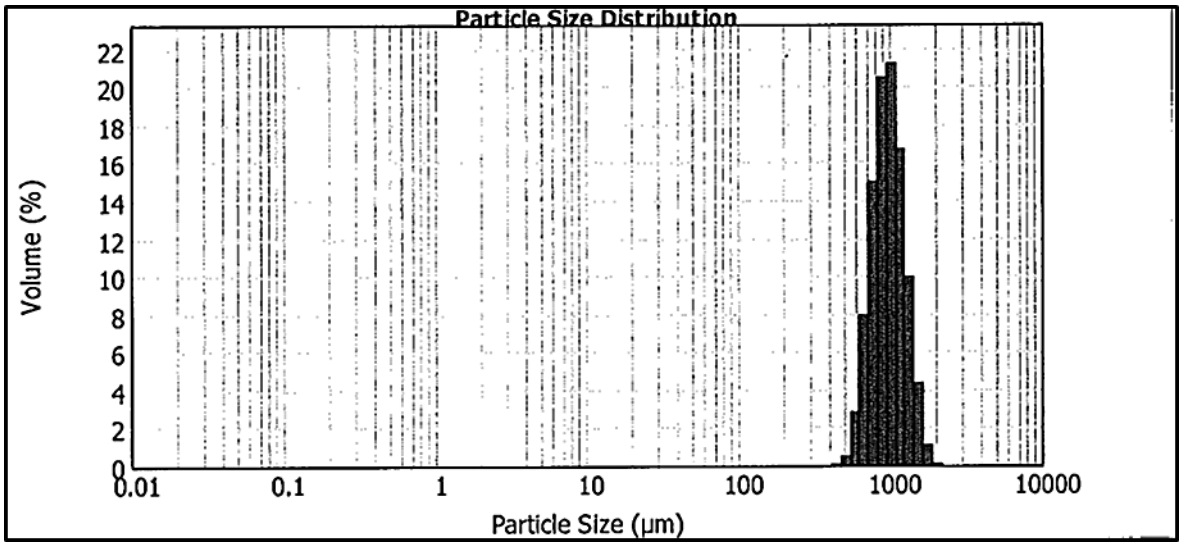

Figure E.2.7: Size distribution plot for bead formulation E7

ADDENDUM F

STATISTICAL ANALYSIS

Table F.1.1: Statistical analysis results of the transport data for the post hoc Dunnett test: Vitamin C transport (P_{app})

	Dunnett test; variable Papp (Data statistiek Papp (Jaco Bezuidenhout Probabilities for Post Hoc Tests (2-sided) Error: Between MS = .00000, df = 14.000	
Cell No.	Var1	{1} .00000
1	Vitamin C Contro	
2	Vitamin C1	0.000165
3	Vitamin C2	0.000009
4	Vitamin C3	0.002828
5	Vitamin C4	0.000057
6	Vitamin C5	0.000009
7	Vitamin C6	0.000009

Table F.1.2: Statistical analysis results of the transport data for the post hoc Kruskal-Wallis test: Vitamin C transport (P_{app}) done in accordance with a Bonferonni adjustment

	Multiple Comparisons p values (2-tailed); Papp (Data statistiek Papp (Jaco Bezuidenhout)) Independent (grouping) variable: Var1 Kruskal-Wallis test: H (6, N= 21) =18.78788 p =.0045						
Depend.: Papp	Vitamin C Control R:2.0000	Vitamin C1 R:8.6667	Vitamin C2 R:15.333	Vitamin C3 R:5.3333	Vitamin C4 R:10.000	Vitamin C5 R:15.667	Vitamin C6 R:20.000
Vitamin C Control		1.000000	0.178358	1.000000	1.000000	0.146666	0.008000
Vitamin C1	1.000000		1.000000	1.000000	1.000000	1.000000	0.530964
Vitamin C2	0.178358	1.000000		1.000000	1.000000	1.000000	1.000000
Vitamin C3	1.000000	1.000000	1.000000		1.000000	0.869089	0.079626
Vitamin C4	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
Vitamin C5	0.146666	1.000000	1.000000	0.869089	1.000000		1.000000
Vitamin C6	0.008000	0.530964	1.000000	0.079626	1.000000	1.000000	

Table F.1.3: Statistical analysis results of the transport data for the post hoc Kruskal-Wallis test: Vitamin C transport compared only with the control group (C7) (*statistically significant differences compared to the control, $p \leq 0.05$)

Formulation	p -Values
C1	1
C2	0.045*
C3	1
C4	1
C5	0.037*
C6	0.002*

Table F.2.1: Statistical analysis results of the percentage retention data for the post hoc Dunnett test: Vitamin E retention (%)

	Dunnett test; variable % Retention (Data statistiek Retensie % (Jaco Bezuidenh Probabilities for Post Hoc Tests (2-sided) Error: Between MS = .06440, df = 13.000	
Cell No.	Var1	{1} .85983
1	Vitamin E Contrc	
2	Vitamin Contrc	1.00000(
3	Vitamin E1	0.94388:
4	Vitamin E2	0.40324:
5	Vitamin E3	0.99993:
6	Vitamin E4	0.80914:
7	Vitamin E5	0.00114:
8	Vitamin E6	0.00031(

Table F.2.2: Statistical analysis results of the percentage retention data for the post hoc Kruskal-Wallis test: Vitamin E retention (%) done in accordance with a Bonferonni adjustment

	Multiple Comparisons p values (2-tailed); % Retention (Data statistiek Retensie % (Jaco Bezuidenhout)) Independent (grouping) variable: Var1 Kruskal-Wallis test: H (7, N= 21) =16.22944 p =.0231							
Depend.: % Retention	Vitamin E Control R:3.0000	Vitamin Control R:4.5000	Vitamin E1 R:8.0000	Vitamin E2 R:12.667	Vitamin E3 R:5.3333	Vitamin E4 R:10.000	Vitamin E5 R:17.667	Vitamin E6 R:19.333
Vitamin E Control		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.633536
Vitamin Control	1.000000		1.000000	1.000000	1.000000	1.000000	0.562711	0.247086
Vitamin E1	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	0.707952
Vitamin E2	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000
Vitamin E3	1.000000	1.000000	1.000000	1.000000		1.000000	0.417637	0.160169
Vitamin E4	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
Vitamin E5	1.000000	0.562711	1.000000	1.000000	0.417637	1.000000		1.000000
Vitamin E6	0.633536	0.247086	0.707952	1.000000	0.160169	1.000000	1.000000	

Table F.2.3: Statistical analysis results of the percentage retention data for the post hoc Kruskal-Wallis test: Vitamin E retention compared only with the control group (E7)

Formulation	p - Values
E1	1
E2	1
E3	1
E4	1
E5	1
E6	0.158