

Development of lipid matrix tablets containing a double-fixed dose combination of artemether and lumefantrine

C.A. Wilkins

 **orcid.org/0000-0003-0546-3189**
(BPharm)

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Supervisor: Dr J.M. Viljoen

Co-Supervisor: Prof L.H. du Plessis

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Student number: 24186899

<http://dspace.nwu.ac.za/>

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*“If you think you’re too small to make a difference
then you’ve never spent the night with a mosquito”*

ï African proverb



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ACT: Artemisinin-based Combination Therapy

AP-BL: Apical to Basolateral

BCS: Biopharmaceutical Classification System

BP: British Pharmacopoeia

CCK: Cholecystokinin

COD: Critical Orifice Diameter

DSC: Differential Scanning Calorimetry

DTG: Differential Weight Loss Thermograms

GIT: Gastrointestinal Tract

HPLC: High Performance Liquid Chromatography

HPMC: Hydroxypropyl Methylcellulose

HSM: Hot-Stage Microscopy

KRB: Krebs-Ringer Bicarbonate

MCC: Microcrystalline Cellulose

MDT: Mean Dissolution Time

RBC: Red Blood Cell

RSD: Relative Standard Deviation

SD: Standard Deviation

SODF: Solid Oral Dosage Form

TEER: Trans-Epithelial Electrical Resistance

TGA: Thermogravimetric Analysis

WHO: World Health Organisation



Malaria remains one of the most serious vector-borne and prominent life-threatening diseases in sub-Saharan Africa. Africa has the heaviest malarial disease burden in the world, accounting for 91% of the estimated 445 000 deaths globally in 2016. Despite numerous efforts to minimise the morbidity and mortality of malaria, it was deliberated the fourth most prevalent cause of death, responsible for 10% of child deaths in sub-Saharan Africa in 2017.

In order to combat this disease, the World Health Organisation (WHO) encourages the development of artemisinin-based combination therapy (ACT) and has recommended the use of ACT as first-line treatment for uncomplicated *P. falciparum* in malaria endemic countries. However, one of the main complications in the development of ACT, is that most of the antimalarial drugs are poorly aqueous soluble and therefore, are poorly biopharmaceutically available for absorption into the systemic circulation. Thus, the primary challenge is to design a dosage form that is able to enhance the solubility of both drugs. For this reason, this study investigated the incorporation of a double-fixed dose combination of 20 mg artemether and 120 mg lumefantrine into lipid matrix tablets as lipid-based formulations are proposed to enhance the solubility of highly lipophilic drugs by providing a microenvironment into which they can partition.

Pre-formulation studies characterised the physical properties of the active ingredients as well as the solid lipid dispersions prepared by means of the hot fusion method. Moreover, their powder flow properties were scrutinised. Thereafter, lipid matrix tablets were directly compressed in accordance with a full factorial design of experiments. The physical tablet properties (mass variation, friability, disintegration, hardness and tensile strength) were subsequently evaluated and only viable formulations progressed for further analysis regarding swelling, erosion and drug release.

Reviewing the physical characteristics of the manufactured lipid matrix tablets, it could clearly be seen that only the formulations comprising either MicroceLac® 100 or CombiLac® as fillers were viable. These resulting 18 formulations underwent dissolution profile characterisation and were compared to the commercially available product, Coartem®. Overall, formulation SA0.5C1 was identified as the optimal formulation (regarding physical and dissolution properties), fitting the Korsmeyer-Peppas with T_{lag} dissolution model capable of releasing 97.21% artemether whilst Coartem® fitted the Peppas-Sahlin 2 with T_{lag} drug release profile and displayed only 86.12% artemether drug release over a period of 12 h in the tested dissolution media. *In vitro* permeability studies were conducted as a proof of concept for the lipid based formulations utilising the study.

optimised formulation, which rendered 3.35% artemether and 4.88% lumefantrine drug transport, respectively.

Therefore, in conclusion, preliminary evaluation of the formulated lipid matrix tablets containing a double-fixed dose combination of artemether and lumefantrine proved capable of increasing the active ingredients solubility whilst demonstrating modified drug release. The optimal lipid matrix tablet formulation, SA0.5C1, requires further *in vivo* analysis to comment on the formulations potential to increase the biopharmaceutical availability of the two incorporated highly lipophilic active ingredients.

Keywords: Malaria; Artemisinin-based combination therapy; Double-fixed dose combination; Artemether; Lumefantrine; Lipid matrix tablets; Lipid-based formulations

CHAPTER 1: INTRODUCTION, AIM AND OBJECTIVES



1.1. Introduction

1.1.1. Malaria – The Silent Killer

Malaria is one of the most serious vector-borne diseases (Sokhna *et al.*, 2013) and the most important parasitic disease in humans (Rosenthal, 2012). Furthermore, Africa has the heaviest malarial disease burden in the world, accounting for 90% of the reported 216 million cases globally in 2015. The World Health Organisation (WHO) recorded an estimated 445 000 deaths globally in 2016, of which the WHO African region accounted for up to 91% (WHO, 2017). By March 2017, South Africa had recorded a total of 9 478 malaria cases and 76 deaths for the year (Communicable diseases communiqué, 2017).

Malaria is caused by four species of protozoa belonging to the genus *Plasmodium* – *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* (Paget, 2011). *Plasmodium* has a complex life cycle, initiated through infection of the adult female *Anopheles* mosquito as a vector where sexual reproduction of the parasite occurs; and then infecting a human host where asexual reproduction follows in the red blood cells as well as in the liver (Ingraham & Ingraham, 2007). Of the four different *Plasmodium* species affecting humans, *P. falciparum* and *P. vivax* are the most prevalent, with *P. falciparum* being the most dangerous. *P. vivax* and *P. ovale* develop hypnozoites which can remain dormant in the liver for months or years; and cause a relapse of symptoms after an asymptomatic period has elapsed (Ingraham & Ingraham, 2007).

Two primary challenges exist in the fight against malaria, with the key challenge being the lack of sufficient, predictable and sustained funding (WHO, 2015a; WHO, 2017). The second challenge is more difficult to overcome given its biological nature (WHO, 2015a; WHO, 2017), namely the development of mosquito resistance to insecticides (vector control and prevention strategies) and of the parasite itself to antimalarial drugs (treatment) which has the potential to cause an increase in malaria incidence and mortality (Sokhna *et al.*, 2013).

The WHO has recommended the use of artemisinin-based combination therapy (ACT) as first-line treatment for uncomplicated *P. falciparum* in malaria endemic countries (WHO, 2015a; WHO, 2017). ACT is the preferred treatment against *P. falciparum* as it allows for the administration of shorter- and longer-acting drugs simultaneously (Mutabingwa, 2005; WHO, 2015a; WHO, 2017). Since the implementation of ACT in 2002, artemisinin-derivatives have formed the cornerstone of antimalarial treatment as they are highly effective due to their elevated parasite killing rates, limited adverse effects and absence of significant resistance (Nosten & White, 2007; White, 1997; WHO, 2015a). Artemisinins are fast-acting and rapidly eliminated from plasma. These derivatives

are often combined with an antimalarial drug that exhibits a slower elimination rate so that parasites remaining after the initial period of artemisinin-derivative treatment may be eradicated (Schrader *et al.*, 2012). The rationale for combining an artemisinin-derivative together with another structurally unrelated antimalarial drug is based upon their differing mechanisms of action, resistance mechanisms and elimination rates (Peters, 1969). By incorporating two drugs with different mechanisms of action, there is a significant reduction in the risk of parasitic resistance developing as the two partner drugs will also have different resistance mechanisms (Premji, 2009).

Artemether-lumefantrine combination is a WHO approved double-fixed dose combination and is currently available on the market as Coartem® (Prabhu *et al.*, 2016). It has been adopted as first-line therapy for the treatment of uncomplicated malaria in over 20 African countries (WHO, 2015b). The administration of Coartem® with the concurrent consumption of a fat rich meal is recommended to aid drug absorption (du Plessis *et al.*, 2015; Kossena *et al.*, 2007; Novartis, 2009). The known symptoms of malaria include headache, fever, chills, nausea, vomiting and loss of appetite (Centres for Disease Control, 2015) and these symptoms often make patients unwilling and unable to take their much-needed medication as prescribed.

Furthermore, treatment failure has been associated with incomplete absorption of the drugs (Pawar *et al.*, 2016a). This could, in part, be attributed to the fact that both artemether and lumefantrine are highly lipophilic drugs and therefore have poor aqueous solubility (Ma *et al.*, 2014). Thus, the primary challenge is to design a dosage form that is able to enhance the solubility of both drugs. For this reason, this study will look at lipid matrix tablets as a dosage form as it was proposed by Christian *et al.* (2017) that the stability and solubility of these drugs could be enhanced in the presence of lipids or oils. The presence of lipids in the duodenum stimulates the secretion of bile salts, together with phosphatidylcholine and cholesterol from the gall bladder and pancreatic fluids (Kossena *et al.*, 2007). Ingested exogenous lipids combine with bile salts and phospholipids to form chylomicrons and a series of colloidal species (Porter *et al.*, 2007). Hofmann and Borgström (1964) described the intestinal mixed micellar phase containing bile salts and phospholipids as responsible for solubilising poorly water soluble, lipophilic drugs and providing a concentration gradient for lipid absorption. This is as a result of endogenous bile salts and phospholipids combining with exogenous, formulation-derived lipids to form a lipidic microenvironment into which lipophilic drugs may partition/solubilise (Humberstone *et al.*, 1996).

1.1.2. Artemether

Artemether (Figure 1.1) is a first-generation, short-chain oil soluble derivative (White, 2008) of the antimalarial artemisinin drug class, which was originally isolated from the Chinese plant *Artemisia annua* (Wang *et al.*, 2015). Artemether has been proven effective against both acute uncomplicated, and severe *P. falciparum* infections; as well as *P. vivax* and cerebral malaria (Pawar *et al.*, 2016a). Artemether possesses higher antimalarial activity compared to its parent drug (Christian *et al.*, 2017; Li *et al.*, 2014) and is included in the essential medicines list of the WHO to treat both chloroquine-sensitive and -resistant strains of *P. falciparum* (Pawar *et al.*, 2016b). This drug is schizontocidal and exhibits a rapid onset of action (Pawar *et al.*, 2016a), thereby easing the initial parasitic burden and malarial symptoms (Prabhu *et al.*, 2016). It is predominantly metabolised by cytochrome P450 (CYP450) enzymes, specifically CYP3A4 and CYP2B6 (Honda *et al.*, 2011) in the liver to the demethylated derivative, dihydroartemisinin (Pawar *et al.*, 2016b) which also displays *in vivo* activity against the *P. falciparum* parasite (Hilhorst *et al.*, 2014).

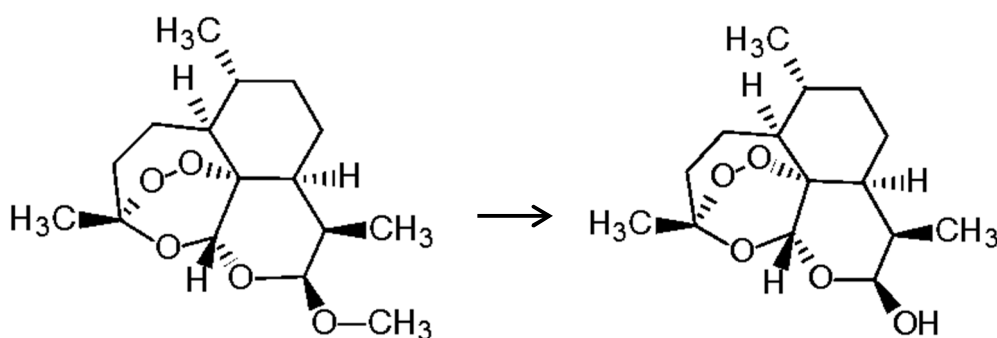


Figure 1.1: Chemical structure of the first generation artemisinin derivative artemether and its demethylated derivative, dihydroartemisinin adapted from Karunajeewa, 2012

According to the Biopharmaceutical Classification System (BCS), artemether is a class II drug, signifying poor aqueous solubility with moderate to high permeability (Lindenberg *et al.*, 2004). It is highly lipophilic ($\log P = 3.5$) and has a melting point of 86 to 90°C (Fule *et al.*, 2013). Artemether has poor aqueous solubility (Mazzone *et al.*, 2014), however, it is highly soluble in acetone and dichloromethane; and shows higher stability when dissolved in oils (Christian *et al.*, 2017). It is generally well tolerated with low toxicity, and therefore, the partner drug used in the ACT will ultimately determine the side-effect profile (Wang *et al.*, 2015). This drug furthermore exhibits poor oral bioavailability (Fule *et al.*, 2013) owing to its poor aqueous solubility, but has improved bioavailability when compared to its parent drug, artemisinin (Hilhorst *et al.*, 2014). Therefore, research is being conducted on developing novel formulation techniques such as lipid-based formulations employing hot fusion, as a method of manufacture, to improve the bioavailability of poorly aqueous soluble drugs (Crowley & Gryczke, 2015).

1.1.3. Lumefantrine

Lumefantrine is a highly lipophilic drug belonging to the arylamino-alcohol class of drugs (Nosten *et al.*, 2012) and is currently recommended by the WHO as first-line treatment against multidrug resistant strains of *P. falciparum* when used in combination with artemether in a 6:1 ratio (Khuda *et al.*, 2014; Van *et al.*, 1998). It is a racemic compound (Figure 1.2) with both enantiomers having equal antimalarial activity against the *P. falciparum* and *P. vivax* asexual stages of parasitic reproduction (Nosten *et al.*, 2012). Lumefantrine is a blood schizontocide (Garg *et al.*, 2017) but exhibits no antimalarial activity against the pre-erythrocytic liver stage of the disease meaning it is not active against hypnozoites or gametocytes (Nosten *et al.*, 2012).

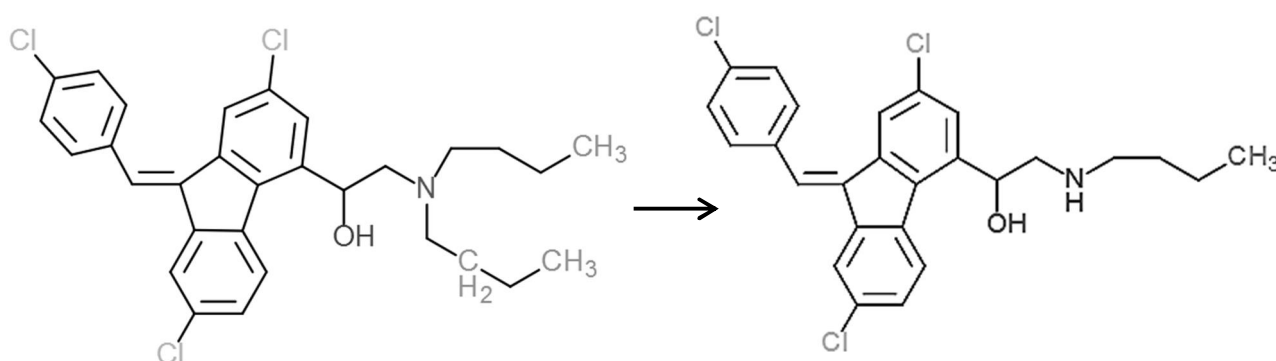


Figure 1.2: Chemical structure of WHO approved ACT partner drug lumefantrine and its metabolite desbutyl-lumefantrine adapted from Song *et al.*, 2016

According to the BCS, lumefantrine is a class II drug as it is poorly water soluble with moderate to high permeability (Lindenberg *et al.*, 2004). Lumefantrine has a melting point of 128 to 131°C (Kotila *et al.*, 2013). This drug is absorbed and eliminated slowly (Thomson *et al.*, 1998) rendering it a suitable candidate to use in ACT as it will eliminate any remaining parasites after the short-acting artemisinin derivative (in this study: artemether) has reduced the initial parasite burden and initial malarial symptoms (Prabhu *et al.*, 2016). There is an initial lag time of up to 2 h before lumefantrine is absorbed, with peak plasma concentrations being achieved 6 to 8 h after administration (Khuda *et al.*, 2014). Lumefantrine is metabolised by CYP3A4 to desbutyl-lumefantrine, but is primarily eliminated as the parent drug (Nosten *et al.*, 2012) and has a terminal elimination half-life of 2 to 3 days in healthy volunteers; and 4 to 6 days in malaria infected patients (Ezzet *et al.*, 2000). It exhibits food-enhanced absorption especially after a fatty meal, and it has been reported that the oral bioavailability can be increased up to 16-fold when lumefantrine is co-administered with lipids (du Plessis *et al.*, 2015; Ezzet *et al.*, 2000). Therefore, due to the high lipophilicity and poor aqueous solubility of lumefantrine, resulting in erratic absorption and subsequently poor bioavailability (Gahoi *et al.*, 2012; Garg *et al.*, 2017), this study will formulate lipid matrix tablets in an attempt to overcome these drawbacks.

1.1.4. Lipid Matrix Tablets

Lipids are one of the most versatile excipient classes available to the pharmaceutical industry, providing the formulator with a wide selection of either natural or synthetic lipids as potential options to improve absorption as well as to control drug release (Feeney *et al.*, 2016; Pouton & Porter, 2008). Lipid-based formulations have proven useful in enhancing the absorption, and consequently the bioavailability of BCS class II drugs such as artemether and lumefantrine (Feeney *et al.*, 2016; Jannin *et al.*, 2008; Pouton, 2006). Lipid-based drug delivery systems are reportedly able to enhance gastrointestinal absorption of lipophilic drugs as they trigger gall bladder contractions and increase biliary and pancreatic secretions which retain the drug in a solubilised form (Elgart *et al.*, 2012). Furthermore, the utilisation of long chain fatty acids enhances the lymphatic uptake of lipid-based drug delivery systems which can assist in avoiding hepatic first-pass metabolism of the drug (Patravale & Prabhu, 2014).

Lipid matrix tablets are classified as monolithic tablets, meaning that drug-plasma levels may be optimised due to the controlled release of the active ingredients from the dosage form (Nisha *et al.*, 2012). Drug release from lipid matrices can occur via two release mechanisms namely, (i) pore diffusion or (ii) erosion (Nisha *et al.*, 2012). The hot fusion method of manufacture ensures that the active ingredients are homogeneously mixed into the inert, melted lipid base, which solidifies upon cooling and coats individual drug particles as well as forms a matrix which is able to control the release of drugs based on the pores formed within the matrix (Abd-Elbary *et al.*, 2013). Furthermore, the formulation derived lipids form a microenvironment which aids in the solubilisation of the drugs, allowing the active ingredients to reach the absorptive membrane of the enterocyte and consequently enhance the absorption of lipophilic drugs (Dahan & Hoffman, 2014).

There are a number of matrix-forming materials available depending on the properties of the drugs to be incorporated into the lipid matrix tablets. For this study, lipids with differing melting points and matrix-forming properties were selected. The two non-swellable, hydrophobic lipids, stearic acid and cetyl alcohol (Özyazici *et al.*, 2006), were investigated together with glycerol monostearate and coconut oil. Stearic acid and cetyl alcohol were chosen for this study due to their hydrophobic matrix-forming properties and the differences in their melting points (i.e. 66 and 69.4°C and 48 and 53°C, respectively) (Acme-Hardesty, 2017; Nisha *et al.*, 2012; Sharma *et al.*, 2002). Stearic acid (Log *P* = 8.23) is a saturated carboxylic acid (Lohan *et al.*, 2016) and is less expensive than other inorganic phase change materials (Zhang *et al.*, 2014b), rendering it a suitable excipient for this study. Cetyl alcohol is a synthetic, fatty alcohol and exists as a waxy, flaky white solid at room temperature (O'Neil, 2011). It is produced from the end-products of the petroleum industry or from vegetable oils such as palm oil or coconut oil. Glycerol monostearate, on the other hand, is a white, wax-like solid and has a melting point of 56 and 58°C (O'Neil, 2011).

Glycerol monostearate is classified as a partial glycoside accounting for its frequent employment in lipid-based formulations (Pizzol *et al.*, 2014).

Coconut oil is emerging as an attractive excipient choice as it is an abundant natural source that is relatively inexpensive, making it an ideal candidate to investigate for artemisinin-based combination therapy, which is predominantly prescribed in low income regions. Coconut oil has well established importance in the food and cosmetic industry (Ivii *et al.*, 2017) and has been investigated as a pharmaceutical excipient option due to its antioxidant properties and health benefits (Famurewa *et al.*, 2017). The advantage of using coconut oil over other natural oils is due to its high saturated fat content, rendering it resistant to rancidification. It is a liquid at temperatures above 25°C and can last for up to six months at room temperature without spoiling (Kempton, 2005). The low melting point of coconut oil could be problematic during the solidification stage when preparing lipid-based formulations via hot fusion, however, the potential benefits make it a worthy candidate to investigate further.

1.1.5. Direct Compression

Direct compression is a simple process in which the active ingredients are blended with the excipients prior to tableting and consists of fewer preparation steps than wet granulation (Rojas *et al.*, 2014). This process is able to overcome the associated drawbacks of wet granulation such as clump formation; incompatibilities between the active ingredients and the wetting solvent; and poor compaction performance (Lakio *et al.*, 2016). Direct compression is suitable for thermolabile and hydrolabile drugs which are not suitable for wet granulation given the process steps involved in the wet granulation method. One challenge associated with direct compression is that the compression mixture needs to be able to flow effectively through the hopper into the tablet die in order to ensure tablets of consistent weight. Therefore, the flowability and compressibility of the excipients together with their quality and consistency, plays a vital role in the success of direct compression (Thoorens *et al.*, 2014).

The four fillers selected for this study are MicroceLac® 100, CombiLac®, RetaLac® and Pharmacel® 101. MEGGLE® describes MicroceLac® 100, CombiLac® and RetaLac® as compatible, well-suited and recommended for manufacturing of tablets via direct compression (Meggle 2016a, b, c). DFE Pharma recommends using Pharmacel® 101 for direct compression as it is composed of microcrystalline cellulose making its compaction effective and therefore ideal as a direct compression excipient (DFE Pharma, (n.d.)).

The release characteristics of the active ingredients are significantly influenced by the mechanical properties of the manufactured tablets, thereby making the method of manufacture an important choice. Tablet strength and porosity of the matrix tablets will dictate the diffusion rate of water via

pores into the matrix and thus ultimately determine the release rate of the active ingredients from the matrix tablets (Petrović *et al.*, 2012).

1.2. Research Problem

Malaria remains one of the most prominent life-threatening diseases in sub-Saharan Africa. In order to combat this disease, the WHO encourages the development of artemisinin-based combination therapy (WHO, 2016a). However, one of the main complications in the development of ACT, is that most of the antimalarial drugs are poorly aqueous soluble and therefore, are poorly biopharmaceutically available for absorption into the systemic circulation.

Both artemether and lumefantrine are considerably poorly water soluble; and thus, the rationale for developing lipid matrix tablets is two-fold. Firstly, lipid-based formulations act on the premise that the presence of exogenous lipid will cause the formation of colloidal species into which the active ingredients can partition and thereby increase their solubilisation within the microenvironment. Additionally, hot fusion is reported to augment the dissolution rate and subsequent bioavailability of such compounds by increasing the surface area and saturation solubility (Ambike *et al.*, 2004); and thus, the plausibility of this will be investigated. Secondly, it needs to be established if the lipid coating of the drug particles and preparation of a lipid matrix tablet will achieve sustained or modified drug release, as the formulation strategy employed is dependent upon matrix systems which can control the diffusion speed of water via pores into the matrix (Nisha *et al.*, 2012; Petrović *et al.*, 2012).

Furthermore, patient non-compliance is an on-going problem associated with the 7-day treatment regimen required by monotherapies (Carborne *et al.*, 2014; WHO, 2015a) coupled with an increased risk for resistance emergence (Premji, 2009). Therefore, the WHO have called for the use of ACT to minimise this risk (WHO, 2016a). Modified release dosage forms capable of potentially reducing the dosing frequency and duration of therapy are desirable as it could ultimately improve patient compliance (Zhang *et al.*, 2014a) and have the added benefit of avoiding dose dumping (Patel *et al.*, 2011). This study will therefore attempt to address the above-mentioned problems by developing a lipid-based formulation containing a double-fixed dose combination of artemether and lumefantrine, utilising the hot fusion method to produce lipid matrix tablets formed via direct compression with modified release properties.

1.3. Aim and Objectives

The aim of this study is to incorporate a double-fixed dose combination of the two antimalarial drugs, artemether and lumefantrine, into a lipid matrix tablet that will offer modified release of the active ingredients. The lipid matrix tablets will be prepared using the hot fusion method and thereafter the formulation will be tableted by means of direct compression. Four different lipid

bases as well as four different filler types will be investigated utilising a full factorial design of experiments to identify the optimal formulation combination according to the factors investigated in this study, which will render tablets that adhere to the criteria of the British Pharmacopoeia.

The objectives set for this study, are to:

- Characterise the four different fillers (MicroceLac® 100, CombiLac®, RetaLac® and Pharmacel® 101) according to their physical properties and morphologies.
- Prepare the predetermined lipid dispersions in accordance with the factorial design by means of the hot fusion method.
- Prepare lipid-matrices containing the double-fixed dose combination by means of hot fusion using either stearic acid, cetyl alcohol or glycerol monostearate as the inert lipid base in different ratios (0.5:1, 0.75:1, 1:1) to the double-fixed dose combination; as well as investigate the plausibility of using coconut oil as a lipid base in the same lipid:drug ratios.
- Characterise the double-fixed dose, solid lipid dispersions by means of differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), X-ray diffraction studies, light microscopy and thermal microscopy.
- Formulate various double-fixed dose, lipid matrix tablets utilising a full factorial design of experiments.
- Assess the physical properties (mass variation, friability, disintegration, thickness, diameter, hardness, and tensile strength) and the surface morphology of the double-fixed dose, lipid matrix tablets prepared by means of direct compression.
- Conduct a drug content assay as to assess the % drug content in both the lipid dispersions and lipid matrix tablets after preparation.
- Evaluate and compare the various double-fixed dose, lipid matrix tablets produced from the various formulations as well as the commercial product, Coartem®, in terms of drug release behaviour in different pH environments.
- Conduct *in vitro* permeability experiments with the selected optimal double-fixed dose, lipid matrix tablet formulation across the jejunum section of porcine intestinal tissues, using Sweetana-Grass diffusion chambers. Where after, statistical analysis will be performed to compare the transport results in order to determine if any active compounds were absorbed in significant concentrations.





2.1. Malaria

2.1.1. A Disease without Borders

Malaria is a vector-borne infectious disease prevalent in tropical and subtropical regions and is endemic in 104 countries and territories (Snow, 2005; WHO, 2016a). Approximately 80% of malarial cases and 90% of deaths were estimated to occur in Africa in 2015 (WHO, 2015a). Despite numerous efforts to minimise the morbidity and mortality of malaria, it was the fourth most prevalent cause of death, responsible for 10% of child deaths in sub-Saharan Africa (Garg *et al.*, 2017). It has been noted by Lin *et al.* (2016) that young children in stable transmission areas endure the highest malarial morbidity and mortality burden. In 2014, the mortality rate of malaria in children equated to a child dying every two minutes (WHO, 2016a).

The biological challenge is complicated by the diversity of malaria vectors and differences in their behaviours, as well as the possibility of more than one species being prevalent in a region (Sokhna *et al.*, 2013; WHO, 2016b). In countries where *P. vivax* is present, it is more difficult to reduce the malarial burden due to the dormant hypnozoite stage formed in the liver, which is presently undetectable, and leads to a relapse of symptoms and ultimately contributes further to disease transmission (WHO, 2015a).

Malaria is an entirely preventable and treatable disease provided that the currently recommended interventions and treatment regimens are adhered to and correctly implemented (WHO, 2016a). In recent years, the progress in reducing the morbidity and mortality of malaria can be attributed to the distribution of long-lasting insecticide-treated bed nets, indoor residual spraying and artemisinin-based combination therapy (Sokhna *et al.*, 2013). This progress is however threatened by the development of parasitic resistance to antimalarial drugs. Antimalarial drug resistance is an ever-increasing public health problem which hinders the control of the disease (Nnamani *et al.*, 2014). Resistant parasites have emerged as a result of non-judicious use of existing malarial moieties, inadequate patient compliance, lack of widespread availability to artemisinin-based therapies, the use of monotherapies and substandard or counterfeit forms of the drugs on the market (Patel *et al.*, 2013; Newton *et al.*, 2002; Wilson & Fenoff, 2011).

Malaria furthermore contributes to the poverty cycle as the highest global disease burden is concentrated mainly in Africa. It disproportionally affects the lowest income and most vulnerable populations which have limited access to adequate health care services and has a detrimental impact on education through missed days of school due to illness (WHO, 2015b). The WHO has stated that the world is at a critical point in the fight against malaria and an opportunity and urgent

need exists to decrease the morbidity (214 million clinical episodes in 2015) and mortality (438 000 deaths in 2015) by identifying approaches that aim to reduce transmission (WHO, 2015a; WHO, 2016a).

In an effort to win the fight against malaria, the WHO has called for the development and implementation of artemisinin-based combination therapies (ACT) in order to combat emerging resistance. The choice of ACT is dependent upon the therapeutic efficacy of the specific combination in the region of intended use (Nnamani *et al.*, 2014). From a resistance prevention perspective, the drugs used in combination should have similar elimination rates to provide optimum mutual protection against resistance (Nosten & White, 2007). However, there are benefits to the artemisinin partner drug having a slower elimination rate, and this approach is often used with ACT. Slower elimination rates allow for a shorter therapeutic regimen to be followed by the patient which could enhance patient compliance and reduce the emergence of resistance due to patient non-compliance. The residual sub-therapeutic levels of the partner drug could also act as a prophylactic dose which is of clinical importance in high-transmission regions, but potentially increases the risk of the partner drug developing resistance (Nosten & White, 2007). Although it may be argued that the remaining parasitic load exposed in isolation to the partner drug (with a slower elimination rate) used in ACT may be too minimal to cause the occurrence of resistance, it remains a noteworthy consideration, as it may create considerable selection pressure (Eastman *et al.*, 2011).

It is therefore imperative to utilise existing drugs judiciously and incorporate them into novel drug delivery systems with the intention to diminish dose-induced side effects, achieve enhanced aqueous solubility, attain active targeting of infected tissues, increase bioavailability, and above all, provide patient friendly dosing regimens to enhance compliance and thereby decrease the emergence of resistance due to patient non-compliance (Nnamani *et al.*, 2014).

2.1.2. Parasitic Life Cycle

The malaria parasite (*plasmodium*) life cycle is complex and involves two hosts namely, the adult female *Anopheles* mosquitoes as a vector where sexual reproduction of the parasite occurs; and the infected human host where asexual reproduction follows in the red blood cells (Ingraham & Ingraham, 2007). The two most prominent malarial vectors in Africa are the *Anopheles gambiae* and *Anopheles funestus* mosquitoes, both of which are strongly anthropophilic. The female *Anopheles* mosquito requires a blood meal for the successful development of her eggs and is primarily responsible for the transmission of the parasite to humans (Centres for Disease Control, 2018).

Figure 2.1 demonstrates how during a blood meal, a *plasmodium*-infected female *Anopheles* mosquito injects sporozoites ❶ into the bloodstream of the human host. These sporozoites are

produced during the sporogonic stage of sexual reproduction occurring in the midgut of the mosquito and migrate to the salivary gland once the oocyte ruptures. Once the human host is inoculated, it is thought to take 30 min for sporozoites to travel to, and enter hepatic cells (Paget, 2011). During the exo-erythrocytic stage, sporozoites differentiate within hepatic cells into schizonts ② which contain multiple uninucleate merozoites. The hepatic schizont ruptures, releasing merozoites into the bloodstream ③ where after asexual multiplication occurs in erythrocytes ④. Ring stage trophozoites mature into blood schizonts ⑤ that release more merozoites ⑥ or alternately differentiate into gametocytes ⑦. These erythrocytic stage parasites are responsible for clinical manifestations of the disease due to the hosts immune system being triggered resulting in proinflammatory molecules being released, accounting for the associated symptoms of fever and chills.

The gametocytes (Male: microgametocytes; Female: macrogametocytes) are ingested by an *Anopheles* female mosquito during a blood meal ⑧, thereby continuing the transmission-infection cycle. During the sporogonic stage, the ingested macrogametocytes and microgametocytes penetrate the mosquito's stomach wall ⑨ and produce a zygote ⑩ which elongates into an ookinete ⑪. The ookinetes invade the midgut wall of the mosquito and develop into oocytes ⑫ which rupture and release sporozoites that travel to the salivary gland of the mosquito ⑬. Inoculation of a new human host perpetuates the malaria parasite life cycle (Paget, 2011).

2.1.3. Classification of Anti-Malarial Drugs

Anti-malarial drugs can be classified according to their target stage in the parasitic life cycle. There are four basic categories into which anti-malarial drugs may be categorised; with some anti-malarial drugs having more than one target stage. Figure 2.1 lists a few examples of anti-malarial drugs in each of the following categories:

- **Tissue schizontocides:** Prevents invasion of malaria parasites into red blood cells in the pre-erythrocytic stage.
- **Blood schizontocides:** Eliminates parasites in the human red blood cells, thus affecting the erythrocytic stage.
- **Gametocytocides:** Eliminates sexual forms of the parasites in hepatic circulation preventing re-uptake and consequently infection of the mosquitoes.
- **Sporontocides:** Inhibits sporogony from occurring within the mosquito (Vinetz *et al.* 2018).

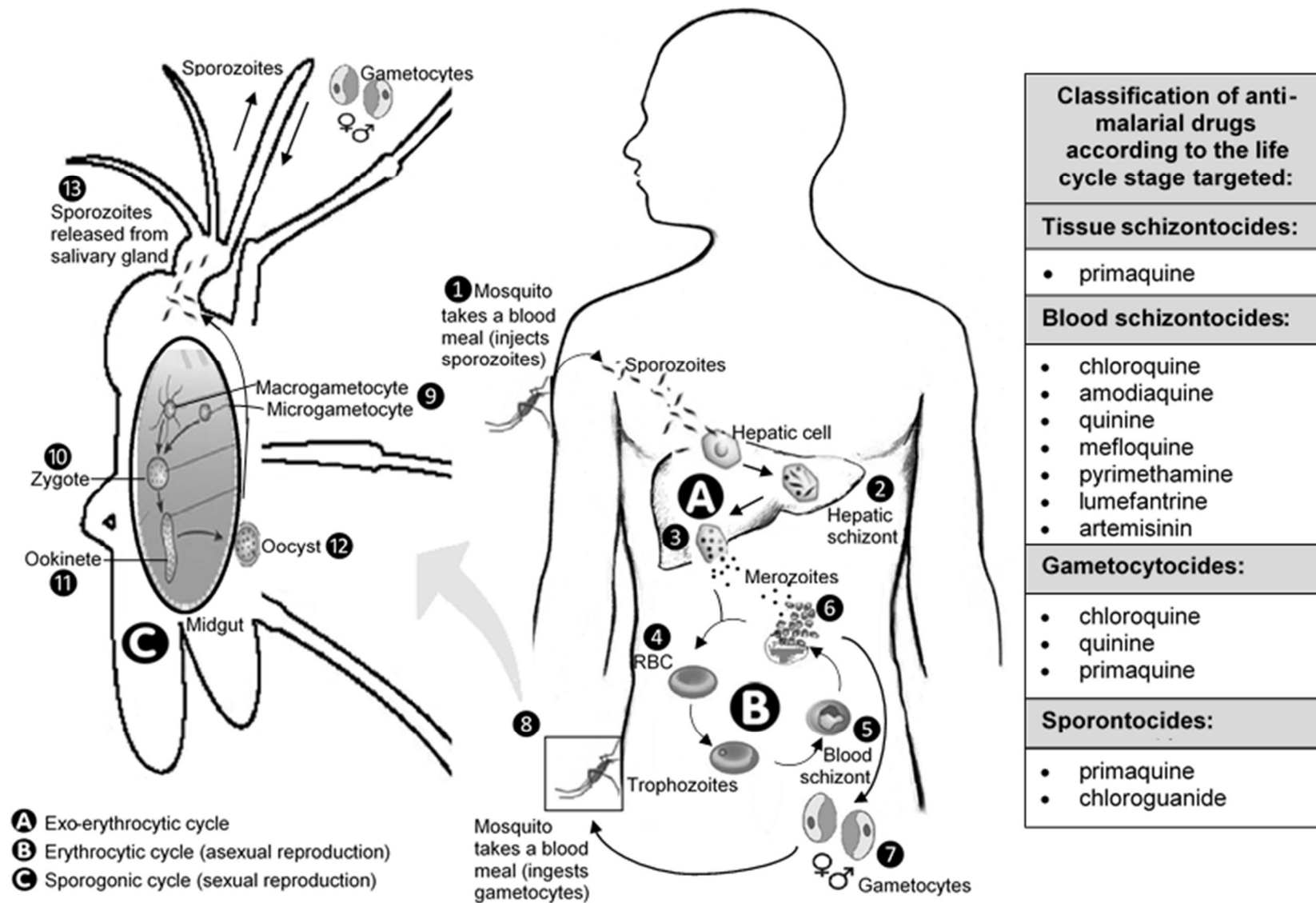


Figure 2.1: Malaria parasite life cycle and examples of anti-malarial drugs per category (Vinetz *et al.* 2018)

2.1.4. Artemisinin-Based Combination Therapy

As stated previously, the WHO recommends artemisinin-based combination therapy (ACT) as mainstay treatment for *P. falciparum* uncomplicated malaria due to the fact that the artemisinin antimalarial drugs are currently the most effective. With no alternative treatment expected to enter the market in years to come, it is vital that their efficacy is preserved. In order to do so, fixed dose combination treatment has been suggested. Fixed dose formulations combine two active ingredients with different mechanisms of action into one, co-formulated dosage form. This is strongly preferred to co-packing of the individual drug components, as fixed dose formulations facilitate adherence and reduce the potential use of individual components as monotherapy. The use of artemisinin and its derivatives has been discontinued as oral monotherapy since this promotes the development of artemisinin resistance (WHO, 2015a).

The following ACTs are recommended by the WHO (2015b) to treat children and adults with uncomplicated *P. falciparum* malaria (except pregnant women in their first trimester):

- Artemether + lumefantrine
- Artesunate + amodiaquine
- Artesunate + mefloquine
- Dihydroartemisinin + piperazine
- Artesunate + sulfadoxine-pyrimethamine

Treatment with one of the above-mentioned ACT regimens should provide three days of treatment. A three-day course with an artemisinin derivative will cover two asexual cycles which ensures that only a small percentage of parasites will remain in the bloodstream for clearance by the partner drug (WHO, 2015b). Artemether-lumefantrine combination is given twice daily for 3 days (total, six doses) and is approved for the use in adults, children and infants with a body weight above 5 kg. The first two doses should ideally be given 8 h apart. A single double-fixed dose tablet contains 20 mg artemether and 120 mg lumefantrine which is prescribed according to body weight as stated in Table 2.1.

Table 2.1: Guidelines for the treatment of uncomplicated malaria with artemether and lumefantrine (2015b)

Body Weight (kg)	Dose (mg) artemether + lumefantrine given twice daily for 3 days
5 – <15	20 + 120 (equivalent to 1 tablet)
15 – <25	40 + 240 (equivalent to 2 tablets)
25 – <35	60 + 360 (equivalent to 3 tablets)
>35	80 + 480 (equivalent to 4 tablets)

2.2. Solid Oral Dosage Forms

2.2.1. Classification of Tablets According to Release Profiles

Tablets are defined by the British Pharmacopoeia as solid preparations each containing a single dose of one or more active ingredient(s) (BP, 2018). Within this definition, tablets may be divided into several categories according to their target site or release behaviour. Some broad categories and examples are provided below to afford an overview of solid oral dosage forms (SODFs). Note the placement of lipid matrix tablets within this functional hierarchy.

Immediate release tablets: refers to conventional tablets that start to disintegrate and release a drug immediately after ingestion, i.e. no lag time exists (Aulton, 2018).

Modified release tablets: defined by the BP (2018) as coated or uncoated tablets that contain special excipients or are prepared by special procedures, or both, designed to modify the rate, the place, or the time at which the active component(s) are released. Modified release tablets can be further divided as follows:

- **Delayed release:** release of the active ingredient at a desired time is achieved via a barrier coating that delays the penetration of water into the tablet until the tablet has reached the desired target site in the intestinal environment. An example is enteric coated tablets (BP, 2018).
- **Extended release:** may be subdivided into controlled release or sustained release tablets.
 - **Controlled release tablets:** designed to release active ingredient(s) at a predetermined rate in order to maintain a constant plasma-drug concentration over a specific period of time and will maintain constant drug levels in blood or tissue for a period of time (McConnell & Basit, 2018).
 - **Sustained release tablets:** a portion of the drug is released immediately to achieve an initial therapeutic effect where after the remaining maintenance dose is released over a prolonged period. However, the therapeutic level is not constantly maintained. Sustained release can be achieved via multiparticulate tablets (crystals, pellets, granules, particles) or monolithic tablets (tablets coated with an inert polymer or matrix tablets) (Bauer *et al.*, 1998).

Sustained release tablets can be classified further according to the drug release mechanism employed as follows (McConnell & Basit, 2018):

- **Diffusion-controlled release systems:** the diffusion of dissolved drug through a polymeric barrier is the rate-determining step. This can be achieved via:

- **Reservoir systems:** the water-soluble polymer material encases a core of drug and the drug will partition into the membrane and exchange with fluids surrounding the tablet. Example: Membrane systems.
 - **Matrix systems:** the drug is dispersed as solid particles within a matrix formed by a water-insoluble polymer or in a matrix forming a gel when in contact with water. Example: Monolithic tablets.
- **Dissolution-controlled release systems:** dissolution of the drug in gastrointestinal juices will control the release of the drug. Encapsulation dissolution control or matrix dissolution control may be employed to achieve a dissolution-controlled release system. An example is gastro-resistant tablets.
 - **Erosion-controlled release systems:** disintegration of the tablet matrix is as a result of degradation and is thus the release rate-determining step. This is a chemically controlled process and both reservoirs and matrices may be used.
 - **Osmotic-controlled release systems:** the flow of liquid into the dosage form, driven by the difference in osmotic pressure between the inside and outside of the tablet is the release rate-determining step. Osmotic-controlled release systems can be designed as single-unit or multiple-unit systems depending on the number of release orifices. This is a solvent activated process (Langer & Peppas, 1983).

The characteristics of the retardant material employed in the formulation of matrix systems will ultimately determine the release characteristics of the sustained release dosage form (Ninama *et al.*, 2015). Figure 2.2 serves as a summary of the different types of SODFs available as discussed above and highlights the release mechanism categories where lipid matrix tablets in particular may be utilised.

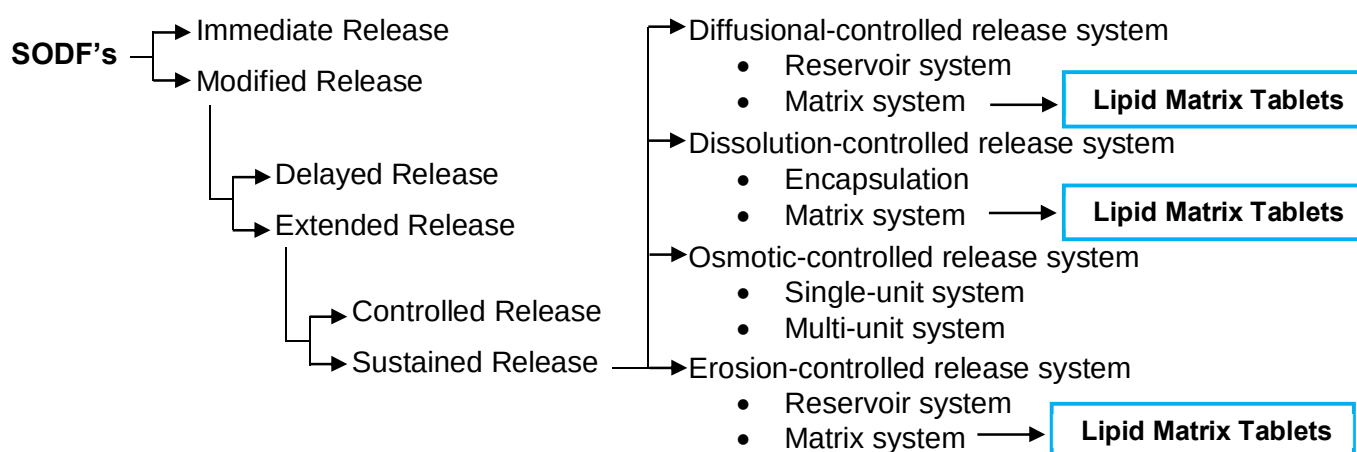


Figure 2.2: Classification of solid oral dosage forms according to release mechanisms indicating the placement of lipid matrix tablets within this functional hierarchy

2.2.2. Classification of Matrix Systems According to Retardant Material Used

2.2.2.1. Hydrophilic matrices

The rate controlling material in a hydrophilic matrix system is water soluble and swells in an aqueous environment. A gelling agent, normally a hydrophilic polymer, forms the matrix system. Non-ionic soluble cellulose such as hydroxypropyl methylcellulose (HPMC) and water-soluble, polysaccharide containing natural gums, e.g. xanthum gum and locust bean gum are often utilised to form hydrophilic matrices (Ninama *et al.*, 2015). The resulting tablet has drug particles interspersed between polymer particles which swell upon exposure to fluid, producing a gel matrix. This gel allows drug particles to be released either through dissolution of the gel or erosion thereof. The rate at which water can diffuse through the dry tablet and the resulting hydrated gel, is the rate-determining step. Polymers, for example HPMC and polyethylene oxide, form very viscous solutions due to their structure that is more dynamic as opposed to true gels such as cross-linked alginic acid (McConnell & Basit, 2018).

2.2.2.2. Hydrophobic matrices

Hydrophobic matrices were one of the earliest forms of oral extended drug release dosage forms. Premarin® tablets were the first to introduce the concept in the 1950s (Ninama *et al.*, 2015; Nisha *et al.*, 2012). A hydrophobic or inert polymer is used to form the matrix system wherein the drug is embedded and is compressed to form a tablet. Sustained release is obtained as the active ingredient must diffuse through a network of channels that exist between the compacted polymer particles. Hydrophobic matrices remain intact during transit throughout the gastrointestinal tract (GIT). The rate-controlling step in these formulations is the penetration of liquid into the matrix and the hydration of the interspersed channels within the polymer, allowing drug to diffuse out (McConnell & Basit, 2018). The following hydrophobic polymers are used to produce hydrophobic, non-swellable matrices: stearic acid, lauryl alcohol, cetyl alcohol, cetostearyl alcohol, carnauba wax, beeswax and microcrystalline wax. These matrices become inert in the presence of gastrointestinal fluid and diffusional-release is a possible drug-release mechanism (Ninama *et al.*, 2015).

2.2.2.3. Lipid matrices

These matrices are prepared from lipid waxes and related materials. Drug release characteristics for these systems are more sensitive to digestive fluids than totally insoluble polymers as drug release occurs via both pore diffusion and erosion. Carnauba wax in combination with either stearyl alcohol or stearic acid is a common retardant base combination employed to achieve sustained release. Other examples include, natural oils and fats, as well as semi-synthetic triglycerides (Ninama *et al.*, 2015).

2.2.2.4. Biodegradable matrices

Biodegradable matrices are denoted by polymers comprising monomers linked via their functional groups which have unstable linkages in the backbone of the structure. Enzymes created by the surrounding living cells or non-enzymatic degradation biologically erode the polymer into oligomers and monomers that can be metabolised or excreted (Munday & Cox, 2000). Natural polymers such as proteins and polysaccharides are examples of materials used to form biodegradable matrices.

2.2.2.5. Mineral matrices

These matrices consist of polymers which are obtained from seaweeds such as a species of brown seaweed (*Phaeophyceae*) that is utilised to obtain alginic acid (Ninama *et al.*, 2015). It swells when placed in water but does not dissolve (BP, 2018) therefore accounting for its use in mineral matrices.

2.3. Targeted Drug Delivery

Modified release dosage forms are designed to release the drug content after a certain period of time has elapsed and are therefore able to offer site-specific targeting in the GIT. Site-specific targeting is achieved due to a lag time that exists between the initial administration of the dosage form and the release of the active ingredient(s) to achieve a pharmacological effect. This type of dosage form is able to overcome the associated drawbacks of the GIT such as transit time, enzyme activity and instability of the active ingredient(s) in gastric fluids (McConnell & Basit, 2013). Figure 2.3 indicates possible target sites when designing modified release dosage forms.

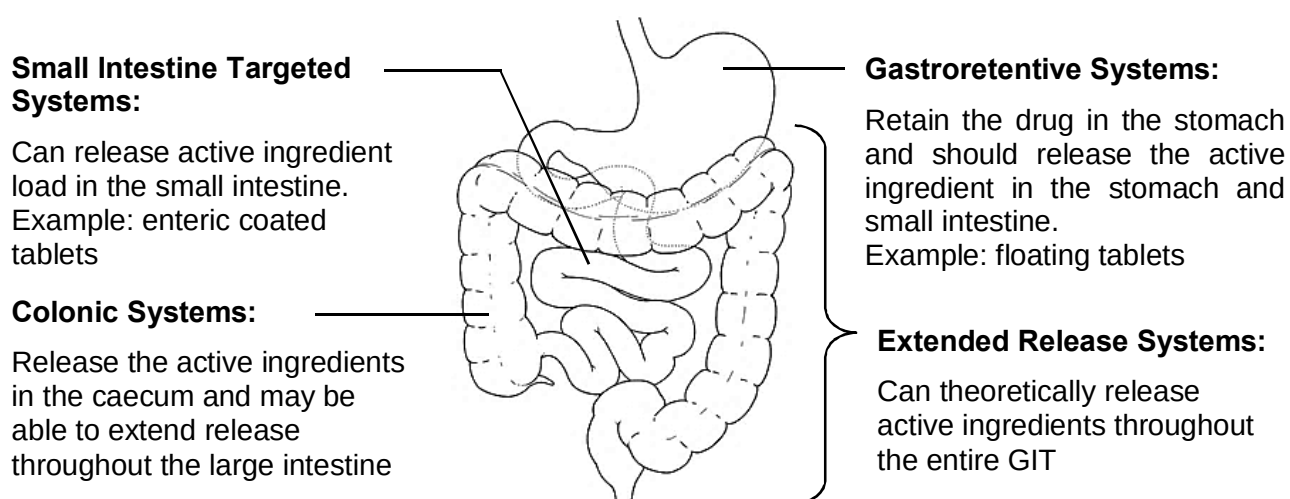


Figure 2.3: Various target sites within the gastrointestinal tract for delayed release dosage forms adapted from McConnell & Basit, 2018

As seen in Figure 2.3, it is theoretically possible for extended release dosage forms to target the entire GIT. For this reason, *in vitro* permeability studies will be conducted on sections of the proximal jejunum excised from porcine intestines as a proof of concept study to assess the validity of the proposed model and identify a possible starting point of drug absorption.

Additionally, sustained release dosage forms are able to avoid the steep plasma peaks associated with immediate release dosage forms and are able to reduce dosing frequency; thereby ultimately increasing patient compliance, which is much needed in the attempt to combat the development of malarial resistance. The current commercially available double-fixed dose combination product for malarial treatment, Coartem®, requires a 3-day treatment regimen with twice daily dosing of four tablets per dose in adults (Carborne *et al.*, 2014; WHO, 2015b). This high dosing frequency, inadequate patient adherence to prescribed antimalarial regimens, and subsequent incomplete courses of treatment, promote the development of drug resistance (Nnamani *et al.*, 2014).

Immediate release dosage forms require multiple dosing which results in periods of time where the plasma drug concentration may indeed exceed the maximum safe concentration, leading to toxicity (Figure 2.4). Thereafter, plasma drug concentrations rapidly decrease and fall below the minimum effective concentration ensuing in subtherapeutic drug concentrations. In this regard, modified release dosage forms offer benefits to health care practitioners and patients alike, with the advantage of controlled plasma-drug concentrations over an extended period of time, thereby reducing dosing frequency and side effects simultaneously (McConnell & Basit, 2018). This reduction in dosing frequency and subtherapeutic levels simultaneously combats the occurrence of malarial resistance as patient compliance is increased and periods where the *plasmodium* parasites are exposed to subtherapeutic levels are decreased.

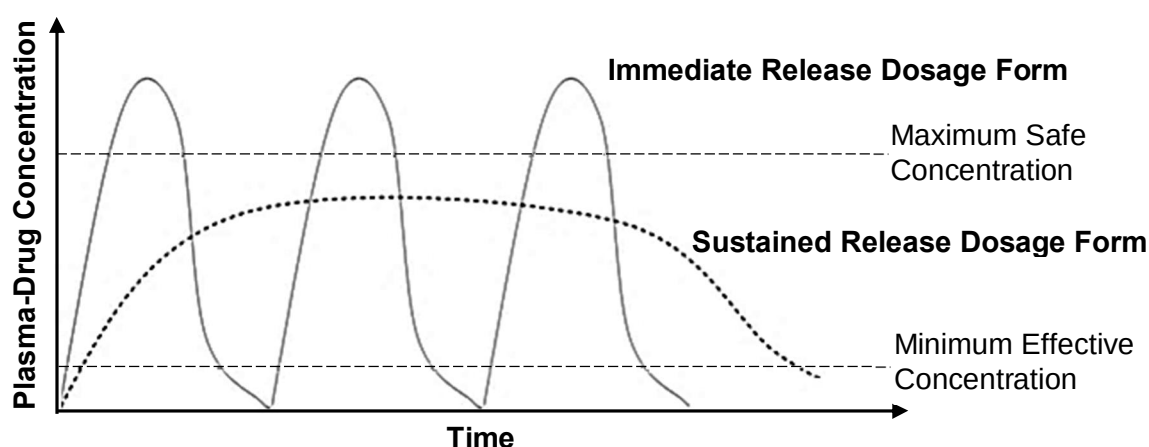


Figure 2.4: A hypothetical plasma-drug profile vs. time graph conveying the effects of immediate release and sustained release dosage forms adapted from McConnell & Basit, 2018

Moreover, modified release dosage forms, specifically the lipid matrix tablets investigated in this study, can be particularly advantageous when working with highly lipophilic drugs for example, as

it may be possible to achieve site-specific targeting with the added advantage of reducing dosing frequency. By being able to release the active ingredient(s) lower down in the GIT, the principles of lipid-based formulations may be fully utilised. Lipid-based formulations employ the body's normal physiological response to the presence of lipids and are built on the premise that the presence of lipids in the small intestine prompts the release of bile that in turn forms a microenvironment in which lipophilic drugs can partition, solubilise and thereby hopefully enhance absorption (Pouton & Porter, 2008). This concept is further explored and explained in Section 2.4.

2.4. Lipid-Based Formulations

The oral route of drug administration is still considered by many as the preferred route of drug administration due to the fact that these dosage forms are convenient for patients and consequently enhance compliance. The incorporation of BCS class II or IV drugs into a formulation can present significant formulation difficulties as lipophilic, poorly water-soluble drugs exhibit reduced absorption from an aqueous environment (Mu *et al.*, 2013). Since the realisation that the co-administration of lipophilic drugs with a fatty rich meal can enhance the drug bioavailability, there has been an increased interest in the development of lipid-based formulations in order to enhance drug solubilisation in the GIT (Charman *et al.*, 1993; Pouton, 2006). To understand the benefits and mechanisms of action of lipid-based formulations, it is important to discuss the physiological response of the body to lipid ingestion.

The presence of chyme containing lipids in the duodenum stimulates the release of the intestinal hormone cholecystokinin (CCK) which relaxes the hepatopancreatic sphincter and stimulates contraction of the gallbladder. This causes the release of bile containing bile salts, phospholipids (primarily phosphatidylcholine) and cholesterol into the small intestine via the common bile duct. Pancreatic fluids containing pancreatic lipase/co-lipase are simultaneously released from the pancreas (Feeney *et al.*, 2016; Porter *et al.*, 2007). The endogenous gastrointestinal fluids (i.e. that secreted in bile) have an inherent capacity to solubilise lipids. Lipid digestion initiates, to a lesser extent in the stomach, via acid-stable lipases and is the source of initial lipid emulsification. Subsequently, the process continues in the small intestine via pancreatic lipases and esterases. In the intestinal milieu, bile components mix with phospholipids to form bile-phospholipid mixed micelles resulting in a range of colloidal species (Feeney *et al.*, 2016; Mu *et al.*, 2013; Porter *et al.*, 2007). The physiological purpose of these colloidal species is to aid in the solubilisation of poorly water-soluble lipid digestion products. In this way, lipid-based formulations exploit the natural process of lipid digestion and are thereby able to provide a microenvironment into which the drug can partition via the formation of exogenous lipid carriers (Porter *et al.*, 2007). Given the high affinity of these drugs for a lipid environment, the colloidal species are ideal for the drug to transfer into where after the digestion products of the lipid substrate, containing drug, are then shuttled to the enterocytes of the intestinal wall for absorption (Mu *et al.*, 2013). Hofmann and

Borgström (1964) were the first to examine the physiological effects of chemical digestion of exogenous lipids and described the bile-phospholipid mixed micelles as responsible for both the solubilisation of the poorly water-soluble drugs as well as the formation of a concentration gradient for lipid absorption.

The administration of the currently available commercial product, Coartem® with the concurrent consumption of a meal rich in fat, is well documented and recommended to invoke the 'food effect' (du Plessis *et al.*, 2015; Kossena *et al.*, 2007; Novartis, 2009). The 'food effect' may enhance the absorption of co-administered lipophilic drugs due to the stimulation of biliary release from the gallbladder which in turn forms a solubilising milieu of acid and homogenised fat to promote dietary lipid absorption (Feeney *et al.*, 2016). Stone *et al.* (2002) studied the effects of administering large quantities of fats equivalent to that consumed with a large fat-rich meal and reported that maximum gallbladder contraction occurs with 10 to 25 g lipid consumption. However, limited data is available on the effect of a small quantity of lipid consumption typically associated with that which could be incorporated into a pharmaceutical solid oral dosage form. On the other hand, Kossena and colleagues (2007) have shown that lipid quantities as low as 2 g are capable of stimulating sufficient bile salt concentrations to invoke intraluminal lipid processing and drug solubilisation. This equates to two capsules containing a lipid-based formulation in the fasted state being adequate to achieve the desired physiological response (Porter *et al.*, 2007; Kossena *et al.*, 2007).

The above physiological responses to lipid ingestion as well as the mechanisms of intestinal drug transport from lipid-based formulations are schematically represented in Figure 2.5. Lipid-based formulations increase absorption from the GIT by accelerating the dissolution process through facilitation of the formation of colloidal species (solubilised phases), changing drug up-take, efflux and disposition by altering enterocyte-based transport (Pramod *et al.*, 2010; Wilson & Mahony, 1997), as well as enhancing drug transport to the systemic circulation and intestinal lymphatic systems (Charman *et al.*, 1993; Driscoll, 2002; Porter *et al.*, 2007). The promising mechanisms of drug uptake, as depicted in Figure 2.5, include: facilitating transcellular absorption of the drug due to increased membrane fluidity ❶; allowing paracellular transport of the drug by opening tight junctions ❷; increasing intracellular concentration and residence time by surfactants due to inhibition of P-glycoprotein and/or cytochrome (CYP) P 450 enzymes ❸; and, lipid stimulation of lipoprotein/chylomicron production for lymphatic uptake ❹.

The lymphatic system plays an important role in the transport of drugs to the systemic circulation given its extensive drainage network that extends throughout the body in proximity to the circulatory system (Kalepu *et al.*, 2013; Kim *et al.*, 2013). The primary function of the lymphatic system is to return products such as sugar, oxygen, proteins and lymphocytes that have leaked

into the interstitial space, to the blood as well as the transport of essential immune cells around the body. Additionally, it may also contribute to the absorption of products from lipid digestion, in particular long chain fatty acids and lipophilic vitamins. The advantages of this route over direct systemic absorption associated with most solid oral dosage forms, is the avoidance of first-pass metabolism in the liver and degradation of the drug in the GIT. The stomach fluid has a pH range of 1.0 in the fasted state and 3 to 4 in the fed state. This highly acidic environment often leads to the degradation or inactivation of orally administered drugs. Moreover, before entering the systemic circulation, drugs are absorbed into the portal venous system and subjected to first-pass metabolism in the liver. Both of these ultimately lower the bioavailability of a drug (Kim *et al.*, 2013). Furthermore, lymphatic transport has the added advantage of offering site specific targeting of diseases that manifest in the lymph (Kalepu *et al.*, 2013).

The association of lipophilic drugs with chylomicrons within the enterocytes is a prerequisite to access lymph (Attil-Qadri *et al.*, 2013; Porter *et al.*, 2007). Highly lipophilic drugs with a Log P >5 and long chain triglycerides with a solubility >50 mg/kg can potentially utilise the lymphatic system as these drugs will transit across enterocytes and associate with enterocyte lipoproteins to form chylomicrons (Charman & Stella, 1986).

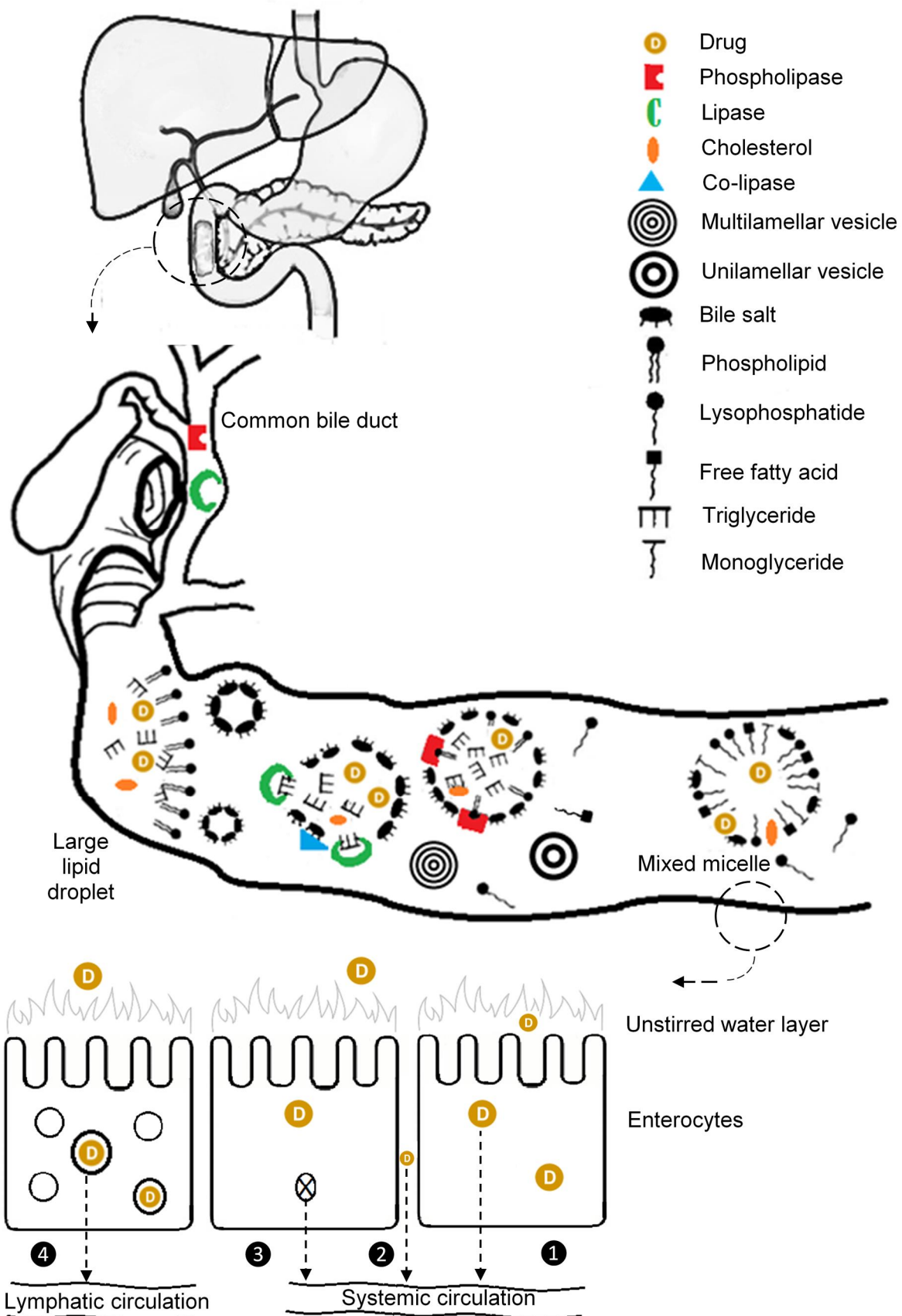


Figure 2.5: Schematic representation of the physiological response to lipid presence in the small intestine and mechanisms of intestinal drug transport from lipid-based formulations adapted in part from Kalepu *et al.*, 2013

The drug containing chylomicrons enter the mesenteric lymph duct, move to the thoracic duct, and finally enter into the systemic circulation at the junction of the left jugular and left subclavian veins. The result of this physiological response and the corresponding anatomy, means highly lipophilic drugs can avoid first-pass metabolism (Kim *et al.*, 2013). Caliph and colleagues (2000) demonstrated that the degree of lymphatic transport for the highly lipophilic antimalarial drug, halofantrine (log P 8.5; triglyceride solubility >50 mg/kg), was strongly correlated to the triglyceride content in the lymph which suggests that lipid digestion products are needed to stimulate the production of chylomicrons. Therefore, lipid-based formulations may be employed to mimic the physiological conditions favourable for lymphatic drug delivery.

2.5. Biorelevant Dissolution Studies

The development of an oral drug delivery system, particularly one incorporating a poorly water-soluble drug compound, requires careful consideration regarding the rate limiting step for drug absorption. An important application of dissolution tests is to predict the *in vivo* performance of a solid oral dosage form. By identifying the limiting factor (solubility, dissolution rate or permeability) as described by the Biopharmaceutics Classification System, it may be possible to design a dissolution study tailored to the needs of the formulated dosage form and respective drug components (Jantratid *et al.*, 2008; Kleberg *et al.*, 2010).

Many dissolution studies use a simple aqueous buffer solution which is not representative of all the physiological aspects in the GIT. Furthermore, the extent of inadequate aqueous solubility for BCS class II and IV drugs is often overstated as this solubility is determined in compendial dissolution media proposed by the Pharmacopoeias; a test consisting primarily of purified water and buffers. The degree of aqueous solubility is therefore misleading as these tests do not account for the significantly higher solubilising capacity of the human intestine as a result of the presence of bile salts (Kleberg *et al.*, 2010).

In order to more accurately predict *in vivo* performance, it is necessary to mimic more of the physiological occurrences and conditions within the GIT. This can be achieved through the utilisation of biorelevant media which considers both the physiological conditions of the GIT and the properties of the drug and dosage form. Predictions of a dosage forms intraluminal performance requires adequate simulation of the stomach and proximal part of the small intestine which requires the incorporation of gastrointestinal fluid properties such as composition, volume, pH, gastric emptying (particularly important for non-disintegrating systems), gastrointestinal enzymes and the presence or absence of food (Zoeller & Klein, 2007).

Sunesen *et al.* (2005) reported that compendial dissolution media often fails to yield *in-vitro-in-vivo* correlations for class II drugs as a result of the absence of relevant physiological parameters. Thus concluding that when optimising a dissolution media's composition, it is crucial that the

physiological relevance is of prime consideration, i.e. biorelevant media is utilised. Additionally, Wang *et al.* (2009) proposed the use of biorelevant media with the incorporation of lipolytic products for lipid-based formulations, particularly those dosage forms containing BSC class II or IV compounds due to drug solubilisation and formulation properties that have a substantial effect on the *in-vitro-in-vivo* correlation. For these reasons, this study will conduct dissolution studies utilising biorelevant media as to mimic the physiological conditions required by lipid-based formulations.

2.6. Lipid Bases Employed in this Study

As previously mentioned, the lipid bases to be utilised in the production of the lipid-based dispersions in this study are stearic acid, cetyl alcohol, glycerol monostearate and coconut oil. The lipid bases employed influence the characteristics of the lipid dispersions formed and so careful consideration was needed in their selection.

Stearic acid is a saturated long-chain fatty acid and is found in many animal, and plant fats and oils. It is a commonly used amino acid in the manufacturing of hair and skin care products as it is the major component of cocoa butter and Shea butter. It is added to aid thickening and hardening of the other ingredients that are utilised in the production of soaps and this characteristic may assist with tablet hardness. Stearic acid is also able to act as a surfactant, meaning it is able to lower the surface tension of oils. This may prove beneficial during tablet dissolution and assist with the absorption of the highly lipophilic drugs artemether and lumefantrine. Stearic acid additionally has lubricating properties (Wang *et al.*, 2010) and is the most frequently used fatty acid boundary lubricant (Morin & Briens, 2013). In general, fatty acids are more effective die lubricants than alcohol based lubricants (Morin & Briens, 2013). These lubricating properties could be highly beneficial during the tableting process as there is a concern that the lipid components in the solid dispersion may cause the formed tablets to stick to the punches and jeopardise tablet integrity. Stearic acid has the highest melting point of the three selected lipids for this study (Table 2.2) and is classified as a non-swellable, hydrophobic matrix forming material (Nisha *et al.*, 2012).

Cetyl alcohol has the lowest melting point of the selected synthetic lipid bases and is also classified as a non-swellable hydrophobic matrix forming material (Nisha *et al.*, 2012). It is a fatty alcohol that was originally isolated from whale oil but is now produced from the reduction of palmitic acid obtained from palm oil. Given that the method of manufacture of the lipid dispersions is hot fusion, a thermal process, the thermal stability and respective melting points of each component to be incorporated into the lipid dispersions were important considerations. Lipid bases with differing melting points were selected in order to evaluate whether the lipid base

melting point would affect manufacturing or the release kinetics of the formulated lipid matrix tablets.

Table 2.2: Physiochemical properties of the three lipid bases utilised in this study (O'Neil, 2011)

		Stearic Acid	Cetyl Alcohol	Glycerol Monostearate
Chemical Properties	Molecular weight (g/mol)	284.484	242.447	420.394
	Hydrogen donating count	1	1	5
	Hydrogen accepting count	2	1	7
	Rotatable bond count	16	14	20
Physical Properties	Smell	Slightly tallow	odourless; faint odour	Mild, fatty odour
	Colour	white; slightly yellow	white	white; almost white
	Appearance	White crystals or powder	Waxy solid; Flakes or granules	Hard, waxy solid either powder, beads or flakes
	Stability	Slowly volatilises at 90; 100°C	Incompatible with strong oxidising agents, strong acids	Stable under ordinary conditions
	Melting point (°C)	69; 72 67; 70	49.3 47; 52	54; 66 56; 58
	Soluble in	Acetone, chloroform, carbon disulfide	Ether, benzene, chloroform, acetone	Hot organic solvents: alcohol, benzene, ether, acetone
	Insoluble in	Water	Water	Water
	Density (g/cm ³)	0.83; 0.94	0.8187	0.970
	Log P	8.23	6.83	7.46
	pKa	4.75	16.20	13.62

Glycerol monostearate, on the other hand, is a glycerol ester of stearic acid and is classified as a monoacylglycerol of the glyceride family. It occurs naturally in the body as a product of the breakdown of fats by pancreatic lipase and is also found in fatty meals. As described in Section 2.4, the body's natural physiological response to the presence of lipids forms the rationale for lipid-based formulations. Glycerol monostearate appears as an endogenous lipid in the body

and therefore its appearance in the GIT in an exogenous form will hopefully mean it will be easily processed in the same way as the endogenous form. This could ultimately be beneficial for the absorption of artemether and lumefantrine when incorporated into this lipid base. Raw glycerol monostearate powder is usually in the β -form, however, once heated and allowed to cool it assumes the α -form. The α -form, however, has a less dense crystal structure compared to the β -form which can affect powder flow and tablet properties (Xia *et al.*, 2013). This will be evaluated during pre-formulation studies.

As previously stated, Charman & Stella (1986) reported that long chain triglycerides with a solubility >50 mg/kg and lipophilic drugs with a Log P value >5 will transit across enterocytes and associate with enterocyte lipoproteins to form chylomicrons. These chylomicrons containing the active ingredients then enter the lymphatic system followed by the systemic circulation. Glycerol monostearate is a glyceride and may therefore facilitate the uptake of the active ingredients into chylomicrons. Additionally, all three synthetic lipid bases have a Log P value >5 which may further facilitate the uptake of the active ingredients, especially because the lipid components form part of the mixed micelle systems that aid in the transportation of the drug into the bloodstream.

2.7. Hot Melt Extrusion

Hot melt extrusion is the process of applying heat and pressure to melt a material such as a polymer, which is forced through an orifice in a continuous process. This process is well known and used to produce products of uniform shape and size. It is a method that dates back to the 1930s within the plastic, rubber and food industries and has been utilised in the pharmaceutical industry since the 1970s (Kolter *et al.*, 2012).

In the pharmaceutical industry, co-rotating twin screw extruders are used to prepare solid dispersions. The polymeric material is pumped through rotating screws at temperatures above its glass transition temperature and sometimes the melting temperature in order to achieve molecular mixing of the active ingredients incorporated into the polymeric material. The molecular mixing can result in the formation of an amorphous product with uniform shape and density which thereby increases the dissolution profile of the poorly water-soluble drug (Patil *et al.*, 2016). This method may be utilised to enhance the active ingredients' bioavailability (Particle sciences, 2011). In this study, the manual application of the above described process, whereby heat was applied directly to the physical mixture of the active ingredients and lipid base until they melted, was employed and is referred to as hot fusion (Kalaiselvan *et al.*, 2006; Kalia & Poddar, 2011).

The advantage of hot fusion and hot melt extrusion is that it is a solvent free, fast and continuous process (Kolter *et al.*, 2012). It is able to overcome poor bioavailability due to inadequate drug solubility by forming a solid solution or dispersion. Drugs or excipients susceptible to hydrolysis may be incorporated into a dosage form prepared via hot melt extrusion and it therefore offers an

alternative form of manufacture to wet granulation. It can further overcome unreliable sustained release action and poor stability or tolerability of the drug in the stomach by preparing a single or multiple unit sustained release or enteric dosage forms, respectively (Kolter *et al.*, 2012).

The most obvious drawback of this process is its elevated temperatures. Active ingredients chosen to be incorporated into solid dispersions prepared via hot fusion or hot melt extrusion need to be thermally stable. The temperatures that the active ingredients are exposed to during this process are substantially higher compared to the drying step in wet granulation. Moreover, a pre-requisite for the polymeric material is appropriate thermoplastic behaviour (Kolter *et al.*, 2012).

There are three primary applications for hot fusion and hot melt extrusion in the pharmaceutical industry which includes bioavailability enhancement, controlled release and safer medication. This method of solid dispersion preparation was selected for this study for the benefits of all of the above-mentioned applications. The lipid matrix tablets intended to be formulated in this study are based on the rationale that the method of manufacture (hot fusion) together with the inclusion of a lipid base (lipid-based formulations), will synergistically increase the bioavailability of the incorporated active ingredients, for which poor bioavailability has been a significant problem.





3.1. Introduction

This chapter provides a detailed explanation of the various methods utilised in this study to manufacture and evaluate the formulated lipid matrix tablets containing a double-fixed dose combination of artemether (20 mg) and lumefantrine (120 mg). Initially, pre-formulation studies were conducted to verify the active ingredients and to assist in the selection of appropriate excipients to manufacture the lipid matrix tablets. Excipients were selected for evaluation according to their unique properties and characteristics identified in literature. The flow properties and thermal profiles of the active ingredients, fillers and the manufactured solid lipid dispersions were successively characterised.

Subsequently, a full factorial design of experiments was employed to evaluate the formulation variables and determine the significance of an observed response between the numerous variables. Once lipid matrix tablets were manufactured via direct compression, the physical properties of the different tablet formulations were evaluated. All formulations that failed to meet the BP (2018) specifications regarding tablet physical properties were removed from further testing which encompassed drug content assay, swelling and erosion experiments.

Thereafter, dissolution studies were conducted on the remaining, viable formulations in order to compare these different formulations and establish which of them could be considered as the most ideal or optimum for delivery of artemether and lumefantrine in a double-fixed dose combination. The absorption profiles of the optimum formulation(s) were additionally compared to the commercially available product, Coartem® in order to identify whether lipid-based formulations could enhance the solubilisation of these lipophilic drugs in different pH environments. Next, *in vitro* permeability studies of the optimum formulation were conducted as a proof of concept across jejunum sections of porcine intestinal tissue, in order to determine if any active compounds were absorbed in significant concentrations and whether significant absorption occurred in the jejunum. In doing so, it was possible to assess whether the formulated research problem had been resolved. A schematic illustration of the summarised experimental plan followed for this study is provided in Figure 3.1.

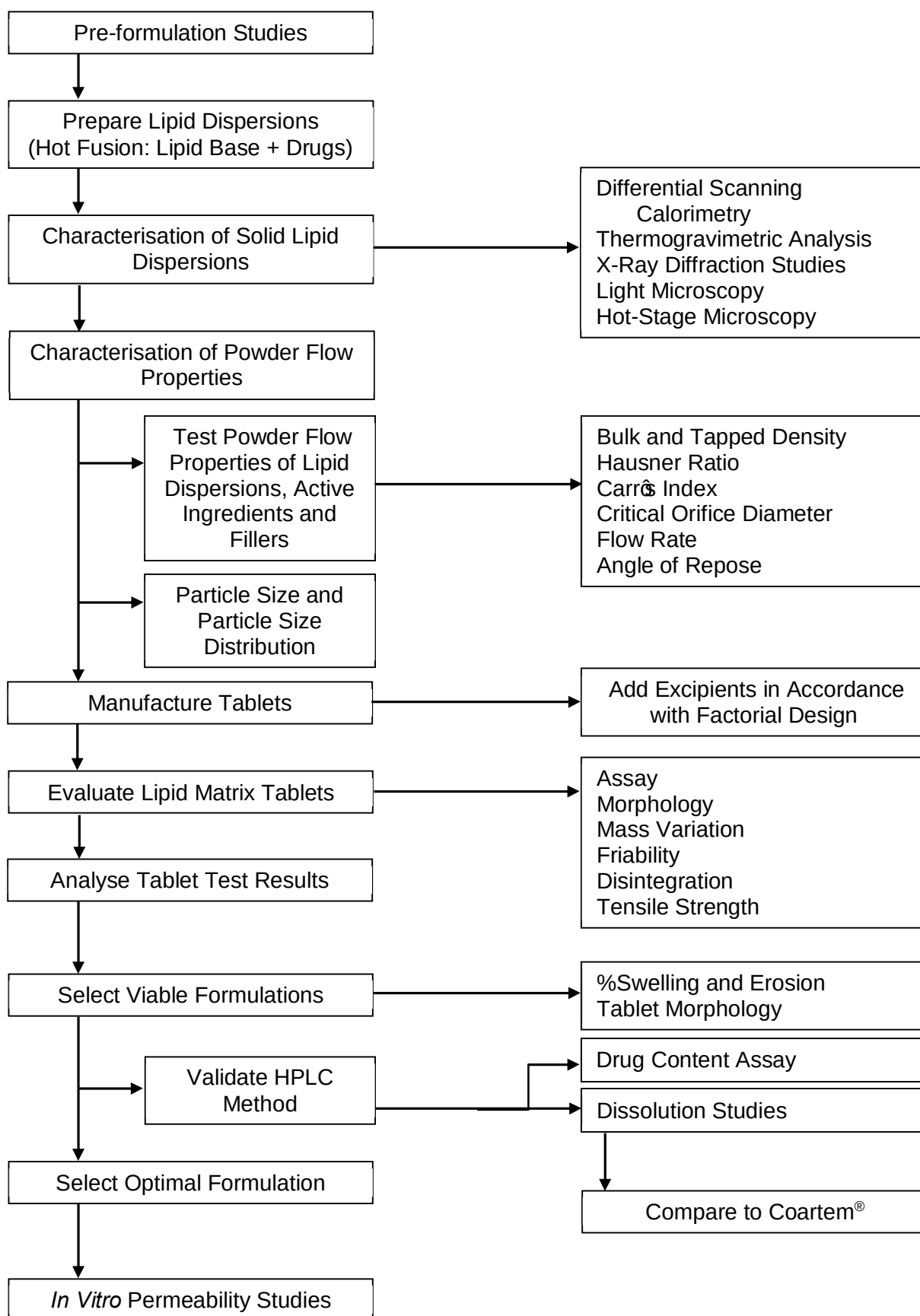


Figure 3.1: Schematic illustration of the summarised experimental plan followed in this study

3.2. Materials

The materials utilised in this study including their lot numbers and respective manufacturers are summarised in Table 3.1. All the materials were of analytical grade and stored correctly throughout the duration of this study.

Table 3.1: List of materials, lot numbers and respective manufacturers

Material	Lot Number	Manufacturer
Artemether	120702	Kindly donated by Prof. Wilna Liebenberg & DB FINE CHEMICALS (PTY) LTD
Lumefantrine	20161213	Kindly donated by the MRC Flagship Programme Cipla (Pty) Ltd, India
MicroceLac® 100	L1411A4971	Meggle Group, Wasserburg, BG Excipients & Technology
CombiLac®	L1434	Meggle Group, Wasserburg, BG Excipients & Technology
RetaLac®	L416300	Meggle Group, Wasserburg, BG Excipients & Technology
PharmaceL® 101	100043	DFE Pharma, Goch: Germany
Stearic acid	32192	Associated Chemical Enterprises (Pty) Ltd. SA
Glycerol monostearate	31566i 31i 1	Associated Chemical Enterprises (Pty) Ltd. SA
Cetyl alcohol	36653i 82i 4	Merck (Pty) Ltd. Gauteng, South Africa
Coconut oil	31CR11/05/16	Lifestyle Food, Dischem Group, Glen Austin, South Africa
Magnesium stearate	21203	Kirsch Pharma, South Africa
Hydrochloric acid	UN1789	Merck (Pty) Ltd. Gauteng, South Africa
Sodium hydroxide	7569	Associated Chemical Enterprises (Pty) Ltd. SA
Tri-sodium orthophosphate anhydrous	Multiple batches used	Associated Chemical Enterprises (Pty) Ltd. SA
Bile salts	SLBH4108V	Sigma-Aldrich
Phosphatidylcholine	0001410262	Sigma-Aldrich
Methanol	Multiple batches used	Associated Chemical Enterprises (Pty) Ltd. SA
Acetonitrile		Merck (Pty) Ltd. Gauteng, South Africa
Octane-sulphonic acid	K48694707	Merck (Pty) Ltd. Gauteng, South Africa
Coartem®	KF522	Novartis (Pty) Ltd. Johannesburg, South Africa

3.3. Pre-formulation Studies

Pre-formulation studies form the first step in the rational development of a dosage form as it provides the formulator with useful information regarding the physicochemical characteristics of the active ingredients and excipients intended for manufacture (Chaurasia, 2016). Once the active ingredients had been verified via IR spectra analysis and the fillers selected, pre-compression parameters were evaluated. Pre-compression parameters included characterisation of the active ingredients thermal analysis profiles consisting of differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), X-ray diffraction studies, hot-stage microscopy, light microscopy, particle size and particle size distribution, as well as powder flow properties.

3.3.1. Infrared Spectroscopy of Active Ingredients

Infrared (IR) spectroscopy studies the interaction of infrared light with matter and determines the functional groups which may be used to identify unknown samples, examine the quality of a sample or determine the amount of compounds in a mixture. IR spectra characterises vibrational bands by their frequency, intensity and band shape (Larkin, 2018). The identities of the active ingredients for this study were verified via analysis utilising an IR-spectroscope (Bruker Alpha Sample Compartment RT-DLaTGS, USA) and OPUS 7 spectroscopy software. An Attenuated Total Reflection (ATR) mode was used as this technique is easier to use than conventional transmission modes and is able to evaluate all sample types (solids, liquids, powders, pastes and pellets) on the ATR crystal. This technique has the added advantage that no dilution of the sample is required. The analysis was conducted by comparing 2 mg samples of the active ingredients to reference standards in order to verify the identity of the batches of artemether and lumefantrine to be utilised in this study and to ensure no contaminants were present. The resulting reference standard and active ingredients IR spectrums were compared by means of data overlays in order to assess the peaks and intensities generated, with a correspondence in peak position and relative size indicating a positive identification. The spectral range was 4000–400 cm^{-1} with a 2 cm^{-1} resolution.

3.3.2. Selection of Fillers

In order to produce lipid matrix tablets by means of direct compression, four fillers, namely: MicroceLac® 100, RetaLac®, CombiLac® and PharmaceL® 101, were selected based on their varying compositions and tableting properties. MicroceLac® 100 comprises 75% β -lactose monohydrate and 25% microcrystalline cellulose (MCC), and is designed for direct compression. MicroceLac® 100 has good flowability and compactibility compared to the individual component characteristics (MEGGLE®, 2014a). It is well suited for both low- and high-dosage formulations and its good flow rate renders it a suitable choice of filler for this study. RetaLac® consists of hypromellose and milled β -lactose monohydrate in a 1:1 ratio. It has a monoparticulate structure

with superior flowability and blendability; and is specifically designed for the direct compression of modified release dosage forms (MEGGLE®, 2014b). RetaLac® is able to control the release of the active ingredient(s) from the dosage form primarily by controlling the diffusion rate through its hydrophilic matrix (MEGGLE®, 2014b). CombiLac® comprises a ternary combination of 70% β-lactose monohydrate, 20% MCC and 10% white, native corn starch. It was specifically designed to ease the development and manufacture of solid oral dosage forms (MEGGLE®, 2014c). In comparison to its individual components, CombiLac® provides enhanced compactibility and flowability which is necessary for increased production rates and decreased mass variation. Lastly, Pharmacel® 101 is manufactured by DFE Pharma® and is a pure MCC filler. It is recommended by DFE Pharma® for direct compression as MCC compacts effectively, thereby suggesting it as an ideal filler choice for this study (DFE Pharma®, (n.d.)).

3.4. Preparation of Lipid Dispersions by Means of the Hot Fusion Method

Double-fixed dose lipid dispersions containing 20 mg artemether and 120 mg lumefantrine were prepared by means of the hot fusion method (Obaidat & Obaidat, 2001) as seen in Figure 3.2. The individual lipid base (glycerol monostearate, stearic acid, cetyl alcohol or coconut oil) was weighed utilising a Mettler Toledo® balance (Mettler, Germany, Model MS205DU) and melted by continual stirring in a porcelain dish in a water bath maintained at 75°C (± 0.5°C). The accurately weighed fixed dose combination of artemether and lumefantrine was added to the melted lipid in the predetermined lipid:drug weight ratio of either: 0.5:1; 0.75:1 or 1:1, and continually stirred until a homogenous mixture was obtained (Obaidat & Obaidat, 2001; Özyazici *et al.*, 2006). The molten mass was allowed to cool down and solidify at room temperature (approximately 25°C ± 0.5°C) where after the hardened mass was crushed and screened through a size 30 mesh or 595 µm sieve. All the resulting powders (solid lipid dispersions) were stored at room temperature in glass containers sealed with Parafilm®, until utilised.

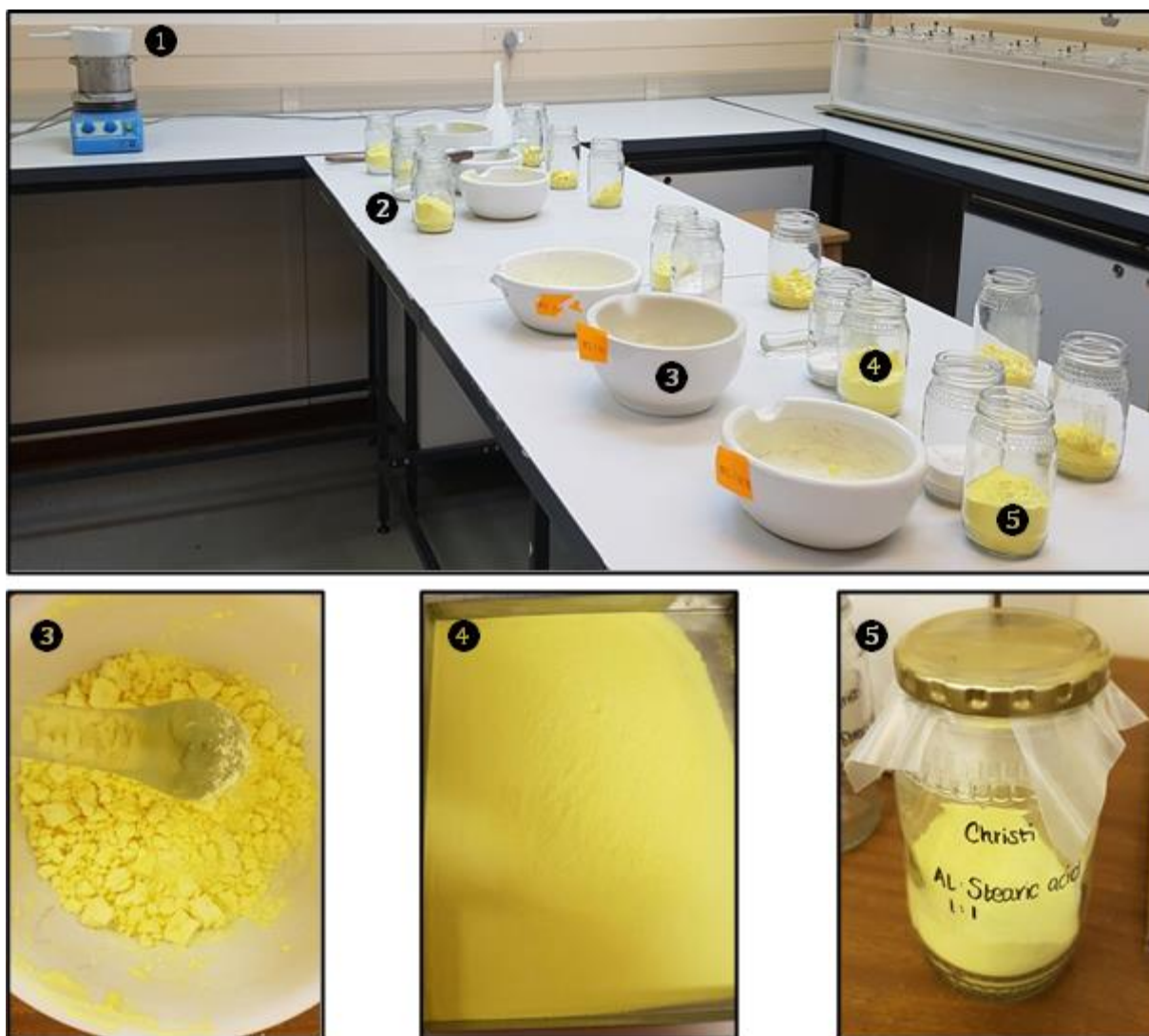


Figure 3.2: Preparation of solid lipid dispersions by means of the hot fusion method. The appropriate lipid:drug ratio is weighed out ❶ and prepared via hot fusion in a porcelain dish in a water bath ❷. The resultant molten mass is allowed to cool and is crushed ❸. The resultant powder is screened through a sieve ❹ which is stored in a glass container sealed with Parafilm® ❺

3.5. Characterisation of Lipid Dispersions

3.5.1. Differential Weight Loss Thermogram (DTG)

A differential weight loss thermogram (DTG) incorporates both differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) simultaneously. DSC is a popular thermoanalytical technique that is used to determine information such as the enthalpies of conformational change, melting point, purity of samples, heat of fusion, possible polymorphs, thermal stability and decomposition of the material being analysed (Sedov *et al.*, 2016). DSC was a tool utilised to determine the melting and recrystallisation behaviour as well as the difference in the heat flow rate of the tested samples under controlled temperature conditions (Hou *et al.*, 2003; Höhne *et al.*, 2003) with the presence of any endothermic and/or exothermic reactions being indicative of an interaction between the tested components (Byrn *et al.*, 1999). TGA is utilised to investigate chemical reactions in solid and liquid states at elevated temperatures. Under controlled heating conditions the structural changes and percentage weight loss can be studied (PerkinElmer, 2010). TGA is a quantitative measurement that monitors sample mass loss normally associated with decomposition, dehydration and/or oxidation as it is exposed to increasing temperatures (Klanjnik *et al.*, 2010).

A DTG is capable of simultaneously measuring the mass loss (TGA) and heat flow (DSC) of a sample during the heating process. With this combined technique, information about the mass loss, melting point, glass transition, solid-state transformation(s), loss of solvents and degradation of a sample could be obtained (Brown, 2001).

For this study, DSC- and TGA-thermograms of the following samples were obtained: individual active ingredients, lipid bases and artemether/lumefantrine/lipid dispersions in pre-established ratios utilising a Mettler DTG 3⁺ (Mettler Toledo, Greifensee, Switzerland). Each sample of 5 mg was weighed and placed in an open aluminium pan (100 µl). Samples were heated at 10°C.min⁻¹ under a nitrogen purge with a gas flow equalling 30 cm³.min⁻¹ until a temperature of 150°C was obtained (Lemmer, 2012; de Kock, 2005). The temperature of the system was determined according to the melting points of the active ingredients and excipients tested.

3.5.2. X-Ray Powder Diffraction Studies

X-Ray Powder Diffraction studies are used to qualitatively identify the crystalline structure of a solid sample, verify if amorphous forms exist and to differentiate between them (Connolly, 2012). An X-ray diffractometer (PANalytical, Almelo, The Netherlands) utilising a Ni-filtered CuK α radiation at a wavelength of 1.542 Å was used to analyse the X-ray spectra of the following solid powder samples: artemether; lumefantrine; lipid bases and artemether/lumefantrine/lipid dispersions in pre-determined ratios. The following settings were applied: tube current at 30 mA; accelerating potential at 45 kV; continuous scanning speed was set to 2°/min over a range of 4°

40°C; the divergence slit, anti-scatter slit and detector slit were adjusted to 2 mm, 0.6 mm and 0.2 mm, respectively (Abd-Elbany *et al.*, 2013).

3.5.3. Light Microscopy

A Nikon Eclipse 50i microscope (Nikon, Tokyo, Japan) fitted with a Nikon DS-Fi1 camera (Nikon, Tokyo, Japan) was utilised to capture images of the active ingredients, a physical mixture of artemether/lumefantrine, lipid bases and lipid dispersions in the varying ratios at the ambient temperature of approximately 25°C. These images were used to investigate morphology as well as the form of the active ingredients and lipid bases prior to incorporation into the lipid dispersions which were then compared to the form and components identifiable in the lipid dispersions. A polaroid lens was used to identify the presence of crystalline structures should birefringence be present while polymorphic forms were identified by a lack of colour interference (Stieger *et al.*, 2012).

3.5.4. Hot-Stage Microscopy

The hot-stage microscope employed in this study was a Nikon Eclipse 50i microscope (Nikon, Tokyo, Japan) fitted with a Nikon DS-Fi1 camera and a Linkam THMS600 heating stage (Linkam scientific, Surrey, UK) equipped with a T95 LinkPad temperature controller (Linkam scientific, Tadworth, UK). Samples of the individual active ingredients, lipid bases, and lipid dispersions in varying ratios were heated at a heating rate of 5°C.min⁻¹; and a series of images captured with the corresponding temperature recorded. The initial image in each series was captured at 25°C to represent the samples form at ambient room temperature where after the onset of melting of the lipid base was captured along with the temperature at which the individual active ingredient components of the lipid dispersions melted completely.

3.6. Characterisation of Powder Flow Properties

The fillers, active ingredients as well as the double-fixed dose, lipid dispersions were characterised in accordance with the British Pharmacopoeia specifications where applicable (BP, 2018). All these tests were conducted in triplicate. The parameters described in this section were used to evaluate and compare the selected powder excipients and lipid dispersions of this study.

3.6.1. Particle Size and Particle Size Distribution

Particle size can significantly influence the flow properties of powders and is therefore an important parameter to measure. The particle size and particle size distribution of the selected fillers, active ingredients and lipid dispersions were analysed by means of laser diffraction in a Malvern® Mastersizer-2000 instrument, equipped with both a Hydro 2000 SM small volume sample dispersion unit and a Hydro 2000 MU dispersion unit (Malvern® Instruments, UK).

In order to obtain accurate measurements, the samples had to be completely insoluble in the dispersion medium. MicroceLac® 100, RetaLac® and CombiLac® utilised cyclohexane (Peña *et al.*, 2000) as a dispersion medium whereas Pharmacel® 101 was dispersed in ethanol (BP, 2018; Yamamoto *et al.*, 1976). The lipid dispersions in their varying ratios as well as both active ingredients utilised distilled water as the dispersion medium. The selected dispersion mediums pertaining to the relevant samples were run initially as a control reference to account for any fluctuations the medium itself may cause during particle size measurements.

Once the corresponding dispersion medium had been chosen for the sample, 6 ml was added to the sample to disperse the particles. A sufficient amount of sample powder was added to the dispersion medium to obtain an obscuration between 10 and 20%. Particle size analysis only commenced once the desired obscuration had been reached. Each measurement consisted of a total of 12 000 snaps. Each sample's particle size and particle size distribution was measured in triplicate in order to calculate the mean value.

3.6.2. Powder and Lipid Dispersion Bulk and Tapped Densities

A 100 g powder sample was weighed (M) and transferred into a 250 ml graduated cylinder to calculate the bulk (ρ_b) and tapped (ρ_t) densities. The bulk density refers to the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. The tapped density, on the other hand, is obtained by mechanically tapping a sample and noting the volume or mass reading once no further change is observed. The initial volume of the powder sample was noted and recorded as the bulk volume (V_b) where after the cylinder was connected to the Tap Density Tester (Erweka®, SVM 121/221, Germany) and vibrated for 3 min at 200 taps/min; the resultant volume was recorded as the tapped volume (V_t). The bulk (ρ_b) and tapped (ρ_t) densities (g.ml^{-1}) were calculated using Equation 3.1 and 3.2, respectively.

$$\rho_b = \frac{M}{V_b} \quad [3.1]$$

$$\rho_t = \frac{M}{V_t} \quad [3.2]$$

3.6.3. Compressibility (Hausner Ratio and Carr's Index)

The Hausner ratio and Carr's index are measures of the propensity of a powder to be compressed. The Hausner ratio and the percentage compressibility (Carr's index) were calculated utilising the bulk (ρ_b) and tapped (ρ_t) densities obtained (BP, 2018). A small difference in bulk and tapped densities obtained indicates a powder that is freer flowing, and interparticulate interactions are less significant. A large difference in densities indicates a poorer flowing powder with more frequent and larger interparticulate interactions. These interactions and differences are reflected

in the calculation of the Hausner ratio and Carr's index utilising Equation 3.3 and 3.4, respectively; and were categorised according to the BP (2018) classifications.

$$\text{Hausner ratio} = \frac{\rho_t}{\rho_b} \quad [3.3]$$

$$\text{Carr's index} = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \quad [3.4]$$

3.6.4. Critical Orifice Diameter

The smallest diameter through which powder freely flows is defined as the critical orifice diameter (Buys, 2006). The critical orifice diameter (COD) of the active ingredients, powder excipients and lipid dispersions were determined using the apparatus and method developed by Buys (2006). The apparatus consists of copper disks with central orifices, ranging from 1.5 to 32 mm. A stack of interlocking copper disks with varying sized openings were arranged with the widest orifice placed on top of the stack to form a smooth funnel through the centre. The assembly was mounted on a tripod, 10 cm above the horizontal work surface and a disc containing a shutter placed at the bottom of the stack was used to conduct each round of testing. To conduct each test, a 100 g powder sample was placed into the cylinder where after, starting from the bottom of the copper disk stack; each individual disk was lifted until powder flowed freely through the orifice. This was noted as the COD of the sample. The test was conducted in triplicate for each powder sample.

3.6.5. Flow Rate

The flow rate (F) was calculated by measuring the time (t) it takes a 100 g powder sample (M) to flow through a funnel with a 10 mm diameter orifice set at a height of 100 mm above the horizontal work surface. A shutter was placed over the funnel outlet which was removed once the funnel was filled with a specific powder sample, allowing the powder to flow freely. The time taken for the 100 g sample to flow completely through the funnel opening was recorded and the flow rate calculated using Equation 3.5. This test was performed in triplicate for each powder sample in order to calculate the mean value.

$$F = \frac{M}{t} \quad [3.5]$$

Where the flow rate (F) was measured in g.s⁻¹; the mass (M) was measured in grams and the time taken (t) in seconds.

3.6.6. Angle of Repose

A 100 g powder sample was transferred into a stainless steel funnel fixed with a shutter covering a 10 mm diameter orifice at the opening of the funnel. Using the fixed-height method (Lavoie *et al.*, 2002; BP, 2018), the shutter of the funnel, set to a height of 10 cm above the horizontal work surface, was opened allowing the powder to flow freely to form a heap on a stainless-steel surface beneath the funnel. A graduated ruler was used to measure the height (h) and radius (r) of the formed powder heap. Employing Equation 3.6, the angle of repose (ϕ) was calculated and the test performed in triplicate in accordance with the BP (2018).

$$\tan \phi = \frac{h}{r} \quad [3.6]$$

3.7. Formulation of Lipid Matrix Tablets

3.7.1. Full Factorial Design

To study the effects of different formulation factors on the physical and dissolution properties of the lipid matrix tablets formulated in this study, a full factorial design was employed as an aid. Factorial design, a form of experimental design, is a methodical and statistically important method used to determine the significance of a response based upon the observed interactions between numerous variables on varying levels (Eriksson *et al.*, 2008). Prior to the development and implementation of factorial design, trial-and-error experiments were conducted to define the effect of one variable on one response. It has subsequently been shown that by conjoining the study of several factors simultaneously, it is possible to reduce the number of experiments performed. Additionally, factorial design can be utilised to obtain the main effects of independent factors or interaction effects of both factors to explain an outcome. Formulations can be optimised using this impartial, methodical guide to assess the effect of several excipients and their varying concentrations on the performance of formulations. Therefore, by using this statistical tool, it is possible to eliminate speculation and guesswork from testing.

In this study, four formulation variables were evaluated, namely, type of lipid base; in varying concentrations of lipid:drug; type of filler; and varying lubricant (magnesium stearate) concentrations. These factors were assessed at different levels, i.e. three types of lipid bases (glycerol monostearate, stearic acid and cetyl alcohol), three lipid:drug ratio per lipid base (0.5:1; 0.75:1; 1:1), four filler types (MicroceLac® 100, RetaLac®, CombiLac®, Pharmacel® 101), and the inclusion of varying lubricant concentrations (0%, 1%, 1.25%). The addition of a lubricant (magnesium stearate) was deemed necessary as during pre-formulation studies it was noted that the formed tablets were sticking to the tablet die, possibly due to the lipid components, resulting in aesthetical defects and problems with mass variation results. The formulation factors and the level at which they were assessed are summarised in Table 3.2.

Table 3.2: Formulation factors, variables and levels investigated in this study

Factors	Variables	Levels
Lipid Base	Glycerol monostearate	3
	Stearic acid	
	Cetyl alcohol	
Lipid:Drug Ratio per Lipid Base	0.5:1	3
	0.75:1	
	1:1	
Filler Type	MicroceLac® 100	4
	RetaLac®	
	CombiLac®	
	Pharmacel® 101	
Lubricant (Magnesium Stearate)	0%	3
	1%	
	1.25%	

A full factorial design (Table 3.3) was utilised to assess the above-mentioned formulation factors and levels in predetermined combinations. Abbreviations were allocated to the combination of factors measured to aid in-text referencing. The assigned code begins with a two-letter designation indicating the type of lipid employed as the base of the lipid dispersion. This is followed by a number indicating the ratio of the aforementioned lipid base to the double-fixed dose combination of artemether and lumefantrine. Thereafter, a single letter indicates the filler type, with the last numerical value representing the percentage lubricant concentration.

Therefore, the code CA0.5M1 signifies cetyl alcohol (CA) as the lipid base in a 0.5:1 lipid:drug ratio; MicroceLac® 100 (M) as filler; and 1% magnesium stearate as lubricant. Similarly, SA0.75R1.25 signifies stearic acid (SA) as the lipid base in a ratio of 0.75:1 with the double-fixed dose; RetaLac® as filler (R); and 1.25% magnesium stearate as lubricant. Glycerol monostearate is denoted as (GM); CombiLac® as (C) and Pharmacel® 101 as (P).

Table 3.3: Factorial design utilised in this study to develop lipid matrix tablets

			Glycerol Monostearate			Stearic Acid			Cetyl Alcohol		
			0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1
MicroceLac® 100	Magnesium Stearate	0%	GM0.5M0	GM0.75M0	GM1M0	SA0.5M0	SA0.75M0	SA1M0	CA0.5M0	CA0.75M0	CA1M0
		1%	GM0.5M1	GM0.75M1	GM1M1	SA0.5M1	SA0.75M1	SA1M1	CA0.5M1	CA0.75M1	CA1M1
		1.25%	GM0.5M1.25	GM0.75M1.25	GM1M1.25	SA0.5M1.25	SA0.75M1.25	SA1M1.25	CA0.5M1.25	CA0.75M1.25	CA1M1.25
RetaLac®		0%	GM0.5R0	GM0.75R0	GM1R0	SA0.5R0	SA0.75R0	SA1R0	CA0.5R0	CA0.75R0	CA1R0
		1%	GM0.5R1	GM0.75R1	GM1R1	SA0.5R1	SA0.75R1	SA1R1	CA0.5R1	CA0.75R1	CA1R1
		1.25%	GM0.5R1.25	GM0.75R1.25	GM1R1.25	SA0.5R1.25	SA0.75R1.25	SA1R1.25	CA0.5R1.25	CA0.75R1.25	CA1R1.25
CombiLac®		0%	GM0.5C0	GM0.75C0	GM1C0	SA0.5C0	SA0.75C0	SA1C0	CA0.5C0	CA0.75C0	CA1C0
		1%	GM0.5C1	GM0.75C1	GM1C1	SA0.5C1	SA0.75C1	SA1C1	CA0.5C1	CA0.75C1	CA1C1
		1.25%	GM0.5C1.25	GM0.75C1.25	GM1C1.25	SA0.5C1.25	SA0.75C1.25	SA1C1.25	CA0.5C1.25	CA0.75R1.25	CA1R1.25
Pharmacel® 101		0%	GM0.5P0	GM0.75P0	GM1P0	SA0.5P0	SA0.75P0	SA1P0	CA0.5P0	CA0.75P0	CA1P0
		1%	GM0.5P1	GM0.75P1	GM1P1	SA0.5P1	SA0.75P1	SA1P1	CA0.5P1	CA0.75P1	CA1P1
		1.25%	GM0.5P1.25	GM0.75P1.25	GM1P1.25	SA0.5P1.25	SA0.75P1.25	SA1P1.25	CA0.5P1.25	CA0.75P1.25	CA1P1.25

3.7.2. Preparation of Powder Mixtures

Utilising the full factorial design, the various artemether/lumefantrine solid lipid dispersions were mixed with the relevant excipients using a Turbula® mixer (T2C, W.A., Bachofen AG maschinenfabrik, Bâle, Switzerland). The formulation ingredients excluding lubricant were accurately weighed, placed into sealed glass containers and mixed for 5 min at 69 rpm. Thereafter, the lubricant was added to the premixed formulation and mixed further for an additional 2 min at 69 rpm to form powder mixtures.

3.7.3. Direct Compression of Powder Mixtures

Each powder mixture containing the double-fixed dose combination of artemether and lumefantrine in a solid lipid dispersion together with excipients as defined by the full factorial design, was compressed by means of direct compression into 10 mm tablets of approximately 500 mg in weight using concaved faced punches and a Korsch® XP1 single station tablet press (Korsch®, Germany). The optimum compression force per formulation was determined to be the force that yielded tablets that could be manufactured, handled and tested with ease; and thereafter was set and remained constant during tableting. The stroke rate was set to a very low stroke speed of 20 strokes/min in order to compensate for any increase in temperature of the tablet press that may occur during tableting at higher speeds as to prevent the lipids from melting.

3.8. Evaluation of Artemether/Lumefantrine Lipid Matrix Tablets

To ensure objectivity when evaluating the lipid matrix tablet formulations and remove operator bias, the tablets were evaluated in accordance with the BP (2018) criteria set for evaluating conventional tablets. The official BP tests, namely: mass variation; friability; dissolution and disintegration were evaluated together with the unofficial BP tests, crushing strength, thickness, diameter and tensile strength (BP, 2018). Additionally, morphology, %swelling and erosion and a tablet assay were conducted to supplement the BP tests data.

3.8.1. Mass Variation

Mass variation of the tablets manufactured in this study per formulation was determined in accordance with the BP (2018) specifications for tablets with an average weight exceeding 250 mg. A sample of 20 tablets was randomly selected per formulation and each tablet individually dusted using an art brush to remove excess powder from the surface of the tablet. The tablets were individually weighed using a Precisa® analytical balance (Precisa®, Zurich, Switzerland) and the weight recorded in order to calculate the average mass, standard deviation (SD) and percentage relative standard deviation (%RSD). No more than two tablets may exhibit more than 5% deviation from the average sample tablet mass calculated (BP, 2018).

3.8.2. Friability

The friability of the randomly selected tablets corresponding to a weight as near as possible to 6.5 g from each formulation was tested using a friabilator (ERWEKA® GmbH, Heusenstamm, Germany). Each tablet was dusted with an art brush to remove excess powder and the ten tablets were collectively weighed prior to testing. The resultant weight of the 10 tablets collectively was noted as the initial weight (W_1). These tablets were placed into the friabilator and rotated for 4 min at 25 rpm. Thereafter, the tablets were removed, dusted again and intact tablets re-weighed to determine the end weight (W_2). The tablets were inspected for any cracks or chips that may have occurred and broken tablets were not permitted to contribute towards the end weight (W_2). The percentage mass loss or friability (%F) was calculated using Equation 3.8.

$$\%F = \frac{W_1 - W_2}{W_1} \times 100 \quad [3.8]$$

3.8.3. Disintegration

A sample of 6 tablets were randomly selected from each formulation and tested in accordance with the BP (2018) specifications. The tablets were individually placed into the compartments of the Erweka® disintegration apparatus (Erweka® model D-63150, Heusenstamm, Germany). The compartments containing the tablets were mechanically lowered into a water bath held at a constant temperature of 37°C ($\pm 0.5^\circ\text{C}$) for the duration of the 15 min test. After the 15 min testing period had elapsed, the tablets were inspected to see if minimal signs of degradation were present as one of the objectives of this study was to establish if modified release could be achieved with these formulations. The BP (2018) states that conventional tablets (immediate release dosage forms) should disintegrate completely within 15 min in order to pass the test and so it was decided that tablets that remained intact after the 15 min had elapsed showed signs of potential modified release.

3.8.4. Crushing Strength, Diameter, Thickness and Tensile Strength

Ten randomly selected tablets from each formulation were analysed utilising the Pharma Test® (model PTB 311, Switzerland) apparatus, where each tablet was crushed at a rate of 0.1 cm.min⁻¹. These tablets were compressed diametrically between the platens of the diametric-compression test apparatus and the crushing strength recorded in newton (N). The thickness and diameter were simultaneously recorded in millimetres.

Tensile strength differs to crushing strength as it takes the tablets geometry into consideration which allows for the comparison of the force that tablets of different sizes can withstand. For each formulation, the tensile strength was calculated utilising Equation 3.7 as well as the standard deviation and percentage relative standard deviation (%RSD).

$$\sigma_x = \left(\frac{10F}{D^2} \right) \left[\left(\frac{2.84H}{D} \right) - \left(\frac{0.126H}{W} \right) + \left(\frac{3.15W}{D} \right) + 0.01 \right]^{-1} \quad [3.7]$$

Where, σ_x is the tensile strength (N.mm⁻²); F, is the crushing strength/hardness (N); D, is the diameter (mm); H, is the tablet thickness (mm); and, W, is the centre cylinder thickness measured in millimetres (Shang *et al.*, 2013).

3.9. High Performance Liquid Chromatographic Method for Artemether and Lumefantrine Lipid Matrix Tablets

3.9.1. Validation of the Method

In order to analyse the active compounds and conduct the assay for this study, a validated method for high performance liquid chromatography (HPLC) able to detect artemether and lumefantrine had to be developed. The method was validated by conducting specificity; linearity; accuracy; precision; as well as quantification limit tests, which all had to comply with the ICH guidelines (Costa *et al.*, 2008).

3.9.1.1. Specificity

Specificity testing is conducted to ensure that the active ingredient(s) may still be detected in the presence of expected components such as excipients, other active ingredients, impurities and degradants. A 100 ml standard solution containing 400 μ g artemether and 300 μ g lumefantrine was prepared. A blank (placebo) sample containing only the relevant excipients and no active ingredients was weighed in the appropriate quantities and diluted to 50 ml with methanol (MeOH). A second sample (solvent sample) comprising excipients was spiked with the correct ratio of active ingredients and diluted to 50 ml with MeOH. Samples of approximately 1.5 ml were withdrawn from the blank and spiked sample; and filtered through a 0.45 μ m filter. The solvent and placebo samples should not produce peaks that interfere with the determination of the active ingredients. All peaks should be discernable from those of the two active ingredients, artemether and lumefantrine obtained from the standard solution (ICH Q2(R1), 2005).

3.9.1.2. Linearity

A 100 ml solution containing 400 μ g artemether and 300 μ g lumefantrine was prepared. A sample (5 ml) from the original solution was withdrawn and made up to a volume of 50 ml (dilution one). From dilution one, 5 ml was withdrawn and made up to a volume of 50 ml (dilution two). The two dilutions and the original 100 ml solution served as the three concentrations to be tested. An Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) was utilised. The resultant peaks were plotted against active ingredient concentration in order to conduct a linear regression

analysis. The R^2 value obtained from a minimum of five samples should be ≥ 0.99 (ICH Q2(R1), 2005).

3.9.1.3. Accuracy

A 100 ml standard solution containing 400 μ g artemether and 300 μ g lumefantrine was prepared as well as a 100 ml solution containing 400 μ g artemether, 300 μ g lumefantrine and the excipients included in the study in the correct ratios. From the original solution, 5 ml was withdrawn and made up to a volume of 20 ml (dilution one). From the original solution, 10 ml was withdrawn and made up to a volume of 20 ml (dilution two). The two dilutions and the original 100 ml solution served as the three concentrations to be tested. Again, an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) was utilised. The resultant peaks were used to determine the %mean recovery. The %mean recovery may only deviate by approximately 2% from the actual concentration (100%); therefore, the range for %mean recovery is between 98.0–102.0% (ICH Q2(R1), 2005).

3.9.1.4. Precision

The precision of an analytical procedure is an expression of the closeness of agreement that exists between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. Ten randomly selected tablets were crushed into powder to be used as powder for each sample. A total of 9 samples were analysed (three concentrations and three replicates each) together with a reference standard. The sample powder was diluted to 200 ml with MeOH and ultrasonicated for 5 min. Samples of approximately 1.5 ml were withdrawn from each 200 ml flask and filtered through a 0.45 μ m filter. A %RSD of $\leq 2\%$ is ideal, but $\leq 5\%$ is still considered acceptable (ICH Q2(R1), 2005).

3.9.1.5. Quantification of Limits

The limit of quantification (LOQ) for an individual analytical procedure is defined as the lowest amount of an analyte in a sample which can be quantitatively determined with suitable precision and accuracy (ICH Q2(R1), 2005). The limit of detection (LOD) is a representative value of sensitivity and the ICH further defines the LOD of an analytical procedure as the lowest amount of an analyte in a sample able to be detected, but not necessarily quantified as an exact value (ICH Q2(R1), 2005).

Standard solutions of artemether and lumefantrine were prepared in concentrations of 10.510 μ g/ml and 1.062 μ g/ml, respectively and subsequently injected into the HPLC machine at differing injection volumes of 1.0, 2.5, 5.0 and 7.5 μ g/ml. Six replicates were injected per concentration (Costa *et al.*, 2008). The resultant mean peak areas were used to calculate the %RSD for LOD and LOQ. The ICH (2005) specifies a LOD %RSD value $\leq 80\%$ and a LOQ %RSD $\leq 10\%$ as satisfactory results (ICH Q2(R1), 2005).

3.9.2. Analysis of Active Compounds

High performance liquid chromatography is regarded by Hansen *et al.* (2012) as the most important analytical technique in pharmaceutical analysis. HPLC was used to validate the active ingredients present in the drug content assay as well as determine the artemether and lumefantrine content present in the dissolution and transport studies (Hansen *et al.*, 2012). The analytical sample preparation and validation of artemether and lumefantrine were done in accordance with the methods described by Costa *et al.* (2008). The linearity; precision; accuracy; specificity; as well as quantification limit were verified (Costa *et al.*, 2008). An Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) was utilised for the concentration assays of the active ingredients. The peak observed for the sample was compared to that of a reference sample, with a similar retention time and peak size indicating a positive identification (Hansen *et al.*, 2012). The HPLC system was equipped with a Luna C₁₈-2 column, 150 x 4.6 mm, 5 µm column (Phenomenex, Torrance, CA) and the temperature set to 30°C. The flow rate was set to 1 ml/min and the mobile phase consisted of acetonitrile (85% v/v) and octanesulphonic acid (15% v/v) with a pH of 3.5.

3.10. Selection and Optimisation of Lipid Matrix Tablets

Once all the formulations specified by the factorial design had been formulated and their physical properties evaluated as described in Sections 3.8.1 to 3.8.4, formulations that failed to meet the BP (2018) requirements or failed to produce tablets were disregarded before further testing commenced. This was because the objective of this study was to determine which of the proposed formulations were viable lipid-based formulations suitable to enhance the absorption and subsequent bioavailability of artemether and lumefantrine. Therefore, it was not necessary to perfect formulations that had failed when a number of viable formulations remained. A tablet assay, morphology and %swelling and erosion tests were conducted to supplement the BP tests data as well as dissolutions studies (a BP official test) were subsequently performed on the formulations that met the BP (2018) physical tablet property requirements.

3.10.1. Assay of Tablets

Twenty tablets from each formulation were crushed into a powder using a pestle and mortar. An equivalent amount of one tablet (500 mg) was weighed from the powdered tablets for each sample; and this powder was dissolved into a 100 ml of an appropriate organic medium namely, MeOH and 1 ml orthophosphoric acid in which both active compounds (artemether and lumefantrine) are soluble. It was stirred continuously for 15 min and ultrasonicated in a Labotec Ecobath® (model 103, Labotec, South Africa) for 20 min. The solution was filtered through a 0.45 µm membrane filter attached to a syringe and the resulting filtrate was diluted to 200 ml. This filtrate was subsequently analysed by means of HPLC as described in Section 3.9. Artemether

and lumefantrine absorbance was measured at 210 nm and 303 nm (Sunil *et al.*, 2010), respectively; and the drug content was calculated from the standard curve utilising Equation 3.9.

$$\% \text{Content} = \frac{(\text{Experimental value of active ingredient})}{(\text{Theoretical value of active ingredient})} * 100 \quad [3.9]$$

3.10.2. Morphology

The morphology of the manufactured tablets was examined using a Nikon Eclipse 50i microscope (Nikon, Tokyo, Japan) fitted with a Nikon DS-Fi1 camera. Six tablets from each formulation were randomly selected for testing. Two of the six selected tablets were dissected in half to bare the internal surface of the tablets. Images of both the internal and external tablet surfaces were captured, examined and compared.

3.10.3. Percentage Swelling and Erosion

Stearic acid and cetyl alcohol are classified as non-swellable matrices (Nisha *et al.*, 2012) however, the presence of fillers in the lipid matrix tablets will probably cause some swelling within the tablet to occur. Therefore, the percentage swelling and erosion tests were conducted using a type II dissolution Distek 2500 basket system (2501049, North Brunswick, New Jersey, USA). Six tablets were randomly selected from each formulation and one tablet was placed into each of the six baskets. The conditions of the dissolution system were standardised regarding temperature ($37 \pm 0.5^\circ\text{C}$), volume (600 ml) and media (distilled water). The initial sample tablet weight (T), as well as the weight of the basket, was recorded before the test was initiated. At the predetermined time intervals of 1, 2, 5, 10, 15, 20, 30, 60, 90, 120 and 150 min, or until no further evidence of swelling was present, the dissolution apparatus was stopped and the basket was blotted with filter paper (Singh *et al.*, 2009). The baskets, containing tablets, were re-weighed at each time interval and this weight was noted as the swollen weight (S). Measurements were taken over the allocated time period until the tablets' weight remained constant for 3 consecutive measurements, or until the tablets had completely disintegrated. To assess the percentage erosion, these swollen samples were placed in the EcoTherm Labotec® oven at 40°C to dry the samples until no further loss in mass was measured. This was recorded as the final mass (R) which was used to calculate if any erosion occurred and to what extent. Equation 3.10 and 3.11 were utilised to calculate the percentage swelling and erosion, respectively.

$$\% \text{Swelling} = \frac{S}{T} \times 100 \quad [3.10]$$

$$\% \text{Erosion} = \frac{T - R}{T} \times 100 \quad [3.11]$$

3.10.4. Dissolution

A Distek® dissolution system (model 2500, Distek® Inc., North Brunswick, New Jersey, USA) with an initial stirring rate of 100 rpm was utilised for this study, according to the basket method stated in the BP (2018). Standard conditions were employed and experiments only commenced once the predetermined temperature of 37°C ($\pm 0.5^\circ\text{C}$) and the desired rotational speed (100 rpm) were achieved; at which point a sample of 4 randomly selected tablets from each formulation were placed into one of the 6 baskets (i.e. 4 tablets per basket) and the 6 baskets were introduced into the medium at time zero ($t=0$).

A 600 ml starting dissolution medium of 0.1 M hydrochloric acid (HCl) (pH 1.2) was placed in the dissolution vessel of the Distek® dissolution system for the first 2 h. This 2 h period mimics the average gastric emptying time of the stomach (Obitte *et al.*, 2010). A 5 ml sample of the acidic phase was removed using a syringe connected to a 0.45 μm membrane filter and placed into HPLC vials at predetermined time intervals of 2, 5, 10, 20, 30, 60, 90 and 120 min. The volume of each individual sample withdrawn was immediately replaced with fresh, preheated dissolution medium of the same temperature (i.e. $37^\circ\text{C} \pm 0.5^\circ\text{C}$) and composition (Vertzoni *et al.*, 2004).

After the initial 2 h period had elapsed, 300 ml of 0.2 M trisodium phosphate dodecahydrate buffer (Na_3PO_4) was added to each vessel in order to raise the pH from 1.2 to 6.8 and the volume from 600 ml to 900 ml. This phase of the dissolution study mimics the average intestinal transit time and the neutral to basic environment found in the small intestine and colon. After adding the buffer, 5 min were allocated for adjusting the pH. All pH adjustments were conducted using either a 2 M HCl solution or a 2 M sodium hydroxide (NaOH) solution. Once equilibrated, further samples were withdrawn at time intervals of 150, 180, 240 and 300 min, respectively.

A third dissolution medium was prepared, in which bile salts and phospholipids were added to the existing phosphate buffer medium to simulate the ileocecal pH and contents where the lipid matrix tablets are supposed to release all of the remaining drug (Obitte *et al.*, 2010). This medium consisted of 3 mM bile salts and 0.5 mM phospholipid at pH 7.4 (Vertzoni *et al.*, 2004). The baskets were raised from the solution and the medium and pH changed to simulate the ileocecal environment. Subsequently, samples were drawn at time intervals of 390, 480, 600 and 720 min; and the medium replaced again per sample withdrawal as described. After the last sample was taken, the rotational speed was adjusted to 150 rpm (infinity sample) and stirred for an additional 30 min to ensure maximum drug release. A summary of the dissolution study conditions is provided in Table 3.4. All dissolution profiles were obtained in six-fold and the artemether and lumefantrine concentrations in the samples were measured utilising the validated HPLC method at the Analytical Technology Laboratory, NWU Potchefstroom.

Table 3.4: Summary of the dissolution conditions specified for the conducted dissolution studies

Dissolution Conditions			
Hour	pH	Composition	Rotational Speed
1	1.2	600 mL 0.1M HCl	100 rpm
2			
3	6.8	+300 mL 0.2M Na ₃ PO ₄	
4			
5			
6	7.4	+ 3 mM Bile Salts and 0.5 mM Phospholipids	
7			
8			
9			
10			
11			
12			
Infinity Sample			150 rpm

Dissolution functions, including the mean dissolution time (MDT) and fit factor values were employed to draw meaningful conclusions from the obtained dissolution or drug release data. Additionally, DDSolver software, a freely available menu-driven add-in programme for Microsoft Excel, was utilised to facilitate the modelling of the dissolution data. Dissolution profile modelling of the commercially available product, Coartem[®], was also conducted.

3.11. *In Vitro* Permeability Studies

In vitro drug absorption, which includes permeation across epithelial cell membranes as well as pre-systemic metabolism, is a complicated chain of processes. The success of *in vitro* models determining the intestinal permeability of a certain compound is determined by how closely the model of choice mimics the characteristics of the *in vivo* situation. Excised porcine tissues are frequently utilised in drug transport studies due to their similarity to human tissues in terms of drug transport (Shikanga *et al.*, 2011). Section of porcine jejunum were employed as epithelial models in the permeability experiments of this study. Transport of artemether and lumefantrine across the porcine intestinal mucosal tissue was investigated in Sweetana-Grass diffusion chambers after exposure of the lipid matrix tablets to the apical side. Transport experiments were conducted in triplicate and analysed employing the validated HPLC method (Section 3.9).

3.11.1. Collection of Porcine Intestinal Tissue

Porcine intestinal tissue was obtained from a local abattoir (Potchefstroom Abattoir, Potchefstroom, South Africa) directly after slaughter. Sections of the proximal jejunum were identified (Figure 3.3 and Figure 3.4) and excised from the gastrointestinal tract of pigs.

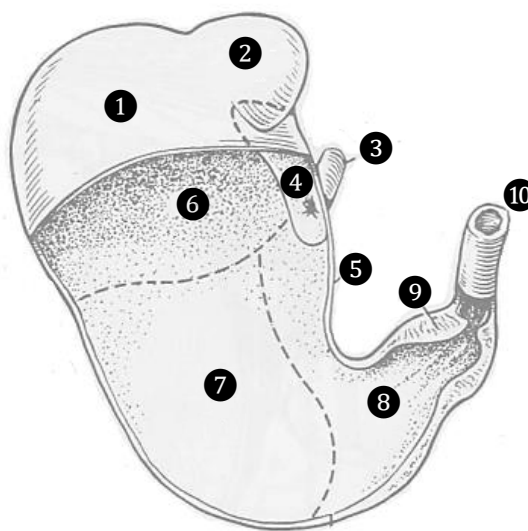


Figure 3.3: Schematic illustration of a partially opened, caudoventral view of a pig stomach as to be able to identify the origin of the duodenum. Other anatomical reference points identified: ① fundus; ② diverticulum; ③ oesophagus; ④ nonglandular mucosa surrounding cardiac opening; ⑤ lesser curvature; ⑥ cardiac gland region of stomach body; ⑦ region of proper gastric glands and remainder of stomach body; ⑧ pyloric gland region of the lesser curvature and pyloric region; ⑨ pylorus; and ⑩ duodenum adapted from Dyce *et al.*, 2002

The respective jejunum segments were inspected for the presence of Peyer's patches (a small mass of lymphatic tissue) as these portions of the intestine should be avoided in transport studies. The selected excised segments were first flushed with ice cold Krebs-Ringer bicarbonate (KRB) buffer and then placed in a cooler box containing cold KRB buffer (pH 7.4) to ensure the maintenance of the cold chain during transportation to the laboratory where the tests were conducted.

The duodenum can be identified where it leaves the pylorus and turns around the caudal aspect of the root of the mesentery, passing between it and the descending colon. Thereafter it dips ventrally to be continued as the jejunum (Figure 3.4). Bile and pancreatic ducts enter the duodenum about 3 cm and 12 cm from the pylorus, respectively. The jejunum is unremarkable and indiscernible from the duodenum. Its coils are only loosely tethered by the long mesentery. The ileum ascends to join the caecum - connected by the ileocecal fold. Sections of the transverse and descending colon hook around the cranial and left aspects of the mesentery root. The ascending colon (arising from the caecum) is prominently elongated in a pig and is coiled to form a cone-shaped mass. The ascending colon ends at a counter clockwise turn and begins to hook around the root of the mesentery to signify the transverse colon. These anatomical references and differences in structure and presentation were used to identify the jejunum segments to be excised.

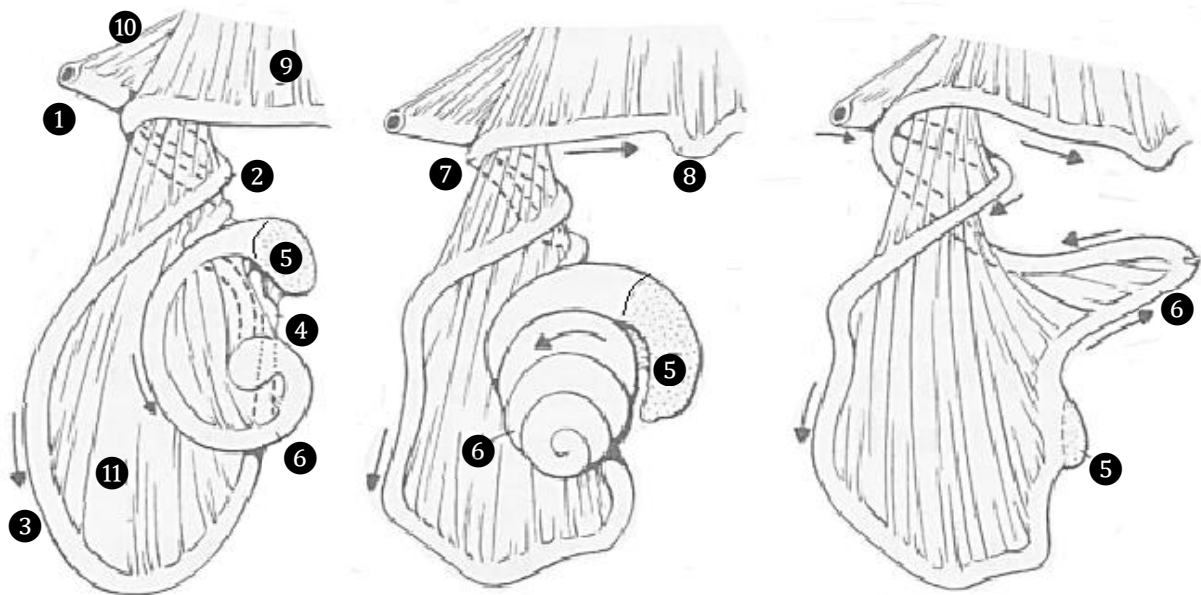


Figure 3.4: Schematic illustration demonstrating the development of the ascending colon in relation to other anatomical reference points including: ① descending duodenum; ② caudal flexure of duodenum; ③ jejunum; ④ ileum; ⑤ caecum; ⑥ ascending colon; ⑦ transverse colon; ⑧ descending colon; ⑨ descending mesocolon; ⑩ mesoduodenum; and ⑪ mesentery adapted from Dyce *et al.*, 2002

3.11.2. Preparation of Porcine Intestinal Tissue

In the laboratory, the excised jejunum segment was pulled over a glass rod in order to remove the serosa layer by means of blunt dissection ❶, as seen in Figure 3.5. A Perspex plate lined with a piece of heavy duty filter paper was positioned on ice upon which the intestinal segments were placed after incision along the mesenteric border with a dissection scalpel ❷. This piece of intestinal tissue was rinsed and kept moist with cold KRB buffer throughout the procedure of cutting the tissue into smaller, 8 cm x 2 cm segments with the apical side facing upwards ❸. These resulting segments were carefully placed upon, and then mounted onto Sweetana-Grass diffusion chamber inserts ❹ (Easy Mount Diffusion Chamber, Physiological Instruments, San Diego, USA) with a transport surface area of 4.52 cm²; the basolateral side facing downwards and the apical side facing upwards ❺. Prior to assembling the two diffusion chamber half-cells, the filter paper was removed ❻. Intestinal sections containing Peyer's patches were discarded. An example of a Peyer's patch is highlighted in Figure 3.5, image ❻ and at magnification 4x ❼. Metal rings were utilised to clamp the two diffusion chamber half-cells together ❽. These combined chambers were placed onto a heating block linked to parallel gas flow ❾.

The mounted basolateral half-cells (Easy Mount Diffusion Chamber, Physiologic Instruments, San Diego, USA) were filled with 7 ml heated KRB buffer (37°C) to simulate the systemic environment. In order to test transport across the jejunum segments, KRB buffer with a pH of 7.4 (37°C) was used to examine transport. Simulated ileocecal medium (pH 7.4; 37°C) was prepared using 3 mM bile salts and 0.5 mM phospholipids to more accurately mimic the intestinal physiological environment and assess transport across the jejunum. The half-cells were linked to parallel gas flow (5% CO₂, 95% O₂) with a flow rate of 15–20 ml/min. The assembled cells were left for 15 min to equilibrate the tissue with the new environment prior to the commencement of the transport study. Thereafter, the KRB buffer was removed from the donor (apical) chamber via aspiration with a vacuum system (Vacusafe®, Hudson, USA) and replaced with 7 ml pre-heated KRB buffer containing the dosage form.

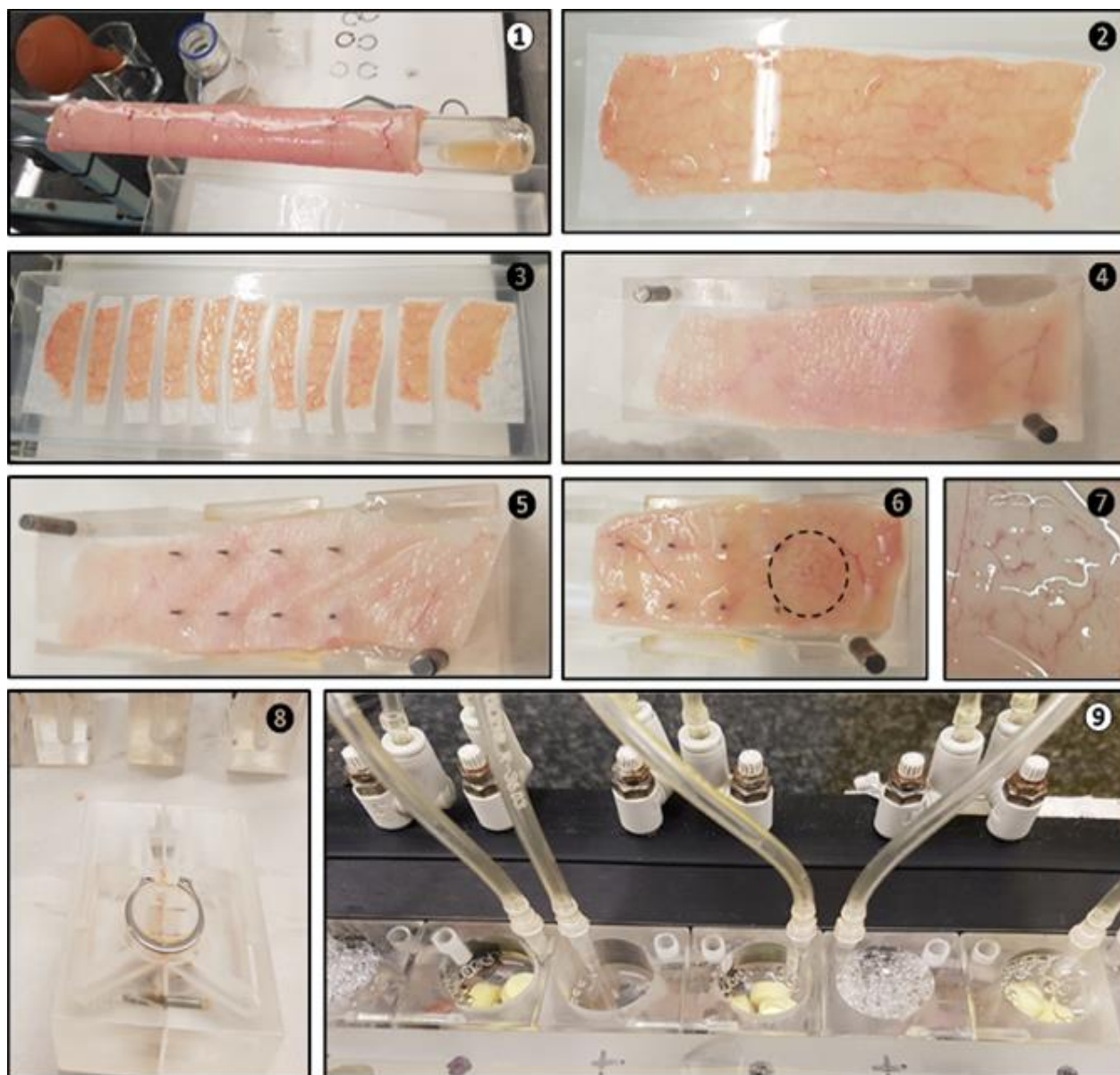


Figure 3.5: Preparation of porcine intestinal tissue for *in vitro* permeability studies including an example of a Peyer's patch ⑥ and at magnification 4x ⑦

3.11.3. Transport Across Intestinal Tissue

Transport of the active compounds from the optimised formulation prepared in this study were analysed in the apical to basolateral (AP-BL) direction utilising the Sweetana-Grass diffusion chamber apparatus (Easy Mount Diffusion Chamber, Physiological Instruments, San Diego, USA) as described above. Aliquots of 1 ml were sampled from each receiver compartment at 20 min intervals for a duration of 2 h. These samples were stored in HPLC vials at 4°C pending analysis by means of HPLC. The samples withdrawn were immediately replaced with an equivalent volume of heated (37°C) KRB buffer. All of the transport experiments were conducted in triplicate.

Tissue integrity was continually evaluated throughout the transport studies by means of trans-epithelial electrical resistance (TEER) readings using a Dual Channel Epithelial Voltage Clamp (Warner Instruments, Hamden, Connecticut, USA). TEER is a measurement of the tight junction integrity within the tissue between adjacent intestinal cells. An initial TEER reading was taken directly before the addition of the lipid matrix tablets to the apical chamber of the Sweetana-Grass diffusion chamber, where after a measurement was taken every 20 min from each chamber for the duration of the permeation study and calculated utilising Equation 3.12.

$$\%TEER \text{ reduction} = \frac{(\text{TEER reading at specific time interval})}{\text{initial TEER reading}} * 100 \quad [3.12]$$

The opening of tight junctions will result in a reduction in TEER of the epithelium due to an increase in flow of the ions through the tight junction openings and intercellular spaces (Beneke *et al.*, 2012). A TEER reading <100 Ω cm² is required to fulfil the criteria of tissue viability in porcine jejunum tissue (Westerhout *et al.*, 2014).

Due to the predicted low %transport and the high lipophilicity of the analysed active ingredients, ex-vivo analysis of the tissue samples was furthermore conducted. Each separate tissue sample was removed from the Sweetana-Grass diffusion chamber, rinsed with deionised water and placed into a 15 ml glass vial containing 5 ml of 100% HPLC grade methanol. These vials were mixed using a Vortex® mixer and subsequently placed in an ultrasonic bath for 10 min to cause lysis of the tissue. Upon completion of the lysis process, the vials were centrifuged for 5 min at 3000 rpm to separate the supernatant. Consequently, 500 µl samples were withdrawn from the supernatant and analysed via HPLC. This procedure allowed for the %active ingredients present in the tissue post 120 min to be determined. The resultant peak areas obtained were utilised to calculate the corresponding drug concentrations where after %retention could be determined by means of Equation 3.13.

$$\%Retention = \frac{\text{Concentration extracted from tissue after lysis}}{\text{Concentration in apical chamber}} * 100 \quad [3.13]$$

3.12. Statistical Data Analysis

From the dissolution and *in vitro* permeability studies data collected, the parameters described in the following section were also calculated.

3.12.1. Mean Dissolution Time

The mean dissolution time (MDT) indicates the average time taken for the entire drug dose to be released from the dosage form into solution. It is a statistical moment for the cumulative dissolution process and provides an accurate drug release rate. This parameter reveals the drug release properties with higher values indicating a higher drug release retarding ability of the tested formulation (Reppas & Nicolaidis, 2000). The MDT was calculated for both active ingredients, artemether and lumefantrine, incorporated into the different formulations utilising Equation 3.14.

$$MDT = \frac{\sum_{j=1}^n t_{mid} \Delta X_d}{\sum_{j=1}^n \Delta X_d} \quad [3.14]$$

Where j is the dissolution sample number; n , is the total number of samples; t_{mid} , is midpoint time between j and $j - 1$; and ΔX_d , is the amount of drug dissolved between j and $j - 1$ (Costa & Lobo, 2001).

3.12.1. Fit Factors

Fit factors were used to compare the dissolution profiles of a test- and a control formulation, namely, Coartem®. Additionally, fit factors were employed to compare relevant formulation variables between the optimised formulations prepared in this study. Fit factor, f_1 , is the difference factor and was utilised to determine the percentage error between the two curves by means of Equation 3.15. Indistinguishable curves would be represented by a value of 0; and as that value increases so does the variation between the two curves.

$$f_1 = \frac{\sum_{j=1}^n |R_j - T_j|}{\sum_{j=1}^n (R_j + T_j)/2} \times 100 \quad [3.15]$$

Fit factor, f_2 , is the similarity factor between the two curves and is a logarithmic transformation of the sum of squares error. This factor applies the average sum of squares of the difference in profiles of the test and reference dissolution profiles. If the value is ≥ 50 it indicates that both the test- and control formulation are fairly similar (Costa & Lobo, 2001), with a value of 100 showing that the two samples are identical. Similarly, as f_2 approaches zero, the dissimilarity between the test and reference profiles increases. Equation 3.16 was used to calculate fit factor f_2 .

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{j=1}^n |R_j - T_j|^2 \right]^{-0.5} \right\} \times 100 \quad [3.16]$$

Where, R_j , is the reference assay at time point, t . T_j , is the test assay at time point, t ; and, n , is the number of pull points (Moore & Flanner, 1996).

3.12.2. Apparent Permeability Coefficient

The transport of the active ingredients, artemether and lumefantrine, was determined across the excised porcine intestinal tissue after exposure to the lipid matrix tablets. Volpe (2010) defined the apparent permeability coefficient (P_{app}) as the rate of accumulated drug in the receiver chamber, normalised for the tissue surface area. The percentage drug transported across the excised intestinal tissue was plotted as a function of time. Apparent permeability coefficient values were calculated from graphs utilising Equation 3.17 (Hellum & Nilsen, 2008; Hansen & Nilsen, 2009).

$$P_{app} = \frac{dQ}{dt} * \frac{1}{A.C_0.60} \quad [3.17]$$

Where P_{app} represents the apparent permeability coefficient (cm/s); dQ/dt ($\mu\text{g/s}$) represents the increase in the amount of drug in the receiver chamber within a defined time period, which is equivalent to the slope of the plot on a graph of drug concentration transported versus time; A (cm^2) represents the effective surface area of the excised porcine intestinal tissue between the apical and basolateral chambers; and C_0 is the initial drug concentration in the apical chamber ($\mu\text{g/ml}$).





4.1. Introduction

Pre-formulation studies are an integral part of the drug and dosage form development process as it is an investigation into the relationship between biopharmaceutical principles and the physicochemical parameters of the active ingredient(s). It offers insight into the kinetic profile and stability, compatibility with excipients and influence of method of manufacture on the active ingredient(s). For these reasons, the pre-compression parameters evaluated in this chapter formed the first step in the rational development of this study's dosage form (Chaurasia, 2016).

4.2. Pre-Formulation Studies

Once the most suitable lipid bases had been selected, both active ingredients were verified by means of IR spectroscopy. The powder flow properties of the active ingredients, the selected fillers as well as the lipid dispersions were characterised and are discussed in detail in Section 4.2.3. The particle size and particle size distribution of the active ingredients, fillers and lipid dispersions were analysed as particle size can affect the flow properties as well as the packing and compaction properties of the powders during tableting (Horiba, 2016). Subsequently, thermal analysis profiles of the active ingredients, lipid bases and lipid dispersions in their varying concentrations were investigated by means of light microscopy, differential weight loss thermograms (DTG), thermogravimetric analysis (TGA), X-ray diffraction studies, and hot-stage microscopy.

4.2.1. Selection of Lipid Bases

Four possible lipid bases were investigated during pre-formulation studies in order to identify the most suitable lipid bases to formulate lipid matrix tablets. The proposed lipid bases were glycerol monostearate, stearic acid, cetyl alcohol and coconut oil. Glycerol monostearate, stearic acid and cetyl alcohol are appropriate lipid bases often employed in matrix systems according to literature (Ninama *et al.*, 2015; Nisha *et al.*, 2012). Coconut oil was investigated as a pharmaceutical excipient option due to its antioxidant properties and health benefits (Famurewa *et al.*, 2017). The advantage of using coconut oil over other natural oils is due to its high saturated fat content, rendering it resistant to rancidification (Kempton, 2005). The melting points of the lipid bases were of importance due to the hot fusion method of manufacture. Differences in melting points of cetyl alcohol (49°C), stearic acid (66°C), glycerol monostearate (70°C) and coconut oil (25°C) were investigated and found to be sufficient. Lipid dispersions were prepared via hot fusion in the pre-determined ratios and assessed in order to identify the most appropriate lipid bases for this study.

Cetyl alcohol, stearic acid and glycerol monostearate produced reproducible lipid dispersions that solidified within 24 h and were able to be crushed into a powder, ready to be tableted in accordance with the previously mentioned method (Section 3.4). It was however, not possible to produce a lipid dispersion comprising coconut oil that solidified sufficiently in order to be crushed into a solid powder lipid dispersion. The nature and physicochemical properties of coconut oil meant it could not be freeze dried and did not solidify comprehensively enough to be incorporated as a lipid base. Therefore, coconut oil was removed as a possible lipid base option and cetyl alcohol, stearic acid and glycerol monostearate were selected as lipid bases to be further investigated in this study.

4.2.2. IR Spectroscopy of Active Ingredients

An Alpha Sample Compartment RT-DLaTGS spectroscope (Bruker Alpha Sample Compartment RT-DLaTGS, USA) was utilised to analyse the absorbance wavelengths of both active ingredients, artemether and lumefantrine. The resulting IR spectrums were compared to that of a reference standard by means of data overlays of the spectrums. Both of the active ingredients were identified and verified to be pure active ingredients with no presence of contamination as the peaks and intensities of the samples correlated to that of their respective reference standards. The absence of additional peaks at differing intensities ruled out the presence of contaminants as can be seen below in Figures 4.1 and 4.2.

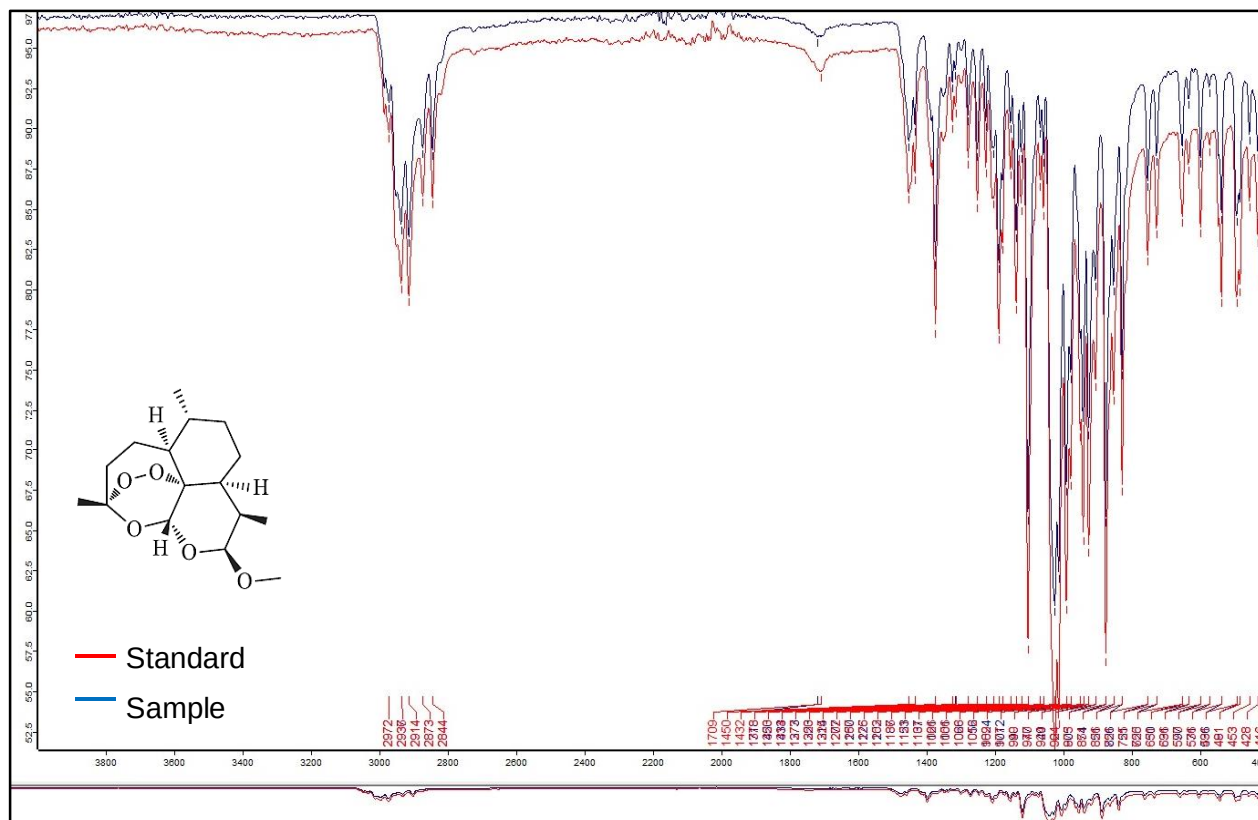


Figure 4.1: Infrared spectra overlay of the active ingredient artemether (blue) and a reference standard (red) with the chemical structure shown as an inset

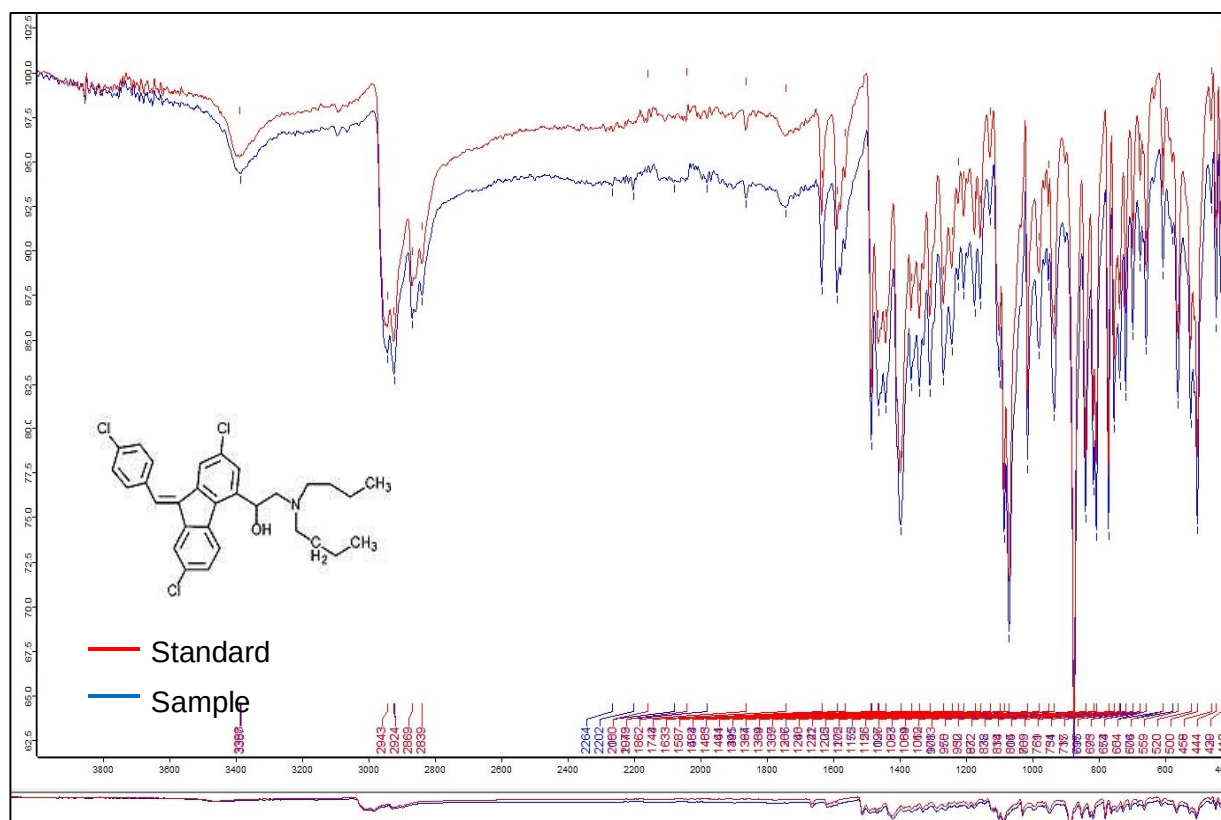


Figure 4.2: Infrared spectra overlay of the active ingredient lumefantrine (blue) and a reference standard (red) with the chemical structure shown as an inset

4.2.3. Evaluation of Powder Flow Properties

The selected fillers, active ingredients and lipid dispersions were characterised according to the methods previously described in Section 3.6. Table 4.1 designates the values and limits used by the BP (2018) to characterise powder flow properties. Three parameters are defined by the BP (2018) to be used in order to assess powder flow, namely: Carr's index; angle of repose and Hausner ratio. Each of these parameters has a prerequisite scale as per the BP (2018), which classifies and distinguishes between the powder flow properties ranges of very poor to excellent.

Table 4.1: Prerequisite scale as per BP (2018) utilised for the characterisation of the three powder flow parameters: Carr's index, angle of repose and Hausner ratio

	Carr's Index (%)	Angle of Repose (°)	Hausner Ratio
Excellent	1 ÷ 10	25 ÷ 30	1.00 ÷ 1.11
Good	11 ÷ 15	31 ÷ 35	1.12 ÷ 1.18
Fair	16 ÷ 20	36 ÷ 40	1.19 ÷ 1.25
Passable	21 ÷ 25	41 ÷ 45	1.26 ÷ 1.34
Poor	26 ÷ 31	46 ÷ 55	1.35 ÷ 1.45
Very Poor	32 ÷ 37	56 ÷ 65	1.46 ÷ 1.59

4.2.3.1 Powder Flow Properties of the Active Ingredients and Prepared Lipid Dispersions

Tablets are manufactured by the filling of the tablet die with powders or granules, thereby rendering powder flow an important parameter to measure as it determines tablet weight, hardness and content uniformity (Gopireddy *et al.*, 2016). The powder flow properties of the individual active ingredients were measured as well as that of the lipid dispersions in order to characterise the flow properties of the active ingredients and identify the impact of their incorporation into lipid dispersions. The powder flow results obtained for the active ingredients and lipid dispersions are recorded in Tables 4.2 and 4.3, respectively, and the values presented in parenthesis indicate the %relative standard deviation (%RSD).

Table 4.2: Powder flow characterisation results pertaining to the two active ingredients

	Artemether	Lumefantrine
Bulk Density (g/ml)	0.600 (0.007)	0.495 (0.010)
Tapped Density (g/ml)	0.741 (0.022)	0.739 (0.011)
Hausner Ratio	1.235 (0.023)	1.493 (0.009)
Carr's Index (%)	18.997 (0.100)	33.002 (0.018)
COD (mm)	8 ÷ 12	16 ÷ 20
Flow Rate (g/s)	38.101 (0.204)	3.326 (0.044)
Angle of Repose (°)	31.157 (0.013)	39.108 (0.010)

**% RSD indicated in parenthesis*

Carr's index and the Hausner ratio are both calculated utilising the bulk and tapped densities which are a representation of the differences in particle size within a powder sample. A powder exhibiting good flow would have a bulk and tapped density close in value and would therefore have a small Carr's index. Alternatively, a powder with poor flow properties would display a greater difference in bulk and tapped densities resulting in higher interparticle interactions and ultimately a larger Carr's index. Particle size and shape as well as cohesivity influence the results obtained for these two parameters as they reflect the influence of tapping on powder packing (Prescott & Barnum, 2000). From Table 4.2 it is clear that artemether depicts bulk and tapped density values close in value, whereas lumefantrine portrays a wider range between these two

values, thus, predicting that artemether will probably show more acceptable flowability compared to lumefantrine.

Considering the other flow property results, artemether demonstrated fair to good powder flow according to the compendial classification of the BP (2018). It has significantly superior flow when compared to lumefantrine which exhibited very poor flow with a Hausner ratio of 1.493 ± 0.009 and a Carr's index of $33.002 \pm 0.018\%$. The critical orifice diameter (COD) results furthermore highlighted the differences in flowability between the two active ingredients with artemether having a COD of 8–12 mm, whereas lumefantrine depicted a notably higher COD of 16–20 mm, thereby demonstrating its poor flowability. Lumefantrine demonstrated 'ratholing' the formation of a vertical cavity within a powder mass above an outlet during flow rate and angle of repose testing. A rathole forms as a result of the cohesive strength of the powder and negatively influences flow (Carson & Marinelli, 1994; Couper *et al.*, 2012; Prescott, 2001; Prescott & Barnum, 2000), which could account for the poor flow rate (3.326 ± 0.044 g/s) and fair angle of repose ($39.108 \pm 0.010^\circ$) observed for lumefantrine (BP, 2018).

The formation of lipid dispersions resulted in the coating of the active ingredients with one of the selected lipid bases. For this reason, the powder flow properties of the lipid dispersions were characterised and conclusions further drawn on the impact of the resultant lipid dispersions on powder flow properties compared to that of the individual active ingredients. The lipid dispersions, regardless the type of lipid base employed, improved the flow properties overall compared to that of the lumefantrine as seen in Table 4.3. This overall improvement was however not achieved when artemether is compared to the various lipid dispersions. The following rank order in terms of both the Hausner ratio and Carr's index could be assigned to the different lipid:drug ratios where stearic acid was employed as the lipid base: 0.5:1 > 0.75:1 > 1:1.

Stearic acid in both 0.5:1 and 1:1 ratios (denoted as SA0.5 and SA1) exhibited excellent flow when characterised in term of angle of repose, obtaining angles of 23.838° and 24.057° , respectively; however, stearic acid in a 0.75:1 ratio (represented as SA0.75) demonstrated only a passible angle of repose result of 42.468° . SA0.5 and SA0.75 revealed an equally superior flow rate (27.169 ± 0.366 and 27.788 ± 0.255 g/s, respectively) compared to that of SA1 (18.385 ± 0.176 g/s). SA0.75 caused the most substantial increase in flow rate compared to that of lumefantrine alone (3.326 ± 0.044 g/s). It can therefore be concluded that the inclusion of the two active ingredients into a stearic acid lipid dispersion overall resulted in a notably improvement in flowability. Furthermore, SA1 demonstrated the highest Carr's index ($35.885 \pm 0.065\%$) compared to SA0.5 and SA0.75 lipid dispersions, and was classified as having very poor compressibility. SA0.75 demonstrated only passable compressibility, whereas SA0.5 was classified as having good flow properties with a Carr's index value of $14.636 \pm 0.014\%$. Therefore, it can clearly be established that the different lipid:drug ratios of the stearic acid lipid dispersions

do influence Carr's index. The presence of a lower lipid content (i.e. SA0.5) resulted in the lowest Carr's index of the three stearic acid lipid dispersions, indicating that the lipid components are poorly compressible, but exhibit superior flow compared to the individual active ingredient's flow properties.

Significant differences were not present between the lipid:drug ratios where glycerol monostearate (denoted as GM) was employed as the lipid base. The 0.5:1 lipid:drug ratio, GM0.5, expressed a Hausner ratio of 1.777 ± 0.014 which is classified according to the BP (2018) as good. GM0.75 and GM1 both fall into the fair category with a Hausner ratio of 1.201 ± 0.034 and 1.209 ± 0.028 , respectively. Considering Carr's index, GM0.5 may be classified as having good flowability; whereas both GM0.75 and GM1 are categorised as displaying fair flow. Similarities in powder flow properties between the differing ratios were further confirmed by the COD, whereby all three glycerol monostearate:drug ratios obtained a COD of 8–12 mm. The angle of repose results achieved for these lipid:drug ratios were able to highlight the differences within the ratios, namely GM0.5 being classified as passable ($41.013 \pm 0.025^\circ$); GM0.75 is considered as having good flowability ($33.171 \pm 0.023^\circ$); and GM1 showed only fair flow characteristics ($37.119 \pm 0.242^\circ$). Furthermore, GM0.75 and GM1 exhibited equivalent flow rates of 18.326 ± 0.120 g/s and 18.813 ± 0.111 g/s, correspondingly. On the other hand, GM0.5 displayed a slower flow rate of 14.170 ± 0.090 g/s, which is still respectable compared to that obtained for all the cetyl alcohol lipid dispersions.

Cetyl alcohol (CA) depicted the least favourable COD and flow rate results of the three lipid bases investigated. The angle of repose results obtained were however more favourable, with both the CA0.5 and CA0.75 lipid dispersion being classified as possessing good flowability; and CA1 as fair. The cetyl alcohol lipid dispersions exhibited increased flowability with an increase in lipid content. This was confirmed with both the Hausner ratio and Carr's index results which ensued in the following rank order being assigned to the cetyl alcohol lipid dispersions: 1:1 > 0.75:1 > 0.5:1. Interestingly, this order was the inverse of both glycerol monostearate and stearic acid lipid dispersions, which proves that the type of lipid base as well as the ratio in which it is incorporated, plays a substantial role in the flow properties of the lipid dispersions. When assessed in terms of flow rate and COD, cetyl alcohol lipid dispersions were meaningfully inferior compared to glycerol monostearate and stearic acid lipid dispersions.

Table 4.3: Lipid dispersions powder flow characterisation results

	Stearic Acid			Glycerol Monostearate			Cetyl Alcohol		
	0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1
Bulk Density (g/ml)	0.457 (0.014)	0.453 (0.019)	0.445 (0.019)	0.480 (0.010)	0.495 (0.021)	0.424 (0.011)	0.483 (0.021)	0.492 (0.014)	0.464 (0.011)
Tapped Density (g/ml)	0.536 (0.016)	0.600 (0.018)	0.695 (0.015)	0.565 (0.015)	0.594 (0.013)	0.512 (0.038)	0.652 (0.024)	0.591 (0.021)	0.545 (0.026)
Hausner Ratio	1.171 (0.002)	1.324 (0.009)	1.561 (0.037)	1.177 (0.014)	1.201 (0.034)	1.209 (0.028)	1.351 (0.043)	1.200 (0.034)	1.176 (0.017)
Carr's Index (%)	14.636 (0.014)	24.469 (0.127)	35.885 (0.065)	15.044 (0.119)	16.667 (0.174)	17.188 (0.096)	25.880 (0.119)	16.604 (0.174)	14.946 (0.096)
COD (mm)	12ĭ 16	8ĭ 12	12ĭ 16	8ĭ 12	8ĭ 12	8ĭ 12	20ĭ 24	24ĭ 28	20ĭ 24
Flow Rate (g/s)	27.169 (0.366)	27.788 (0.255)	18.385 (0.176)	14.170 (0.090)	18.326 (0.120)	18.813 (0.111)	3.351 (0.012)	5.013 (0.006)	1.856 (0.127)
Angle of Repose (°)	23.838 (0.053)	42.468 (0.715)	24.057 (0.040)	41.013 (0.025)	33.171 (0.023)	37.119 (0.242)	33.463 (0.029)	32.668 (0.021)	38.049 (0.020)

*% RSD indicated in parenthesis

Thus, in terms of Hausner ratio and Carr's index, both these expressions increased in compendial classification with an increase in lipid:drug ratio. The 0.5:1 lipid:drug ratio, regardless the type of lipid base, exhibited the highest flow rate, with an average of 14.897 g/s. This ratio furthermore portrayed the smallest average angle of repose thereby having the highest compendial classification of the three lipid drug ratios regarding angle of repose. Of the three lipid bases investigated, stearic acid exhibited on average the highest flow rate and the lowest Hausner ratio, with the average values being categorised as having good flow characteristics. Glycerol monostearate depicted the most uniform and smallest average Carr's index of 16.299% and was therefore classified as having fair flowability. Lastly, of the three lipid bases, cetyl alcohol obtained an average angle of repose result of 34.727°, earning a compendial classification of good flowability, whereas glycerol monostearate was classified as possessing fair flow, whilst stearic acid was categorised as excellent in this regard.

4.2.3.2 Powder Flow Properties of Selected Fillers

The selected fillers for this study, MicroceLac® 100, RetaLac®, CombiLac® and Pharmacel® 101 were also evaluated in terms of bulk and tapped density, Hausner ratio, Carr's index, COD, flow rate and angle of repose. The subsequent results acquired are summarised in Table 4.4; and the values presented in parenthesis indicate the %RSD. Comparisons were further drawn between the filler powder flow property results obtained and the published results from the respective manufacturers of these fillers.

Table 4.4: Powder flow characterisation results obtained for the investigated fillers

	CombiLac®	RetaLac®	Pharmacel® 101	MicroceLac® 100
Bulk Density (g/ml)	0.489 (0.003)	0.312 (0.004)	0.352 (0.038)	0.488 (0.005)
Tapped Density (g/ml)	0.567 (0.009)	0.427 (0.022)	0.481 (0.025)	0.644 (0.004)
Hausner Ratio	1.159 (0.010)	1.369 (0.021)	1.369 (0.049)	1.320 (0.006)
Carr's Index (%)	13.702 (0.061)	26.923 (0.058)	26.853 (0.136)	24.226 (0.019)
COD (mm)	3i 8	3i 8	12i 6	3i 8
Flow Rate (g/s)	76.074 (0.050)	48.788 (0.073)	22.969 (0.111)	65.577 (0.093)
Angle of Repose (°)	15.609 (0.004)	24.230 (0.011)	24.999 (0.041)	46.840 (0.007)

**% RSD indicated in parenthesis*

From the results obtained, the following rank order was allocated to the fillers in terms of best flowability: CombiLac® > MicroceLac® 100 > RetaLac® = Pharmacel® 101, according to the parameters specified by the BP (2018) in Table 4.1. CombiLac® was observed to have good to excellent flow as it displayed the lowest Hausner ratio (1.159 ± 0.010), Carr® index ($13.702 \pm 0.061\%$) and angle of repose ($15.609 \pm 0.004^\circ$) when compared to the other fillers evaluated in this study. This classification is consistent with the claims made by MEGGLE® (Meggle, 2016c).

Although RetaLac® and Pharmacel® 101 exhibited an excellent angle of repose ($24.230 \pm 0.011^\circ$ and $24.999 \pm 0.041^\circ$, respectively), the flowability of these fillers in general was found to be equally poor when characterised in terms of the Hausner ratio and Carr® index. Both RetaLac® and Pharmacel® 101 depicted a poor Hausner ratio of 1.369, and a Carr® index of $26.923 \pm 0.058\%$ and $26.853 \pm 0.136\%$, individually. The findings for RetaLac® regarding the Hausner ratio and Carr® index were coherent with MEGGLE® who reported a Hausner ratio of 1.35 and a Carr® index of 26.09% (Meggle, 2016a). Pharmacel® 101 comprises only microcrystalline cellulose, which according to Saha and Shahiwala (2009) accounts for this filler's poor fluidity on account of its relatively small particle size.

MicroceLac® 100 demonstrated passible flow in terms of the Hausner ratio and Carr® index, which again was consistent with MEGGLE®'s conclusions (Meggle, 2014b). There was however a discrepancy regarding angle of repose results obtained in this study (46.840°) with MEGGLE®'s findings of 34° (Meggle, 2014b). The angle of repose refers to the constant three dimensional cone-shaped powder heap that forms when powder flows onto a surface. It is used as an indication of the strength of interparticle forces within a powder sample; with a flatter powder heap indicating weaker forces (BP, 2018). MicroceLac® 100 consists of 75% α -lactose monohydrate and 25% microcrystalline cellulose, both of which are relatively highly hygroscopic. The hygroscopicity of the components could account for a small percentage water uptake during storage which would strengthen the interparticle forces due to the presence of van der Waals forces and hydrogen bonding (Nokhodchi, 2005; Sandler *et al.*, 2010). Additionally, the particles will swell with the uptake of water causing them to be more tightly packed, rendering poorer flow as the particles cannot move as freely past each other, in so doing, effecting and accounting for the discrepancy observed with the angle of repose results (Armstrong *et al.*, 2014). MicroceLac® 100 exhibited adequate flow during the tableting process validated by complete and consistent filling of the tablet die which was further substantiated by minimal variance in the mass of the tablets produced with this filler. Therefore, although poor angle of repose results were obtained, powder flow during the tableting process was not hindered.

CombiLac® and MicroceLac® 100 exhibited superior flow rates (76.074 ± 0.505 g/s and 65.577 ± 0.093 g/s, respectively) compared to that of RetaLac® and Pharmacel® 101 (48.788 ± 0.073 g/s and 22.969 ± 0.111 g/s, individually). This could be attributed to particle shape and size characteristics that influence flow and compaction; and will be further examined in Section 4.2.4. Larger, more spherical particles will typically flow more easily than smaller particles; hence, the particle size and particle size distributions were evaluated in order to supplement the flow property results. Additionally, poor powder flowability was revealed during tableting with Pharmacel® 101 where incomplete filling of the tablet die was observed. This in turn affected the mass variation results for all formulations containing Pharmacel® 101, causing most of the formulations to fail in meeting the BP (2018) specifications. Each individual filler obtained the same compendial classification of powder flow for their respective Hausner ratio and Carr® index results, thereby highlighting the uniformity in results obtained.

4.2.4. Particle Size and Particle Size Distribution

Particle size influences many properties when working with particulate material and is a valuable indicator when assessing quality and performance. Particle size analysis is utilised in the pharmaceutical industry to determine the size of active ingredient and excipient particles as this can influence important dosage form characteristics such as content uniformity, dissolution and absorption rates. The size, shape and distribution of particles impact powder behaviour during tablet die filling and compaction which influences the physical properties of tablets. It is furthermore a valuable tool in operations that aim to reduce particle size to a desired specification such as milling or crushing (Horiba, 2016).

The particle size and particle size distribution of the four fillers selected for this study, together with the two active ingredients and the prepared lipid dispersions were determined using a Malvern Mastersizer 2000. Results acquired are provided in Annexure A and a summary of the most important factors for consideration and discussion are summarised in Table 4.5. Although the active ingredients were incorporated into solid lipid dispersions that would take on new properties compared to each individual component, the active ingredients were still assessed in order to provide a broad understanding of their individual flow properties, particle size and particle shape.

The results are presented in terms of particle volume distribution curves (Annexure A) which indicate the particles in a given size range by percentage of the total sample volume. The D-values provided describe the distribution width as the percentage mass below the diameter of interest and are expressed as the number after the 'D'. For example, the $d_{0.1}$ -value is the diameter at which 10% of a sample's mass is comprised of smaller particles and the $d_{0.9}$ -value is the diameter at which 90% of a sample's mass consists of smaller particles, when all the particles in

a sample are arranged in order of ascending mass. The $d_{0.5}$ -value is likewise referred to as the "mass median diameter" as it divides the sample equally by mass, with exactly half of the population lying above and below this value (Innopharma, 2016; Wolfrom, 2011).

Table 4.5: Summary of average particle size and particle size distribution results

	$d_{0.1}$-value (μm)	$d_{0.5}$-value (μm)	$d_{0.9}$-value (μm)	Span Value	Mean Volume Particle Size (μm)
Artemether	77.985 (± 0.129)	227.535 (± 0.187)	562.091 (± 0.098)	2.151 (± 0.094)	279.002 (± 0.131)
Lumefantrine	40.374 (± 0.046)	137.751 (± 0.076)	327.857 (± 0.089)	2.084 (± 0.039)	164.411 (± 0.069)
MicroceLac[®] 100	49.028 (± 0.028)	157.762 (± 0.012)	312.115 (± 0.017)	1.567 (± 0.311)	172.029 (± 0.009)
CombiLac[®]	67.148 (± 0.022)	163.734 (± 0.009)	307.729 (± 0.005)	1.469 (± 0.011)	177.561 (± 0.008)
RetaLac[®]	84.016 (± 0.009)	183.077 (± 0.098)	390.747 (± 0.009)	1.589 (± 0.013)	218.075 (± 0.005)
Pharmacel[®] 101	22.193 (± 0.006)	67.004 (± 0.001)	137.703 (± 0.003)	1.724 (± 0.004)	74.869 (± 0.003)
Stearic acid 1:1	109.660 (± 0.005)	356.898 (± 0.029)	717.419 (± 0.035)	1.703 (± 0.014)	389.479 (± 0.031)
Stearic acid 0.75:1	106.248 (± 0.023)	340.259 (± 0.032)	749.150 (± 0.037)	1.889 (± 0.007)	390.118 (± 0.035)
Stearic acid 0.5:1	105.126 (± 0.022)	357.247 (± 0.021)	726.948 (± 0.022)	1.741 (± 0.002)	391.754 (± 0.022)
Glycerol monostearate 1:1	54.690 (± 0.040)	194.000 (± 0.022)	517.079 (± 0.021)	2.384 (± 0.038)	245.032 (± 0.012)
Glycerol monostearate 0.75:1	70.850 (± 0.030)	288.800 (± 0.017)	640.187 (± 0.016)	1.971 (± 0.002)	327.124 (± 0.016)
Glycerol monostearate 0.5:1	49.617 (± 0.179)	217.812 (± 0.113)	390.117 (± 0.138)	2.355 (± 0.031)	185.156 (± 0.166)
Cetyl alcohol 1:1	36.583 (± 0.011)	252.202 (± 0.034)	740.215 (± 0.035)	2.789 (± 0.007)	324.341 (± 0.033)
Cetyl alcohol 0.75:1	35.065 (± 0.056)	196.517 (± 0.106)	744.071 (± 0.054)	3.623 (± 0.061)	308.847 (± 0.063)
Cetyl alcohol 0.5:1	20.625 (± 0.001)	117.443 (± 0.041)	664.266 (± 0.198)	4.002 (± 0.015)	280.939 (± 0.118)

**% RSD indicated in parenthesis*

Skewness and kurtosis of samples were used to describe the distribution symmetry and sharpness of a peak compared to a normal distribution, correspondingly, as to provide meaning to the span value, which is a simple indication of the width of the distribution. Examples of normal distribution graphs as well as skewness and kurtosis are provided in Figure 4.3. The span value

is calculated using the $d_{0.1}$ -, $d_{0.5}$ - and $d_{0.9}$ -values and provides a comparable value with which particle size distributions of differing samples may be compared (Wolfrom, 2011).

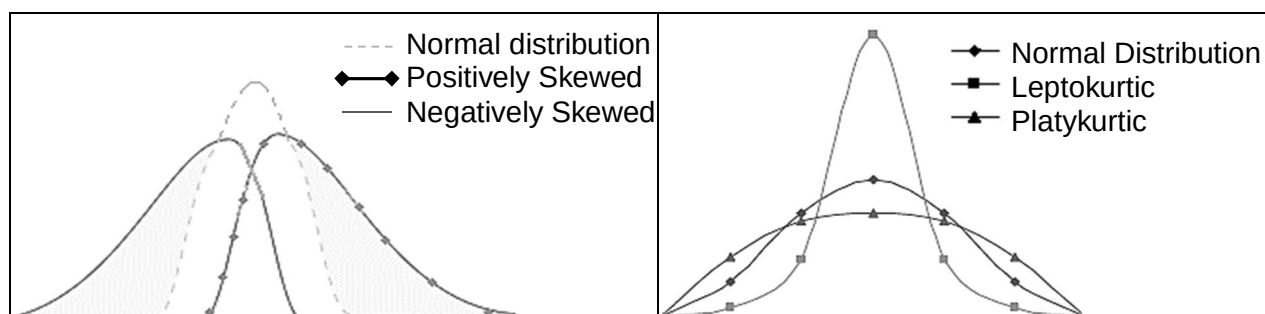


Figure 4.3: Examples of normal distribution, skewness and kurtosis

Both artemether and lumefantrine displayed a large kurtosis as the distribution peaks were very narrow and sharp (Annexure A). This leptokurtic distribution was confirmed by the mean volume particle size of lumefantrine ($164.411 \pm 0.069 \mu\text{m}$), as seen in Table 4.5, being close to its $d_{0.5}$ -value of ($137.175 \pm 0.076 \mu\text{m}$). Lumefantrine exhibited a markedly smaller mean volume particle size compared to that of artemether ($279.002 \pm 0.13 \mu\text{m}$), indicating that lumefantrine has a higher percentage of smaller particles that could attribute to its poor powder flow properties. Smaller particles generally relate to poor flowability as these smaller particles have a larger contact surface area with surrounding particles, which can result in an increase in the bonding forces between the particles (Aulton, 2018).

MicroceLac® 100 and CombiLac® exhibited a similar mean volume particle size ($172.029 \pm 0.009 \mu\text{m}$ and $177.561 \pm 0.08 \mu\text{m}$, respectively) which explains the similarity in their flow properties. Both curves were negatively skewed, and displayed mean volume particle size values similar to their respective $d_{0.5}$ -values thereby signifying that there was a higher percentage of smaller particles present in the population. This can aid the tableting process to a degree, given that smaller particles can fill gaps between larger particles, in so doing supporting compaction of the powder. MicroceLac® 100 has superior flow properties due to its nearly spherical particle shape as a result of the co-spray-drying manufacturing process. This ultimately reduces blend segregation and improves the content uniformity of the finished dosage form (Meggles, 2016b). CombiLac® features suitable powder flow characteristics necessary to enhance weight uniformity of dosage forms and increase throughput in direct compression (Meggles, 2016c).

RetaLac® revealed flow properties that did not adhere to the general rule that larger particles flow more freely compared to smaller particles, thus having increased flowability. The poor flowability depicted by RetaLac® can be attributed to the shape rather than the size ($218.075 \pm 0.005 \mu\text{m}$) of the RetaLac® particles. RetaLac® is irregular in shape, consisting of spheroidal and oblong rods as opposed to spherical particles (Meggles, 2016a), which impede flow. Irregularly shaped

particles may be subjected to mechanical interlocking as well as adhesive and cohesive forces, all of which can be detrimental to powder flow (Aulton, 2018). Furthermore, the findings of this study regarding the flow properties of RetaLac® were consistent with the results reported by MEGGLE® which stated that RetaLac® typically has a $d_{0.5}$ -value ranging from 100 to 200 μm (Meggle, 2016a) which was in accordance with the value obtained during this study ($183.077 \pm 0.098 \mu\text{m}$).

Pharmacel® 101, on the other hand, exhibited the highest span value of 1.724 ± 0.004 compared to that of the other investigated fillers. The high variance in particle size distribution, as indicated by the span value, may account for the poor flow properties observed with Pharmacel® 101. It moreover exhibited the smallest mean volume particle size ($74.869 \pm 0.003 \mu\text{m}$) of the fillers which further explains its poor flowability as small particles experience higher interparticle interactions on account of their increased contact surface area, ultimately leading to poorer flow properties (Aulton, 2018; Hou & Sun, 2007).

The use of electron-micrographs confirms and clearly highlights the effect of particle shape on powder flow properties in addition to the effect of particle size. When the four fillers were examined at 700X magnification (Figure 4.4) similarities in the spherical shape of CombiLac® and MicroceLac® 100 could clearly be seen. These relatively spherical agglomerates, formed by the co-spray-drying process of manufacture (Meggle, 2014b; Meggle, 2014c), exhibited similar flow rates (Table 4.4). CombiLac® exhibited superior flow compared to MicroceLac® 100, which can be attributed to the larger more uniform agglomerates as confirmed by particle size analysis. RetaLac® is a co-pressed excipient comprising equal parts of hypromellose and milled β -lactose monohydrate which account for this powder's porous and irregular structure. These elongated rod-like structures are supposed to enhance compactibility during tableting due to the brittle, plastic fracture deformation properties of the filler powder (Meggle, 2014a). Lastly, Pharmacel® 101 consisted of elongated particles that were more sharply shaped shards rather than spherical particles. The elongated and jagged surface of both RetaLac® and Pharmacel® 101, together with the particle size and particle size distribution accounts for the poor powder flow properties associated with these two fillers.

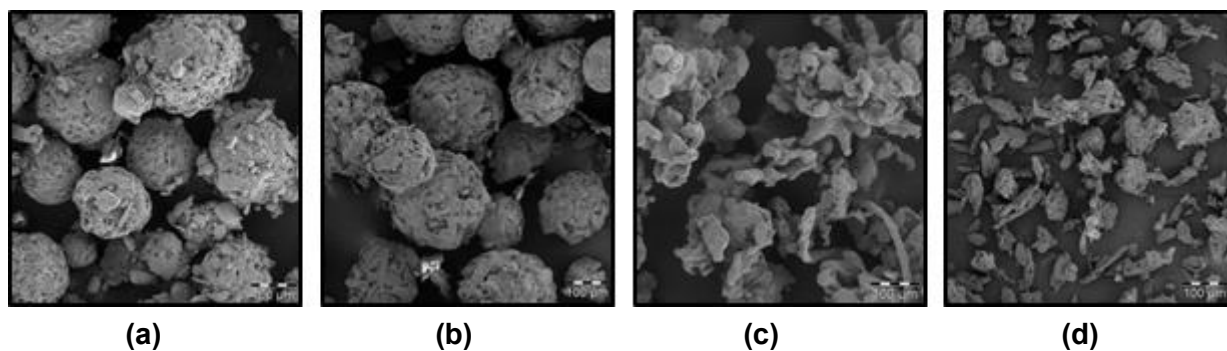


Figure 4.4: Electron-micrographs at 700X magnification of filler powder samples (a) CombiLac® (b) MicroceLac® 100 (c) RetaLac® and (d) Pharmacel® 101 adapted with permission from Nieman, 2017

The following rank order could be allocated to the fillers regarding span: CombiLac® > MicroceLac® 100 > RetaLac® > Pharmacel® 101, which remarkably correlates to the rank order allocated to the flow properties of these fillers. This suggests that the smaller degree of particle size distribution (indicated by the span value) supports flow characteristics as the potential for segregation was minimised coupled with the positive effects of more spherical particles.

Of the three lipid bases characterised, stearic acid lipid dispersions, regardless the lipid:drug ratio, exhibited the highest mean volume particle size, with SA0.5 demonstrating the highest value ($391.754 \pm 0.022 \mu\text{m}$). However, although stearic acid lipid dispersions depicted larger mean particle sizes, it illustrated a smaller span compared to the glycerol monostearate lipid dispersions, meaning that the larger particles were distributed over a smaller range. All particle volume distribution curves of the stearic acid and glycerol monostearate lipid dispersions were negatively skewed and leptokurtic. The larger particle size of the stearic acid lipid dispersions positively influenced the flowability of these lipid dispersions as seen in Table 4.3. The three stearic acid lipid dispersions in varying lipid:drug ratios displayed notably superior flow properties compared to that of glycerol monostearate and cetyl alcohol lipid dispersions. SA0.75 depicted a flow rate of $27.788 \pm 0.255 \text{ g/s}$ compared to $18.326 \pm 0.120 \text{ g/s}$ for GM0.75 and $5.013 \pm 0.006 \text{ g/s}$ for CA0.75. Additionally, the narrow particle size distribution of stearic acid contributed further to its superior flow as a wide particle size distribution can lead to segregation due to the gravitational effects on small particles that flow between larger particles (Aulton, 2018). The stearic acid lipid dispersions thereby practically conveyed theoretical understanding and predictions on the correlation between particle size and particle size distribution and flow properties.

Cetyl alcohol lipid dispersions exhibited the largest span compared to glycerol monostearate and stearic acid lipid dispersions. The span of the cetyl alcohol lipid dispersions increased with a decrease in lipid concentration. However, this trend was not observed with stearic acid nor glycerol monostearate lipid dispersions as no significant variations in the span values obtained

and correlating lipid:drug ratio were present. All three of the particle size distribution curves for cetyl alcohol lipid dispersions were bimodal. This phenomenon suggests that there were two particle size ranges present, where the majority of the cetyl alcohol lipid dispersions particles fell. It offers insight into the fragmenting behaviour of the solidified mass after melting which caused two distinct particle size groups to form.

4.2.5. Differential Weight Loss Thermogravimetric Analysis

Differential weight loss thermogravimetric analysis (DTG) was employed to identify the percentage mass loss as well as the flow of heat in the form of thermograms. DTG was able to measure both parameters simultaneously and the results are recorded in Annexure B. The presence of a trough on a DSC graph indicates an endothermic reaction which signifies the point where melting occurred (Figure 4.5a). The sample weight does not decrease significantly over the course of an endothermic melting point. Conversely, a peak on a DSC thermogram indicates an exothermic event such as crystallisation or decomposition (Figure 4.5b), whilst base line shifts are indicative of glass transitioning (Figure 4.5c). The mass loss associated with TGA only occurs if a substance containing a volatile fraction is present or there is a percentage moisture loss.

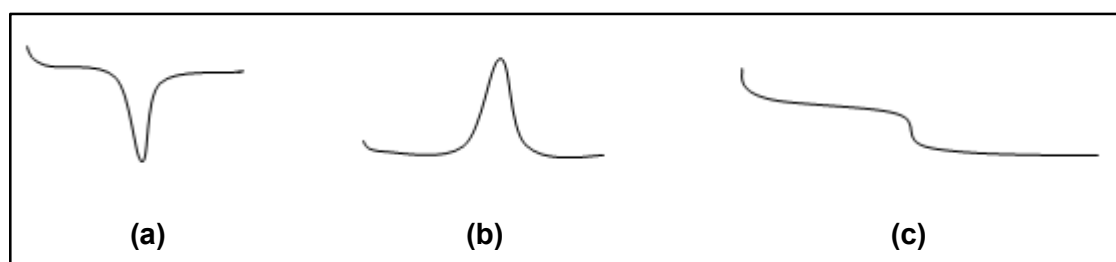


Figure 4.5: DSC thermogram interpretation of (a) endothermic melting peak (b) ideal exothermic reaction and (c) glass transition

Artemether (Figure B.1; Annexure B) depicted an endothermic reaction with a maximum endothermic melting peak occurring at 93.8°C. No water loss or degradation transpired which would have been characterised by an exothermic reaction (peak). This is in concurrence with the recorded onset of melting temperature range of 89–90°C for artemether in literature (Fule *et al.*, 2013). TGA displayed a 3.9251% loss in mass after 12 min. Although this value is slightly higher than the 3% specified by the USP, it was measured after melting occurred, which could account for this increased percentage mass loss. Lumefantrine (Figure B.2) depicted an onset of melting at 128.83°C with a maximum peak melting temperature at 141.15°C. Literature reports the onset of melting of lumefantrine in the range of 128–131°C (Kotila *et al.*, 2013) and thus the findings of this study were deemed acceptable.

DTG analysis of the lipid base raw materials (glycerol monostearate, stearic acid and cetyl alcohol, independently) were conducted to serve as reference standards for the prepared lipid

dispersions prepared. Glycerol monostearate (Figure B.4), stearic acid (Figure B.8) and cetyl alcohol (Figure B.12) displayed maximum melting at 85.53°C, 79.64°C and 49°C, respectively; which is also in accordance with literature (Acme-Hardesty, 2017; O'Neil, 2011; Sharma *et al.*, 2002). TGA thermograms of glycerol monostearate, stearic acid and cetyl alcohol revealed 0.7660, 1.0930 and 1.5294% mass loss, correspondingly, which can be ascribed to moisture loss in all three thermograms. The DSC thermograms of the lipid dispersions were only able to demonstrate the melting points of the lipids as these melting points occurred before those of the artemether and lumefantrine; and further indicated that no interactions transpired.

4.2.6. X-ray Powder Diffraction Studies

X-ray powder diffraction (XRPD) studies were conducted to offer insight into the crystallinity of the lipid dispersions manufactured in this study as well as of the individual components of the lipid dispersions (the active ingredients and lipid bases). XRPD was conducted to assess the effect of the hot fusion method on the individual components regarding crystalline and amorphous forms. The intensity counts for each individual component as well as the manufactured lipid dispersions are presented in Table 4.6. The presence of a plateau or hump formation on the graph is an indication of the amorphous nature of a sample, whereas a series of peaks and an intensity count above 40 000 depict crystalline structures (Byrn *et al.*, 1999). The respective graphs of each sample, indicating the crystallinity (sharp peaks) or amorphous form (plateau) are recorded in Annexure C.

Artemether (Figure C.1; Annexure C) and lumefantrine (Figure C.2) were found to be relatively crystalline given the sharp peak formed, and intensity counts above 100 000 and 80 000, respectively. This was in agreement with prior findings of light microscopy (Section 4.2.5) where both active ingredients were identified as crystalline. Light microscopy and HSM studies (Section 4.2.8) were conducted in order to supplement and confirm the XRPD data obtained.

Table 4.6: X-ray powder diffraction study results indicating intensity count

	Intensity Count
Artemether	>100 000
Lumefantrine	>80 000
Glycerol monostearate raw material	>60 000
Stearic acid raw material	>100 000
Cetyl alcohol raw material	>200 000
Glycerol monostearate 1:1	40 000
Glycerol monostearate 0.75:1	40 000
Glycerol monostearate 0.5:1	>60 000
Stearic acid 1:1	>60 000
Stearic acid 0.75:1	>60 000
Stearic acid 0.5:1	>60 000
Cetyl alcohol 1:1	>60 000
Cetyl alcohol 0.75:1	>60 000
Cetyl alcohol 0.5:1	>60 000

All of the lipid dispersions, regardless the lipid base employed, depicted crystalline structures with intensity peaks in excess of 40 000. It was visible with both light microscopy and HSM that the lipid coating of the drug particles that occurred after hot fusion was amorphous. The amorphous form of the lipid dispersion was not visible with XRPD as both artemether and lumefantrine retained their crystalline structure after hot fusion which accounts for the high crystallinity observed for all the lipid dispersions (i.e. >40 000 counts). Therefore, the lipid base that was present in the amorphous form after hot fusion only served as a coating of the drug particles and was not sufficient enough to obscure the crystallinity of the two active ingredients present. This result was ideal as it demonstrated that the physical characteristics of both artemether and lumefantrine were not affected by the hot fusion method. The reduction in intensity counts between the individual components and the lipid dispersions, however, serves as a representation of the amorphous lipid covering present in the lipid dispersions once hot fusion occurred.

4.2.7. Light Microscopy

Utilising a Nikon clinical microscope, images of the individual active ingredients, the individual lipid base raw materials, as well as the lipid dispersions of varying concentrations were captured and are presented in Figure 4.6. These images offer insight into the morphology of the various components of the lipid dispersions; and the degree to which the lipid base coated the two active ingredients particles.

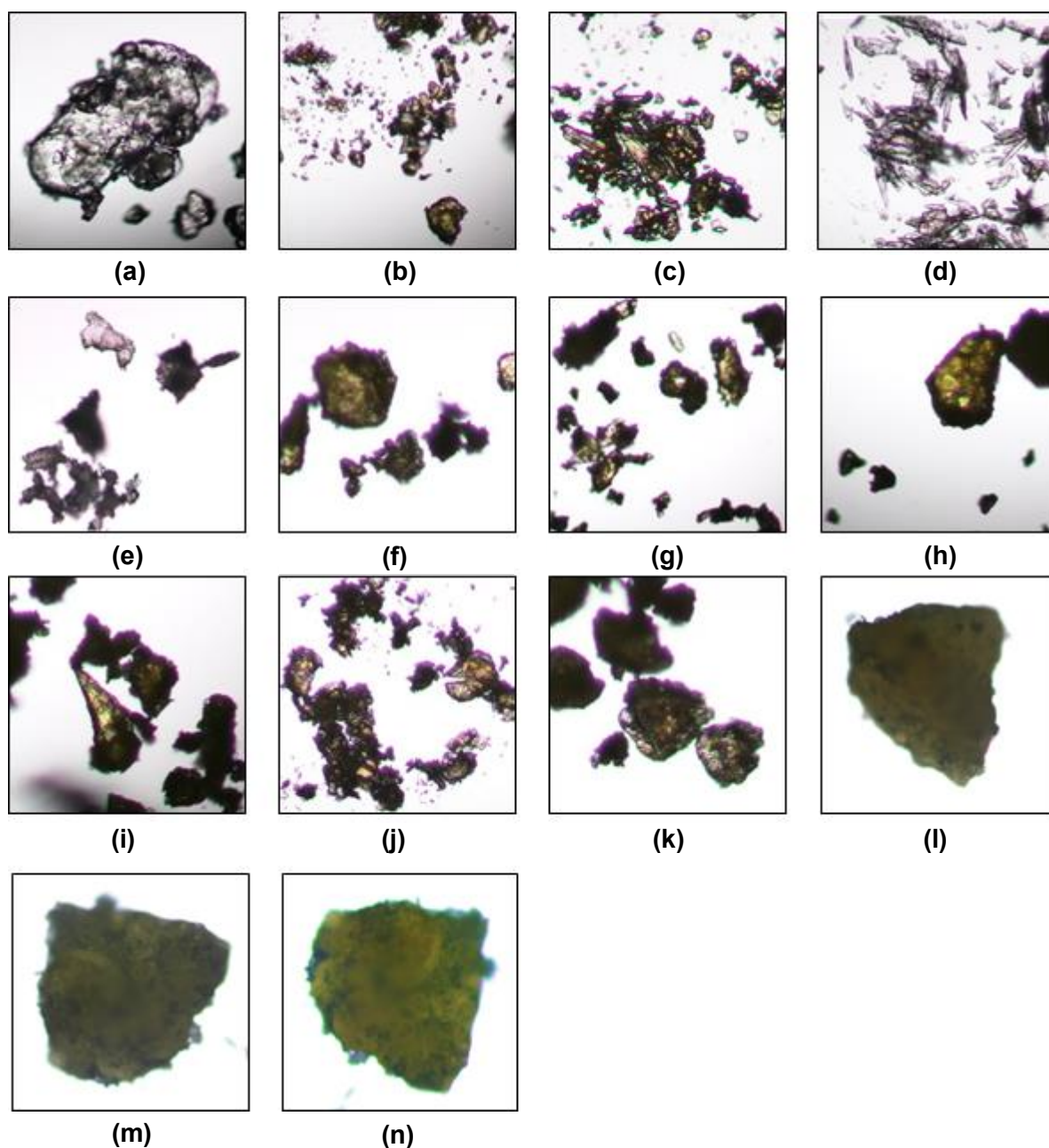


Figure 4.6: Light microscopy images at magnification 10x of (a) Artemether (b) Lumefantrine (c) GM raw material (d) SA raw material (e) CA raw material (f) GM0.5 (g) GM0.75 (h) GM1: (i) SA0.5 (j) SA0.75 (k) SA1 (l) CA0.5 (m) CA0.75 (n) CA1

The crystalline structures of artemether, lumefantrine, glycerol monostearate, stearic acid and cetyl alcohol can clearly be seen in Figure 4.6 (a–e). Crystalline structures reflect light and can appear clear or birefringence is observed. Lumefantrine (Figure 4.6b) is crystalline and yellow in colour, rendering it easy to differentiate from other components within the lipid dispersions. It is evident from Figure 4.6 (f–n) that once the lipid base melted and was allowed to solidify again to form the lipid dispersions, the lipid bases coated the drug particles and were subsequently observed as being amorphous. This is represented by the dark areas visible in Figure 4.6 (f–n) due to the fact that amorphous forms lack interference colours (Stieger *et al.*, 2012). In conclusion, all of the lipid bases displayed adequate coating of the drug particles.

4.2.8. Hot-Stage Microscopy Results

Hot-stage microscopy (HSM) studies were conducted complimentary to DTG and XRPD analysis as to supplement this information. Both active ingredients, together with the lipid base raw materials and the prepared lipid dispersions in varying concentrations were evaluated by means of a series of images captured using a Nikon Eclipse 50i microscope (Section 3.5.4). The initial image of each sample was taken at 25°C to capture each samples form at ambient room temperature. Thereafter, the samples were heated at a heating rate of 10°C/min and an image of the onset of melting of the respective components was captured and the corresponding temperature recorded. A number of images were taken after the onset of melting of the lipid base to observe the temperature at which the individual components of the lipid dispersion melted. A polaroid lens was utilised to highlight birefringence of crystalline structures during melting. Initially the polaroid lens was not utilised as the lipid base coating the active ingredients within the lipid dispersions was amorphous, depicted by no colour interference (Stieger *et al.*, 2012). Given the irregular size and shape of the lipid dispersion particles, it was not always possible to focus the entire image in the initial photo as the particle had an inconsistent height. Once the lipid base had melted, the polaroid lens was employed to clearly identify the crystalline structures of the active ingredients.

Artemether (Figure 4.7) and lumefantrine (Figure 4.8) were individually captured and identified to have crystalline structures. Artemether depicted an onset of melting at 86°C and had completely melted at 89°C, which was consistent with literature as Fule *et al.* (2013) reported melting between 86–90°C. The yellow colour of lumefantrine could clearly be seen and was valuable during the melting process of the lipid dispersions as it allowed lumefantrine to easily be distinguished from other components within the lipid dispersions. Lumefantrine experienced onset of melting at 120°C and had almost completely melted at 130°C. Kotila *et al.* (2013) reported the melting point of lumefantrine to be between 128–131°C. The slight difference in melting point can be attributed to this study recording the onset of melting (120°C) compared to the maximum melting point

recorded by Kotila *et al.* (2013). Furthermore, a difference in machinery could additionally account for this slight variance in results.

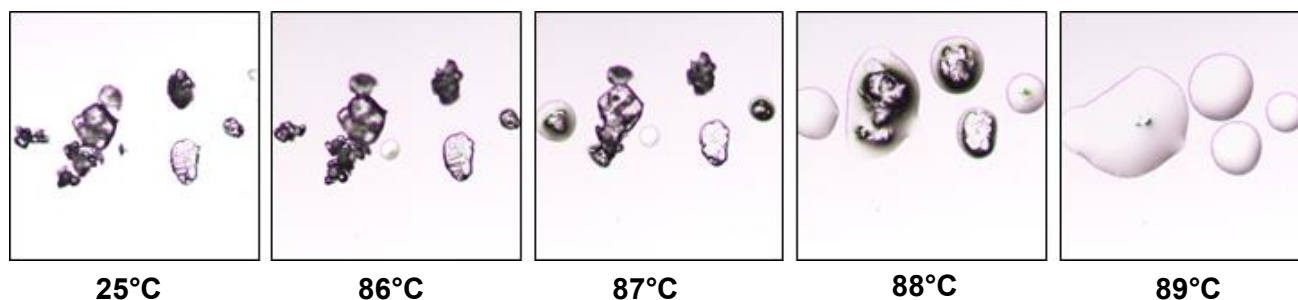


Figure 4.7: HSM micrographs of artemether at increasing temperatures at magnification 10x

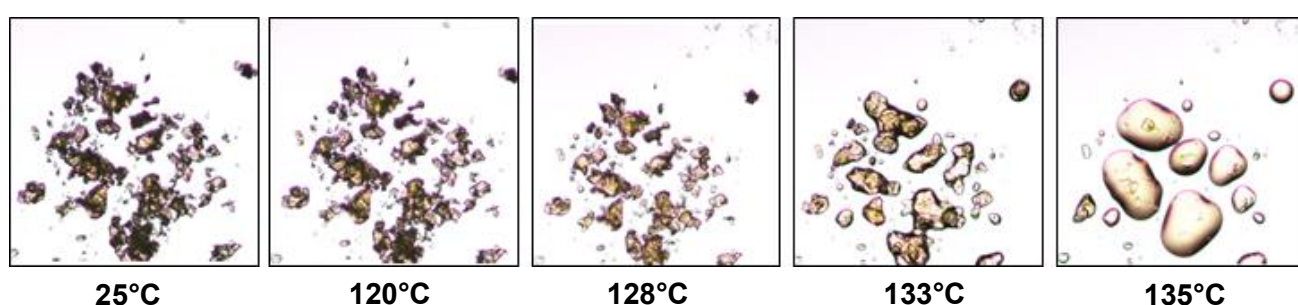


Figure 4.8: HSM micrographs of lumefantrine at increasing temperatures at magnification 10x

The three lipid bases utilised in this study were initially analysed as raw materials and then examined within the lipid dispersions in order to obtain an indication of a change in form. Stearic acid (Figure 4.9), glycerol monostearate (Figure 4.10) and cetyl alcohol (Figure 4.11) all appeared to be crystalline in their raw form as the particles presented clear, with birefringence under a polaroid lens (Stieger *et al.*, 2012). According to literature, stearic acid has a melting point of 66–69°C (Acme-Hardesty, 2017) which is higher than the results attained in this study as the onset of melting was recorded as 58°C. Glycerol monostearate displayed an onset of melting at 65°C, which is in accordance with literature that stated that this lipid's melting point to be 54–65°C (Castor international, 2017). Of the three lipid bases investigated, cetyl alcohol demonstrated the lowest onset of melting at 51°C; and it was almost completely melted at 52.5°C. O'Neil (2011) reported the melting range to be between 46–52°C; and the results of this study concurred with those findings.

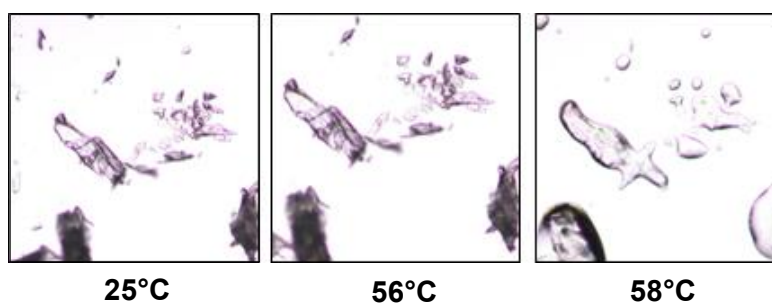


Figure 4.9: HSM micrographs of stearic acid at increasing temperatures at magnification 10x

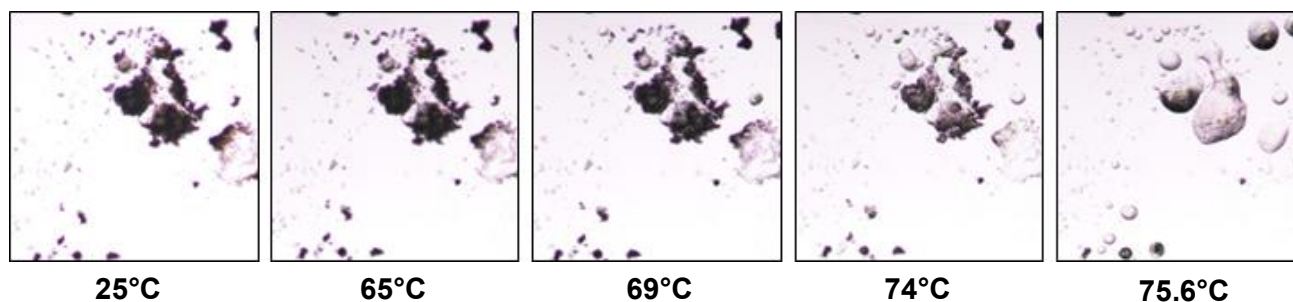


Figure 4.10: HSM micrographs of glycerol monostearate at increasing temperatures at magnification 10x

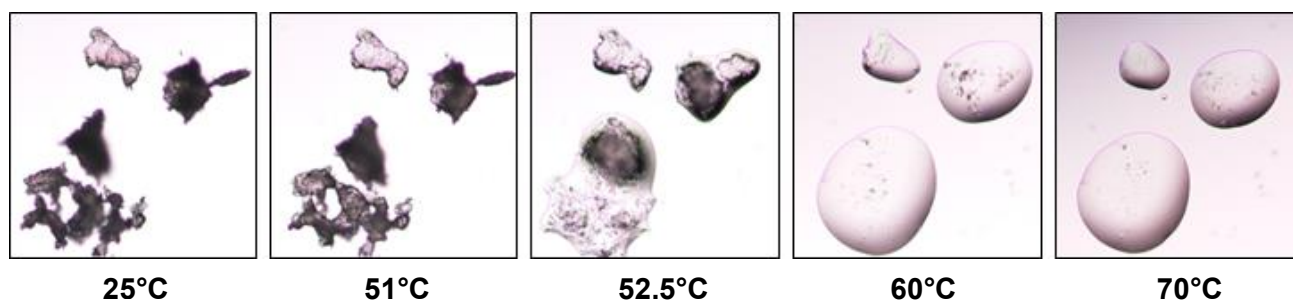


Figure 4.11: HSM micrographs of cetyl alcohol at increasing temperatures at magnification 10x

Due to the careful selection of each component regarding its melting point, it was possible to clearly identify each component at different temperatures during the heating process. All three lipid bases exhibited melting points lower than that of artemether, with lumefantrine depicted the highest melting point. At 25°C none of the lipid dispersions demonstrated any colour interaction, thereby signifying the amorphous property of the lipid base post hot fusion. As the temperature approached the melting point of the specific lipid base, there was visible spreading of the lipid and the crystalline structures of both artemether and lumefantrine became visible, and identifiable, as seen in Figures 4.12–4.20. The artemether crystals began to move and subsequently melt as the temperature approached approximately 85°C. At 90°C, only lumefantrine crystals remained as all artemether crystals had already melted; and the point of onset of melting could clearly be observed for the remaining lumefantrine crystals.

The stearic acid lipid dispersions demonstrated a lipid:drug concentration dependent onset of melting. The SA1 lipid dispersion (Figure 4.12) depicted the highest onset of melting at 55°C and as the lipid concentration in the dispersions decreased, the onset of melting similarly lowered. SA0.75 (Figure 4.13) experienced an onset of melting at 48°C, whereas SA0.5 (Figure 4.14) portrayed the lowest onset of melting at 46°C. This linear relationship that exists between stearic acid lipid:drug concentration and onset of melting may be due to the quantities of active ingredients present. The highly crystalline structures of artemether and lumefantrine possess a large number of covalent bonds and subsequently have high melting points (Brown & Brown, 2000; Smith & Williams, 1983). Therefore, when present in higher concentrations such as in SA0.75 and SA1, the active ingredients absorb energy in the form of heat and require more energy to initiate melting due to the presence of strong covalent bonds (Kohli, 2009). Thus requiring that more heat is required to initiate melting of the lipid base as the crystalline structures of the active ingredient are absorbing a large portion of the heat transfer during experimentation accounting for the differences in onset of melting temperatures (Brown & Brown, 2000; Smith & Williams, 1983).

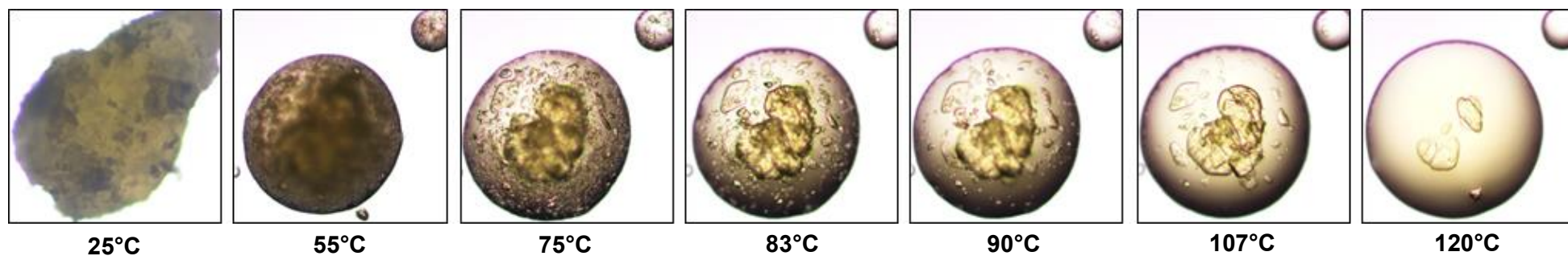


Figure 4.12: HSM micrographs of stearic acid 1:1 lipid dispersion at increasing temperatures at magnification 10x

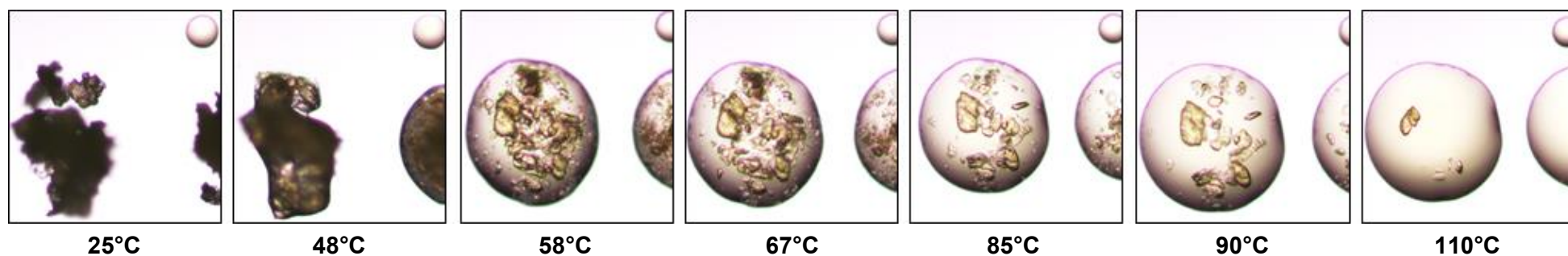


Figure 4.13: HSM micrographs of stearic acid 0.75:1 lipid dispersion at increasing temperatures at magnification 10x

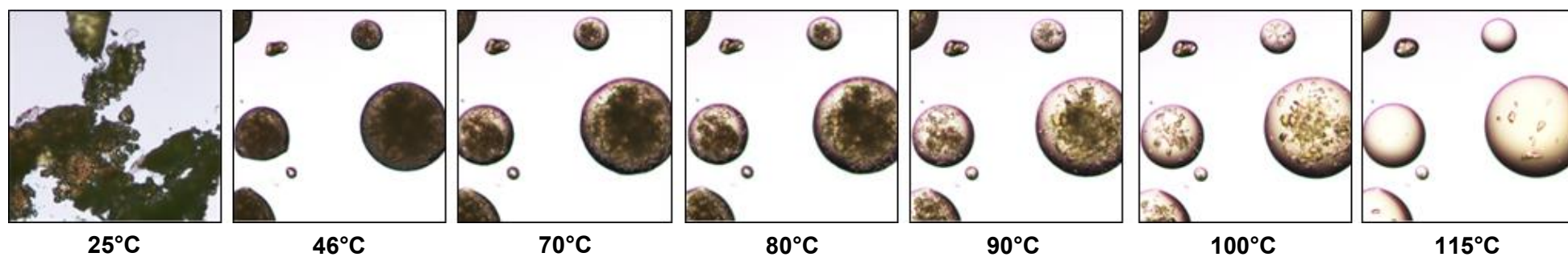


Figure 4.14: HSM micrographs of stearic acid 0.5:1 lipid dispersion at increasing temperatures at magnification 10x

Lipid dispersions containing both glycerol monostearate and cetyl alcohol depicted onset of melting within the temperature ranges recorded in literature. According to Castor International (2017), glycerol monostearate has an onset of melting between 54–65°C. GM1 (Figure 4.15) depicted a slightly higher onset of melting (68°C) which could be as a result of the presence of the two highly crystalline active ingredients that absorb a portion of the heat transfer, as previously explained. GM0.75 and GM0.5 displayed onset of melting at 65°C and 64°C, respectively; which is within the recorded and expected temperature range. Cetyl alcohol has a recorded onset of melting between 48–53°C (Sharma *et al.*, 2002); and all three cetyl alcohol lipid dispersions investigated in this study compared well with this noted melting range. The overall trend observed, regardless of the lipid base employed, was that as the active ingredient concentration increased, there was a slight increase in the onset of melting temperature of the lipid bases; with GM1 displaying an onset of melting above the recorded temperature range for the lipid base raw material.

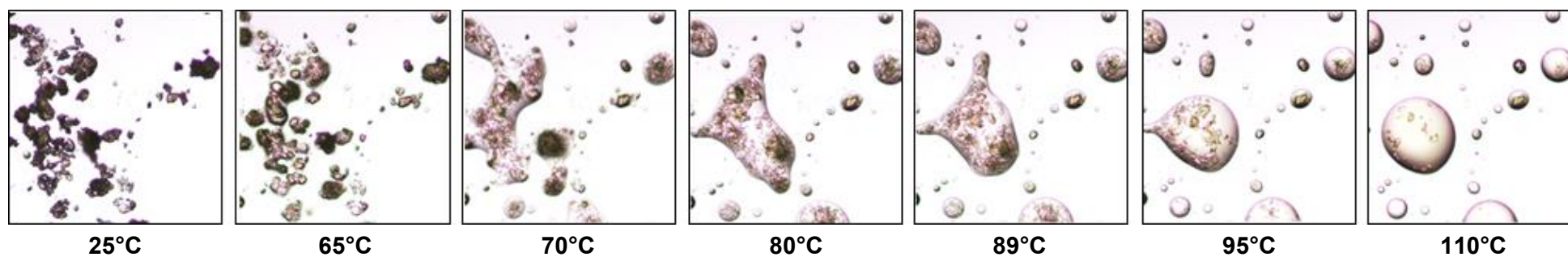


Figure 4.15: HSM micrographs of glycerol monostearate 1:1 lipid dispersion at increasing temperatures at magnification 10x

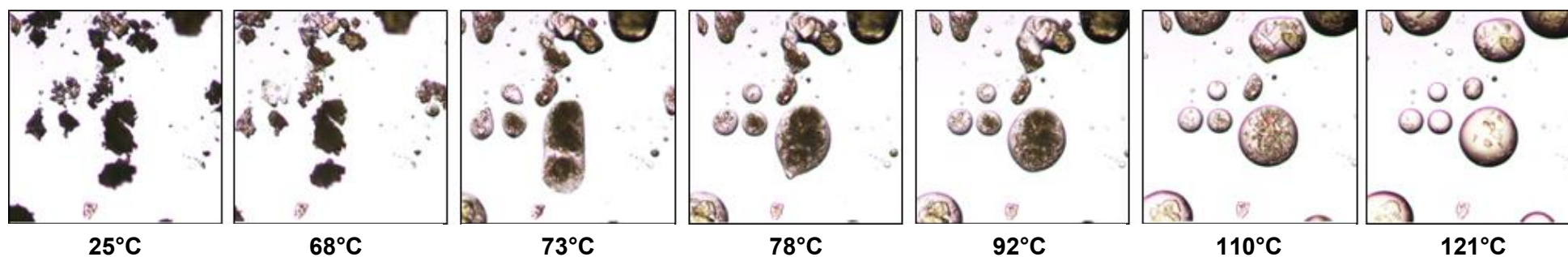


Figure 4.16: HSM micrographs of glycerol monostearate 0.75:1 lipid dispersion at increasing temperatures at magnification 10x

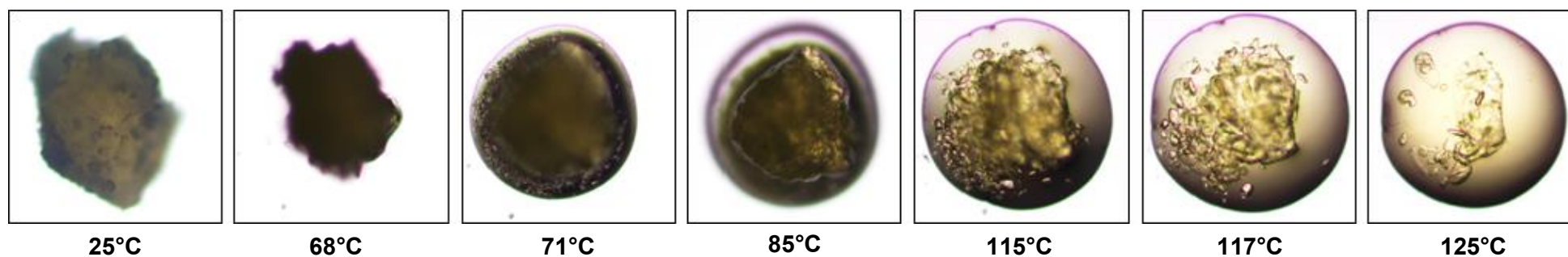


Figure 4.17: HSM micrographs of glycerol monostearate 0.5:1 lipid dispersion at increasing temperatures at magnification 10x

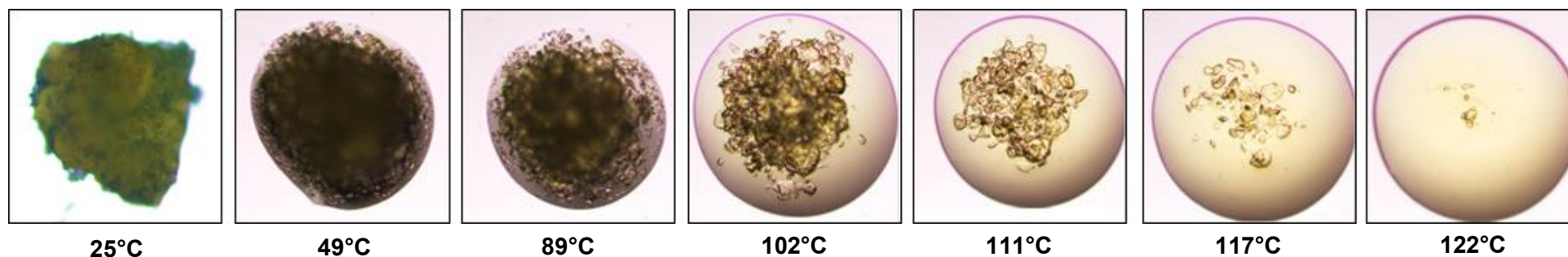


Figure 4.18: HSM micrographs of cetyl alcohol 1:1 lipid dispersion at increasing temperatures at magnification 10x

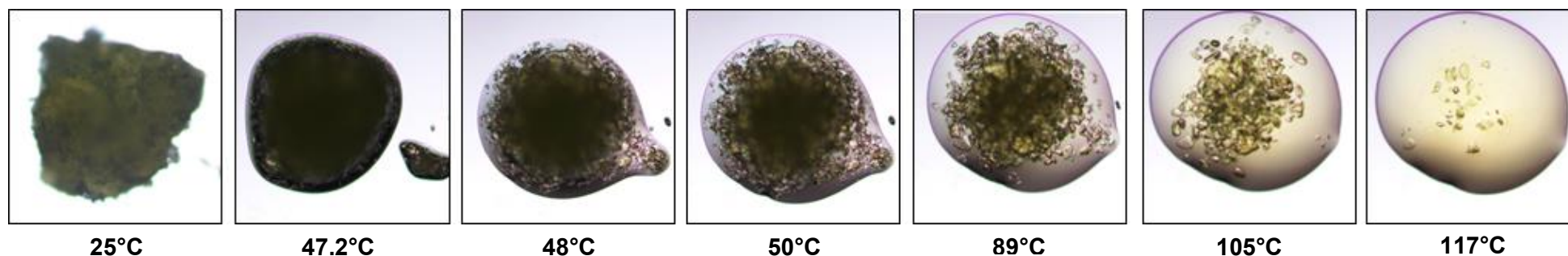


Figure 4.19: HSM micrographs of cetyl alcohol 0.75:1 lipid dispersion at increasing temperatures at magnification 10x

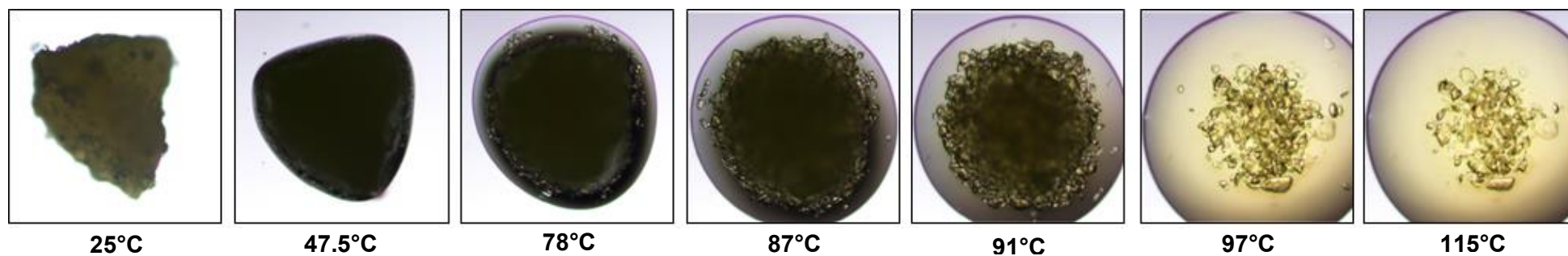


Figure 4.20: HSM micrographs of cetyl alcohol 0.5:1 lipid dispersion at increasing temperatures at magnification 10x

4.3. Pre-Formulation Conclusion

Pre-formulation studies revealed the crystallinity of both active ingredients prior to as well as post hot-fusion. This was confirmed via light microscopy, DSC, XRPD and HSM. Thus the method of manufacture did not alter the physicochemical characteristics of the double-fixed dose combination.

The prepared lipid dispersions, fillers and active ingredients were evaluated in terms of particle size, particle size distribution and flow properties in order to offer insight into potential powder flow problems that may be detrimental to the manufacture of lipid matrix tablets by means of direct compression. This analysis revealed that the lipid dispersions provided an overall improvement in powder flow characteristics, specifically in comparison to lumefantrine. The following rank order was interestingly assigned to the fillers regarding both particle size and particle size distribution together with powder flow characteristics: CombiLac® > MicroceLac® 100 > RetaLac® > Pharmacel® 101; thereby practically conveying theoretical understanding and predictions on the correlation between particle size, particle size distribution and flow properties. It was therefore hypothesized that CombiLac® and MicroceLac® 100 would assist in the manufacturing of superior lipid matrix tablets compared to the other two fillers based upon their flow properties and correlation to particle size and shape.

During HSM studies, it was noted that the high crystallinity of the two active ingredients caused a slight increase in the point of onset of melting of the lipid bases which is explained by the absorption of energy in the form of heat by the crystal lattice of the two active ingredients. Conclusion of these pre-formulation tests resulted in the successful completion of the first four objectives set out for this study and offered useful insights into the lipid dispersion and filler powder flow characteristics which would prove all important for the upcoming manufacturing of the lipid matrix tablets.





5.1. Introduction

Utilising a full factorial design, the lipid matrix tablets were manufactured and the physical tablet properties (mass variation, friability, disintegration, hardness, thickness, diameter and tensile strength) were evaluated. Formulations that complied with the above-mentioned tests were further evaluated in terms of morphology, swelling, erosion, assays and drug dissolution characteristics. This chapter also includes the validation of the HPLC method utilised in this study. Dissolution studies comparing the absorption of this study's optimised formulations to that of the commercially available product, Coartem®, were also carried out. All of these experiments were conducted following the BP (2018) standards. Lastly, once the optimal formulation from this study had been identified, *in vitro* permeability studies were conducted as a proof of concept.

5.2. Physical Tablet Property Test Results

Formulations for this study were manufactured utilising a full factorial design of experiments and tablets were tableted to a weight of approximately 500 mg. All of the manufactured formulations were evaluated in accordance with the BP (2018) guidelines and specifications. Once the formulations were tableted, they were allowed to stand for a minimum period of 24 h prior to evaluation in order for plastic and elastic deformation to first occur. All formulations were left for an equal period of time in order to ensure uniformity of the test conditions. Results acquired from the physical property experiments (mass variation, disintegration, friability and tensile strength) are recorded in Annexure D. The same abbreviations used previously for the full factorial design are utilised to describe the various formulations, with the now added suffixes for fillers: C: CombiLac®; M: MicroceLac® 100; R: RetaLac®; P: PharmaceL® 101; as well as 1% and 1.25% magnesium stearate denoted as 1 and 1.25. Therefore, the code CA0.5M1 signifies cetyl alcohol (CA) in a 0.5:1 ratio with the double-fixed dose combination of artemether and lumefantrine; MicroceLac® 100 (M) as filler; and 1% magnesium stearate as lubricant.

All formulations containing MicroceLac® 100 and CombiLac® as fillers, regardless of the lipid base or lipid:drug ratio, passed all of the physical property tests conducted. Conversely, all 18 PharmaceL® 101 formulations manufactured failed all of the set criteria and it can therefore be concluded that PharmaceL® 101 is not a suitable filler when used in combination with the factors investigated in this study. RetaLac® was able to produce a few formulations capable of passing some of the physical property tests; however, there was not one RetaLac® formulation that passed all four physical property tests. For this reason, it was concluded that RetaLac® was similarly not able to produce lipid matrix tablets with the necessary physical characteristics utilising the given

factors of this study and consequently, RetaLac® lipid matrix tablet formulations together with formulations comprising Pharmacel® 101 were discarded from further evaluation. It is important to note that whilst glycerol monostearate and stearic acid lipid dispersions in combination with RetaLac® or Pharmacel® 101 produced poor tablets, all cetyl alcohol formulations containing RetaLac® or Pharmacel® 101 were unable to produce any tablets at all, indicating that neither are viable lipid base-filler combinations.

Due to the utilisation of a full factorial design to assess each factor systematically and impartially, a summary table (Table 5.1) comparing all of the factors evaluated at each level, and the corresponding response was drawn up. Pharmacel® 101 exhibited very poor flowability during powder flow characterisation which was evident during the tableting process as well. All Pharmacel® 101 formulations, irrespective of the lipid base, experienced incomplete filling of the tablet die during tableting. This in turn resulted in relatively high percentage relative standard deviations (%RSDs) during mass variation trials. While stearic acid formulations containing Pharmacel® 101 depicted a slight reduction in mass variation, it was not sufficient enough for the formulations to pass the test in accordance with the BP (2018) specifications. From Table 5.1 it is furthermore clear that Pharmacel® 101 depicted the highest %RSD for mass variation (3.758%) followed by RetaLac® (2.708%). Formulation SA0.5P1.25 portrayed the highest percentage friability of 77.85% (Annexure D) and only 3 of the 18 Pharmacel® 101 formulations, namely GM0.75P1, GM1P1.25 and SA0.75P1, passed the friability criteria. Pharmacel® 101 produced tablets with markedly low tensile strength, on average 0.309 N.mm^{-2} , and several tablet formulations (disregarding the lipid base or lipid:drug ratio) crumbled upon handling. Many of these lipid matrix tablets were too soft to obtain any hardness or tensile strength results and subsequently no clear conclusions could be drawn. The Pharmacel® 101 formulation with the highest tensile strength was GM1P1.25 (0.720 N.mm^{-2}) whilst SA1P1 (0.116 N.mm^{-2}) displayed the most fragile lipid matrix tablets overall.

The effects of poor powder flow resulting in inconsistent tablet weights, a subsequent lack of content uniformity and ultimately weak tablets, were further confirmed during disintegration tests. All of the Pharmacel® 101 formulations disintegrated completely within seconds, with SA1P1 displaying the longest disintegration time of 2.04 min. Disintegration results were not in agreement with the objectives outlined for this study as this study aimed to achieve a modified release dosage form, requiring disintegration times in excess of the 15 min specified by the BP (2018) for conventional tablets. However, all other lipid base and filler combinations produced disintegration results in agreement with the objective set to achieve modified release. For this reason, disintegration was not discussed per factor as only Pharmacel® 101 yielded formulations unable to meet the set objective regarding disintegration.

Table 5.1: Comparison of all formulation factors investigated in the full factorial design regarding average mass variation, friability and tensile strength results

	Mass Variation		Friability (%)	Tensile Strength (N.mm ⁻²)
	Mass (mg)	%RSD		
Pharmacel® 101	497.804	3.758	13.941	0.309
RetaLac®	487.692	2.708	12.460	0.172
MicroceLac® 100	494.939	1.390	0.429	1.850
CombiLac®	494.533	1.712	0.519	1.978
Glycerol Monostearate	494.558	2.898	5.672	0.958
Stearic Acid	497.377	1.804	7.742	1.104
Cetyl Alcohol	485.833	1.719	0.997	2.097
0.5:1	493.363	2.768	12.746	1.306
0.75:1	493.355	1.948	2.554	1.253
1:1	495.105	1.959	1.394	1.174
MgSt 1%	493.388	2.013	3.798	1.244
MgSt 1.25%	494.493	2.436	7.298	1.246

RetaLac® formulations depicted the second highest %RDS in view of mass variation assessment results (2.708%) attained for the selected fillers as well as the lowest average tensile strength (0.172 N.mm⁻²). RetaLac® lipid matrix tablets portrayed an average friability of 12.460%, with GM0.5R1.25 demonstrating the highest friability (76.022%) and GM1R1 the lowest (1.005%). Although GM1R1 achieved the lowest %Friability for formulations containing RetaLac®, it still did not comply with the BP (2018) specification of less than 1% mass loss during testing. Once again, no clear trend could be established between any of the factors for lipid matrix tablet formulations comprising this filler. RetaLac® was selected as a filler option based on its individual components, namely hypromellose and D-lactose monohydrate co-pressed excipients which control drug release predominantly by diffusion through the hydrophilic matrix formed. This gel layer was clearly visible during disintegration evaluation and all RetaLac® containing formulations did not disintegrate within the specified 15 min time limit, which was ideal as this study aimed to achieve modified release. Prolonged disintegration times (longer than 15 min) were considered acceptable, as this would assist in obtaining modified release characteristics. Unfortunately, despite RetaLac® formulations passing the disintegration tests and forming a gel matrix system, the lack of appropriate tablet properties such as hardness and friability, resulted in RetaLac®'s inability to produce lipid matrix tablets of suitable integrity, rendering it inappropriate for further investigation.

It was furthermore observed that when all the factors were considered and the averages calculated per factor (Table 5.1), the poor results of RetaLac® and Pharmacel® 101 lipid matrix tablet formulations were skewing the overall data obtained. Therefore, a fair and accurate comparison could not be conducted. Table 5.2 was thus included so that an accurate comparison between MicroceLac® 100 and CombiLac® lipid matrix tablet formulations could be formulated without interference from the skewed data. This was necessary as only the formulations containing MicroceLac® 100 and CombiLac® were considered for further testing during this study, and an accurate understanding of the physical properties of these formulations was required before the next phase of analysis could commence.

Table 5.2: Comparison of average results obtained for mass variation, friability and tensile strength considering MicroceLac® 100 and CombiLac® formulations only

	Mass Variation		Friability (%)	Tensile Strength (N.mm ⁻²)
	Mass (mg)	%RSD		
MicroceLac® 100	494.939	1.390	0.429	1.850
CombiLac®	494.533	1.710	0.519	1.978
Glycerol Monostearate	498.592	1.912	0.209	1.613
Cetyl Alcohol	485.833	1.719	0.997	2.097
Stearic Acid	499.783	1.027	0.217	2.031
0.5:1	495.775	2.088	0.459	2.084
0.75:1	496.500	1.411	0.430	1.923
1:1	491.930	1.158	0.534	1.735
MgSt 1%	497.130	1.411	0.460	1.914
MgSt 1.25%	492.339	1.694	0.489	1.914

From Table 5.2 it can be seen that there were no significant differences between the %RSD-values for mass variation, the friability or tensile strength results attained for either MicroceLac® 100 or CombiLac® formulations.

The type of lipid base employed was compared and evaluated as a factor in the form of a dual-scaled axis graph (Figure 5.1) in terms of %friability and tensile strength. The average results obtained for glycerol monostearate revealed a friability of 0.209%, a tensile strength of 1.613 N.mm⁻², as well as the highest %RSD for mass variation (1.912%) of the lipid bases employed. Glycerol monostearate displayed significantly lower average tensile strength values for formulations containing MicroceLac® 100 and CombiLac® in comparison to the other lipid bases. Cetyl alcohol, on the other hand, exhibited the highest average tensile strength (2.097 N.mm⁻²) and also portrayed the highest %friability (0.997%); indicating that the increased

tensile strength caused the tablets to be very brittle, which led to an increased %mass loss (friability) during friability evaluation. Although the average friability of cetyl alcohol was still in compliance with the BP (2018) specifications, it was meaningfully higher than the average friability results of glycerol monostearate and stearic acid (0.209 and 0.217%, respectively). Stearic acid depicted the smallest average %RSD for mass variation, with a tensile strength of 2.031 N.mm⁻² and a satisfactorily low %friability of only 0.217%. As stated and hypothesized in Chapter 2, stearic acid is often employed as a thickening and hardening agent in the cosmetic industry which may account for the increased tablet hardness presented as superior tensile strength in comparison to the other lipid bases. In terms of the physical tablet characteristics, the following rank order could be allocated to the type of lipids investigated: stearic acid > glycerol monostearate > cetyl alcohol.

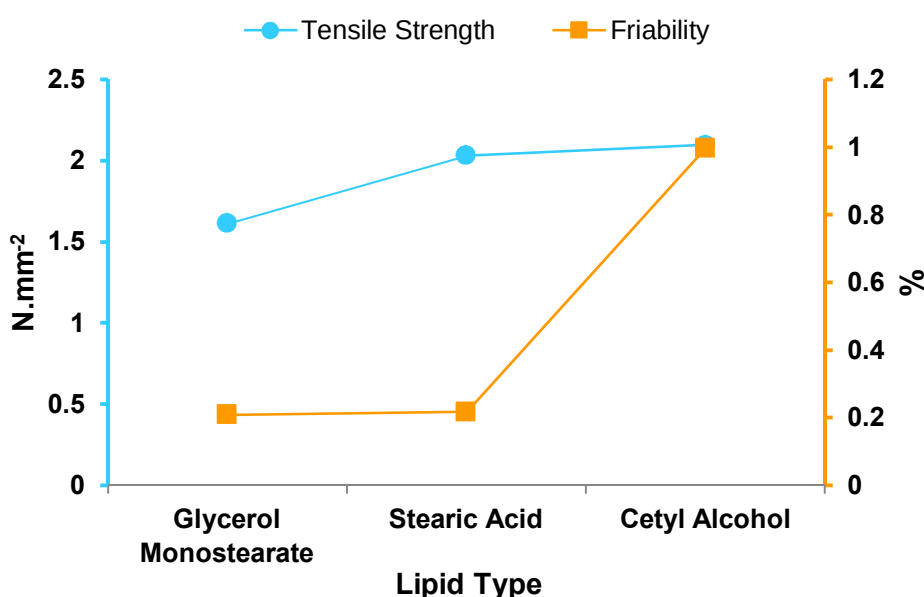


Figure 5.1: Comparison and evaluation of lipid type factor considering tensile strength on the left y-axis and friability on the right y-axis

A distinct correlation between the ratio, friability and tensile strength could be established when the lipid:drug ratios were considered. This correlation was plotted as a dual-scaled axis graph (Figure 5.2) and revealed that an increase in lipid concentration caused a subsequent decrease in the tensile strength and an increase in the %friability. This correlation between tensile strength and %friability can be attributed to harder tablets, with higher tensile strength, having stronger interparticle forces and tight compaction thereby rendering the tablets less friable. In terms of the more acceptable physical tablet characteristics, the following rank order can be allocated to the lipid:drug ratios investigated in this study: 0.75:1 > 0.5:1 > 1:1.

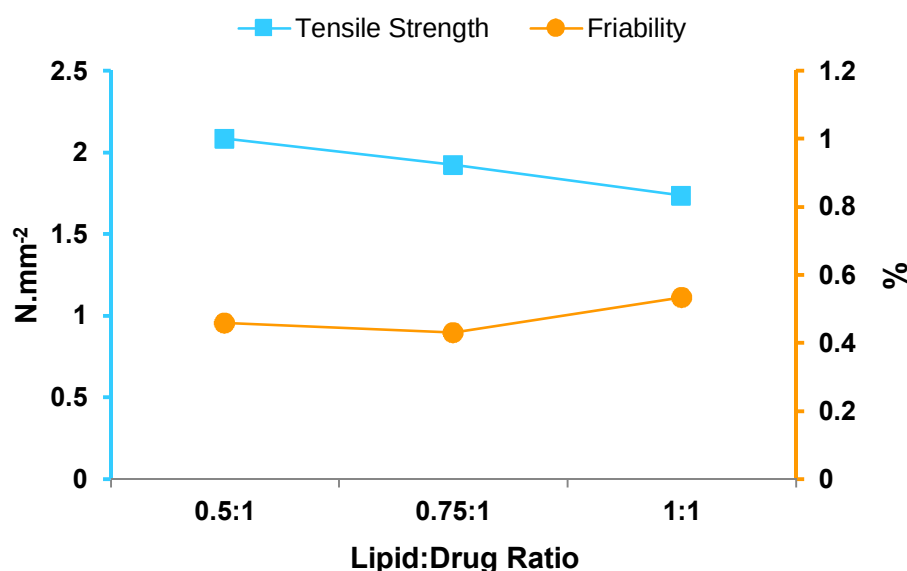


Figure 5.2: Comparison and evaluation of lipid:drug ratio factor considering tensile strength on the left y-axis and friability on the right y-axis

The 0.5:1 lipid:drug ratio produced tablets with the highest tensile strength (2.084 N.mm^{-2}) and an adequately low friability (0.459%), however, it exhibited the highest %RSD-values for mass variation (2.088%). The lower lipid content meant that there was more filler present in the 0.5:1 formulations, which could account for the higher %RSD-values due to the compaction properties of the filler as well as the fact that the fillers have smaller particle size distributions compared to the lipid dispersions. MicroceLac® 100 and CombiLac® presented with a mean volume particle size of $172.029 \mu\text{m}$ and $177.561 \mu\text{m}$, respectively; whereas the 0.5:1 lipid dispersions of cetyl alcohol, stearic acid and glycerol monostearate exhibited significantly higher mean volume particle sizes of $280.939 \mu\text{m}$, $391.754 \mu\text{m}$ and $185.156 \mu\text{m}$, respectively. Larger particles, such as those of the lipid dispersions, flow more freely than smaller particles (the fillers) which have larger surface areas to form adhesive bonds (Aulton, 2018). Moreover, larger particles are subjected to gravitational force (Aulton, 2018) during tableting and thus the difference in mean volume particle size between the lipid dispersions and the fillers could account for the larger %RSD-values observed with the smaller lipid:drug ratio (0.5:1). The 0.75:1 ratio produced an intermediary tensile strength value (1.923 N.mm^{-2}) and the lowest %friability (0.430%). This indicates that although the 0.5:1 ratio depicted %friability in accordance with the BP (2018) standards, the increased tensile strength caused the tablets to be too hard and these tablets subsequently chipped accounting for the slightly higher %friability compared to that of the 0.75:1 ratio. The 1:1 ratio demonstrated the lowest tensile strength (1.735 N.mm^{-2}) and the highest %friability, implying that the reduction in tablet hardness caused formulations containing the 1:1 lipid:drug ratio to be more friable.

During pre-formulation studies, it was identified that a lubricant (magnesium stearate) was required to aid the compaction process due to its ability to reduce wall friction during tablet ejection (Sheskey *et al.*, 1995), improve powder flow (Podczeck & Miah, 1994) and reduce the risk of the pharmaceutical formulation adhering to exposed metal surfaces during tableting (Sabir *et al.*, 2001; Yamamura *et al.*, 2009). Formulations containing 0% magnesium stearate adhered to the tablet punches during tableting resulting in non-uniform appearance (Figure 5.3), tablet weight and physical tablet properties. The inclusion of magnesium stearate at the two levels of 1% and 1.25% did not produce a substantial difference in results between formulations containing MicroceLac® 100 and CombiLac® as seen in Table 5.2 and Figure 5.3. The higher level (1.25%) was however necessary during the production of formulations containing RetaLac® and Pharmacel® 101, as these tablet formulations adhered to the surface of the tablet die, particularly the upper punch which applies the compression force.

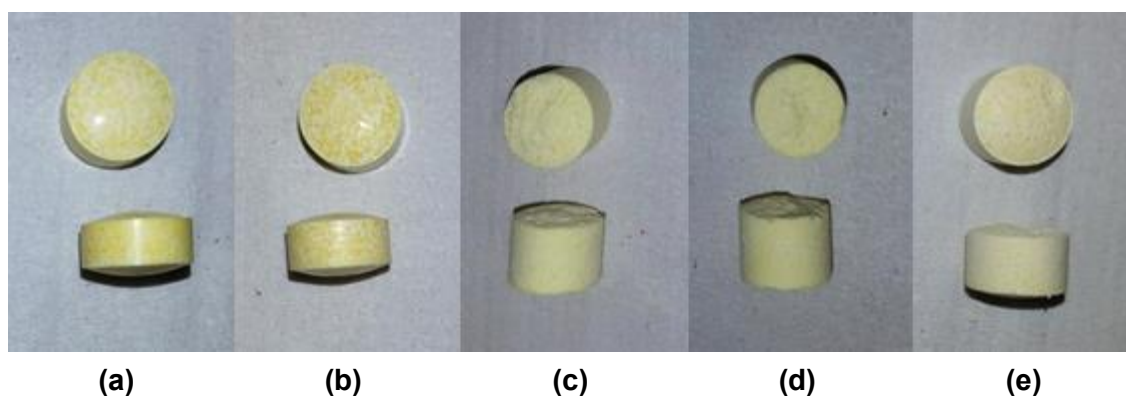


Figure 5.3: Photographs demonstrating the impact of magnesium stearate concentration on the different formulations manufactured containing (a) MicroceLac® 100 at 1% lubricant, (b) CombiLac® at 1% lubricant, (c) RetaLac® at 1% lubricant, (d) RetaLac® at 1.25% lubricant, and (e) Pharmacel® 101 at 1.25% lubricant

With reference to MicroceLac® 100 and CombiLac®, both concentrations of magnesium stearate produced acceptable %RSD-values for mass variation of 1.411% and 1.694%, respectively; and an equal tensile strength of 1.914 N.mm⁻². This could indicate that the difference in magnesium stearate concentration was possibly not sufficient to produce a significant effect regarding MicroceLac® 100 and CombiLac®, despite producing a visibly noticeable difference with the other two fillers. Formulations containing RetaLac® and Pharmacel® 101 experienced a higher variance in %RSD-values for mass variation as well as increased friability with inclusion of the higher lubricant level (1.25%). However, this magnesium stearate concentration overall produced more aesthetically appealing tablets that adhered less to the tablet punches.

The effect of an increase in magnesium stearate concentration can clearly be seen with the results presented in Table 5.1, which considered all four fillers. An increase in magnesium stearate concentration resulted in an increase in %RSD-values for mass variation and an even more pronounced increase in the %friability. The occurrence of an increase in magnesium stearate concentration associated with a decrease in tablet hardness, resulting in increased friability, is well documented (Bossert & Stamm, 1980; Dansereau & Peck, 1987; Bolhuis & Hollzer, 1996; Kikuta & Kitamori, 1994; Sheskey *et al.*, 1995). This phenomenon correlates strongly with an increased mixing time and for this reason magnesium stearate was only added and blended for 2 min once all the other excipients had been blended for 5 min to balance the benefits of magnesium stearate as a processing aid with potential drawbacks to product quality (Wang *et al.*, 2010). Since only formulations containing MicroceLac® 100 and CombiLac® would progress to the next phase of testing, it was decided to select the lowest functioning concentration (i.e. 1% magnesium stearate) to be kept as a constant moving forward to the next experimental stage as the investigated difference in magnesium stearate concentration had negligible effects on these two fillers.

5.3. Full Factorial Design of Experiments

A full factorial design was utilised as a pilot study to evaluate all of the proposed filler, lipid type and lipid dispersion formulation combinations possible and thereafter evaluate the physical properties of the manufactured formulas. The purpose of conducting the pilot study was to identify significant responses relating to the investigated factors. Lipid matrix tablet formulations that did not comply with the criteria set by the BP (2018) for the physical tablet property experiments were eliminated before further tests were conducted as they did not meet the objectives set for this study. These non-complying formulations could however be investigated further in a future study to identify the factors that could be enhanced and possible additional factors that would need to be investigated in order to optimise these formulations.

RetaLac® and Pharmacel® 101 failed to meet the BP (2018) specifications regarding the tablet physical property tests and therefore these two factors were removed from the factorial design before further testing commenced. During pre-formulation studies, it was found that 0% magnesium stearate did not yield viable tablets and so this level was removed from the factorial design. Additionally, there was no statistically significant effect observed between the 1% and 1.25% magnesium stearate concentrations with regards to physical property tests when comparing MicroceLac® 100 and CombiLac® formulations, and so the lower level of 1% magnesium stearate was selected and kept constant for the next phase of testing. The remaining formulation factors to be investigated further as determined by the pilot study are presented in Table 5.3 below. Additionally, formulations that complied with the physical tablet property test criteria are highlighted in Table 5.4 and were further evaluated in terms of, %swelling and erosion, an assay and dissolution profiles.

Table 5.3: Formulation factors, variables and levels investigated further in this study

Factors	Variables	Level
Lipid Base	Glycerol monostearate	3
	Stearic acid	
	Cetyl alcohol	
Lipid:Drug Ratio per Lipid Base	0.5:1	3
	0.75:1	
	1:1	
Filler Type	MicroceLac® 100	2
	CombiLac®	

Table 5.4: Full factorial design utilised to identify formulations requiring further evaluation as highlighted below

			Glycerol Monostearate			Stearic Acid			Cetyl Alcohol		
			0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1
MicroceLac® 100	Magnesium Stearate	1%	GM0.5M1	GM0.75M1	GM1M1	SA0.5M1	SA0.75M1	SA1M1	CA0.5M1	CA0.75M1	CA1M1
		1.25%	GM0.5M1.25	GM0.75M1.25	GM1M1.25	SA0.5M1.25	SA0.75M1.25	SA1M1.25	CA0.5M1.25	CA0.75M1.25	CA1M1.25
RetaLac®		1%	GM0.5R1	GM0.75R1	GM1R1	SA0.5R1	SA0.75R1	SA1R1	CA0.5R1	CA0.75R1	CA1R1
		1.25%	GM0.5R1.25	GM0.75R1.25	GM1R1.25	SA0.5R1.25	SA0.75R1.25	SA1R1.25	CA0.5R1.25	CA0.75R1.25	CA1R1.25
CombiLac®		1%	GM0.5C1	GM0.75C1	GM1C1	SA0.5C1	SA0.75C1	SA1C1	CA0.5C1	CA0.75C1	CA1C1
		1.25%	GM0.5C1.25	GM0.75C1.25	GM1C1.25	SA0.5C1.25	SA0.75C1.25	SA1C1.25	CA0.5C1.25	CA0.75C1.25	CA1C1.25
Pharmacel® 101		1%	GM0.5P1	GM0.75P1	GM1P1	SA0.5P1	SA0.75P1	SA1P1	CA0.5P1	CA0.75P1	CA1P1
		1.25%	GM0.5P1.25	GM0.75P1.25	GM1P1.25	SA0.5P1.25	SA0.75P1.25	SA1P1.25	CA0.5P1.25	CA0.75P1.25	CA1P1.25

5.4. High Performance Liquid Chromatography Validation

The results achieved during the validation of the proposed high performance liquid chromatography (HPLC) method are reported to verify the appropriateness and effectiveness of this analytical method. This method was used to detect the active ingredients during the drug content assay, dissolution and *in vitro* permeability studies data analysis. Therefore, it was necessary to validate this analytical method before the commencement of these experiments.

5.4.1. Specificity

Specificity tests for both active ingredients were conducted in accordance with Section 3.9.1.1 and produced the chromatogram presented in Figure 5.4. From this overlay it is clear that no interferences were present as there are two discernible peaks which represent the two active ingredients. Retention times of 2.055 min and 3.665 min were recorded for lumefantrine and artemether, respectively.

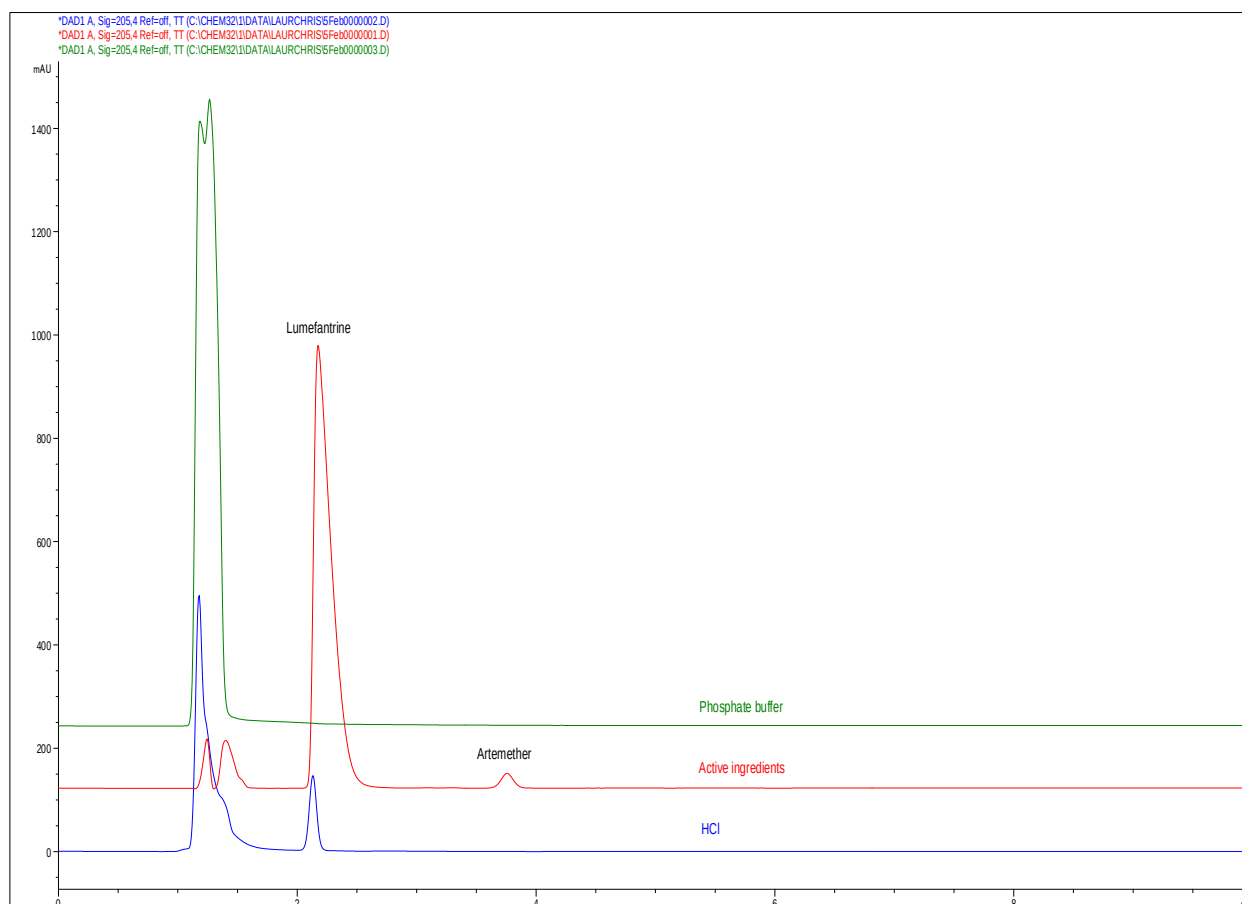


Figure 5.4: High performance liquid chromatography chromatogram depicting specificity of artemether and lumefantrine

5.4.2. Linearity

A standard solution containing both lumefantrine and artemether was prepared and injected at different injection volumes of 2.5; 5.0; 7.5 and 10.0 μ l. The lumefantrine and artemether peak areas of the standard solution are listed in Table 5.5 and Table 5.6, respectively. According to the method described in Section 3.9.1.2, the standard solution was prepared and dilutions of the standard solution were injected in duplicate per injection volume in order to determine the peak area. The chromatographic data obtained was used to determine if a linear relationship exists between peak area and concentration when utilising the proposed HPLC method.

Table 5.5: Peak areas obtained for lumefantrine linearity validation

Lumefantrine Linearity Data					
Standard Solution (μ g/ml)	Injection volume	Final Concentration (μ g/ml)	Peak Area		Average Peak Area
4.016	2.5	1.004	32.300	32.400	32.350
	5.0	2.008	63.700	64.000	63.850
	7.5	3.012	95.000	95.000	95.000
	10.0	4.016	128.500	127.600	128.050
40.160	2.5	10.040	262.100	258.600	260.350
	5.0	20.080	516.800	515.900	516.350
	7.5	30.120	773.500	771.400	772.450
	10.0	40.160	1033.300	1029.900	1031.600
401.600	2.5	100.400	2379.800	2374.000	2376.900
	5.0	200.800	4760.000	4761.500	4760.750
	7.5	301.200	7099.500	7098.600	7099.050
	10.0	401.600	9479.500	9537.800	9508.650

From Table 5.5 it can be seen that the peak areas increased with an increase in lumefantrine concentration of the standard solution. The peak area values obtained for the duplicate injections at each injection volume were very similar which represents a high level of repeatability. From this data, a linear regression graph for lumefantrine (Figure 5.5) was plotted and the regression statistical analysis data is represented in Table 5.7.

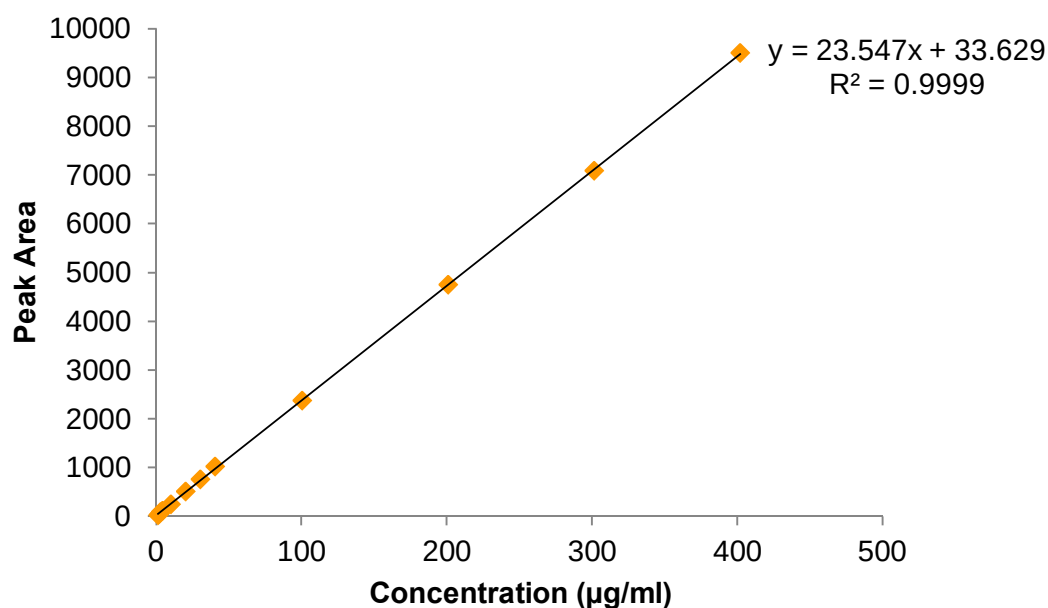


Figure 5.5: Linear regression graph for lumefantrine validation indicating correlation coefficient (R^2) and linear equation

The artemether peak area values attained for the duplicate injections performed at each injection volume demonstrated a high level of repeatability (Table 5.6). This data was utilised to plot a linear regression graph for artemether (Figure 5.6) and the regression statistical analysis data is represented in Table 5.7.

Table 5.6: Peak areas obtained for artemether linearity validation

Artemether Linearity Data					
Standard Solution (µg/ml)	Injection volume	Final Concentration (µg/ml)	Peak Area		Average Peak Area
3.104	2.5	0.776	1.900	1.000	1.445
	5.0	1.552	1.500	1.500	1.500
	7.5	2.328	2.100	2.300	2.200
	10.0	3.104	3.000	2.800	2.900
31.040	2.5	7.760	7.100	5.800	6.450
	5.0	15.520	13.800	12.100	12.950
	7.5	23.280	20.400	18.700	19.550
	10.0	31.040	24.500	26.500	25.500
310.400	2.5	77.600	52.700	53.400	53.050
	5.0	155.200	105.000	104.200	104.600
	7.5	232.800	156.400	155.800	156.100
	10.0	310.400	213.400	222.700	218.050

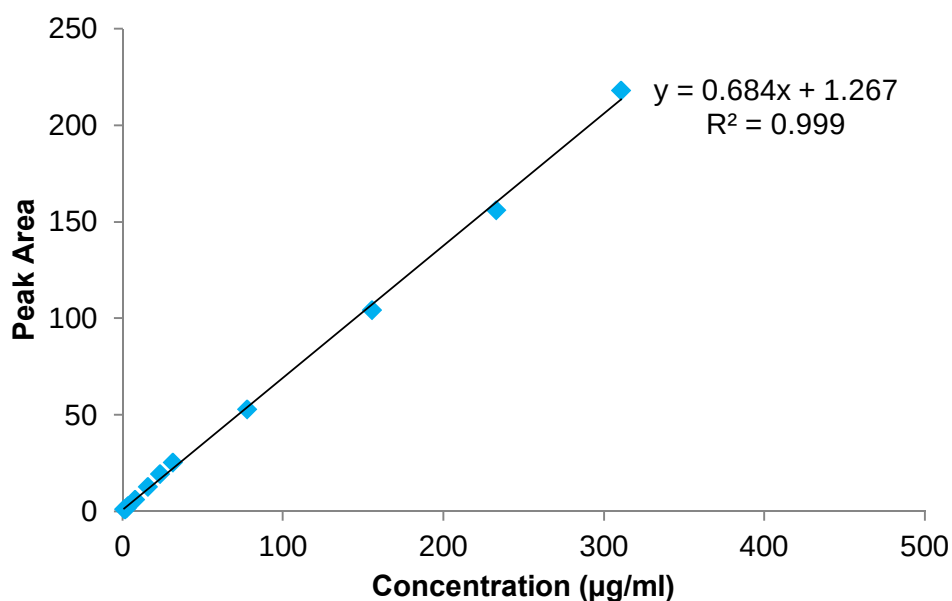


Figure 5.6: Linear regression graph for artemether validation indicating correlation coefficient (R^2) and linear equation

In order for an HPLC method to be validated, the linear regression analysis of peak area and concentration should yield a correlation coefficient (R^2 -value) of at least 0.99. From the data presented in Table 5.7, it is clear that both lumefantrine and artemether met the required criteria with a correlation coefficient R^2 -value of 0.9999 and 0.9991, respectively. Therefore, both active ingredients depicted linearity and the HPLC method was deemed suitable for the analysis of lumefantrine and artemether.

Table 5.7: Linearity regression statistical analysis data for lumefantrine and artemether

Lumefantrine	Regression Statistical Analysis Data	Artemether
0.999911	R^2	0.999101
33.629	Intercept	1.267
23.547	Slope	0.684

5.4.3. Accuracy

A standard solution of lumefantrine and artemether was prepared and subsequently diluted in order to compare the results to a sample solution containing the active ingredients and excipients to be used in the study. Accuracy is performed to identify the occurrence of any interactions. The data obtained during accuracy determination for lumefantrine and artemether are tabulated in Table 5.8 and Table 5.9, respectively.

Table 5.8: Peak areas and %recovery obtained for lumefantrine accuracy validation

Lumefantrine Accuracy Data					
Concentration Spike (µg/ml)	Peak Area		Mean Peak Area	Recovery	
				(µg/ml)	%
305.000	26201.90	25785.30	25993.600	305.100	100.03
	26111.70	26155.70	26133.700	306.700	100.57
	25849.20	26128.40	25988.800	305.000	100.02
152.500	13309.10	13493.50	13401.300	157.300	103.15
	13272.10	13469.50	13370.800	156.900	102.91
	13769.40	13519.60	13644.500	160.200	105.02
76.250	5663.00	5450.90	5556.950	65.200	85.54
	6013.90	6038.00	6025.950	70.700	92.76
	6044.20	6009.00	6026.600	70.700	92.77
Mean % Recovery = 98.10					
SD = 6.00					
% RSD = 6.10					

Table 5.9: Peak areas and %recovery obtained for artemether accuracy validation

Artemether Accuracy Data					
Concentration Spike (µg/ml)	Peak Area		Mean Peak Area	Recovery	
				(µg/ml)	%
402.900	118.800	116.900	117.850	408.000	101.30
	114.400	117.600	116.000	401.600	99.70
	114.300	114.700	114.500	396.400	98.40
201.450	59.000	59.300	59.150	204.800	101.70
	59.800	58.800	59.300	205.300	101.90
	59.100	57.800	58.450	202.400	100.40
100.725	30.200	30.800	30.500	105.600	104.80
	30.900	30.500	30.700	106.300	105.50
	31.700	29.200	30.450	105.400	104.70
Mean %Recovery = 102.00					
SD = 2.30					
%RSD = 2.30					

For an HPLC method to be deemed accurate, the mean percentage (%) recovery of the analyte should be between 98.0–102.0% according to the International Conference of Harmonisation (ICH Q2(R1), 2005). This HPLC method yielded a 98.1% mean recovery for lumefantrine and a 102.0% mean recovery for artemether. Consequently, the results obtained validate that the method of analysis is appropriate and acceptable.

5.4.4. Precision

The ICH defines precision as an analytical procedure in which the closeness of agreement between a series of measurements obtained from multiple samples of the same homogenous sample within the prescribed conditions, is expressed (ICH Q2(R1), 2005). Intra- and inter-day precision were analysed in order to ascertain repeatability under the same operating conditions over a short time interval and intermediate precision expressed on different days. The %RSD obtained for intra-day repeatability testing and inter-day precision should be $\leq 2.0\%$. Results captured for intra-day precision testing of lumefantrine and artemether are displayed in Table 5.10 and Table 5.11, respectively. The inter-day results portraying reproducibility for lumefantrine and artemether are shown in Table 5.12 and Table 5.13, respectively. Additionally, the inter-day precision between days's results are recorded in Table 5.14.

Table 5.10: Intra-day results obtained for lumefantrine precision validation

Lumefantrine Intra-Day Precision Data							
Mass (mg)	Concentration	$\mu\text{g/ml}$	Peak Area		Mean Peak Area	Recovery	
						$\mu\text{g/ml}$	%
201.600	48.400	241.920	4066.442	4067.300	4067.371	269.100	111.20
200.900	48.200	241.080	4040.911	4032.600	4036.756	267.100	110.80
201.100	48.300	241.320	4034.015	4029.700	4031.858	266.700	110.50
Mean %Recover = 110.90							
SD = 0.30							
%RSD = 0.30							

Table 5.11: Intra-day results obtained for artemether precision validation

Artemether Intra-Day Precision Data							
Mass (mg)	Concentration	$\mu\text{g/ml}$	Peak Area		Mean Peak Area	Recovery	
						$\mu\text{g/ml}$	%
201.600	8.100	40.320	12.605	10.400	11.504	41.100	102.10
200.900	8.000	40.180	11.263	11.200	11.232	40.200	100.00
201.100	8.000	40.220	11.083	11.100	11.092	39.700	98.70
Mean %Recover = 100.20							
SD = 1.40							
%RSD = 1.40							

Six solutions at 100% of the target concentration should demonstrate repeatability with a %RSD $\leq 2.0\%$. Lumefantrine and artemether both complied with a respective %RSD of 0.3% and 1.4%. Furthermore, reproducibility is obtained at a %RSD $\leq 8.0\%$. Lumefantrine complied (0.197%) and the result attained for artemether (0.038%) was deemed acceptable. Therefore, from the results obtained, the method was found to be precise.

Table 5.12: Inter-day results obtained for lumefantrine precision validation

Lumefantrine Inter-Day Precision Data											
	Mass (mg)	Concentration	$\mu\text{g/ml}$	Peak Area		Mean Peak Area	Recovery		Mean %Recovery	%SD	%RSD
							$\mu\text{g/ml}$	%			
Day 1	301.800	72.400	326.160	6034.252	6032.900	6033.576	399.200	110.20	109.733	51.633	0.576
	301.200	72.300	361.440	5903.435	5902.100	5902.768	390.500	108.00			
	301.200	72.300	361.440	6065.014	6058.900	6061.957	401.100	111.00			
Day 2	250.000	60.000	300.000	4987.787	4980.800	4984.294	329.800	109.90	111.133	0.981	0.011
	250.600	60.100	300.720	5059.505	5046.600	5053.051	334.300	111.20			
	250.100	60.200	300.840	5110.107	5100.900	5105.503	337.800	112.30			
Day 3	201.600	48.400	241.920	4066.442	4067.300	4067.371	269.100	111.20	110.833	0.287	0.003
	200.900	48.200	241.080	4040.911	4032.600	4036.756	267.100	110.80			
	201.100	48.300	241.320	4034.015	4029.700	4031.858	266.700	110.50			

* *SD* = standard deviation

* *%RSD* = relative standard deviation

Table 5.13: Inter-day results obtained for artemether precision validation

	Artemether Inter-Day Precision Data										
	Mass (mg)	Concentration	µg/ml	Peak Area		Mean Peak Area	Recovery		Mean %Recovery	%SD	%RSD
							µg/ml	%			
Day 1	301.800	12.100	60.360	16.800	14.600	15.700	56.200	93.00	99.000	4.246	0.053
	301.200	12.000	60.240	18.025	16.400	17.213	61.600	102.20			
	301.200	12.000	60.240	17.383	16.900	17.142	61.300	101.80			
Day 2	250.000	10.000	50.000	15.300	13.600	14.450	51.700	103.40	98.533	3.476	0.043
	250.600	10.000	50.120	13.100	14.000	13.550	48.500	96.70			
	250.100	10.000	50.020	13.400	13.300	13.350	17.800	95.50			
Day 3	201.600	8.100	40.320	12.605	10.400	11.504	41.100	102.10	100.267	1.401	0.017
	200.900	8.000	40.180	11.263	11.200	11.232	40.200	100.00			
	201.100	8.000	40.220	11.083	11.100	11.092	39.700	98.70			

* SD = standard deviation

* %RSD = relative standard deviation

Table 5.14: Inter-day precision results obtained for artemether and lumefantrine between days

Artemether	Between Days	Lumefantrine
99.267	Mean %Recover	110.567
3.041	%SD	17.634
0.038	%RSD	0.197

5.4.5. Limit of Quantification and Limit of Detection

The chromatographic data obtained for LOQ and LOD tests conducted for artemether and lumefantrine are represented in Table 5.15 and Table 5.16, respectively. Artemether was determined to have a LOQ of 2.628 $\mu\text{g/ml}$ (%RSD 05) and LOD of 1.051 $\mu\text{g/ml}$ (%RSD 010). Lumefantrine acquired a LOQ of 0.525 $\mu\text{g/ml}$ (%RSD 01) and LOD of 0.105 $\mu\text{g/ml}$ (%RSD 010). Therefore, both active ingredients produced satisfactory LOD and LOQ results by obtaining the specified LOD %RSD value 080% and a LOQ %RSD 010%, respectively (ICH Q2(R1), 2005).

Table 5.15: Artemether limit of quantification and limit of detection data

Artemether LOQ and LOD										
Concentration (µg/ml)	Injection volume	Final concentration (µg/ml)	Peak area						Mean	%RSD
10.510	1.0	1.051	0.491	0.488	0.437	0.491	0.441	0.407	0.459	7.13
	2.5	2.628	1.272	1.186	1.195	1.289	1.269	1.195	1.234	3.46
	5.0	5.255	2.337	2.432	2.164	2.416	2.560	2.365	2.379	5.01
	7.5	7.883	4.000	3.600	4.000	3.900	4.600	4.195	4.049	7.50
LOQ = 2.628 µg/ml (%RSD = 5)										
LOD = 1.051 µg/ml (%RSD = 10)										

Table 5.16: Lumefantrine limit of quantification and limit of detection data

Lumefantrine LOQ and LOD										
Concentration (µg/ml)	Injection volume	Final concentration (µg/ml)	Peak area						Mean	%RSD
1.062	1.0	0.105	3.200	3.200	3.600	3.300	3.400	3.700	3.400	5.63
	2.5	0.263	8.500	8.500	8.400	8.600	8.800	8.300	8.520	1.85
	5.0	0.525	17.100	16.900	17.200	17.100	17.300	17.100	17.120	0.71
	7.5	0.788	25.600	25.600	25.600	25.900	25.700	25.500	25.650	0.49
LOQ = 0.525 µg/ml (%RSD = 1)										
LOD = 0.105 µg/ml (%RSD = 10)										

In conclusion, the HPLC-method proved to be specific for both lumefantrine and artemether. Linearity was established over a range of 80.0–120.0% with an R^2 value of 0.999911 and 0.999101 for lumefantrine and artemether, respectively. The mean %recovery was within the specified acceptance criteria (98.0–102.0%) proving the method to be accurate. Precision testing furthermore substantiated the repeatability and reproducibility of this method as seen in Table 5.17.

Table 5.17: Summary of validation results obtained for the double-fixed dose combination of artemether and lumefantrine lipid matrix tablets HPLC method

Parameter	Acceptance Criteria	Results
Specificity	The solvent and placebo solutions should not generate any peaks that may interfere with the determination of the active ingredients. All other peaks must be discernible from those of the active ingredients.	The chromatogram produced by the solvent and placebo solutions showed no indication of interference between the solvents and the active ingredients. Clear, discernible peaks were produced.
Linearity	The method should be linear over a range of 80.0–120.0%. The correlation coefficient (R^2) value $\geq$ 0.99.	Lumefantrine R^2 : 0.999911 Artemether R^2 : 0.999101
Accuracy	The difference between the percentage mean recovery and the theoretical recovery (100%) must be $\pm$ 2.0%. Recovery range: 98.0–102.0%.	Lumefantrine: 98.10% Artemether: 102.00%
Precision		
A: Repeatability (Intra-day)	Six solutions at 100% of the target concentration should demonstrate repeatability with a %RSD $\leq$ 2.0%.	Lumefantrine %RSD: 0.30% Artemether %RSD: 1.40%
B: Reproducibility (Inter-day)	The %RSD obtained by each analyst should be $\leq$ 3.0%.	Lumefantrine %RSD: 0.197% Artemether %RSD: 0.038%

5.5. Assay

Artemether and lumefantrine content was tested per formulation according to Section 3.10.1. As described previously, the lipid matrix tablets were formulated to contain 120 mg lumefantrine and 20 mg artemether per tablet. The results pertaining to the assay experiments are recorded in Table 5.18 and presented as the calculated %drug content per formulation.

Table 5.18: %Drug content determined per formulation

%Lumefantrine	Formulation	%Artemether
86.16	CA1C1	71.24
80.93	CA1M1	65.67
112.54	CA0.5C1	99.34
117.60	CA0.5M1	126.10
92.94	CA0.75C1	81.31
89.84	CA0.75M1	79.92
93.20	SA1C1	95.00
91.60	SA1M1	78.54
105.46	SA0.5C1	91.50
116.28	SA0.5M1	100.56
131.72	SA0.75C1	72.43
133.37	SA0.75M1	75.34
72.79	GM1C1	60.21
101.60	GM1M1	104.20
74.74	GM0.5C1	67.23
116.56	GM0.5M1	100.77
98.15	GM0.75C1	86.64
91.30	GM0.75M1	82.66

As to be able to ascertain whether noticeable differences were present, a summary table per factor (Table 5.19) was drawn up. No conspicuous differences were detected between the various lipid bases for artemether content. Lumefantrine content however, was found to be the highest when stearic acid was employed as the lipid base. MicroceLac® 100 formulations depicted overall slightly higher drug content for both the active ingredients. The 0.5:1 lipid:drug ratio similarly displayed overall higher drug content for both active ingredients, whereas the 1:1 ratio exhibited the lowest drug content of the three ratios investigated. Thus, as the lipid concentration increased, the overall drug content appeared to decrease.

Table 5.19: %Drug content calculated per factor

	Lumefantrine (%)	Artemether (%)
Cetyl alcohol	96.67	87.26
Stearic acid	111.94	85.56
Glycerol monostearate	92.52	83.62
MicroceLac® 100	104.34	90.42
CombiLac®	96.41	80.55
0.5:1	107.20	97.58
0.75:1	106.22	79.72
1:1	87.71	79.14

The trend observed revealed that the artemether %drug content obtained was typically lower than the theoretical content whilst lumefantrine in comparison demonstrated a noticeably higher %drug content. The variance in target content may have resulted from loss of active ingredients during weighing, handling and the hot-fusion method of manufacture, which not only caused dust formation, but the method of production furthermore requires particle size reduction and therefore the use of a sieve accounting for loss of active ingredients.

5.6. Tablet Morphology

Photos were taken of the outer and inner surfaces of the selected viable formulations (Figure 5.7). No significant observable differences were obtained during inspection of MicroceLac® 100 and CombiLac® formulations.

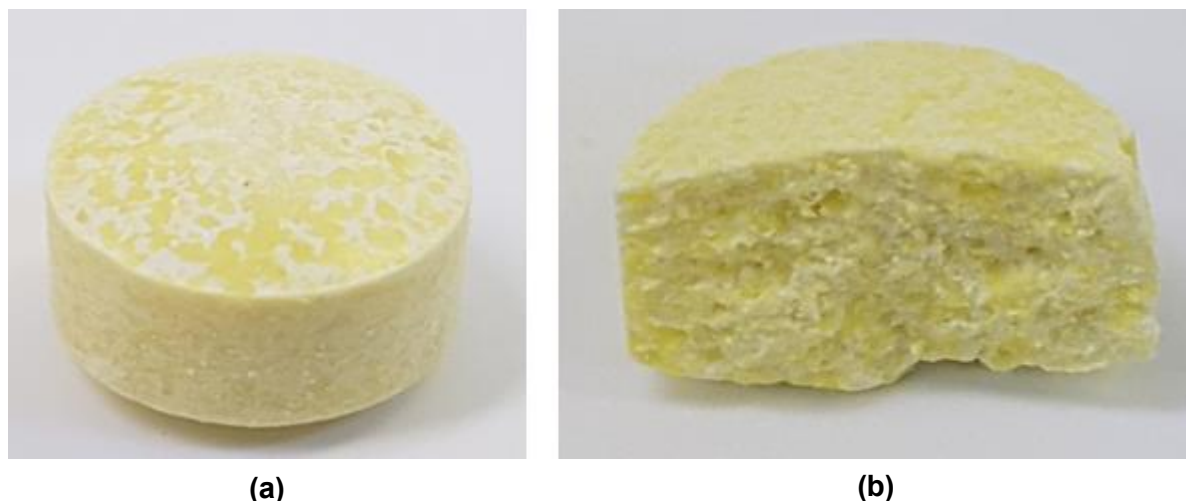


Figure 5.7: Example of the (a) external and (b) internal surface morphologies of a lipid matrix tablet produced in this study

The mottled appearance of the tablet is produced from the lipid dispersion - coloured yellow due to the presence of lumefantrine and the white colouration of the fillers. Neither the type of lipid base, the ratio thereof, nor the concentration magnesium stearate altered the physical appearance of the tablet to such an extent that distinctions could be made between them. The average central cylinder thickness of all the lipid matrix tablets produced, irrespective of the formulation factors, was approximately 4 mm. Photos of each formulation are included in Annexure E.

5.7. Swelling and Erosion Studies

Swelling and erosion experiments were conducted on the selected formulations as per the full factorial design, and in accordance with the method described in Section 3.10.3. Figures 5.8i 5.10 graphically represent the swelling results achieved for each lipid base as %swelling vs. time diagrams. Table 5.20 denotes the erosion results acquired. Additionally, Table 5.21 indicates the %swelling and erosion per formulation factor in order to highlight the influence of the formulation factors on the observed responses. The results were initially grouped per lipid base to assess the effects of lipid ratio and filler per lipid base, where after the lipid bases were compared.

SA1C1 (Figure 5.8) depicted the highest swelling capability ($165.333 \pm 13.269\%$) of all the formulations comprising stearic acid (SA); and the SA:drug ratio 1:1, regardless of the filler,

exhibited the highest %swelling. Generally, CombiLac® formulations displayed a higher %swelling compared to MicroceLac® 100 formulations (at approximately 1 to 2 min), but nonetheless experienced significantly more erosion compared to MicroceLac® 100 formulations. After 2 min, formulations containing CombiLac® already displayed visible erosion. Stearic acid formulations in combination with MicroceLac® 100 depicted relatively low erosion values as indicated by the stabilised %swelling recorded in Figure 5.8.

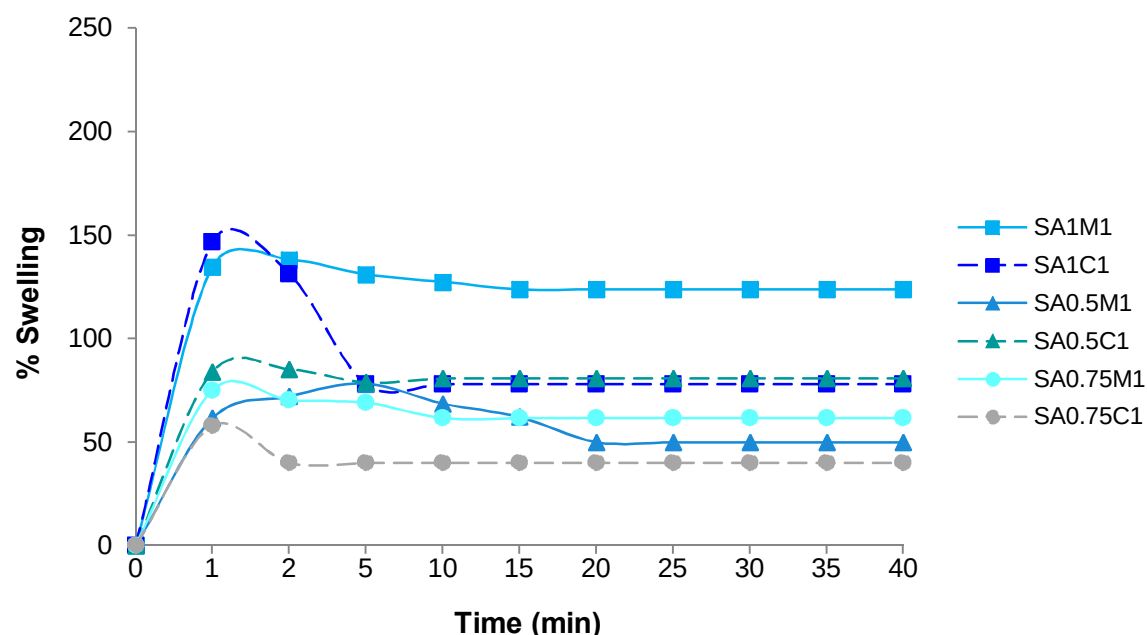


Figure 5.8: Percentage swelling recorder for the respective stearic acid formulations over time

CombiLac® differs from MicroceLac® 100 in composition by only one ingredient, namely, white native corn starch (10%). Its presence in CombiLac® appears to have a significant impact on the swelling properties of the filler (Meggle, 2016c). The heating of starch molecules in the presence of excess water results in its semi-crystalline structure being shattered, causing water molecules to associate by means of hydrogen bonding with the exposed hydroxyl groups on the amylose and amylopectin molecules. This association subsequently causes swelling and an increase in granule size and solubility to transpire (Singh *et al.*, 2003). The different swelling behaviours of the two fillers can therefore mostly be ascribed to this difference in composition as seen in Figure 5.8 and Figure 5.9. Corn starch is utilised as a disintegrant in the pharmaceutical industry based on its swelling mechanism to impart the disintegrating effect as well as a water retention agent in the food industry (Carter, 2009; Singh, 2003). MEGGLE® (2016c) reported corn starch to be a traditional disintegrant capable of ensuring rapid water uptake that is independent to tablet hardness. De Willigen (1976) furthermore reported that corn and wheat starches can swell up to 30 times their original volume, without exhibiting disintegration, which could account for the initial increase in tablet weight prior to the tablets eroding, as observed in this study.

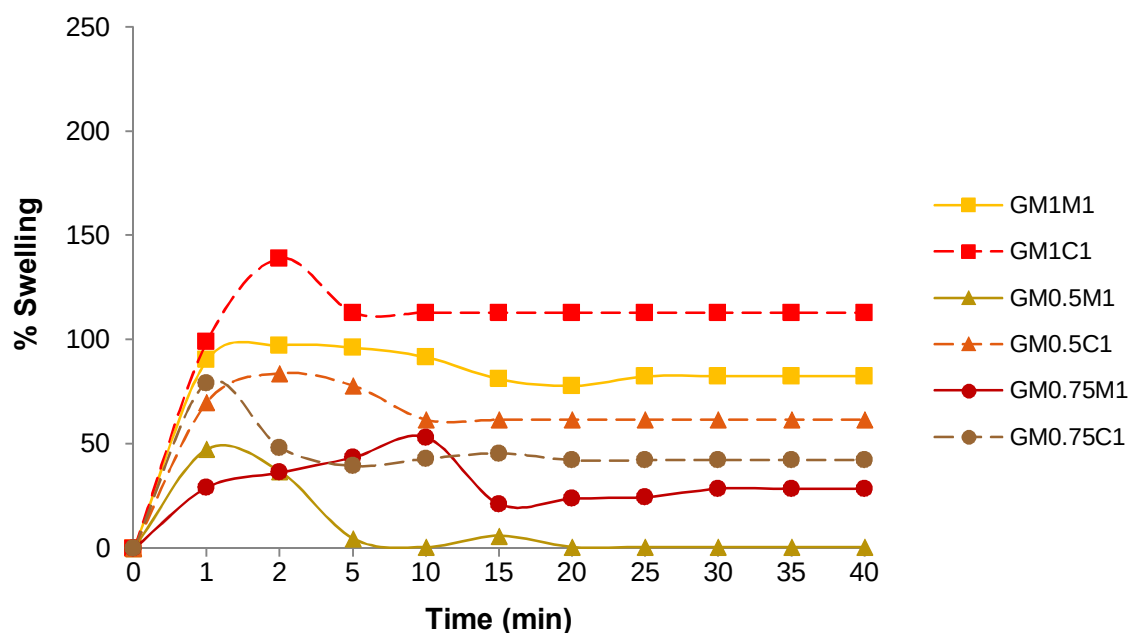


Figure 5.9: Percentage swelling recorded for the respective glycerol monostearate formulations over time

Evaluation of formulations containing glycerol monostearate (GM) revealed that CombiLac® in combination with the 1:1 lipid:drug ratio (GM1C1) portrayed the highest %swelling ($154.719 \pm 42.001\%$) which was also observed when stearic acid was employed as the lipid base. CombiLac® formulations, regardless the GM:drug ratio, once again displayed higher %swelling per lipid:drug ratio compared to MicroceLac® 100. Overall, CombiLac® experienced superior swelling compared to MicroceLac® 100 when GM was employed as the lipid base.

Cetyl alcohol and stearic acid are classified as non-swellable matrix forming materials, however, the fillers present in the lipid matrix tablets were sufficient enough to cause swelling as seen in Figures 5.8 and 5.10. CA1M1 depicted significantly higher swelling compared to the other cetyl alcohol formulations. There was no noteworthy difference in the %swelling of the other cetyl alcohol formulations, however, CA0.5M1 displayed maximum swelling at 15 min which occurred later compared to the other formulations (2–5 min). CombiLac® in a ratio of 1:1 (CA1C1) displayed a different trend in swelling behaviour when in combination with cetyl alcohol compared to when in combination with glycerol monostearate (GM1C1) and stearic acid (SA1C1). This could be an indication that cetyl alcohol's characteristic of being a non-swellable matrix component was more prominent than that associated with stearic acid. It would be expected that the lower lipid:drug ratios would experience a higher %swelling due to a higher filler content per tablet, however as seen in Figure 5.10, this was not the case. A possible explanation for this observation could be the composition of the fillers utilised. MicroceLac® 100 and CombiLac® comprise 75% and 70% D-lactose monohydrate, respectively. Lactose is soluble in water (BP, 2018) and thus the high

percentage lactose incorporated into each filler will dissolve, which in turn will subject these lipid matrix tablets to erosion whilst minimal swelling will transpire due to the non-swellaable characteristics of cetyl alcohol.

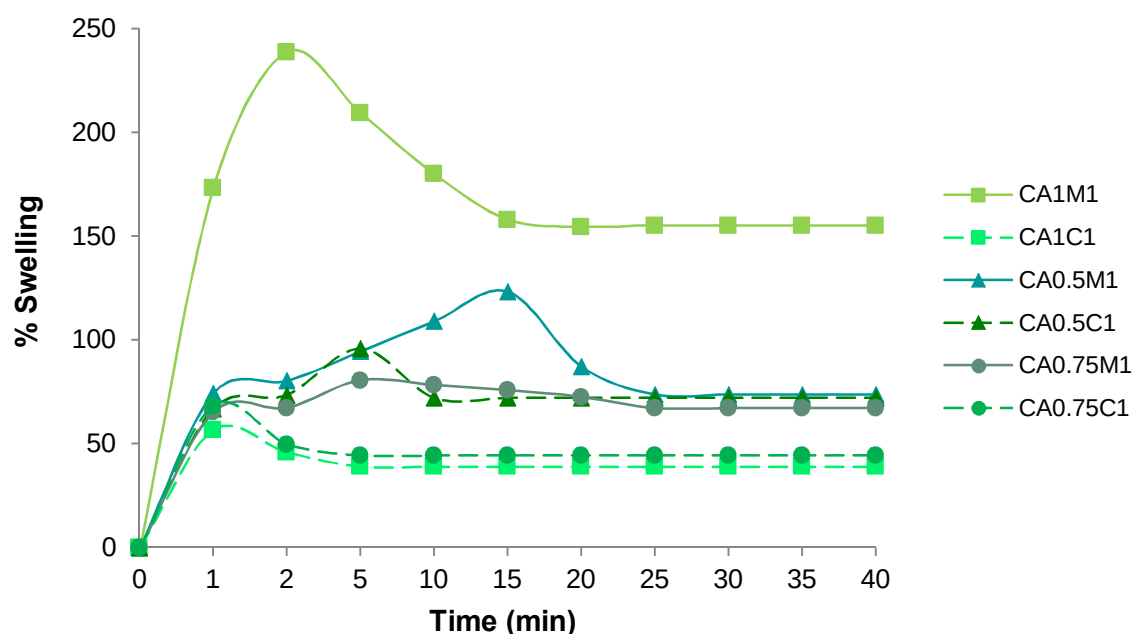


Figure 5.10: Percentage swelling recorded for the respective cetyl alcohol formulations over time

Following swelling analysis, erosion experiments were conducted by subjecting the same samples to oven drying (Section 3.10.3). The lipid matrix tablets were dried until such a time that no variation in mass per tablet was recorded for three consecutive readings. As stated previously, data collected concerning erosion is presented in Table 5.20; the values presented in parenthesis indicate the %RSD.

From Table 5.20 it can be seen that the SA:drug ratios utilising either MicroceLac® 100 or CombiLac® experienced a relatively similar degree of erosion and were not heavily influenced by the filler type as highlighted by SA0.75M1 and SA0.75C1 displaying erosion of 16.871% and 16.000%, respectively. This grouping per drug ratio, with only small differences in erosion per filler, was observed as a trend for stearic acid formulations. A representation of all the formulations assessed as well as their respective percentage error bars are presented in Annexure F.

Regarding glycerol monostearate formulations, GM1C1 experienced substantially higher erosion than GM1M1 as well as achieving the highest %erosion ($46.613 \pm 10.397\%$) for this base. Overall, this trend pertaining to erosion of lipid matrix tablets comprising CombiLac® was true for all three lipid bases and was most profound with cetyl alcohol formulations. It represents the tendency of CombiLac® formulations to be highly susceptibility to erosion which again could be ascribed to

the lactose component (70.0%) in CombiLac®, which is water soluble and would dissolve once in contact with an aqueous media (BP, 2018; Meggle, 2016c).

Table 5.20: %Erosion results summarised per formulation and grouped per lipid base

	Formulation	%Erosion
Stearic acid formulations	SA1M1	4.762 (±65.465)
	SA1C1	14.667 (±7.873)
	SA0.5M1	10.256 (±60.273)
	SA0.5C1	8.974 (±12.372)
	SA0.75M1	16.871 (±17.164)
	SA0.75C1	16.000 (±25.000)
Glycerol monostearate formulations	GM1M1	3.230 (±68.069)
	GM1C1	46.613 (±10.397)
	GM0.5M1	44.599 (±4.721)
	GM0.5C1	11.061 (±27.824)
	GM0.75M1	10.000 (±0.000)
	GM0.75C1	9.401 (±32.756)
Cetyl alcohol formulations	CA1M1	24.082 (±45.693)
	CA1C1	35.823 (±12.217)
	CA0.5M1	21.429 (±75.719)
	CA0.5C1	37.211 (±32.150)
	CA0.75M1	16.095 (±21.029)
	CA0.75C1	17.338 (±6.999)

**%RSD indicated in parenthesis*

Comparison of formulations containing cetyl alcohol revealed that CA0.5C1 exhibited the highest %erosion ($37.211 \pm 32.150\%$) closely followed by CA1C1 ($35.823 \pm 12.217\%$), again signifying that of the two fillers investigated in this study, CombiLac® was more susceptible to erosion than

MicroceLac® 100. The CA0.75M1 illustrated the lowest %erosion ($16.095 \pm 21.029\%$) concerning cetyl alcohol formulations.

In order to produce more in-depth conclusions, it is necessary to evaluate each formulation factor individually as specified in Table 5.21 - values presented in parenthesis indicate the %RSD.

Table 5.21: Summary of the average %swelling and erosion results assessed per formulation factor

	%Swelling & Erosion per Factor	
	% Swelling	% Erosion
Glycerol Monostearate	91.884 (37.294)	20.817 (28.753)
Stearic Acid	104.023 (20.484)	11.922 (31.358)
Cetyl Alcohol	101.876 (20.845)	25.329 (32.301)
MicroceLac® 100	103.805 (23.074)	16.814 (39.793)
CombiLac®	94.717 (29.341)	21.899 (17.823)
1:1	141.317 (27.531)	21.529 (34.952)
0.75:1	53.048 (30.091)	15.833 (17.158)
0.5:1	87.504 (21.002)	22.255 (35.510)

* %RSD indicated in parenthesis

Differentiating between the lipid bases, it is observed that formulations consisting of glycerol monostearate depicted a marginally lower %swelling ($91.884 \pm 37.294\%$), whilst formulations comprising stearic acid and cetyl alcohol experienced a relatively negligible difference in %swelling. On the other hand, erosion profiles of the three lipid bases displayed more clear differences, with cetyl alcohol formulations having the highest average %erosion ($25.329 \pm 32.301\%$) followed by glycerol monostearate formulations. Drug release from hydrophobic and non-swellable matrix tablets generally involves mechanisms of both pore diffusion and matrix erosion (Basak *et al.*, 2008; Nisha *et al.*, 2011). Basak *et al.* (2008) further stated that cetyl alcohol exhibits matrix erosion resulting from high water penetration into the matrix. Additionally, cetyl alcohol has the lowest melting point (49°C) of the lipid bases investigated in this study. The addition of heat causes the particles in a solid to gain energy and vibrate faster, causing the structure to gradually weaken, thereby expanding the solid known as polymer relaxation (Grinsted *et al.*, 1992; Qin *et al.*, 1993). Further heat energy results in particles breaking free of the structure and are able to move around, however, they remain loosely

connected. At this point, the solid is starting to melt to a liquid (Jones, 2000). Therefore, it may be reasoned that the higher average %erosion of cetyl alcohol could be in response to the loose association of its particles resulting from the test conditions ($37 \pm 0.5^\circ\text{C}$) together with the erosion characteristics of the formed matrix. Glycerol monostearate, although showing less signs of swelling was clearly susceptible to reasonably high erosion due to water penetration as it experienced $20.817 \pm 28.753\%$ erosion. Stearic acid, on the other hand, displayed the highest %swelling ($104.023 \pm 20.484\%$) and only minimal signs of erosion ($11.922 \pm 31.358\%$) were present, validating that these lipid matrix tablets were capable of high water up take, but nonetheless able to withstand erosion.

In conclusion, MicroceLac® 100 portrayed the higher %swelling of the two fillers and exhibited substantially less erosion ($16.814 \pm 39.793\%$) which was clearly visible during testing. Many of the CombiLac® formulations eroded completely during the swelling phase of experimenting, whilst most MicroceLac® 100 formulations were still well intact, even after the swelling weight stabilised, with no visual signs of erosion. Moreover, the 1:1 lipid:drug ratio formulations exhibited substantially higher %swelling, which was interesting to observe that the higher lipid content (and thus lower filler content), and more hydrophobic environment of the tablets, played no role in the penetration of water into the lipid matrix tablets which was initially hypothesised. This phenomenon can be explained primarily by filler composition coupled with filler concentration together with the mechanism of drug release, which is predominantly via pore diffusion. Thus, water penetration via capillaries is applicable. Lipid matrix tablet formulations with a lipid:drug ratio of 1:1 contained the smallest percentage filler of the formulations investigated, thereby having the least percentage lactose and starch. As previously stated lactose is highly water soluble and will therefore dissolve, causing erosion of the tablets, whilst the starch component in CombiLac® will swell. It is evident that the lower percentage lactose present in 1:1 ratio formulations caused profound effects on the swelling capability of these formulations in the absence of the erosion occurrence caused by the lower percentage lactose present.

5.8. Dissolution Studies

5.8.1. Introduction

Dissolution studies were conducted on the remaining 18 viable formulations in order to ascertain the drug release potential of each formulation. This was a critical step in the evaluation of the lipid matrix tablets as valuable information pertaining to the percentage drug available for absorption was achieved. The values obtained for each formulation during the assay experiments were noted as 100% of the theoretical drug content; and the data obtained from each dissolution study was related to this corresponding assay value in order to calculate the percentage dissolution.

Mean dissolution time (MDT) represents the average dissolution time of the percentage drug released at all the tested time intervals during a dissolution study. In so saying, a higher MDT value is indicative of a slower drug release profile and offers insights into the level of delayed drug release and retarding potential of the formulation. The MDT values of the tested formulations were utilised to identify and compare the capability of a formulation to offer modified and extended drug release profiles. Additionally, fit factors were calculated for formulations of interest to establish whether statistically significant differences were present between the drug release profiles obtained for the investigated formulations. Should the f_1 -value be >15 , a variation between the two curves is present and is thus said to be statistically significantly different. Fit factor, f_2 , is the similarity factor between the two curves and a value of ≥ 50 indicates that the two-compared formulation are fairly similar, with a value of 100 showing that the two samples are identical (Costa & Lobo, 2001). Both the MDT and fit factor values were employed to draw meaningful conclusions from the obtained dissolution studies data. A summarised table of all the evaluated fit factors is available in Annexure G. Additionally, DDSolver software, a freely available menu-driven add-in programme for Microsoft Excel, was utilised to facilitate the modelling of the dissolution data obtained.

5.8.2. Dissolution Profile Modelling

The selection of a suitable model is essential for the quantitative evaluation and comparison of dissolution profiles and thus three selection criteria were used to determine the goodness of fit of a model as well as the mechanistic plausibility of the model. When fitting release models with the same number of parameters, the coefficient of determination (R^2) can be used to discriminate the most appropriate model. However, when models with a different number of parameters are compared, the adjusted coefficient of determination (R^2_{adj} or R^2_{adjusted}) should be used. It was necessary to utilise R^2_{adjusted} for this study as the R^2 value will always increase with the addition of more parameters, whilst R^2_{adjusted} may decrease when over-fitting has occurred. Therefore, the R^2_{adjusted} with the highest value (closest approaching 1.000) was utilised to indicate the best fit (Costa & Lobo, 2001; Zhang *et al.*, 2010). Moreover, the Akaike Information Criterion (AIC) and

Model Selection Criterion (MSC) were employed to confirm goodness of fit. When comparing models with differing numbers of parameters, the model with a lower AIC value can be considered the more appropriate model (Costa *et al.*, 2003). The MSC value is a modified reciprocal form of the AIC but has been normalised so that it is independent of the scaling of data points (Zhang *et al.*, 2010). Generally, a MSC value higher than two to three is indicative of a good fit (Koizumia *et al.*, 2001; Mayer *et al.*, 1999).

Korsmeyer-Peppas derived a simple equation to describe the drug release from polymeric systems and this relationship is frequently used to describe the release from modified release dosage forms (Costa & Lobo, 2001; Korsmeyer *et al.*, 1983). The release mechanism is identified by fitting the first 60% of drug release to the model. The subsequent drug release exponent (n) value is used to characterise different drug release mechanisms as described in Table 5.22; and k_{KP} is the release constant incorporating structural and geometric characteristics of the drug-dosage form (Zhang *et al.*, 2010).

Table 5.22: Correlation of release exponent (n) values with the indicative drug release mechanism

Exponent n	Mechanism of Drug Release
≤ 0.45	Fickian Diffusion
$0.45 < n < 0.85$	Non-Fickian Diffusion
0.89	Case II transport
≥ 1.00	Super case II transport

Fickian diffusion is characterised by the solvent diffusion rate (R_{diff}) being slower than the rate of polymer relaxation (R_{relax}). It is often observed in polymer networks at a temperature above the glass transition temperature as the polymer is then in a rubbery state and the polymer chains exhibit higher motility that allows for the easier penetration of the solvent (Grinsted *et al.*, 1992; Qin *et al.*, 1993). Due to the fact that solvent absorption studies are often carried out below the temperature of glass transitioning, few examples of Fickian diffusion in polymers are reported in literature. However, Grinsted *et al.* (1992) and Ercken *et al.* (1996) have both reported cases of Fickian diffusion in polymer systems below glass transition temperature given the presence of a plasticiser.

The process of non-Fickian diffusion is mainly observed when the temperature is below that of glass transitioning meaning that the polymer chains are not sufficiently mobile to permit immediate solvent penetration into the polymer core (Grinsted *et al.*, 1992). Two types of non-Fickian diffusion have been defined and differ mainly in terms of solvent diffusion rate. Case II diffusion exhibits a diffusion rate faster than polymer relaxation ($R_{diff} > R_{relax}$) whereas anomalous diffusion

displays solvent diffusion rate and polymer relaxation of similar magnitudes ($R_{\text{diff}} \sim R_{\text{relax}}$). A model that yields an n value higher than 1.00 may be regarded as super case II kinetics arising from a reduction in the attractive forces within a polymer chain (Alfrey *et al.*, 1966).

The binomial equation developed by Peppas and Sahlin is similar in meaning to Korsmeyer-Peppas, as both describe and quantify the contribution of either the erosion or relaxation mechanism coupled with the diffusive mechanism. This model however accounts for the simultaneous effects of Fickian diffusion and case II transport. The substitution of the exponent coefficient (n) from the Korsmeyer-Peppas model in the Peppas-Sahlin model allows for the constants k_1 and k_2 to be calculated (Peppas & Sahlin, 1989; Siepmann & Peppas, 2001) where k_1 is the diffusion constant; k_2 is the relaxation constant associated with dissolution as well as polymer chain relaxation; and m is the diffusion exponent (Costa & Lobo, 2001).

All of the formulations tested fitted one of the above discussed models. The three models that provided the best fit all considered the lag time (T_{lag}) present and differed with regards to the number of parameters measured as shown in Table 5.23.

Table 5.23: Most appropriate models available in DDSolver used for fitting drug release data

Model	Equation	Parameters	References
Korsmeyer-Peppas with T_{lag}	$F = k_{\text{KP}} * (t - T_{\text{lag}})^n$	$k_{\text{KP}}, n, T_{\text{lag}}$	Tarvainen <i>et al.</i> , 2004
Peppas-Sahlin1 with T_{lag}	$F = k_1 * (t - T_{\text{lag}})^m + k_2 * (t - T_{\text{lag}})^{2m}$	$k_1, k_2, m, T_{\text{lag}}$	Ford <i>et al.</i> , 1991
Peppas-Sahlin2 with T_{lag}	$F = k_1 * (t - T_{\text{lag}})^{0.5} + k_2 * (t - T_{\text{lag}})$	k_1, k_2, T_{lag}	Peppas & Sahlin, 1989

5.8.3. Artemether Drug Release

Artemether drug release profiles pertaining to stearic acid formulations revealed the most uniform release profiles of the three lipid bases. A consistent lag time of 120 min was present for all of these formulations and the increase in pH from 1.2 to 6.8 subsequently coincided with artemether drug release (Figure 5.11).

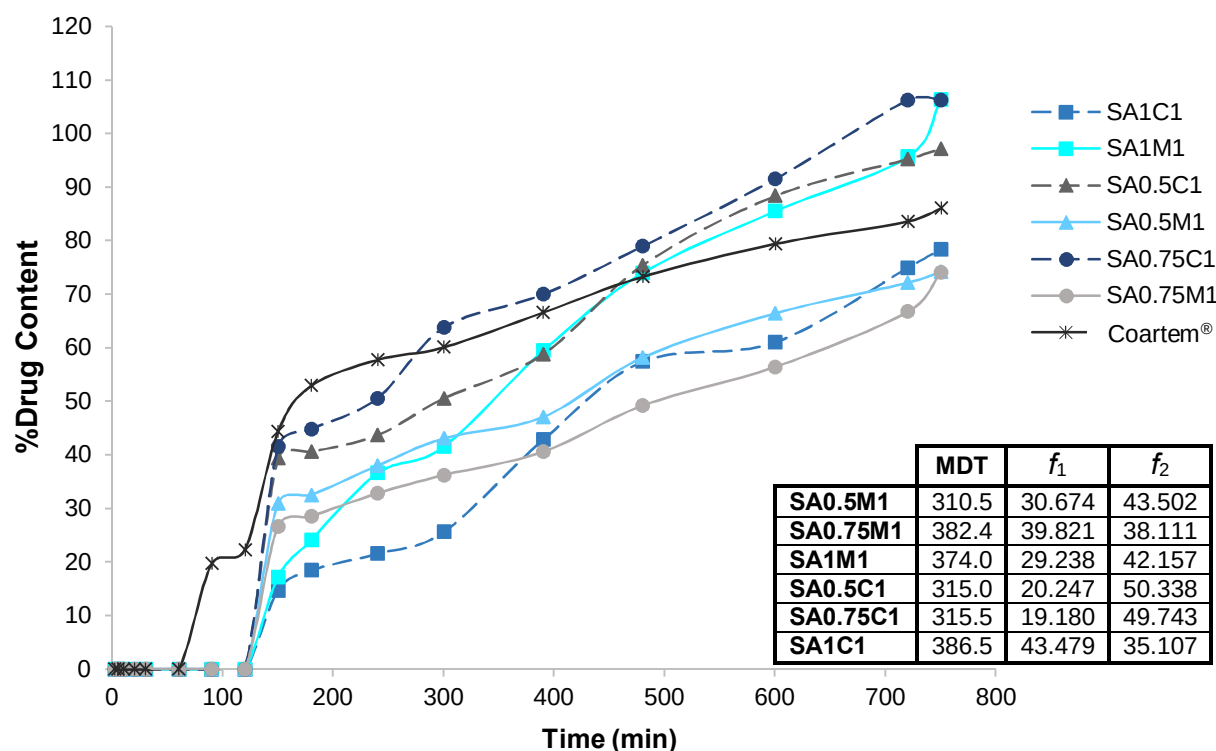


Figure 5.11: Artemether drug release profiles for the respective stearic acid formulations with corresponding MDT (min) together with f_1 - and f_2 -values vs. Coartem® as an inset

All six of the tested stearic acid formulations, irrespective of the filler or lipid:drug ratio, produced a %drug release in accordance with the criteria set in the BP (2018). After the initial 2 h lag period, sustained, modified release was achieved until the last sample was taken at time 750 min. Statistically relevant differences between the lipid:drug ratios were present between SA1C1 and SA0.5C1 (f_1 : 35.663; f_2 : 40.802), SA1C1 and SA0.75C1 (f_1 : 42.160; f_2 : 34.892) as well as SA1M1 and SA0.75M1 (f_1 : 37.049; f_2 : 44.463). Comparison of the same lipid:drug ratios but different filler combinations for stearic acid revealed statistical differences (Annexure G) demonstrating that the filler type together with the lipid:drug ratio was instrumental in drug release.

Most noteworthy, stearic acid was the only lipid base able to produce drug release profiles for all six respective formulations above the acceptance criteria of 75% (BP, 2018). Both cetyl alcohol and glycerol monostearate formulations failed to consistently produce formulations capable of releasing >75% drug content. This is most likely as a result of stearic acid's inherent surfactant

properties (Wang, 2010). Stearic acid furthermore displayed an average MDT of 370.303 min for the six formulations.

All stearic acid formulations bar one, SA0.5C1, showed statistically relevant differences versus Coartem[®] as seen in Figure 5.11 (f_1 : 20.247; f_2 : 50.338). Coartem[®] comprises polysorbital 80 (a surfactant) as well as hypromellose and microcrystalline cellulose as fillers. The presence of a surfactant together with the gel matrix forming properties of hypromellose made for interesting comparisons pertaining to %drug release and the dissolution release kinetics. It is clear that the polysorbital 80 was able to provide sufficient wetting of artemether at a lower pH of 1.2. An increase in pH to 6.8 resulted in a prominent spike in artemether release. Coartem[®] fitted the Peppas-Sahlin 2 with T_{lag} model which could be explained by the erosion of the tablet (visible within the first 2 min of dissolution testing) and simultaneous diffusion of the active ingredients out of the gel matrix formed by the hypromellose content as seen in Figure 5.12a. Coartem[®] released a total of 86.12% of the label claim and presented a MDT of 235.252 min.

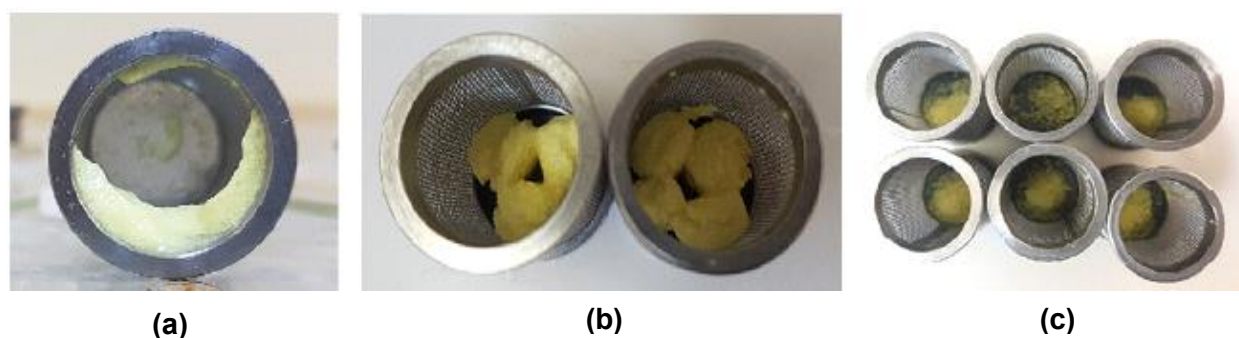


Figure 5.12: Visual representation of (a) hypromellose gel matrix formed by Coartem[®] (b) example of relatively intact formulated lipid matrix tablets after 750 min dissolution study highlighting the different effects of erosion, relaxation or diffusion-controlled drug release mechanisms and (c) example of formulated lipid granules that once coated drug particles within lipid matrix tablets

Stearic acid displayed a lipid:drug ratio dependent drug release trend. The 0.5:1 ratio, regardless the filler, fitted the Korsmeyer-Peppas with T_{lag} model which were indicative of Fickian diffusion due to the n -value being below 0.45. The 0.75:1 ratio demonstrated dissolution profiles fitting the Peppas-Sahlin 1 with T_{lag} model. This model follows non-Fickian diffusion which entails a diffusion rate greater than/equal to the rate of polymer relaxation. The plausibility of this model was visual confirmed by the presence of mostly intact tablets after 750 min dissolution studies capable of acceptable drug release as per BP (2018) requirements; which is observed in Figure 5.12b. Lastly, the 1:1 ratios depicted drug dissolution profiles that conformed to the Peppas-Sahlin 2 with T_{lag} model. This type of dissolution profile is achieved by the erosion or relaxation of the polymer

coupled with drug diffusion. It thus explains the presence of only lipid granules (Figure 5.12c) that once coated the drug particles in the lipid dispersion.

It was observed that cetyl alcohol formulations comprising MicroceLac® 100 depicted a burst release of the artemether after the lag time had elapsed. This is clearly visible in Figure 5.13, especially concerning formulation CA1M1.

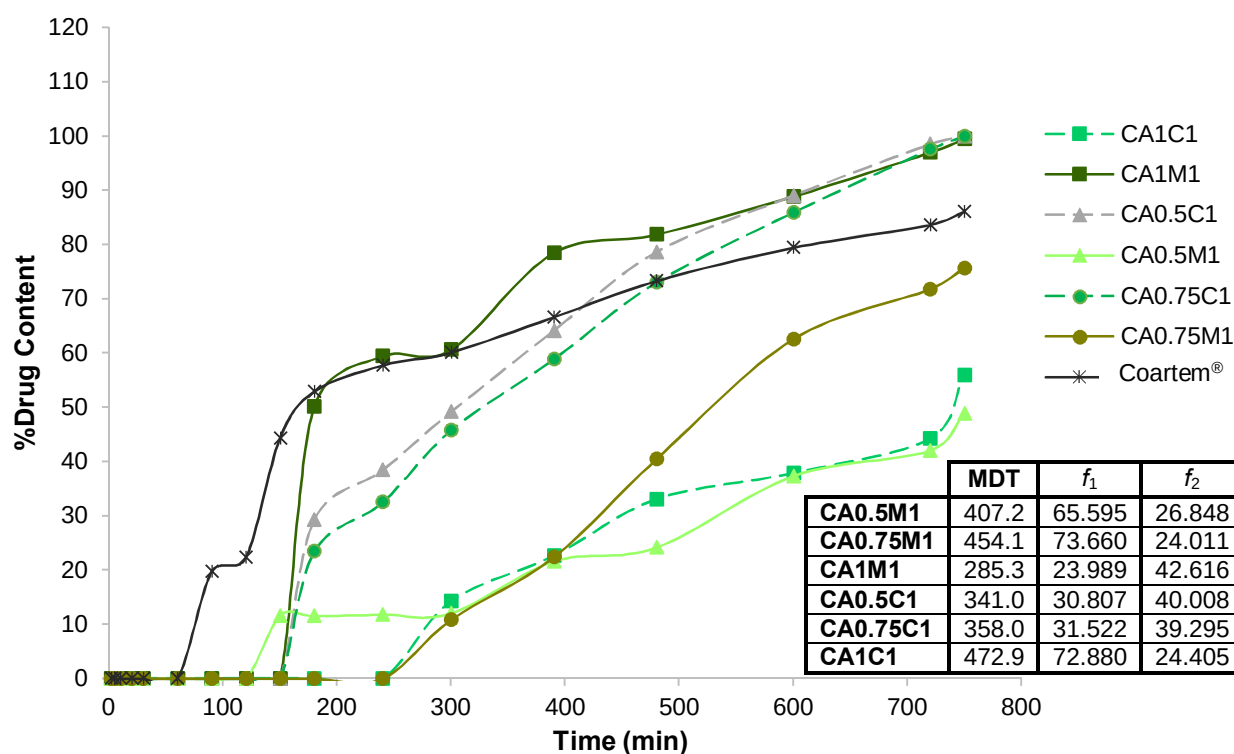


Figure 5.13: Artemether drug release profiles for the respective cetyl alcohol formulations with corresponding MDT (min) together with f_1 - and f_2 -values vs. Coartem® as an inset

CA1M1 demonstrated the most rapid drug release of these formulations in conjunction with the initial change in pH (120 min) as well as a further spike in drug release with the addition of biorelevant media (300 min); and subsequent increase in pH to 7.4. Cetyl alcohol-CombiLac® formulations exhibited similar release profiles in terms of sustained release; and this similarity was confirmed by the fit factors. CA0.5C1 versus CA0.75C1 attained an f_1 -value of 4.584 and an f_2 -value of 82.168, signifying that no statistical differences were present which coincides with these two formulations exhibiting the same lag time. However, significant differences between CA1C1 and the formulations CA0.5C1 and CA0.75C1, respectively, were present which can be explained by the difference in lag time namely: CA1C1 displayed initial drug release at time 240 min whereas both CA0.5C1 and CA0.75C1 showed signs of drug release in excess of 20% at time 180 min.

The dissolution functions, f_1 and f_2 , revealed statistically significant differences between all six cetyl alcohol formulations and Coartem®. Cetyl alcohol formulations portrayed filler dependent dissolution profiles which correlates with the non-swelling properties previously characterised for cetyl alcohol. MicroceLac® 100 formulations best fitted the Korsmeyer-Peppas with T_{lag} model. CA0.5M1 depicted super case II transport, CA1M1 portrayed Fickian diffusion and CA0.75M1 was classified as non-Fickian diffusion as per the n -values. It is therefore clear that the lipid:drug ratio determined the mechanism of drug release associated with the Korsmeyer-Peppas with T_{lag} model. CombiLac® formulations (CA1C1 and CA0.75C1) exhibited drug dissolution in accordance with the Peppas-Sahlin 2 with T_{lag} model whilst CA0.5C1 fitted the Peppas-Sahlin 1 with T_{lag} model.

Cetyl alcohol exhibited the highest average MDT of the three lipid bases indicating that this lipid base retarded drug release the most. The effects of the presence of biorelevant media is perhaps most pronounced when considering formulation CA0.5M1 where it can clearly be seen that drug release was at a constant rate after the increase in pH to simulate that of the small intestine prior to the addition of biorelevant media. Drug release thereafter becomes near linear thereby highlighting the positive influence and necessity of experimenting in biorelevant media. CA0.5M1 and CA1C1 did not meet the acceptance criteria for %drug release set by the BP (2018) as they both released less than 60% of the artemether content.

Glycerol monostearate exhibited the highest variance in lag time as well as %drug release between the respective formulations compared to the other two lipid bases. GM0.75M1 specifically, displayed release characteristics dependent upon the presence of the biorelevant media as seen in Figure 5.14. GM1M1 also highlighted the influence of biorelevant media as there are two distinct spikes in drug release, first when the pH increases at time 120 min and secondly, when the biorelevant media was added at time 300 min. GM0.5C1 released significantly less artemether in comparison with the other formulations. Although its release profile demonstrated sustained release, this formulation failed to meet the BP (2018) criteria in terms of %drug released.

All glycerol monostearate formulations displayed statistically relevant differences when compared (Annexure G). Glycerol monostearate additionally portrayed the lowest average MDT of the lipid bases, denoting that it possessed the least retarding properties. GM0.5M1 and GM0.5C1 did not adhere to the acceptance criteria for %drug release set by the BP (2018) as these formulations released 63.49%, and 36.71% artemether, respectively. Additionally, the dissolution functions, f_1 and f_2 , revealed statistically significant differences between all six glycerol monostearate formulations and Coartem®.

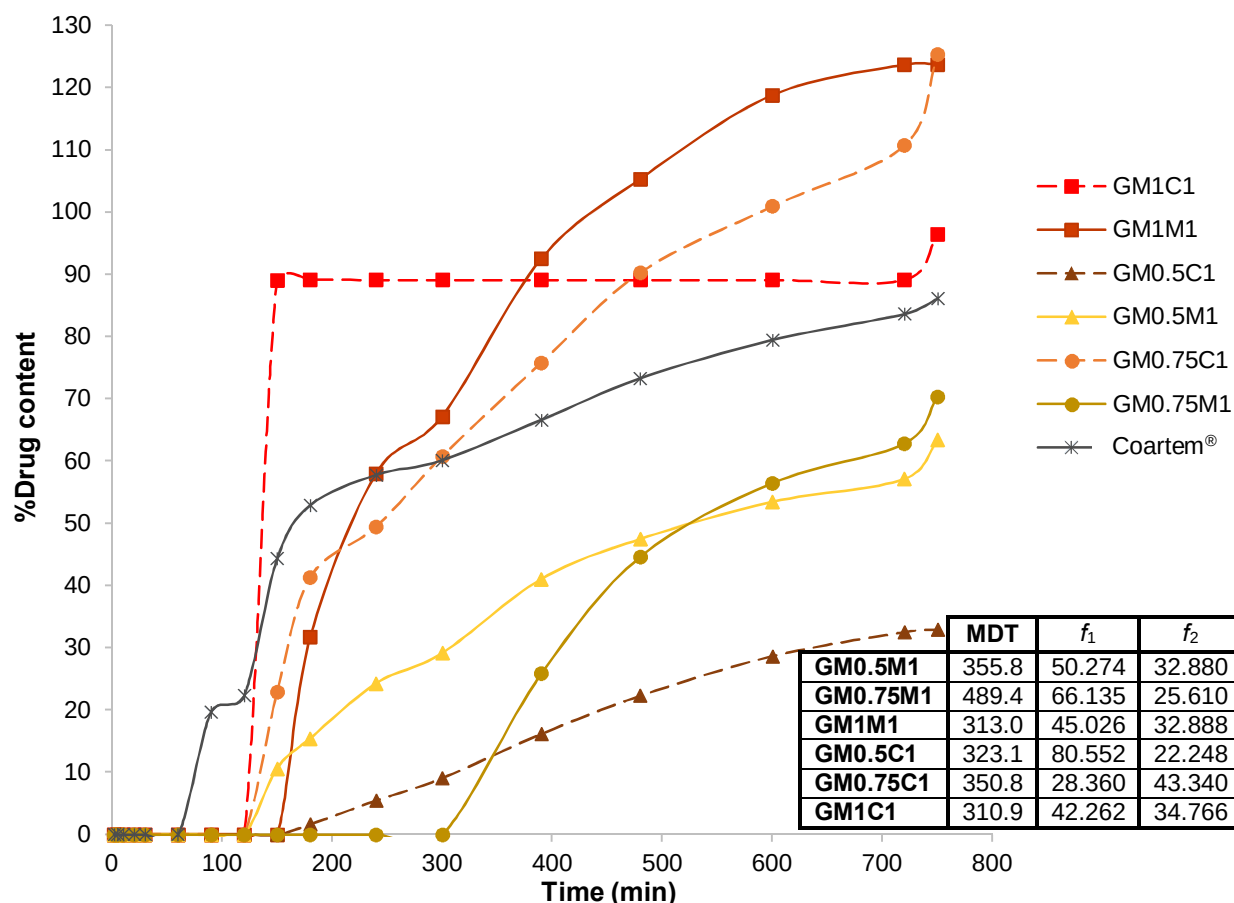


Figure 5.14: Artemether drug release profiles for the respective glycerol monostearate formulations with corresponding MDT (min) together with f_1 - and f_2 -values vs. Coartem® as an inset

No specific overall trend for glycerol monostearate release profiles could be defined. However, GM0.5C1, GM0.5M1 and GM0.75C1 all displayed non-Fickian diffusion fitting the Korsmeyer-Peppas with T_{lag} model. GM0.75M1 and GM1M1 followed Peppas-Sahlin 2 with T_{lag} model, whilst GM1C1 fitted the Peppas-Sahlin 1 with T_{lag} model that also demonstrates non-Fickian diffusion.

The inclusion of biorelevant media proved most valuable and its effects significant. Its influence was most pronounced with cetyl alcohol formulations as highlighted by an R^2 -value of 0.737 prior to its inclusion and a subsequent R^2 -value of 0.969 thereafter. In other words, its inclusion rendered near-linear drug release. This effect was true for all three lipid bases. Stearic acid presented a R^2 -value of 0.897 between time 180 min and 300 min and a R^2 -value of 0.972 between time 400 min and 750 min. Glycerol monostearate approached linear drug release (R^2 -value: 0.972) post biorelevant media addition compared to an initial drug release R^2 -value of 0.941. Therefore, when utilising highly lipophilic active ingredients, biorelevant media is of immense value and a worthwhile inclusion necessary to more accurately predict drug release given the more realistic physiologically mimicked environment provided.

In conclusion, stearic acid formulations provided the most consistent modified artemether release profiles with a predictable lag time of 180 min. The inherent surfactant properties of stearic acid proved beneficial and resulted in the consistently highest initial %drug release in conjunction with an increase in pH from 1.2 to 6.8. Thus, this lipid base was selected as optimal for the formulation of lipid matrix tablets consisting of artemether. On the other hand, glycerol monostearate formulations depicted higher variances in lag times. These formulations overall depicted inadequate artemether release profiles comparatively. Of the two fillers incorporated, CombiLac® was able to produce more formulations capable of releasing the required drug content compared to MicroceLac® 100. Additionally, formulations comprising CombiLac® exhibited less variance in lag times. Regarding lipid:drug ratio, the 0.75:1 ratio fared favourably as it yielded acceptable drug release for all formulations bar one (70.36%). However, in totality, SA0.5C1 yielded the ideal %drug release according to assay value versus theoretical content; and it may therefore be concluded that it is the most promising formulation of the investigated candidates pertaining to drug release kinetics.

5.8.4. Lumefantrine Drug Release

The therapeutic potential of lumefantrine is significantly hindered due to its low and inconsistent bioavailability which arises from its poor aqueous solubility. Low aqueous solubility can chiefly be ascribed to the high intermolecular forces contained within a crystal lattice, high lipophilicity or a combination of these elements. One method employed to augment the dissolution rate and subsequent bioavailability of such compounds is hot melt extrusion. Hot melt extrusion is proposed to enhanced dissolution by increasing the surface area and saturation solubility (Ambike *et al.*, 2004). Enhanced dissolution due to hot melt extrusion was observed for the formulated lipid matrix tablets consisting of stearic acid (97.21% theoretical artemether content release) when compared to drug dissolution from directly compressed Coartem® tablets (86.12% artemether pharmaceutically available). However, lumefantrine was not detected during the dissolution studies conducted for both the Coartem® and formulated lipid matrix tablets; and possible reasons therefore are discussed below.

A study conducted by Fule *et al.* (2013) investigated the effects of three polymers (Soluplus, Kollidon® VA64 and Plasdone S630) in terms of solubilisation and dissolution rate enhancement of lumefantrine utilising hot melt extrusion as a means of manufacture. Two essential factors responsible for the intermixing of drug-polymer particles at a molecular level are the glass transition temperature of both the polymer and drug as well as the melting point of the drug. Dissolution studies were conducted with 1% Benzalkonium chloride (BKC) to maintain sink conditions; given the extremely low aqueous solubility of lumefantrine. The observed increase in dissolution rate provided by hot melt extrusion was ascribed to the amorphous state of the lumefantrine. The higher apparent solubility and increased dissolution rate for amorphous

materials are well known and extensively documented (Blagden *et al.*, 2007; Corrigan, 1985; Hancock & Parks, 2000; Hancock & Zografi, 1997; Janssens & Van der Mooter, 2009). Increased dissolution rates are linked to the lower thermodynamic barrier to dissolution and the formation of a glassy solution wherein the drug is molecularly dispersed within the polymer. The enhancement in solubility is as a result of the disordered structure of the amorphous solid. Crystalline material requires disruption of the crystal lattice as a prerequisite for dissolution whereas an amorphous system has short-range intermolecular interactions that require no lattice energy to be overcome. Fule *et al.* (2013) stated that although BKC was added to provide sufficient wetting, the polymers played a crucial role in solubility enhancement. Solid dispersions with Soluplus yielded dissolution approximately 18-fold higher than lumefantrine alone; with Kollidon® VA64 and Plasdone S630 respectively producing %dissolution of 21-fold and 20-fold higher than lumefantrine alone.

Therefore, from the findings of Fule *et al.* (2013), three fundamental factors can be identified and attributed to the solubility and dissolution rate enhancement of lumefantrine, namely: the utilisation of hot melt extrusion to produce amorphous lumefantrine; secondly, inclusion of 1% BKC in the dissolution media as a wetting agent; and lastly, the positive effects associated with the inclusion of a polymer. Thus, the lack of these factors in the present study could possibly account for lumefantrine's absence in the dissolution studies conducted. Lumefantrine was present in its crystalline form in the manufactured lipid dispersions, as confirmed by HSM and XRPD studies. This is as a result of the lipid dispersions manufacture at a temperature lower than the glass transitioning temperature.

Additionally, biorelevant media was utilised in order to evaluate its efficacy and relevance in dissolution studies of highly lipophilic active ingredients. While its presence was sufficient to basically simulate the conditions in the small intestine and thus alter the solubility and subsequent dissolution profiles of artemether, it is clear that it was not sufficient to overcome the extremely poor aqueous solubility of lumefantrine. This speaks more perhaps to the high lipophilicity of lumefantrine rather than the inadequacy of the biorelevant media. It is important to note that this study incorporated the most basic biorelevant media composition in order to limit the variables present. More complex biorelevant media includes oleic acid (a known surfactant), known to enhance the solubility and dissolution rate of lumefantrine (Kleberg *et al.*, 2010; Patel *et al.*, 2013; Saini *et al.*, 2015). Other possible biorelevant media components include monoglycerides, free fatty acids, monoolein acid, maleic acid, sodium oleate, acetic acid and lecithin (Jantratid *et al.*, 2008; Kleberg *et al.*, 2010; Zoeller & Klein, 2007). Due to the investigation into, and focus on the drug release profiles in a more accurately simulated dissolution media, wetting agents such as BKC, Tween® 80 and sodium lauryl sulphate (SLS) (and their corresponding concentrations) were not investigated. This was due to the fact that improvement and validation of a suitable dissolution

method for the concurrent evaluation of artemether and lumefantrine was not an objective set for this study.

A study conducted by Narayankar *et al.* (2010) included several trials of different dissolution media and concluded that the addition of a surfactant is required for both artemether and lumefantrine. Their findings revealed that while the inclusion of 0.5% SLS to 300 ml sodium dihydrogen phosphate buffer (pH 7.2) provided sufficient artemether solubility, lumefantrine remained insoluble in the media. The increase of SLS concentration to 1% yielded no change in artemether solubility and lumefantrine remained insoluble. Artemether and lumefantrine remained practically insoluble in sodium phosphate buffer containing Tween® 80 in varying concentrations. Finally, Narayankar and colleagues investigated BKC (1%) in 0.1N HCL where in artemether was practically insoluble and lumefantrine was sparingly soluble (Narayankar *et al.*, 2010). They established that the drastically differing solubility of the two drugs warrants the use of two different dissolution media and therefore, suggested that the two drugs to be analysed separately. They further went on to note that after a certain time point, precipitation of lumefantrine was observed and increased with time. To increase the solubility, they investigated either increasing the volume of dissolution media from 1000 ml to 2000 ml, increasing the surfactant quantity, or increasing the experiment time period. Increasing the volume of the dissolution media had practical limitations pertaining to the dissolution apparatus, hence, the increase of BKC from 1% to 2% was chosen. This makes for interesting future prospects and considerations in the refinement of a dissolution media suitable for lumefantrine.

The distribution coefficient (log D value) is a fundamental consideration and explanation for the absence of lumefantrine in the dissolution studies conducted during this study. This value reflects the distribution of a drug between two phases, namely: an organic phase and a water phase. A high value is indicative of a lipophilic drug with predominant distribution into the organic phase. Conversely, a low (i.e. negative) value indicates predominant water distribution and therefore a hydrophilic drug. A log D value may be displayed at a pH of 6 as an indication of a drug's lipophilicity after oral administration whereas a log D value at pH 7.4 displays how lipophilic a drug is at physiological pH. Lumefantrine has a log D value of 8.9 and 10.1 at pH 6 and 7.4, respectively (Schlagenhauf-Lawlor, 2008). This correlates to 4.236×10^{-8} $\mu\text{g/ml}$ lumefantrine available in the water phase of the dissolution media at pH 7.4. Similarly, 6.714×10^{-7} $\mu\text{g/ml}$ lumefantrine would distribute to the water phase at pH 6 of the theoretically available drug content. The limit of detection for lumefantrine utilising the specified HPLC method is 0.105 $\mu\text{g/ml}$ (%RSD ≤ 10). Clearly the concentration lumefantrine that could theoretically partition into the water phase is too low to be detected. Additionally, the concentration of phospholipid present was not sufficient to increase the wettability to such an extent as to render a high enough concentration lumefantrine present in the water phase.

Finally, drug entrapment was considered as one last possible explanation despite the other reasons that validate the absence of lumefantrine. It was concluded that this was not possible as both light microscopy and HSM studies revealed that not every lumefantrine crystal was equally or completely coated by the lipid base. Therefore, contact could be made between lumefantrine and the dissolution media. The log D value explains the high lipophilicity and subsequent reluctance of lumefantrine to distribute into the water phase, resulting in its lack of detection. The lipid provided the ideal environment for lumefantrine as opposed to the aqueous phase but did not cause drug entrapment. Physiologically, the lipid will be broken down, mixed micelles and chylomicrons will form, and these will shuttle drug into the systemic and lymphatic circulation, as explained in Chapter 2, Section 2.4.

5.9. Selection of the Optimised Formulation

Powder flow, physical properties, assay values, and dissolutions profiles were evaluated as a whole in order to be able to identify the optimal formulation produced during this study. Relating to the selection of a lipid base, stearic acid proved superior in all regards. It ranked highest of the three lipid bases during powder flow, particle size and particle size distribution evaluation. The narrow particle size distribution of stearic acid particles practically conveyed theoretical predictions on the correlation between particle size and particle size distribution, and flow properties. Stearic acid depicted the smallest average %RSD for mass variation, with a tensile strength of 2.031 N.mm⁻² and a satisfactorily low %friability of only 0.217%. It outperformed glycerol monostearate regarding %friability and tensile strength correlations, whilst cetyl alcohol only just met the BP (2018) friability requirements. Stearic acid displayed the highest %swelling and only minimal signs of erosion were present, validating that these lipid matrix tablets were capable of high water up take, but nonetheless able to withstand erosion. A trend was observed during assay experiments which revealed that the artemether %drug content obtained was typically lower than the theoretical content compared to that of lumefantrine which demonstrated a noticeably higher %drug content. Stearic acid however, produced sufficient concentrations despite the observed variance in target drug content. Lastly, it displayed the most uniform and predictable release profiles of the three lipid bases during dissolution studies, thereby confirming its selection as the lipid base of choice for this study.

The second factor requiring selection is that of the lipid:drug ratio. Powder flow results proved somewhat inconclusive and portrayed lipid base–lipid ratio dependent relationships. Overall, glycerol monostearate and stearic acid produced the following rank order: 0.5:1 > 0.75:1 > 1:1. However, cetyl alcohol demonstrated the inverse thereby highlighting the influence of the lipid base type. Physical property characterisation of the lipid matrix tablets offered more tangible insights into the effect of lipid:drug ratio. The correlation of %friability with tensile strength portrayed the 0.75:1 ratio as marginally superior to the 0.5:1 ratio, purely based on the 0.5:1 ratio

exhibiting higher %RSD during mass variation testing. It should be noted that the 0.5:1 ratio portrayed the highest tensile strength and lowest friability. The 0.5:1 ratio demonstrated most acceptable %drug content assays and consistent %drug release profiles. Given the similarities in performance of the two ratios (0.5:1 and 0.75:1), the lower level of 0.5:1 was selected.

Inclusion of MicroceLac® 100 and CombiLac® into the lipid matrix tablets yielded tablets with sufficient integrity and a good overall appearance; both performing equally well during physical property characterisation. Tablets consisting of CombiLac® however, portrayed superior powder flow properties, particle size and particle size distribution, and outperformed lipid matrix tablets comprising MicroceLac® 100 during dissolution testing. For this reason, CombiLac® was selected as the optimum filler. As previously discussed, the lowest functioning lubricant concentration (i.e. 1% magnesium stearate) was kept as a constant as the investigated difference in magnesium stearate concentration had negligible effects on these two fillers.

Therefore, it was concluded that stearic acid in a 0.5:1 lipid:drug ratio in combination with CombiLac® and 1% magnesium stearate (SA0.5C1) is the optimal formulation produced during this study within the given parameters.

5.10. *In Vitro* Permeability Studies

Permeability studies of the two highly lipophilic active ingredients entailed their own unique set of challenges, most of which could be explained by a lack of specificity of the model used. To date, and to the best of our knowledge, highly lipophilic drugs incorporated into lipid-based formulations have yet to be tested on the *in vitro* permeability model investigated in this study. Therefore, as a proof of concept, a concurrent permeability study of the two active ingredients from the optimised formulation, SA0.5C1, was conducted across the proximal jejunum of porcine intestinal tissue. Dissolution studies revealed a higher %artemether drug release in comparison to the commercially available product, Coartem®, thus it was not necessary to utilise Coartem® as a control as it had already been established that the lipid-matrix tablets formulated in this study were capable of superior artemether drug release profiles.

The primary drawback of diffusion chambers, specifically Ussing chambers or Sweetana-Grass diffusion chambers, is the underestimation of drug transport, particularly for lipophilic compounds (Nunes *et al.*, 2016). The short comings of this model proved problematic and required a number of adjustments to be made during this proof of concept study. The aqueous KRB environment normally utilised for a transport study resulted in extremely low solubility of both active ingredients in the apical chambers; which in turn ensued in drug concentrations in these chambers being below the limit of detection and quantification. Following, it was decided to introduce bile salts and phospholipids into the transport media so as to increase the drug solubility in the donor chambers as well as to more accurately mimic the ileocecal environment; which is one of the most relevant

parameters for predicting *in vivo* gastrointestinal absorption (Chaves *et al.*, 2014). The addition of simulated ileocecal media proved to be valuable as the solubility of the active ingredients was increased to such an extent that they could both be accurately detected (>LOQ values obtained) in the apical chambers. Additionally, dissolution studies demonstrated that artemether was released from the dosage form for over 12 hours. Therefore, tablets were initially subjected to a 12 h dissolution environment where after the equivalent volume required per donor chamber was removed and used for the *in vitro* permeability study. The simulated dissolution environment was necessary to release the maximum amount of drug prior to exposure to the porcine intestinal tissue as it is only viable for 2 h after collection and preparation (Haslam *et al.*, 2011; Pietzonka *et al.*, 2002).

Although the solubility had been sufficiently increased in the apical chambers, permeation of the drugs into the basolateral chambers was not initially detected. This was ascribed to the high dilution factor which resulted from the removal and replacement of 1 ml per withdrawal for a total of six withdrawals during the transport experiments. In order to combat this problem, as well as the resulting drug concentrations being below the LOD and LOQ values, it was decided to only conduct one withdrawal at time 120 min. The combined effects of the above-mentioned adjustments resulted in the detection of both artemether and lumefantrine in the basolateral chambers. Artemether and lumefantrine achieved mean values of 3.35% and 4.88% transport, respectively and exhibited P_{app} -values of $1.581\text{E-}07\text{ cm.s}^{-1}$ and $1.470\text{E-}06\text{ cm.s}^{-1}$, correspondingly. The overall average %TEER reduction at time 120 min was 7.09% indicating that the tight junctions were not opened and the tissue integrity remained intact and viable.

Due to the high lipophilicity of the drugs investigated, subsequent low aqueous solubility and relatively small percentage transport achieved, it was decided to conduct *ex vivo* permeability studies as to ascertain the %drug retention in the porcine intestinal tissue post *in vitro* permeability studies. This was a valuable and worthwhile exercise as 9.88% artemether and 59.56% lumefantrine drug retention was obtained in the porcine tissue. A possible reason for detection of the two drugs in the tissue could be explained by the movement of the molecules into the intercellular spaces causing them to be trapped in between the relatively small tight junctions (Reitsma *et al.*, 2014). Additionally, the mucus that covers the epithelium is intended to prevent penetration of pathogens into the epithelium. This mucus layer can inadvertently entrap and prevent or restrict particle penetration into the underlying epithelia, ultimately causing particles to be eliminated via clearance mechanisms (Cone, 2009; Walter *et al.*, 2000). During *in vitro* permeability studies, active ingredients do not necessarily have to cross the membrane to indicate their potential for absorption. Entry into the tissue is sufficient evidence of possible *in vivo* performance as capillaries are easily permeable and therefore, the presence of the drugs in the tissue would suggest that they would be able to perfuse into the bloodstream. This would need to

be assessed *in vivo* as no blood flow or sink conditions were present in the model employed. Moreover, pertaining to lipophilic drug transport, a drug reservoir can establish within the stratum corneum layer of the skin. As the drug penetrates further into the skin, it is absorbed into the local capillaries and is then transported into the systemic circulation (Margetts & Sawyers, 2007).

The gastrointestinal tract has two primary yet conflicting roles namely, the absorption of nutrients whilst concurrently serving as an effective protective barrier against harmful substances and bacteria (Barreau & Hugot, 2014; Lennernas, 2013; Powell, 1981). Whilst the mucosal barrier properties of the intestine are partially dependent upon factors such as gastrointestinal secretions and the mucus layer, the epithelium itself plays the predominant role in the barrier properties (Reitsma *et al.*, 2014; Travis & Menzies, 1992). Primary investigations of the human gastrointestinal tract mainly utilise *in vivo* permeability studies which lend themselves to the study of the passage of molecules, but fails to provide information pertaining to the permeability of specific regions within the gastrointestinal tract nor the permeability routes or mechanisms employed (Bjarnason *et al.*, 1995; Meddings & Gibbon, 1998; Travis & Menzies, 1992). In order to circumvent these problems, *in vitro* techniques are frequently employed to examine the permeability routes and mechanisms in laboratory animals which are then further extrapolated to the human situation (Mynott *et al.*, 1997; Pantzar *et al.*, 1993; Schlichting *et al.*, 1995). Given the anatomical, physiological and metabolic differences present between species, it is a reasonable assumption that the barrier properties of the intestinal mucosa may also vary (Kararli, 1995; Taylor & Anderson, 1972; Watkins, 1997). For this reason, Nejdfors *et al.* (1999) conducted mucosal *in vitro* permeability studies in the intestinal tract of the pig, the rat and man to establish species- and regional-related difference. Nejdfors and colleagues (1999) concluded that whilst permeability data from the pig correlated fairly well with those of man, the rat differed thereby rendering it difficult to extrapolate data from rat studies to make predictions in man. This accounts for the discrepancies often seen in *in-vitro-in-vivo* correlations.

Despite the common conception that the Caco-2 cell line is the best known *in vitro* model for permeability studies (Artursson *et al.*, 2001; Hidalgo, 2001; Sambuy *et al.*, 2005), one cell type cannot sufficiently replicate the complete physiology of the human intestine and thus often leads to inaccurate *in-vitro-in-vivo* correlations (Artursson *et al.*, 2001; Artursson & Karlsson, 1991; Cone, 2009; Sun *et al.*, 2008). Therefore, Schimpel *et al.* (2014) investigated the development of an advanced intestinal *in vitro* triple culture permeability model to study the transport of nanoparticles utilising co-cultured Caco-2 cells and mucus-secreting goblet cells with the addition of Raji B cells to stimulate the differentiation of M cells. Epithelial M cells are an important consideration when preparing a model as these cells are located in the epithelium overlying Peyer's patches (with relatively less mucus protection) and also play a dominant role in addition to enterocytes and mucus (Barreau & Hugot, 2014; Ensign *et al.*, 2012; Snoeck *et al.*, 2005).

These epithelial M cells represent a minor population in the follicle-associated epithelium, however, they are responsible for the uptake and trans-epithelial transport of microorganisms and foreign substances to the lymphoid follicles (Kerneis *et al.*, 2000). Provided that one of the major mechanisms of lipid-based formulation absorption is lymphatic transport, the inclusion of M cells in a model is absolutely necessary for model accuracy (Kalepu *et al.*, 2013). The triple culture permeability study conducted by Schimpel and colleagues (2014) obtained data that correlated well with data from *ex vivo* permeability experiments utilising porcine intestinal mucosa thereby demonstrating the reliability of the investigated model. Whilst this alternative *in vitro* permeability model closely replicates the human small intestine physiology to study the transcytosis of nanoparticles, its relevance for lipid-based formulations has yet to be proven and thus should be investigated further to identify a more suitable model for the analysis of lipid-based formulations than the one utilised in this study.

Finally, given the necessity for Replacement, Refinement, and Reduction of animals in research and testing, coupled with the problems associated with the current model and testing techniques, it makes for a strong case to move to synthetic intestines or the development of a new model that is capable of meeting the unique requirements of lipid-based formulations. 3D culture models have become a mainstream approach due to the similarities between *in vivo* morphology and behaviour of cells in 3D culture conditions (Pampaloni *et al.*, 2007) and therefore might be a solution to the model problem.

In conclusion, despite this study detecting a relatively small %transport, there is a need for the development and refinement of models such as synthetic intestines, triple culture permeability models and 3D cultures which are capable of decreasing the use of animals in research (Pampaloni *et al.*, 2007) whilst simultaneously providing the researcher with a more accurate permeability model for the evaluation of lipid-based formulations.

5.11. Chapter Conclusion

Lipid matrix tablets were manufactured in accordance with the full factorial design of experiments and the physical properties of all the manufactured formulations were assessed. Only the 18 formulations comprising either MicroceLac® 100 or CombiLac® were found capable of passing all of the official and unofficial BP (2018) tests and therefore, only these would progress for further testing.

In terms of the physical tablet characteristics, the following general rank order could be allocated to the type of lipids investigated: stearic acid > glycerol monostearate > cetyl alcohol. Additionally, a distinct correlation between the lipid:drug ratio, friability and tensile strength could be established when the lipid:drug ratios were considered. Regarding physical tablet characteristics, the following ranking was assigned: 0.75:1 > 0.5:1 > 1:1.

Subsequently, an assay was conducted utilising the validated HPLC method and revealed that in general artemether content was slightly lower (91%) and lumefantrine content was approximately 110% of the proposed theoretical content. Additionally, the internal and external surface morphologies of the manufactured tablets were inspected and presented a constant mottled appearance.

Stearic acid displayed the highest %swelling with only minimal signs of erosion, validating that these lipid matrix tablets were capable of high water up take, but nonetheless able to withstand erosion. Filler composition played the predominant role in the swelling and erosion test results. Many of the CombiLac® formulations eroded completely during the swelling phase of experimenting, whilst most MicroceLac® 100 formulations were still well intact, even after the swelling weight stabilised, with no visual signs of erosion.

Dissolution studies showed SA0.5C1 to be the optimal formulation regarding artemether drug release (97.21% of theoretical content) and was compared to the artemether dissolution achieved by Coartem® tablets (86.12% artemether pharmaceutically available). This study focused on investigating the relevance of biorelevant media and in no way set out to perfect a dissolution media environment optimal for the concurrent evaluation of artemether and lumefantrine. From this study, it was concluded that the addition of biorelevant media was a valuable inclusion and significantly increased the release of artemether. However, the basic composition of the biorelevant media utilised lacked characteristics capable of solubilising lumefantrine to such an extent that it could be detected. This speaks to the unique needs and high lipophilicity of lumefantrine rather than to an inadequacy of biorelevant media.

After the evaluation of all the above mentioned parameters and consideration of their corresponding responses, it was concluded that stearic acid in a 0.5:1 lipid:drug ratio in

combination with CombiLac® and 1% magnesium stearate (SA0.5C1) was the optimal formulation produced during this study.

Finally, permeability studies of the optimised formulation were conducted as a proof of concept and revealed a lack of specificity of the model used. After a number of adjustments, including the addition of bile salts and phospholipids; and minimising the dilution factor, transport of both artemether and lumefantrine was achieved. Further *ex vivo* analysis of the tissue revealed drug retention for both active ingredients within the tissue. These results in are in no means ideal, however they do highlight the need for the development of an *in vitro* model suited to the specific needs of lipid-based formulations and makes for interesting future prospects.





6.1. Summary of Study Outcomes

This study aimed to incorporate a double-fixed dose combination of the two antimalarial drugs, artemether and lumefantrine, into a lipid matrix tablet that would offer modified release of the active ingredients. This was evaluated on a number of levels, as discussed below, and ultimately revealed that the aim as well as all the objectives set for this study had been achieved.

Lipid dispersions were successfully prepared via the hot fusion method utilising either stearic acid, glycerol monostearate or cetyl alcohol as a lipid base. Investigation into the preparation of a solid lipid dispersion utilising coconut oil as a lipid base proved to be non-viable. The composition of coconut oil (rendering a low melting point of approximately 25°C) meant it would not solidify to such an extent that it could be handled and manufactured into lipid matrix tablets.

Pre-formulation studies included the characterisation of the active ingredients as well as prepared lipid dispersions in terms of their flow properties, particle size and particle size distribution analysis. It was found that the incorporation of the two active ingredients into the lipid dispersions allowed for an overall improvement in flow characteristics compared to the active ingredients in isolation. This improvement in flowability was profoundly significant when considering lumefantrine. Stearic acid lipid dispersions possess the highest flow properties followed by glycerol monostearate and cetyl alcohol. The four different fillers were similarly characterised and remarkably yielded the following general rank order for both powder flow and particle size parameters: CombiLac® > MicroceLac® 100 > RetaLac® Ó Pharmacel® 101. This rank order ultimately conveys theoretical predictions practically regarding the correlation between particle size, particle size distribution and powder flow properties.

DSC, TGA, XRPD, light microscopy and HSM were subsequently carried out on the lipid dispersions and their respective individual components to assess the effect of the hot fusion method on the active ingredients. DSC and XRPD confirmed that both active ingredients retained their crystalline structure after hot fusion. This result was validated by the observations made when employing light microscopy and HSM. Light microscopy revealed that the crystalline active ingredient particles had been coated either partially or completely by the lipid base, which was the ideal outcome. HSM offered further insights and demonstrated that once the lipid base had melted, the crystalline form of both drugs remained and continued to melt at their respective melting point temperatures.

Lipid matrix tablets were subsequently manufactured in accordance with the full factorial design of experiments accounting for a total of 72 formulations. It was established during pre-formulation

studies that the lower level of magnesium stearate (0%) was incongruous for the manufacture of lipid matrix tablets. During tableting, formulations containing MicroceLac® 100 and CombiLac® (with either 1% or 1.25% magnesium stearate) yielded tablets with sufficient integrity and a good overall appearance. RetaLac® and Pharmacel® 101 formulations required the upper level of magnesium stearate (1.25%) in order to produce tablets of which many crumbled upon handling or had significant defaults. Additionally, other RetaLac® and Pharmacel® 101 formulations were unable to produce any tablets.

The physical properties of all the manufactured formulations were assessed in terms of mass variation, friability, disintegration, hardness, thickness, diameter and tensile strength. From these tests, only the 18 formulations comprising either MicroceLac® 100 or CombiLac® were capable of passing all of the above-mentioned tests. For this reason, only formulations containing these two fillers would progress for further testing. It was therefore decided to select the lowest functioning concentration lubricant (i.e. 1% magnesium stearate) to be kept as a constant moving forward to the next experimental stage as the investigated difference in magnesium stearate concentration had negligible effects on these two fillers.

The type of lipid bases employed were compared and evaluated as a factor regarding mass variation (%RSD), %friability, tensile strength and disintegration. Although the average friability of cetyl alcohol was still in compliance with the BP (2018) specifications, it was meaningfully higher than the average friability results obtained for formulations comprising either glycerol monostearate or stearic acid (0.209 and 0.217%, respectively). Stearic acid depicted the smallest average %RSD for mass variation, with a tensile strength of 2.031 N.mm⁻² and a satisfactorily low %friability of only 0.217%. All three lipid bases exceeded the 15 min BP (2018) criteria for disintegration and were therefore deemed as probably having extended drug release properties. In terms of the physical tablet characteristics, the following rank order could be allocated to the type of lipids investigated: stearic acid > glycerol monostearate > cetyl alcohol.

A distinct correlation between the lipid:drug ratio, friability and tensile strength could be established when the lipid:drug ratios were considered. This correlation revealed that an increase in lipid concentration caused a subsequent decrease in the tensile strength and an increase in the %friability. Regarding physical tablet characteristics, the following ranking was assigned: 0.75:1 > 0.5:1 > 1:1.

An assay was conducted utilising the validated HPLC method and revealed that in general artemether content was slightly lower (91%) and lumefantrine content was approximately 110% of the proposed theoretical content. Additionally, the internal and external surface morphologies of the manufactured tablets were inspected. The mottled appearance of the lipid matrix tablets resulted from the lipid dispersions being coloured yellow on account of the presence of

lumefantrine mixed with the white colour of the particular corresponding fillers. This mottled appearance was found to be uniform throughout each individual lipid matrix tablet regardless the formulation variables such as type of lipid base or the lipid:drug ratio incorporated.

Cetyl alcohol and stearic acid are classified as non-swelling matrix forming materials; however, the fillers present in the lipid matrix tablets were sufficient enough to cause swelling. There was evidence that cetyl alcohol's characteristic of being a non-swelling matrix was more prominent than that associated with stearic acid. Stearic acid displayed the highest %swelling and only minimal signs of erosion were present, validating that these lipid matrix tablets were capable of high water up-take, but nonetheless able to withstand erosion. Filler composition played the most predominant role in the swelling and erosion test results. The different swelling behaviours of the two fillers can mostly be ascribed to the presence of corn starch in CombiLac®. The heating of starch molecules in the presence of excess water subsequently causes swelling and an increase in granule size and solubility to transpire (Singh *et al.*, 2003), thereby accounting for the more prolific swelling behaviour of CombiLac®. MicroceLac® 100 portrayed the higher %swelling of the two fillers and exhibited substantially less erosion which was clearly visible during testing. Many of the CombiLac® formulations eroded completely during the swelling phase of testing, whilst most MicroceLac® 100 formulations were still well intact, even after the swelling weight stabilised, with no visual signs of erosion. CombiLac® comprises 70% D-lactose monohydrate which is soluble in water (BP, 2018) and thus the high percentage lactose incorporated coupled with the swelling effects invoked by the presence of 10% corn starch resulted in these formulations being more susceptible to erosion.

Dissolution studies yielded the most interesting results of the study. An objective set for this study was to evaluate and compare the various formulated lipid matrix tablets and assess the impact of the inclusion of biorelevant media. Formulated lipid matrix tablets and optimal drug release formulation, SA0.5C1 (97.21% theoretical artemether content release), were compared to artemether dissolution from directly compressed Coartem® tablets (86.12% artemether pharmaceutically available). Artemether release from SA0.5C1 fitted the Korsmeyer-Peppas with T_{lag} model which is indicative of Fickian diffusion whilst Coartem®-artemether release fitted the Peppas-Sahlin 2 with T_{lag} model accounting for the simultaneous effects of Fickian diffusion and case II transport. This study in no way set out to perfect a dissolution media environment optimal for the concurrent evaluation of artemether and lumefantrine but rather investigated the relevance of basic biorelevant media and whether it would be sufficient to overcome the associated challenges of highly lipophilic drugs. From this study, it can be concluded that the addition of biorelevant media significantly increased the release of artemether and proved to be a valuable inclusion. However, the basic composition of the biorelevant media utilised, lacked characteristics capable of solubilising lumefantrine to such an extent that it could be detected for neither

Coartem® nor the formulated lipid matrix tablets. This speaks to the unique needs and high lipophilicity of lumefantrine rather than to an inadequacy of biorelevant media.

After the evaluation of all the above mentioned parameters and consideration of their corresponding responses, it was concluded that stearic acid in a 0.5:1 lipid:drug ratio in combination with CombiLac® and 1% magnesium stearate (SA0.5C1) was the optimal formulation produced during this study.

To conclude, proof of concept permeability studies utilising the optimised formulation were conducted and revealed a lack of specificity of the model used. After numerous adjustments to the transport model, both artemether and lumefantrine were detected in the basolateral chambers. Artemether and lumefantrine achieved 3.35% and 4.88% transport, respectively. Additionally, *ex vivo* analysis revealed 9.88% and 59.56% artemether and lumefantrine drug retention in the tissue, correspondingly. The proof of concept results highlight the need for the development of an *in vitro* model specific to the needs of lipid-based formulations and makes a worthwhile contribution to the scientific community regarding future focus and experimental method development areas pertaining to highly lipophilic active ingredients specifically, as well as lipid-based drug delivery systems.

6.2. Future Prospects

After completion of this study, the following recommendation are made regarding future investigations and areas of focus:

- Other lipids could be investigated as possible bases for the lipid matrix tablets such as cetostearyl alcohol (Ninama *et al.*, 2015), particularly focusing on the physicochemical properties such as melting point and inherent surfactant properties.
- Additional factors, for example a binder could be included in formulations containing RetaLac® or Pharmacel® 101 to produce tablets of enhanced integrity. RetaLac® is a matrix forming filler which could optimise the delayed release mechanism. Furthermore, attempts to enhance their powder flow properties could be developed, for instance the formation of beads or agglomerates.
- According to Fule *et al.* (2013) the inclusion of Kollidon® VA 64 is capable of increasing the solubility of lumefantrine as much as 21-fold. This could prove an immensely supportive formulation aid given the high lipophilicity and subsequent low aqueous solubility of lumefantrine. Moreover, Kollidon® VA 64 has strong binding mechanisms which could render it the ideal candidate for testing in conjunction with a filler such as RetaLac®. Research into other polymers capable of increasing the solubility of lumefantrine should also be conducted.

- When utilising the hot fusion method of manufacture, it may be beneficial to melt lumefantrine above its glass transition temperature as a means of increasing its solubility (Blagden *et al.*, 2007; Corrigan, 1985; Grinsted *et al.*, 1992; Hancock & Parks, 2000; Janssens & Van der Mooter, 2009). Poor biopharmaceutical absorption associated with lumefantrine stems from its low aqueous solubility and therefore every possible approach should be contemplated to enhance its solubility.
- Another possible approach for enhancing solubility of both the active ingredients from this study would be to include an appropriate surfactant in the tablet formulation. Examples may comprise polysorbital® 80, SLS, HPMC, LATHANOL® LAL powder, to name a few. Compatibility of the excipients would need to be established; as well as powder flow and compressibility studies of the excipients selected for inclusion need to be performed.
- Although the optimisation of dissolution media for the concurrent analysis of artemether and lumefantrine was not an objective set for this study, it certainly warrants further investigation with the following being viable starting points:
 - Inclusion of BKC 2% in dissolution media (Fule *et al.*, 2013);
 - Oleic acid inclusion in biorelevant media (Kleberg *et al.*, 2010; Patel *et al.*, 2013; Saini *et al.*, 2015); and
 - Differing concentrations of components in biorelevant media and the inclusion of other components such as monoglycerides, free fatty acids, monoolein acid, maleic acid, sodium oleate, acetic acid and lecithin (Jantratid *et al.*, 2008; Kleberg *et al.*, 2010; Zoeller & Klein, 2007).
- Assay studies revealed a slightly lower percentage artemether in the lipid matrix tablets compared to the theoretical drug content. A solution to this problem would be to spike the artemether content, but ultimately the cause of the lower artemether drug content should be identified. Moreover, bulk content uniformity testing could be conducted on the powder mixtures before tableting to supplement the assay data.
- The permeability proof of concept study conducted revealed the area with the greatest necessity for further research and method development. As discussed previously, a model catering to the specific need of a) highly lipophilic drugs and b) lipid-based formulations, needs to be established in order to make meaningful conclusions from the *in vitro* data obtained from such experiments. Despite the small percentage transport achieved for both drugs from the formulated lipid matrix tablets, it is still vital to conduct *in vivo* experiments to determine whether the *in vitro* results are comparable *in vivo*.
- Other *in vitro* possibilities to investigate have previously been discussed and include, but are not limited to, triple culture Caco-2 cells, synthetic intestines or 3D cultures. Perhaps the answer of a model possessing the required specificity for highly lipophilic drugs and/or lipid-based formulations lies within these proposed models.

- Finally, stability analysis should be conducted where either accelerated (6 months) or real time (2 years) experiments should be performed. Accelerated stability tests are conducted at elevated temperatures which may be problematic given the relatively low melting temperatures of the various lipid bases incorporated into the different lipid matrix tablets. Temperatures below the lipid melting point would need to be selected, or a lipid with a higher onset of melting temperature would need to be incorporated into these formulations.





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Particle Size and Particle Size Distribution

A.1: Active Ingredient Artemether	A2
A.2: Active Ingredient Lumefantrine	A3
A.3: MicroceLac® 100 Filler Powder	A4
A.4: RetaLac® Filler Powder	A5
A.5: CombiLac® Filler Powder	A6
A.6: Pharmacel® 101 Filler Powder.....	A7
A.7: Glycerol Monostearate 0.5:1 Lipid Dispersion	A8
A.8: Glycerol Monostearate 0.75:1 Lipid Dispersion	A9
A.9: Glycerol Monostearate 1:1 Lipid Dispersion.....	A10
A.10: Stearic Acid 0.5:1 Lipid Dispersion	A11
A.11: Stearic Acid 0.75:1 Lipid Dispersion.....	A12
A.12: Stearic Acid 1:1 Lipid Dispersion	A13
A.13: Cetyl Alcohol 0.5:1 Lipid Dispersion.....	A14
A.14: Cetyl Alcohol 0.75:1 Lipid Dispersion	A15
A.15: Cetyl Alcohol 1:1 Lipid Dispersion	A16

A.1: Active Ingredient Artemether



Result Analysis Report

Sample Name:

Artemether RM

Sample Source & type:

CW+LC

Sample bulk lot ref:

RM (Sample 3)

SOP Name:

Christi Wilkins

Measured by:

Jan Steenekamp

Result Source:

Measurement

Measured:

23 March 2017 12:53:13 PM

Analysed:

23 March 2017 12:53:15 PM

Particle Name:

Titanium Dioxide

Particle RI:

2.741

Dispersant Name:

Water

Accessory Name:

Hydro 2000SM (A)

Absorption:

0.1

Dispersant RI:

1.330

Analysis model:

General purpose

Size range:

0.020 to 2000.000 um

Weighted Residual:

0.831 %

Sensitivity:

Enhanced

Obscuration:

13.24 %

Result Emulation:

Off

Concentration:

0.3474 % Vol

Span :

1.937

Uniformity:

0.603

Result units:

Volume

Specific Surface Area:

0.0345 m²/g

Surface Weighted Mean D[3,2]:

174.103 um

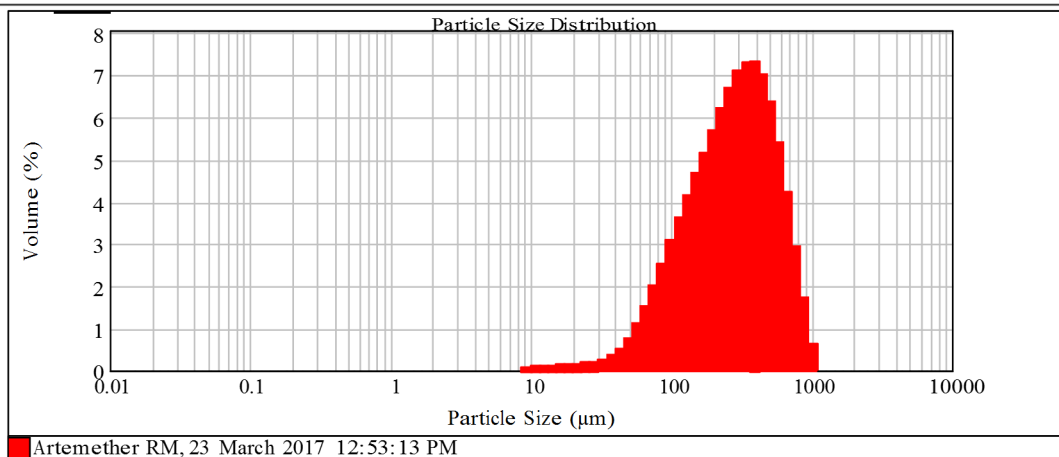
Vol. Weighted Mean D[4,3]:

321.456 um

d(0.1): 89.336 um

d(0.5): 276.506 um

d(0.9): 624.925 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.10	120.226	4.16	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.12	138.038	4.68	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.13	158.489	5.19	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.15	181.970	5.71	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.16	208.930	6.22	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.17	239.883	6.70	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.20	275.423	7.32	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.26	316.228	7.08	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.36	363.078	7.31	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.54	416.869	7.01	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.79	478.630	6.38	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	1.13	549.541	5.43	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	1.55	630.957	4.26	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	2.04	724.436	2.97	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	2.56	831.764	1.76	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	3.10	954.993	0.67	10000.000	0.00
0.091	0.00	0.965	0.00	10.000	0.08	104.713	3.64	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925			

Operator notes:

A.2: Active Ingredient Lumefantrine



MASTERSIZER



Result Analysis Report

Sample Name:

Lumefantrine

SOP Name:

Christi Wilkins

Measured:

23 March 2017 03:00:21 PM

Sample Source & type:

CW+LC

Measured by:

Jan Steenekamp

Analysed:

23 March 2017 03:00:22 PM

Sample bulk lot ref:

RM (Sample 1)

Result Source:

Measurement

Particle Name:

Yellow pigment

Accessory Name:

Hydro 2000SM (A)

Analysis model:

General purpose

Sensitivity:

Enhanced

Particle RI:

2.190

Absorption:

0.1

Size range:

0.020 to 2000.000 μm

Obscuration:

11.48 %

Dispersant Name:

Water

Dispersant RI:

1.330

Weighted Residual:

0.445 %

Result Emulation:

Off

Concentration:

0.1206 %Vol

Span :

1.992

Uniformity:

0.622

Result units:

Volume

Specific Surface Area:

0.0833 m^2/g

Surface Weighted Mean D[3,2]:

71.991 μm

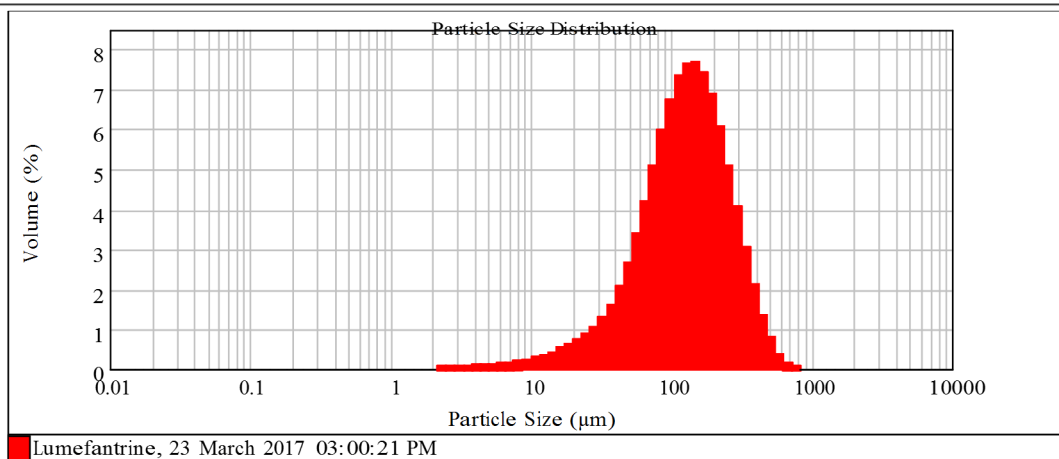
Vol. Weighted Mean D[4,3]:

151.883 μm

d(0.1): 41.482 μm

d(0.5): 126.994 μm

d(0.9): 294.482 μm



Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.38	120.226	7.66	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.46	138.038	7.70	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.56	158.489	7.44	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.66	181.970	6.88	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.77	208.930	6.09	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.05	22.909	0.91	239.883	5.12	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.06	26.303	1.08	275.423	4.09	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.06	30.200	1.65	316.228	3.06	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.07	34.674	1.32	363.078	2.14	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.08	39.811	2.09	416.869	1.38	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.09	45.709	2.67	478.630	0.81	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.10	52.481	3.39	549.541	0.42	5754.399	0.00
0.052	0.00	0.560	0.00	5.754	0.12	60.256	4.22	630.957	0.16	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.16	69.183	5.11	724.436	0.05	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.20	79.433	5.98	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.25	91.201	6.75	954.983	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.31	104.713	7.33	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925	0.00		

Operator notes:

Malvern Instruments Ltd.

Malvern, UK

Tel : +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 5.60

Serial Number : MAL1007548

File name: 23 Mrt 2017.mea

Record Number: 11

2017/07/07 10:24:43 AM

A.3: MicroceLac® 100 Filler Powder



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Result Analysis Report

Sample Name:
Microcelac

Sample Source & type:
Supplier = Jan Nieman

Sample bulk lot ref:
ML 2 (Sample 1)

SOP Name:
Jan Nieman

Measured by:
Jan Steenekamp

Result Source:
Measurement

Measured:
13 May 2016 10:46:10 AM

Analysed:
13 May 2016 10:46:11 AM

Particle Name:
Titanium Dioxide

Particle RI:
2.741

Dispersant Name:
Cyclohexane

Accessory Name:
Hydro 2000SM (A)

Absorption:
0.1

Dispersant RI:
1.426

Analysis model:
General purpose

Size range:
0.020 to 2000.000 um

Weighted Residual:
0.803 %

Sensitivity:
Enhanced

Obscuration:
12.84 %

Result Emulation:
Off

Concentration:
0.2016 %Vol

Span :
1.608

Uniformity:
0.488

Result units:
Volume

Specific Surface Area:
0.0573 m²/g

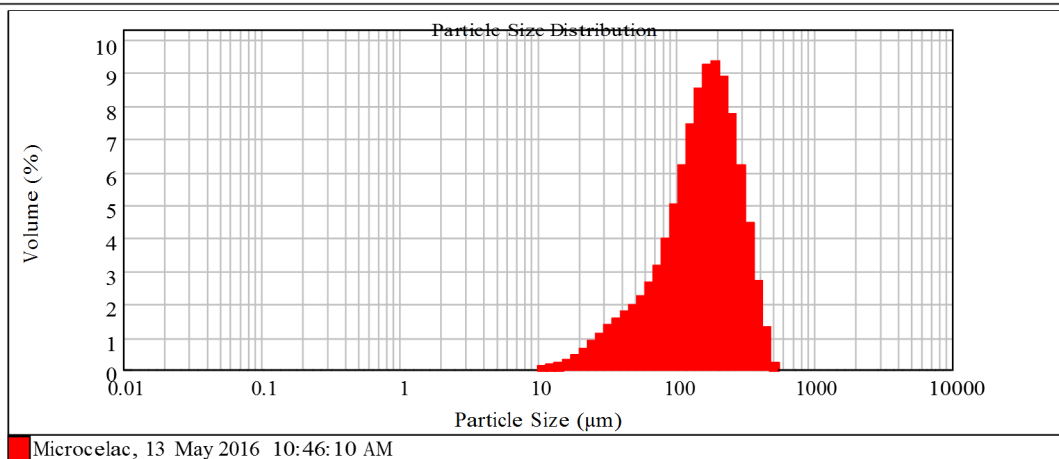
Surface Weighted Mean D[3,2]:
104.755 um

Vol. Weighted Mean D[4,3]:
171.284 um

d(0.1): 50.736 um

d(0.5): 158.897 um

d(0.9): 306.224 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.11	120.226	7.46	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.17	138.038	8.54	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.30	158.489	9.23	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.46	181.970	9.37	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.66	208.930	8.88	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.89	239.883	7.77	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	1.13	275.423	6.22	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	1.37	316.228	4.45	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	1.59	363.078	2.72	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	1.79	416.869	1.33	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	1.99	478.630	0.20	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	2.25	549.541	0.00	5754.399	0.00
0.052	0.00	0.560	0.00	5.754	0.00	60.256	2.63	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	3.19	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	4.00	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	5.02	954.993	0.00	10000.000	0.00
0.091	0.00	0.965	0.00	10.000	0.00	104.713	6.23	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.06	120.226		1258.925	0.00		

Operator notes:

A.4: RetaLac® Filler Powder



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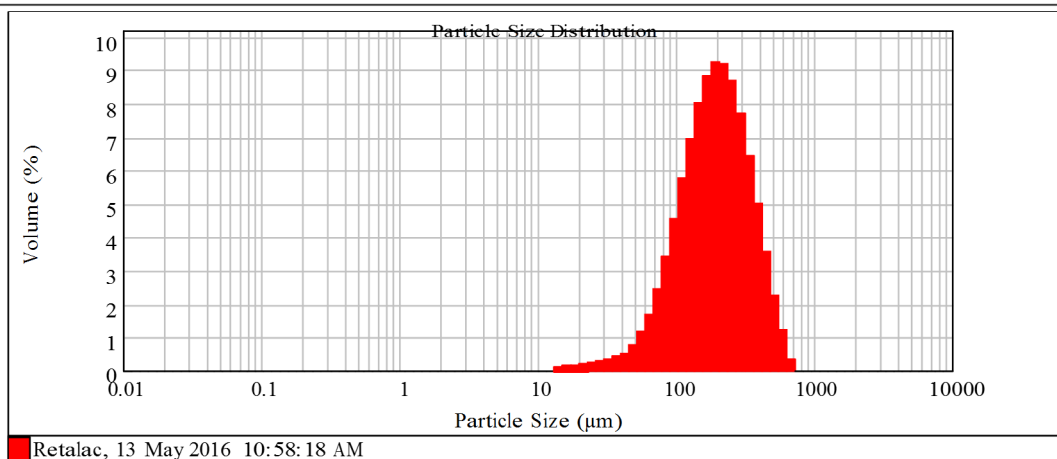
Result Analysis Report

Sample Name: Retalac	SOP Name: Jan Nieman	Measured: 13 May 2016 10:58:18 AM
Sample Source & type: Supplier = Jan Nieman	Measured by: Jan Steenekamp	Analysed: 13 May 2016 10:58:19 AM
Sample bulk lot ref: RL 2 (Sample 1)	Result Source: Measurement	

Particle Name: Titanium Dioxide	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle RI: 2.741	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 13.95 %
Dispersant Name: Cyclohexane	Dispersant RI: 1.426	Weighted Residual: 0.945 %	Result Emulation: Off

Concentration: 0.3144 %Vol	Span : 1.566	Uniformity: 0.483	Result units: Volume
Specific Surface Area: 0.0404 m²/g	Surface Weighted Mean D[3,2]: 148.535 um	Vol. Weighted Mean D[4,3]: 217.350 um	

d(0.1): 84.857 um d(0.5): 192.952 um d(0.9): 387.113 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.00	120.226	6.96	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.06	138.038	8.04	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.11	158.489	8.84	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.15	181.970	9.26	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.19	208.930	9.22	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.23	239.883	8.69	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.27	275.423	7.74	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.33	316.228	6.47	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.41	363.078	5.04	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.55	416.869	3.61	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.79	478.630	2.29	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	1.17	549.541	1.27	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	1.73	630.957	0.32	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	2.49	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	3.44	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	4.55	954.993	0.00	10000.000	0.00
0.091	0.00	0.965	0.00	10.000	0.00	104.713	5.77	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.00	120.226		1258.925	0.00		

Operator notes:

A.5: CombiLac® Filler Powder



MASTERSIZER



Result Analysis Report

Sample Name:

Combilac

SOP Name:

Jan Nieman

Measured:

13 May 2016 10:25:03 AM

Sample Source & type:

Supplier = Jan Nieman

Measured by:

Jan Steenekamp

Analysed:

13 May 2016 10:25:04 AM

Sample bulk lot ref:

CL 2 (Sample 1)

Result Source:

Measurement

Particle Name:

Titanium Dioxide

Accessory Name:

Hydro 2000SM (A)

Analysis model:

General purpose

Sensitivity:

Enhanced

Particle RI:

2.741

Absorption:

0.1

Size range:

0.020 to 2000.000 μm

Obscuration:

13.24 %

Dispersant Name:

Cyclohexane

Dispersant RI:

1.426

Weighted Residual:

1.001 %

Result Emulation:

Off

Concentration:

0.2413 % Vol

Span :

1.458

Uniformity:

0.446

Result units:

Volume

Specific Surface Area:

0.0496 m^2/g

Surface Weighted Mean D[3,2]:

120.926 μm

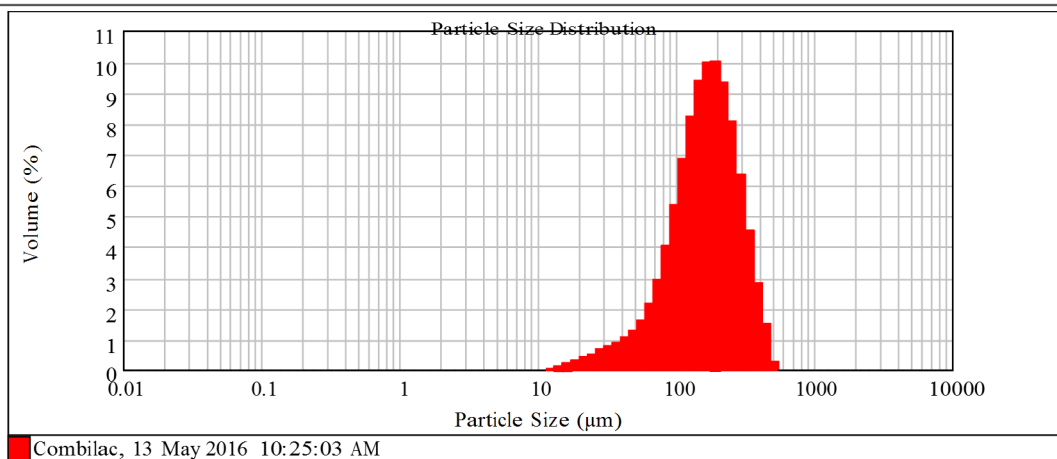
Vol. Weighted Mean D[4,3]:

179.169 μm

d(0.1): 68.700 μm

d(0.5): 165.272 μm

d(0.9): 309.672 μm



Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.05	120.226	8.24	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.11	138.038	9.38	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.17	158.489	9.38	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.28	181.970	10.02	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.40	208.930	10.02	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.53	239.883	9.35	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.67	275.423	8.06	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.80	316.228	6.39	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.93	363.078	4.57	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	1.08	416.869	2.84	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	1.29	478.630	1.50	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	1.64	549.541	0.24	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	2.18	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	2.97	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	4.06	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	5.38	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	6.84	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.00	120.226		1258.925	0.00		

Operator notes:

A.6: Pharmacel® 101 Filler Powder



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Result Analysis Report

Sample Name:

Combilac

SOP Name:

Jan Nieman

Measured:

13 May 2016 10:25:03 AM

Sample Source & type:

Supplier = Jan Nieman

Measured by:

Jan Steenekamp

Analysed:

13 May 2016 10:25:04 AM

Sample bulk lot ref:

CL 2 (Sample 1)

Result Source:

Measurement

Particle Name:

Titanium Dioxide

Accessory Name:

Hydro 2000SM (A)

Analysis model:

General purpose

Sensitivity:

Enhanced

Particle RI:

2.741

Absorption:

0.1

Size range:

0.020 to 2000.000 um

Obscuration:

13.24 %

Dispersant Name:

Cyclohexane

Dispersant RI:

1.426

Weighted Residual:

1.001 %

Result Emulation:

Off

Concentration:

0.2413 %Vol

Span :

1.458

Uniformity:

0.446

Result units:

Volume

Specific Surface Area:

0.0496 m²/g

Surface Weighted Mean D[3,2]:

120.926 um

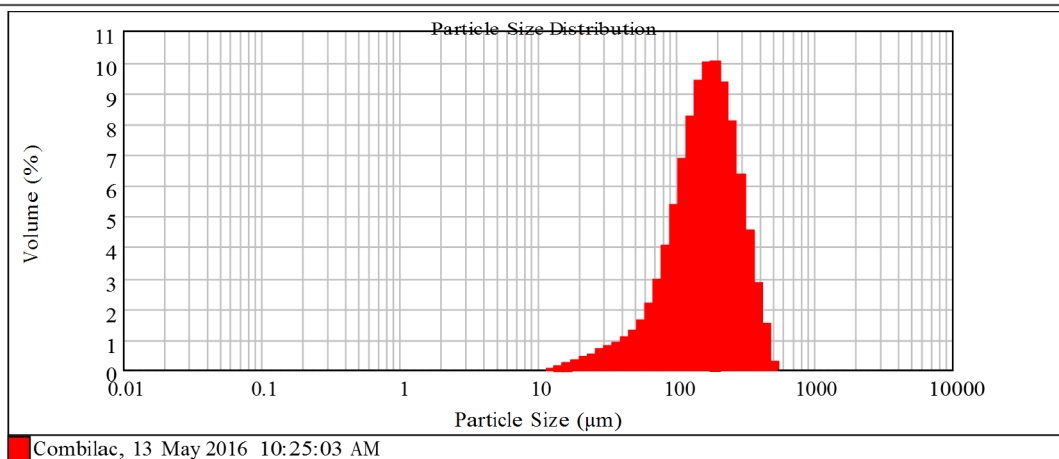
Vol. Weighted Mean D[4,3]:

179.169 um

d(0.1): 68.700 um

d(0.5): 165.272 um

d(0.9): 309.672 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.05	120.226	8.24	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.11	138.038	9.38	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.17	158.489	9.38	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.28	181.970	10.02	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.40	208.930	9.35	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.53	239.883	8.06	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.67	275.423	6.39	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.80	316.228	4.57	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.93	363.078	2.84	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	1.08	416.869	1.50	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	1.29	478.630	0.24	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	1.64	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	2.18	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	2.97	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	4.06	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	5.38	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	6.84	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.00	120.226		1258.925	0.00		

Operator notes:

A.7: Glycerol Monostearate 0.5:1 Lipid Dispersion



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Result Analysis Report

Sample Name:
Monostearate 0.5:1

Sample Source & type:
CW+LC

Sample bulk lot ref:
MS 0.5:1 (Sample 1)

SOP Name:
Christi Wilkins

Measured by:
Jan Steenekamp

Result Source:
Edited

Measured:
23 March 2017 03:14:03 PM

Analysed:
23 March 2017 03:14:04 PM

Particle Name: Yellow pigment	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle RI: 2.190	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 14.42 %
Dispersant Name: Water	Dispersant RI: 1.330	Weighted Residual: 0.797 %	Result Emulation: Off

Concentration:
0.2490 %Vol

Span :
2.289

Uniformity:
0.715

Result units:
Volume

Specific Surface Area:
0.052 m²/g

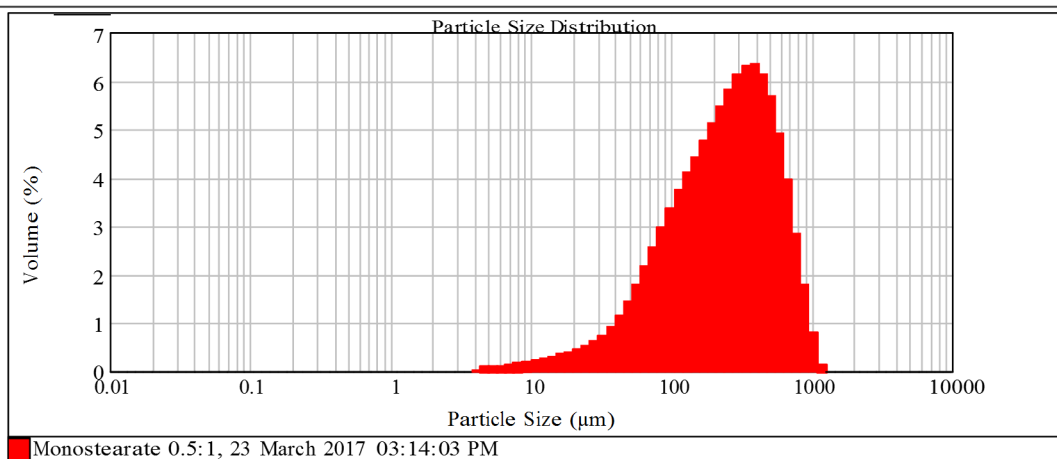
Surface Weighted Mean D[3,2]:
115.490 um

Vol. Weighted Mean D[4,3]:
299.467 um

d(0.1): 59.810 um

d(0.5): 246.161 um

d(0.9): 623.325 um



Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %	Size (µm)	Volume in %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.27	120.226	4.11	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.31	138.038	4.44	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.36	158.489	4.77	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.41	181.970	5.12	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.47	208.930	5.48	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.54	239.883	5.83	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.63	275.423	6.14	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.76	316.228	6.34	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.94	363.078	6.37	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.03	39.811	1.16	416.869	6.16	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.08	45.709	1.45	478.630	5.68	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.09	52.481	1.79	549.541	4.93	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.11	60.256	2.18	630.957	3.96	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.13	69.183	2.58	724.436	2.87	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.16	79.433	3.00	831.764	1.81	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.19	91.201	3.39	954.993	0.83	10000.000	0.00
0.091	0.00	0.965	0.00	10.000	0.23	104.713	3.76	1096.478	0.12		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925			

Operator notes:

A.8: Glycerol Monostearate 0.75:1 Lipid Dispersion



MASTERSIZER



Result Analysis Report

Sample Name:

Combilac

SOP Name:

Jan Nieman

Measured:

13 May 2016 10:25:03 AM

Sample Source & type:

Supplier = Jan Nieman

Measured by:

Jan Steenekamp

Analysed:

13 May 2016 10:25:04 AM

Sample bulk lot ref:

CL 2 (Sample 1)

Result Source:

Measurement

Particle Name:

Titanium Dioxide

Accessory Name:

Hydro 2000SM (A)

Analysis model:

General purpose

Sensitivity:

Enhanced

Particle RI:

2.741

Absorption:

0.1

Size range:

0.020 to 2000.000 μm

Obscuration:

13.24 %

Dispersant Name:

Cyclohexane

Dispersant RI:

1.426

Weighted Residual:

1.001 %

Result Emulation:

Off

Concentration:

0.2413 % Vol

Span :

1.458

Uniformity:

0.446

Result units:

Volume

Specific Surface Area:

0.0496 m^2/g

Surface Weighted Mean D[3,2]:

120.926 μm

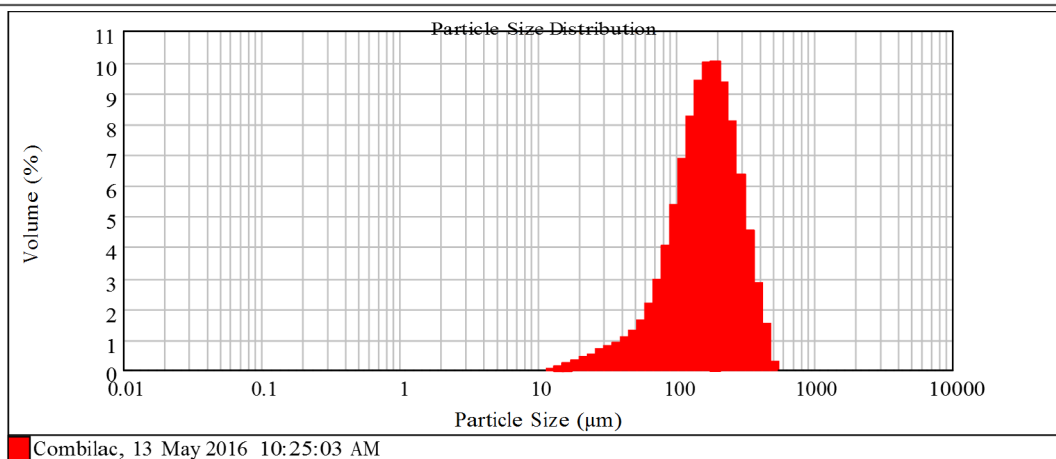
Vol. Weighted Mean D[4,3]:

179.169 μm

d(0.1): 68.700 μm

d(0.5): 165.272 μm

d(0.9): 309.672 μm



Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.05	120.226	8.24	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.11	138.038	9.38	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.17	158.489	9.38	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.28	181.970	10.02	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.40	208.930	9.35	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.53	239.883	8.06	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.67	275.423	6.39	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.80	316.228	4.57	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.93	363.078	2.84	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	1.08	416.869	1.50	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	1.29	478.630	0.24	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	1.64	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	2.18	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	2.97	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	4.06	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	5.38	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	6.84	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.00	120.226		1258.925	0.00		

Operator notes:

Malvern Instruments Ltd.

Malvern, UK

Tel : +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 5.60

Serial Number : MAL1007548

File name: 13 Mei 2016.mea

Record Number: 16

2017/07/07 10:12:02 AM

A.9: Glycerol Monostearate 1:1 Lipid Dispersion



MASTERSIZER



Result Analysis Report

Sample Name:

Monostearate

SOP Name:

Christi Wilkins

Measured:

24 March 2017 09:44:22 AM

Sample Source & type:

CW+LC

Measured by:

Jan Steenekamp

Analysed:

24 March 2017 09:44:23 AM

Sample bulk lot ref:

MS 1:1 (Sample 1)

Result Source:

Measurement

Particle Name:

Yellow pigment

Accessory Name:

Hydro 2000SM (A)

Analysis model:

General purpose

Sensitivity:

Enhanced

Particle RI:

2.190

Absorption:

0.1

Size range:

0.020 to 2000.000 μm

Obscuration:

12.99 %

Dispersant Name:

Water

Dispersant RI:

1.330

Weighted Residual:

0.760 %

Result Emulation:

Off

Concentration:

0.2131 %Vol

Span :

2.300

Uniformity:

0.707

Result units:

Volume

Specific Surface Area:

0.0545 m^2/g

Surface Weighted Mean D[3,2]:

110.136 μm

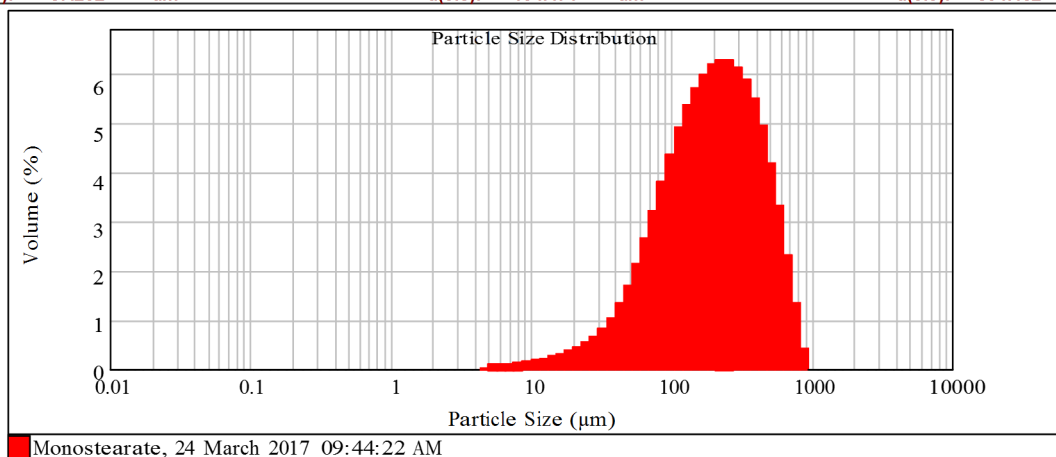
Vol. Weighted Mean D[4,3]:

242.416 μm

d(0.1): 57.202 μm

d(0.5): 194.471 μm

d(0.9): 504.432 μm



Monostearate, 24 March 2017 09:44:22 AM

Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.23	120.226	5.36	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.28	138.038	5.74	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.34	158.489	6.01	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.40	181.970	6.20	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.47	208.930	6.29	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.57	239.883	6.27	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.69	275.423	6.15	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.85	316.228	5.90	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	1.07	363.078	5.51	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	1.35	416.869	4.95	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	1.72	478.630	4.21	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.07	52.481	2.16	549.541	3.33	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.08	60.256	3.24	630.957	2.34	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.10	69.183	3.83	724.436	1.37	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.13	79.433	4.39	831.764	0.45	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.16	91.201	4.91	954.993	0.00	10000.000	0.00
0.091	0.00	0.965	0.00	10.000	0.19	104.713		1096.478			
0.105	0.00	1.096	0.00	11.482		120.226		1258.925			

Operator notes:

A.10: Stearic Acid 0.5:1 Lipid Dispersion



MASTERSIZER



Result Analysis Report

Sample Name:
Stearic acid

SOP Name:
Christi Wilkins

Measured:
24 March 2017 09:56:56 AM

Sample Source & type:
CW+LC

Measured by:
Jan Steenekamp

Analysed:
24 March 2017 09:56:57 AM

Sample bulk lot ref:
SA 0.5:1 (Sample 1)

Result Source:
Measurement

Particle Name: Yellow pigment	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle RI: 2.190	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 11.03 %
Dispersant Name: Water	Dispersant RI: 1.330	Weighted Residual: 1.579 %	Result Emulation: Off

Concentration:
0.3347 %Vol

Span :
1.738

Uniformity:
0.533

Result units:
Volume

Specific Surface Area:
0.0294 m²/g

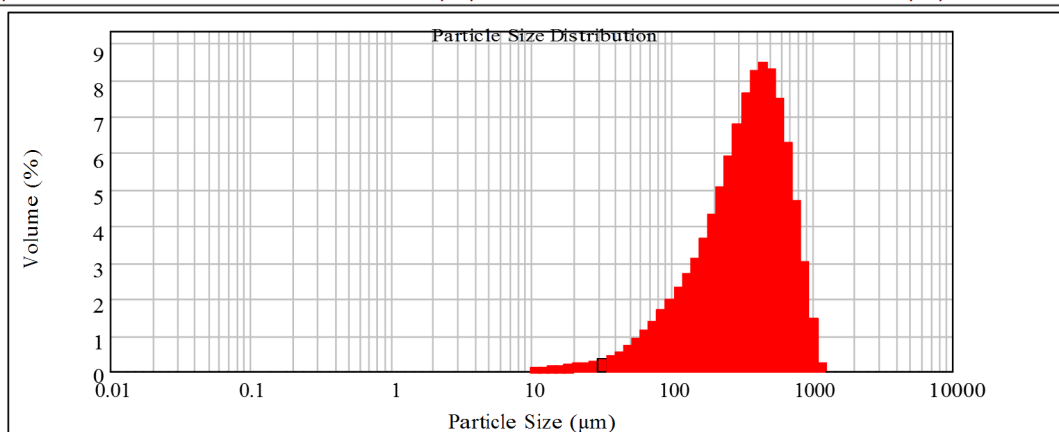
Surface Weighted Mean D[3,2]:
203.816 um

Vol. Weighted Mean D[4,3]:
384.478 um

d(0.1): 102.476 um

d(0.5): 351.657 um

d(0.9): 713.806 um



Stearic acid, 24 March 2017 09:56:56 AM

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.07	120.226	2.89	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.10	138.038	3.13	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.12	158.489	3.65	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.15	181.970	4.30	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.19	208.930	5.06	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.23	239.883	5.92	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.28	275.423	6.81	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.34	316.228	7.63	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.43	363.078	8.23	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.55	416.869	8.49	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.71	478.630	8.26	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.90	549.541	7.51	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	1.13	630.957	6.27	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	1.40	724.436	4.70	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	1.68	831.764	3.02	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	1.99	954.993	1.45	10000.000	0.00
0.091	0.00	0.965	0.00	10.000	0.00	104.713	2.32	1096.478	0.23		
0.105	0.00	1.096	0.00	11.482	0.05	120.226		1258.925			

Operator notes:

A.11: Stearic Acid 0.75:1 Lipid Dispersion



MASTERSIZER



Result Analysis Report

Sample Name:
Stearic acid

SOP Name:
Christi Wilkins

Measured:
24 March 2017 10:06:29 AM

Sample Source & type:
CW+LC

Measured by:
Jan Steenekamp

Analysed:
24 March 2017 10:06:30 AM

Sample bulk lot ref:
SA 0.75:1 (Sample 1)

Result Source:
Measurement

Particle Name:
Yellow pigment

Accessory Name:
Hydro 2000SM (A)

Analysis model:
General purpose

Sensitivity:
Enhanced

Particle RI:
2.190

Absorption:
0.1

Size range:
0.020 to 2000.000 um

Obscuration:
10.74 %

Dispersant Name:
Water

Dispersant RI:
1.330

Weighted Residual:
1.300 %

Result Emulation:
Off

Concentration:
0.3223 % Vol

Span :
1.875

Uniformity:
0.583

Result units:
Volume

Specific Surface Area:
0.0298 m²/g

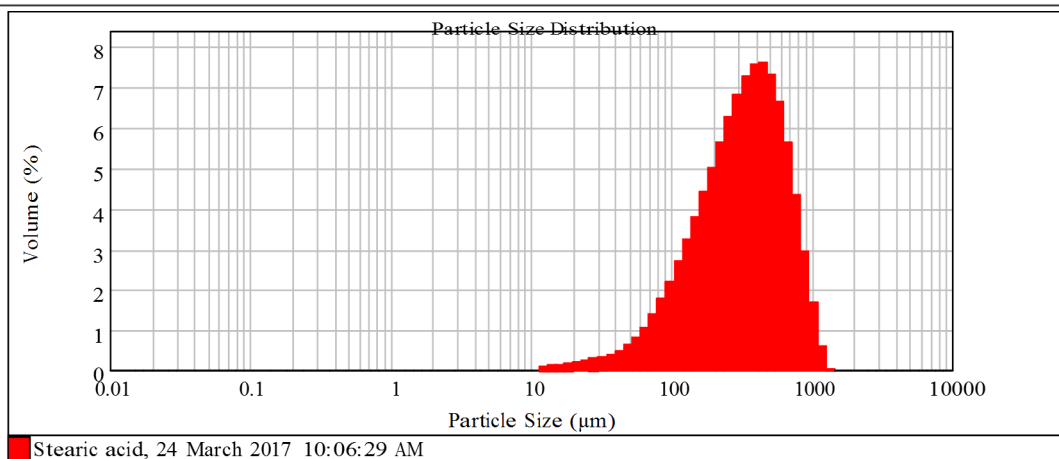
Surface Weighted Mean D[3,2]:
201.615 um

Vol. Weighted Mean D[4,3]:
374.443 um

d(0.1): 103.399 um

d(0.5): 327.617 um

d(0.9): 717.638 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.06	120.226	3.24	1258.925	0.01
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.09	138.038	3.82	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.12	158.489	4.41	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.15	181.970	5.03	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.19	208.930	5.65	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.22	239.883	6.27	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.27	275.423	6.83	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.32	316.228	7.30	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.39	363.078	7.59	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.49	416.869	7.63	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.63	478.630	7.34	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.82	549.541	6.67	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	1.08	630.957	5.64	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	1.39	724.436	4.36	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	1.77	831.764	2.98	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	2.21	954.993	1.68	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	2.71	1096.478	0.62		
0.105	0.00	1.096	0.00	11.482	0.00	120.226		1258.925			

Operator notes:

A.12: Stearic Acid 1:1 Lipid Dispersion



MASTERSIZER



Result Analysis Report

Sample Name:
Stearic acid

SOP Name:
Christi Wilkins

Measured:
24 March 2017 10:13:07 AM

Sample Source & type:
CW+LC

Measured by:
Jan Steenekamp

Analysed:
24 March 2017 10:13:08 AM

Sample bulk lot ref:
SA 1:1 (Sample 1)

Result Source:
Measurement

Particle Name: Yellow pigment	Accessory Name: Hydro 2000SM (A)	Analysis model: General purpose	Sensitivity: Enhanced
Particle RI: 2.190	Absorption: 0.1	Size range: 0.020 to 2000.000 um	Obscuration: 13.90 %
Dispersant Name: Water	Dispersant RI: 1.330	Weighted Residual: 1.245 %	Result Emulation: Off

Concentration:
0.4587 %Vol

Span :
1.679

Uniformity:
0.516

Result units:
Volume

Specific Surface Area:
0.0276 m²/g

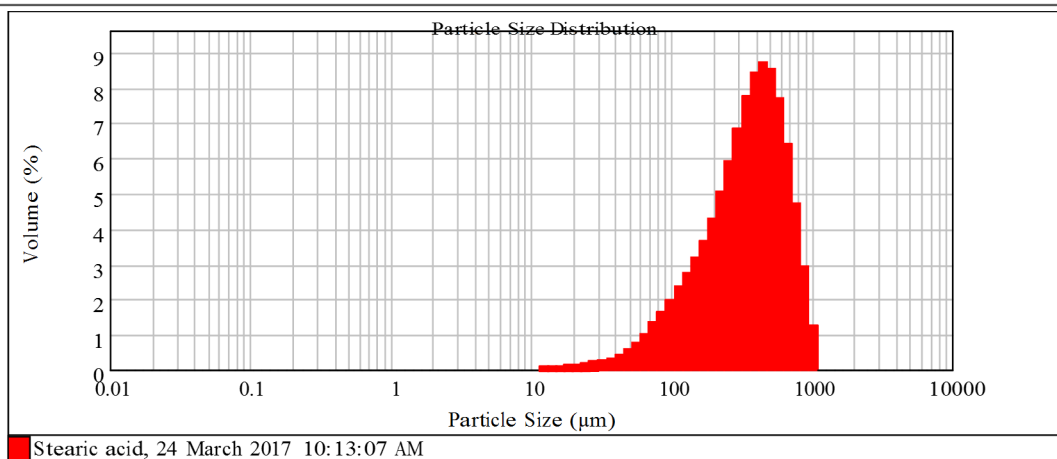
Surface Weighted Mean D[3,2]:
217.631 um

Vol. Weighted Mean D[4,3]:
385.510 um

d(0.1): 109.226 um

d(0.5): 355.664 um

d(0.9): 706.332 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.05	120.226	2.74	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.08	138.038	3.17	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.09	158.489	3.68	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.11	181.970	4.31	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	0.13	208.930	5.06	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	0.16	239.883	5.94	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	0.20	275.423	6.87	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	0.25	316.228	7.75	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.32	363.078	8.43	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.44	416.869	8.74	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.60	478.630	8.53	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.80	549.541	7.73	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	1.06	630.957	6.42	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	1.35	724.436	4.72	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	1.67	831.764	2.97	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	2.01	954.993	1.26	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	2.37	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.00	120.226		1258.925			

Operator notes:

A.13: Cetyl Alcohol 0.5:1 Lipid Dispersion



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Result Analysis Report

Sample Name:

Cetyl alcohol 0.5

SOP Name:

Christi Wilkins

Measured:

28 June 2017 10:50:01 AM

Sample Source & type:

CW

Measured by:

Jan Steenekamp

Analysed:

28 June 2017 10:50:02 AM

Sample bulk lot ref:

C A 0.5 (Sample 3)

Result Source:

Measurement

Particle Name:

Yellow pigment

Accessory Name:

Hydro 2000SM (A)

Analysis model:

General purpose

Sensitivity:

Enhanced

Particle RI:

2.190

Absorption:

0.1

Size range:

0.020 to 2000.000 um

Obscuration:

11.51 %

Dispersant Name:

Water

Dispersant RI:

1.330

Weighted Residual:

0.775 %

Result Emulation:

Off

Concentration:

0.1279 %Vol

Span :

3.968

Uniformity:

1.31

Result units:

Volume

Specific Surface Area:

0.0792 m²/g

Surface Weighted Mean D[3,2]:

75.738 um

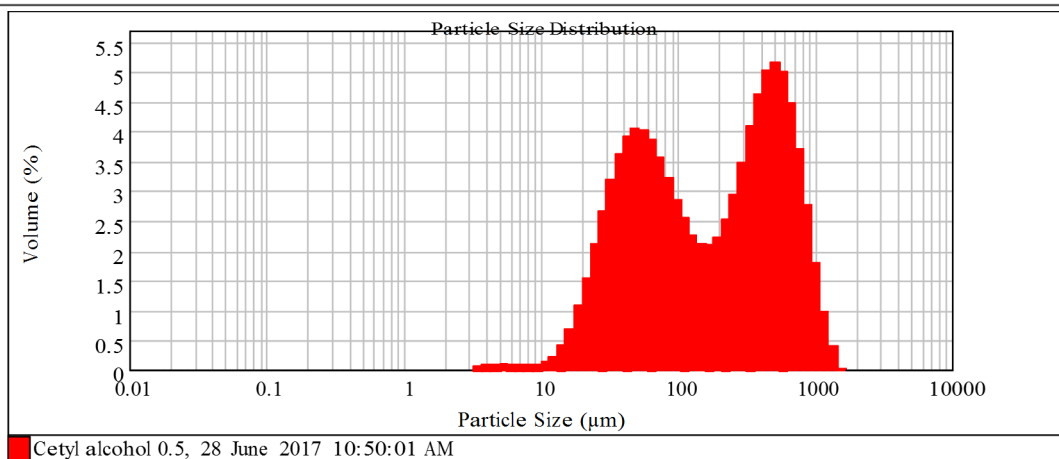
Vol. Weighted Mean D[4,3]:

290.826 um

d(0.1): 30.954 um

d(0.5): 172.801 um

d(0.9): 716.596 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.23	120.226	2.27	1258.925	0.40
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.41	138.038	2.11	1445.440	0.01
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.69	158.489	2.09	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	1.08	181.970	2.22	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	1.56	208.930	2.51	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	2.11	239.883	2.95	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	2.67	275.423	3.49	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	3.19	316.228	4.08	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	3.62	363.078	4.63	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.08	39.811	3.92	416.869	5.02	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.09	45.709	4.05	478.630	5.17	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.09	52.481	4.03	549.541	4.99	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.08	60.256	3.85	630.957	4.49	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.08	69.183	3.57	724.436	3.70	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.07	79.433	3.23	831.764	2.76	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.08	91.201	2.87	954.993	1.81	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.12	104.713	2.53	1096.478	0.98		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925			

Operator notes:

A.14: Cetyl Alcohol 0.75:1 Lipid Dispersion



MASTERSIZER



Result Analysis Report

Sample Name:
Cetyl alcohol 0.75

Sample Source & type:
CW

Sample bulk lot ref:
C A 0.75 (Sample 1)

SOP Name:
Christi Wilkins

Measured by:
Jan Steenekamp

Result Source:
Measurement

Measured:
28 June 2017 11:00:57 AM

Analysed:
28 June 2017 11:00:58 AM

Particle Name:
Yellow pigment

Particle RI:
2.190

Dispersant Name:
Water

Accessory Name:
Hydro 2000SM (A)

Absorption:
0.1

Dispersant RI:
1.330

Analysis model:
General purpose

Size range:
0.020 to 2000.000 um

Weighted Residual:
0.889 %

Sensitivity:
Enhanced

Obscuration:
12.87 %

Result Emulation:
Off

Concentration:
0.1735 %Vol

Specific Surface Area:
0.0661 m²/g

Span :
3.443

Surface Weighted Mean D[3,2]:
90.797 um

Uniformity:
1.14

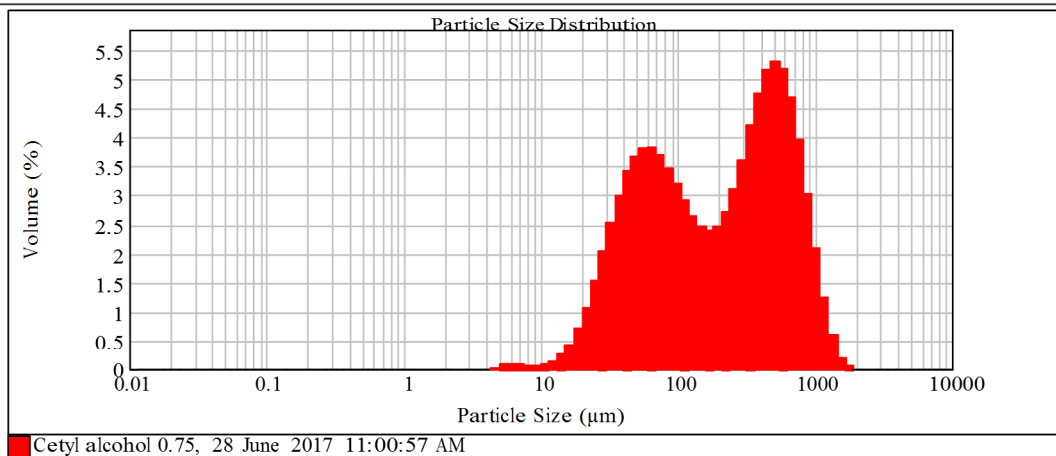
Vol. Weighted Mean D[4,3]:
314.669 um

Result units:
Volume

d(0.1): 36.194 um

d(0.5): 207.824 um

d(0.9): 751.700 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.13	120.226	2.65	1258.925	0.60
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.25	138.038	2.46	1445.440	0.18
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.44	158.489	2.48	1659.587	0.03
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.71	181.970	2.71	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	1.08	208.930	3.11	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	1.53	239.883	3.62	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	2.03	275.423	4.20	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	2.54	316.228	4.75	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	3.01	363.078	5.15	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	3.40	416.869	5.32	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	3.68	478.630	5.18	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.02	52.481	3.82	549.541	4.71	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.07	60.256	3.82	630.957	3.96	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.06	69.183	3.69	724.436	3.04	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.06	79.433	3.47	831.764	2.09	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.06	91.201	3.20	954.993	1.23	10000.000	0.00
0.091	0.00	0.965	0.00	10.000	0.08	104.713	2.91	1096.478			
0.105	0.00	1.096	0.00	11.482		120.226		1258.925			

Operator notes:

A.15: Cetyl Alcohol 1:1 Lipid Dispersion



MASTERSIZER



Result Analysis Report

Sample Name:

Cetyl alcohol 1:1

SOP Name:

Christi Wilkins

Measured:

28 June 2017 11:28:48 AM

Sample Source & type:

CW

Measured by:

Jan Steenekamp

Analysed:

28 June 2017 11:28:49 AM

Sample bulk lot ref:

C A 1:1 (Sample 2)

Result Source:

Measurement

Particle Name:

Yellow pigment

Accessory Name:

Hydro 2000SM (A)

Analysis model:

General purpose

Sensitivity:

Enhanced

Particle RI:

2.190

Absorption:

0.1

Size range:

0.020 to 2000.000 μm
Obscuration:

12.54 %

Dispersant Name:

Water

Dispersant RI:

1.330

Weighted Residual:

1.079 %

Result Emulation:

Off

Concentration:

0.1713 %Vol

Span :

2.769

Uniformity:

0.923

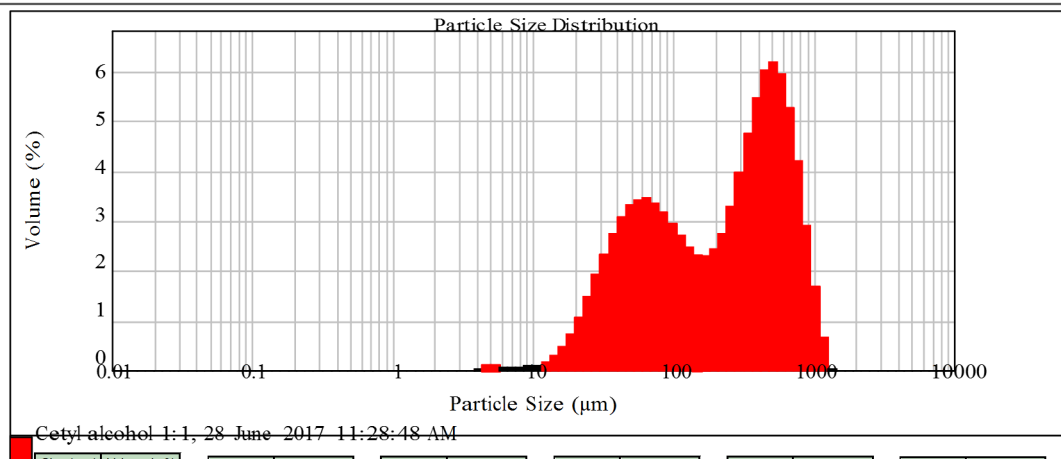
Result units:

Volume

Specific Surface Area:

0.065 m^2/g
Surface Weighted Mean D[3,2]:

92.341 μm
Vol. Weighted Mean D[4,3]:

313.942 μm
d(0.1): 36.460 μm
d(0.5): 244.808 μm
d(0.9): 714.274 μm


Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %	Size (μm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	0.17	120.226	2.48	1258.925	0.01
0.011	0.00	0.120	0.00	1.259	0.00	13.183	0.29	138.038	2.32	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	0.47	158.489	2.29	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.00	17.378	0.74	181.970	2.43	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.00	19.953	1.08	208.930	2.76	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.00	22.909	1.48	239.883	3.29	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.00	26.303	1.92	275.423	3.98	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.00	30.200	2.35	316.228	4.75	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	2.75	363.078	5.48	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.03	39.811	3.08	416.869	6.01	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.07	45.709	3.31	478.630	6.20	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.07	52.481	3.44	549.541	5.96	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.07	60.256	3.45	630.957	5.26	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.07	69.183	3.36	724.436	4.20	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.06	79.433	3.19	831.764	2.93	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.07	91.201	2.96	954.993	1.70	10000.000	0.00
0.091	0.00	0.965	0.00	10.000	0.10	104.713	2.70	1096.478	0.86		
0.105	0.00	1.096	0.00	11.482		120.226		1258.925			

Operator notes:

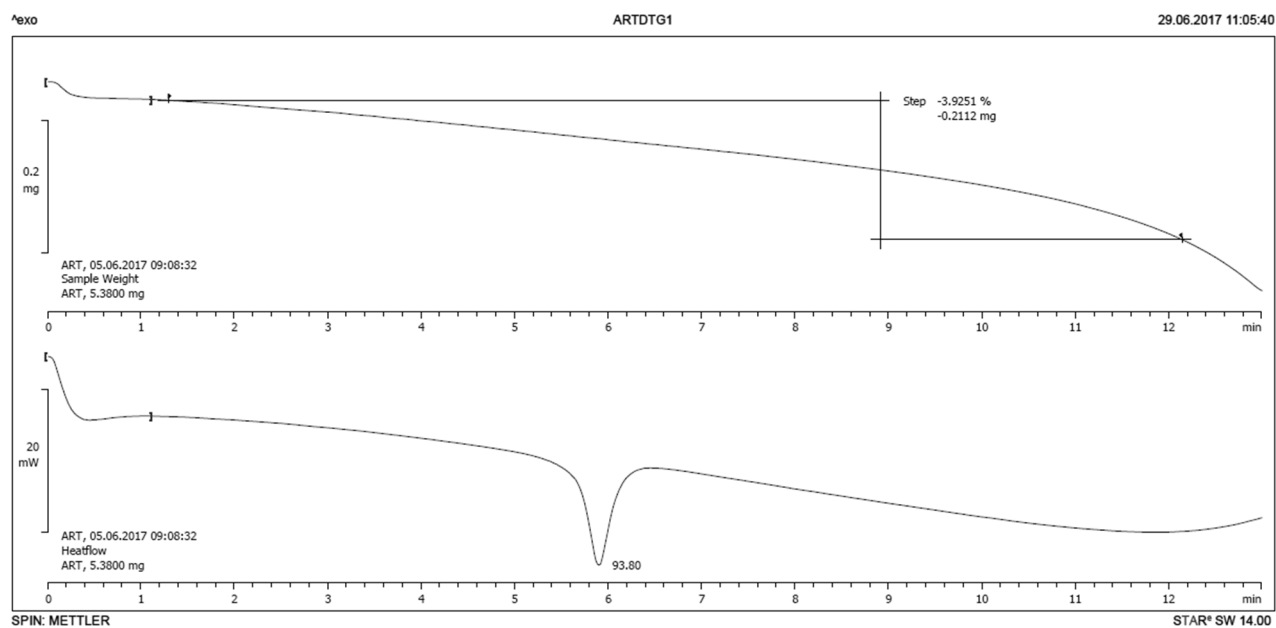


DSC and TGA Results

Figure B.1: DTG curve of Artemether	B2
Figure B.2: DTG curve of Lumefantrine.....	B2
Figure B.3: DTG curve of Artemether/Lumefantrine Physical Mixture.....	B3
Figure B.4: DTG curve of Glycerol Monostearate Raw Material	B3
Figure B.5: DTG curve of Glycerol Monostearate 0.5:1 Lipid Dispersion	B4
Figure B.6: DTG curve of Glycerol Monostearate 0.75:1 Lipid Dispersion	B4
Figure B.7: DTG curve of Glycerol Monostearate 1:1 Lipid Dispersion	B5
Figure B.8: DTG curve of Stearic Acid Raw Material.....	B5
Figure B.9: DTG curve of Stearic Acid 0.5:1 Lipid Dispersion	B6
Figure B.10: DTG curve of Stearic Acid 0.75:1 Lipid Dispersion	B6
Figure B.11: DTG curve of Stearic Acid 1:1 Lipid Dispersion	B7
Figure B.12: DTG curve of Cetyl Alcohol Raw Material	B7
Figure B.13: DTG curve of Cetyl Alcohol 0.5:1 Lipid Dispersion	B8
Figure B.14: DTG curve of Cetyl Alcohol 0.75:1 Lipid Dispersion	B8
Figure B.15: DTG curve of Cetyl Alcohol 1:1 Lipid Dispersion.....	B9

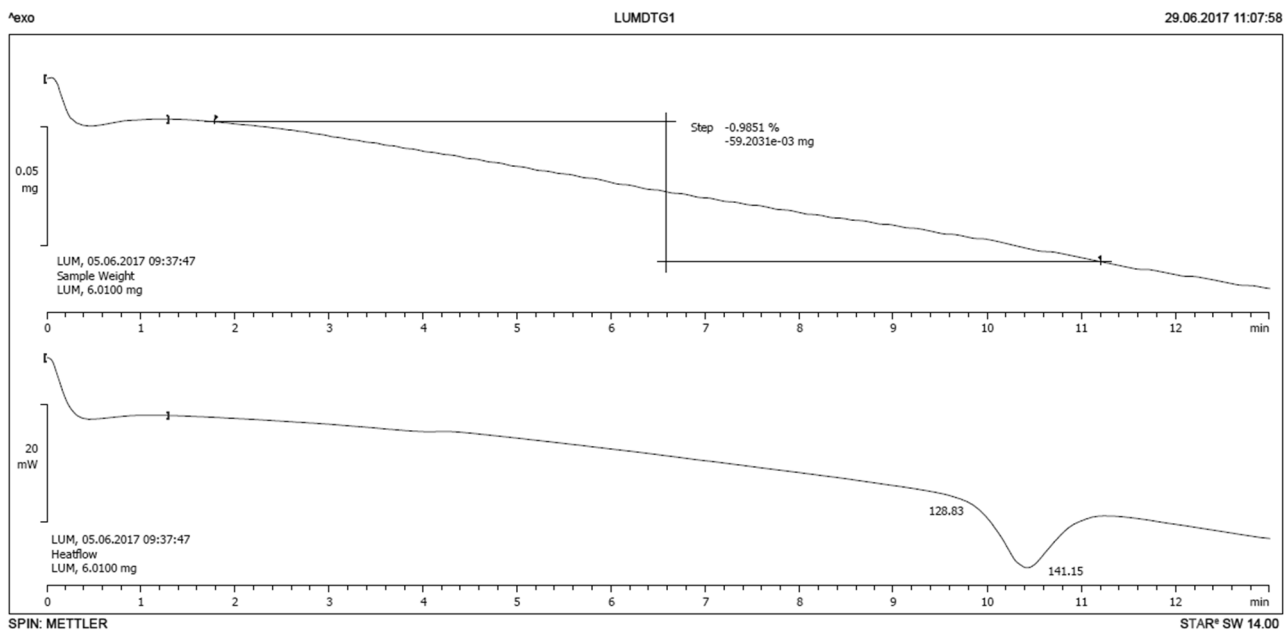
B.1: DTG curve of Artemether

Top graph: Artemether TG; **Bottom graph:** Artemether DTA



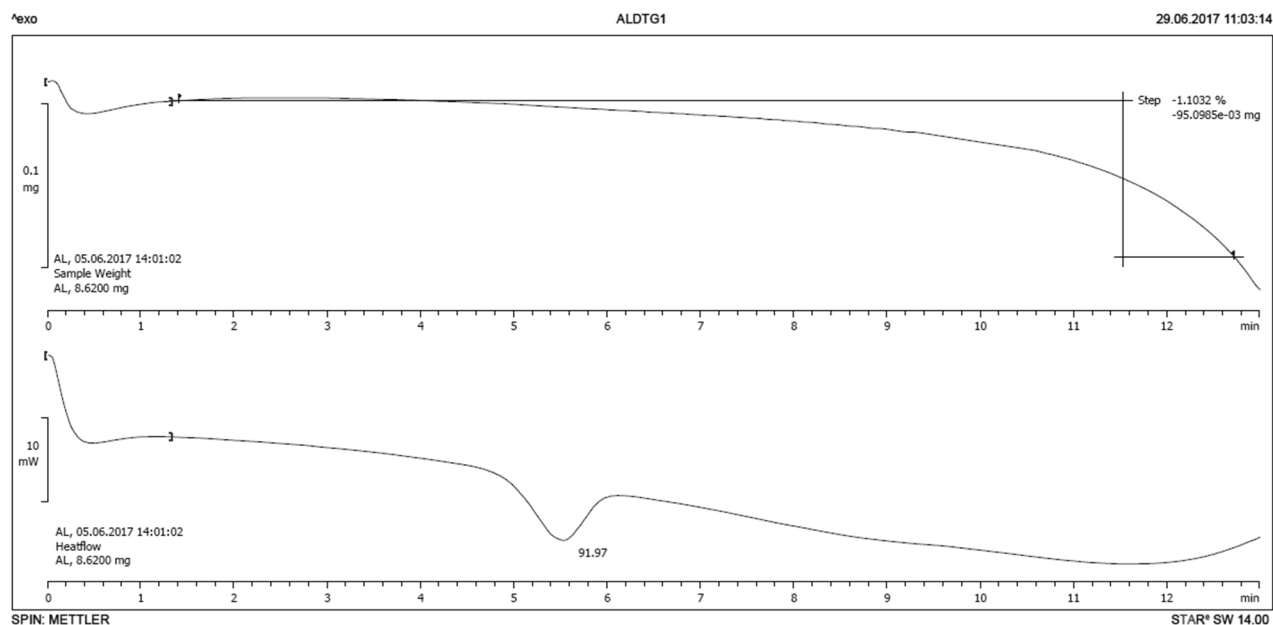
B.2: DTG curve of Lumefantrine

Top graph: Lumefantrine TG; **Bottom graph:** Lumefantrine DTA



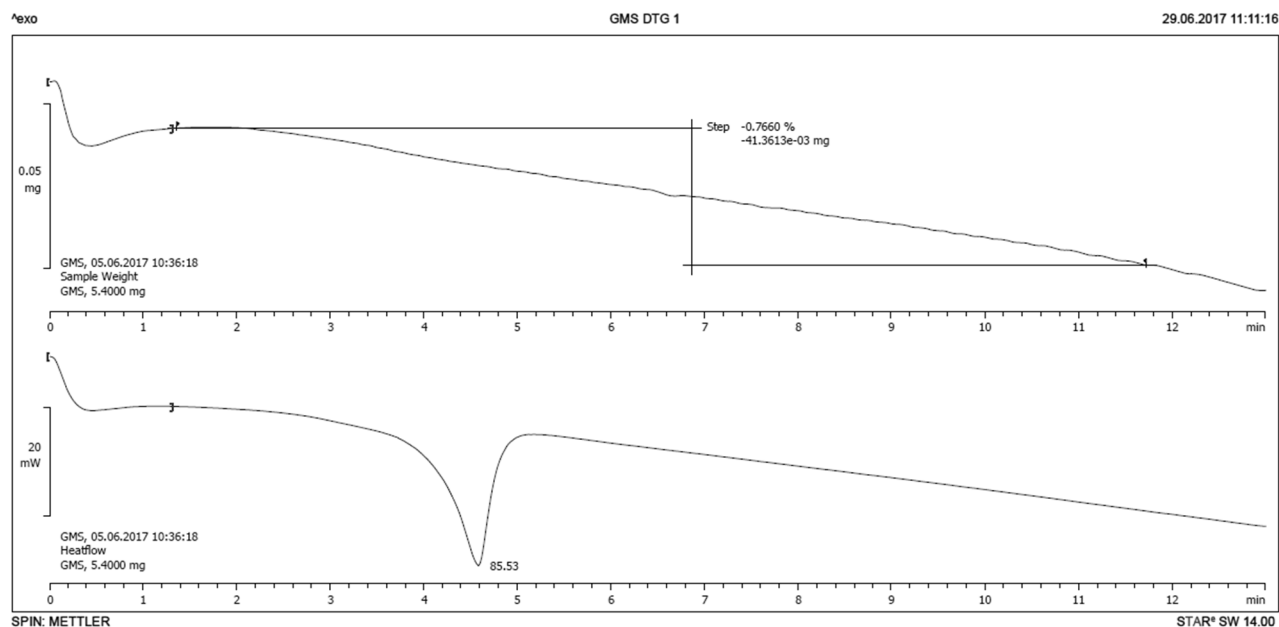
B.3: DTG curve of Artemether/Lumefantrine Physical Mixture

Top graph: Artemether/Lumefantrine TG; **Bottom graph:** Artemether/Lumefantrine DTA



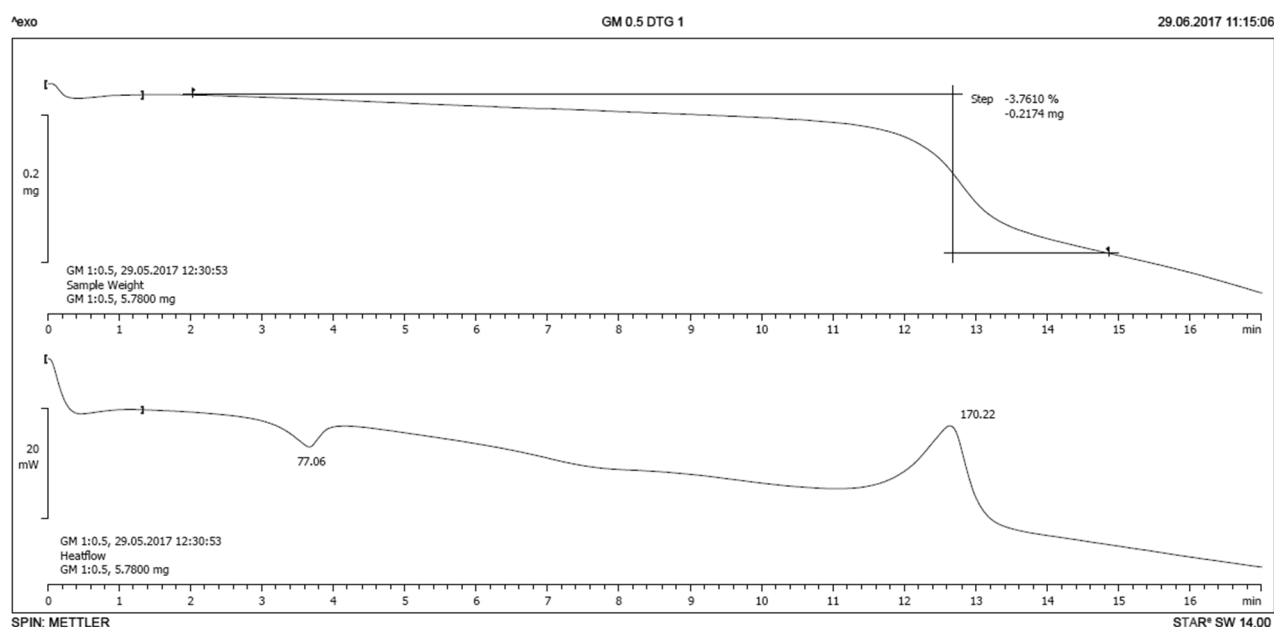
B.4: DTG curve of Glycerol Monostearate Raw Material

Top graph: Glycerol monostearate TG; **Bottom graph:** Glycerol monostearate DTA



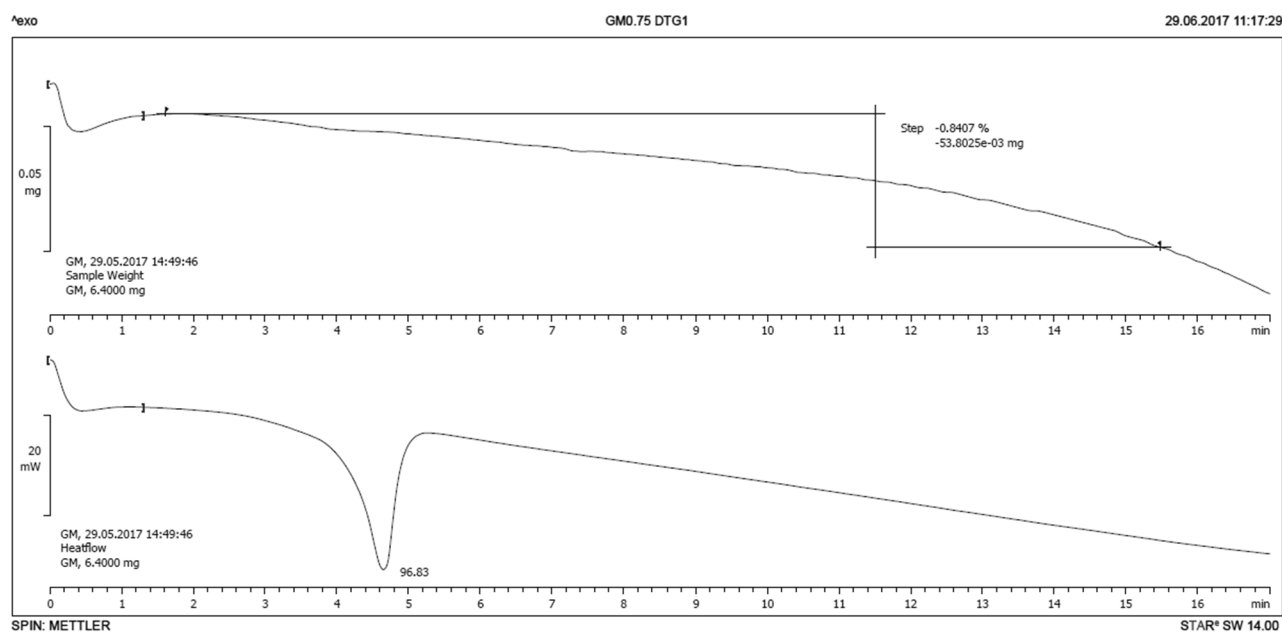
B.5: DTG curve of Glycerol Monostearate 0.5:1 Lipid Dispersion

Top graph: Glycerol monostearate 0.5:1 lipid dispersion TG; **Bottom graph:** Glycerol monostearate 0.5:1 lipid dispersion DTA



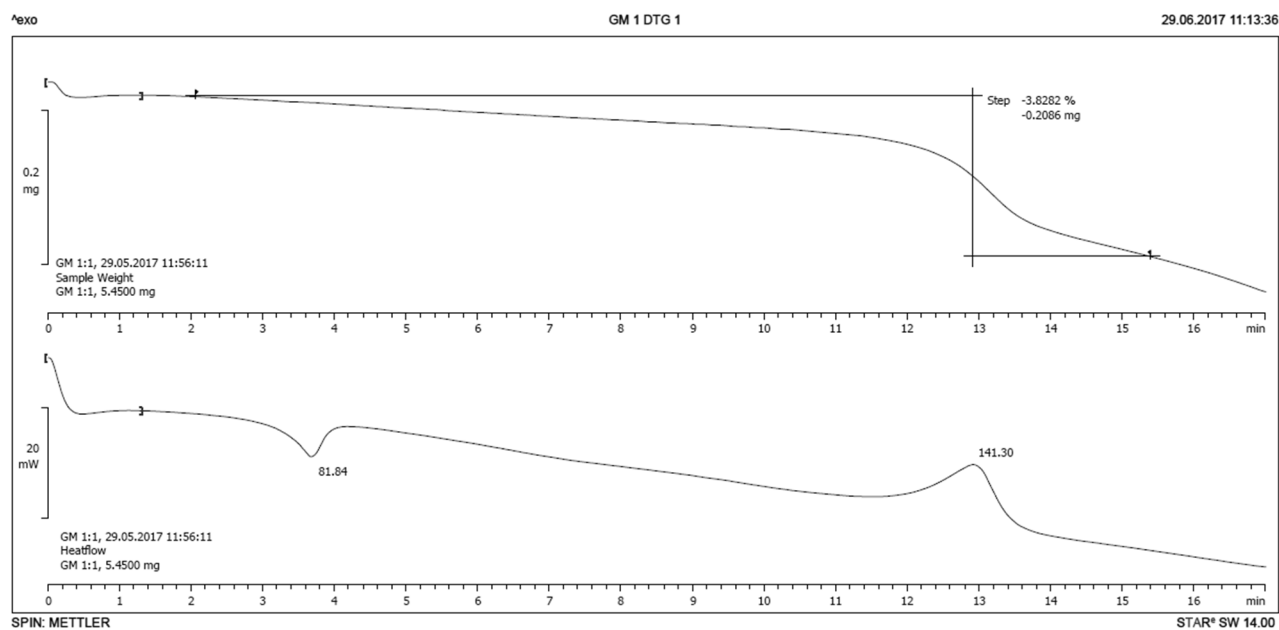
B.6: DTG curve of Glycerol Monostearate 0.75:1 Lipid Dispersion

Top graph: Glycerol monostearate 0.75:1 lipid dispersion TG; **Bottom graph:** Glycerol monostearate 0.75:1 lipid dispersion DTA



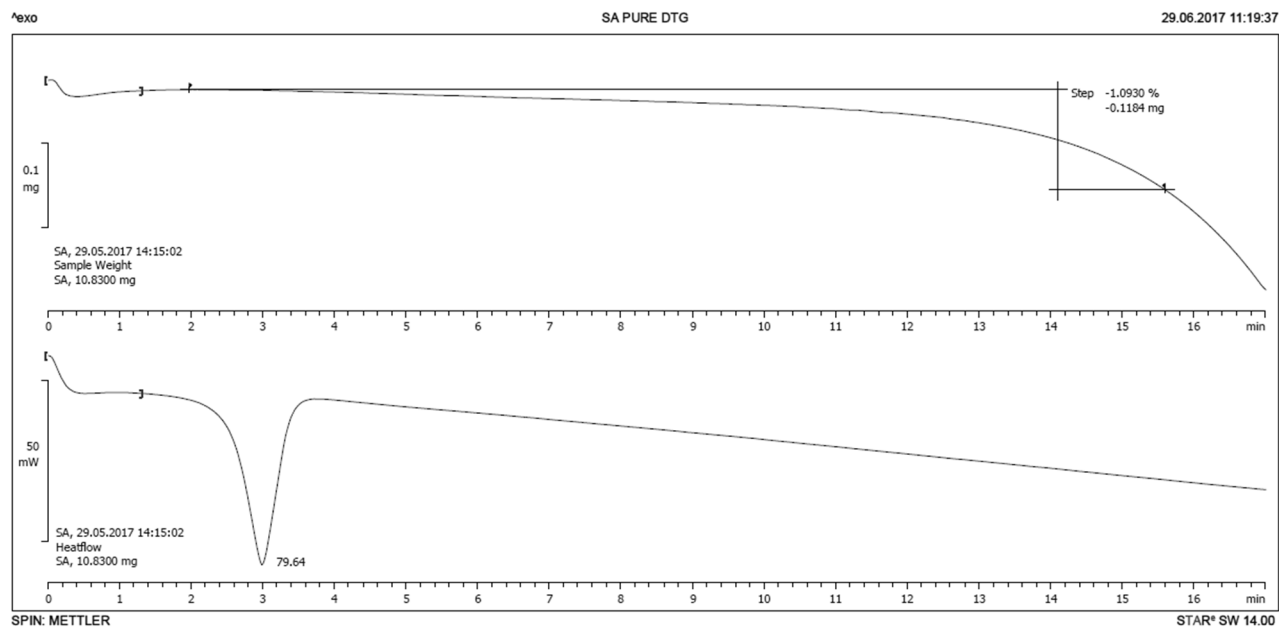
B.7: DTG curve of Glycerol Monostearate 1:1 Lipid Dispersion

Top graph: Glycerol monostearate 1:1 lipid dispersion TG; **Bottom graph:** Glycerol monostearate 1:1 lipid dispersion DTA



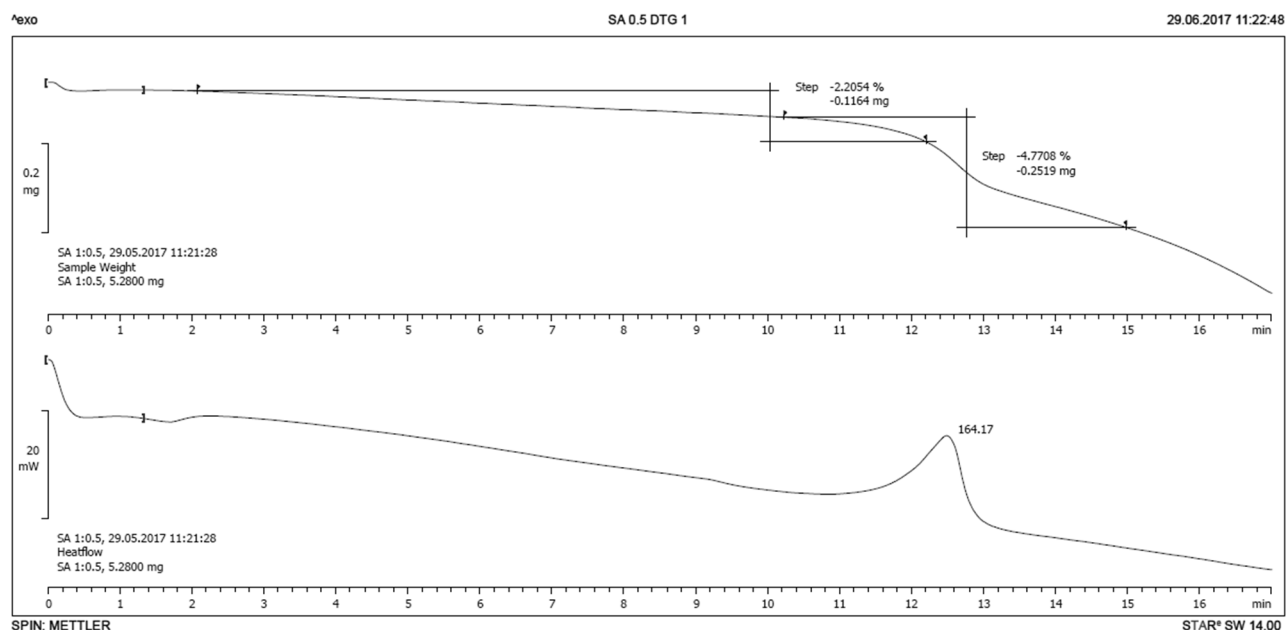
B.8: DTG curve of Stearic Acid Raw Material

Top graph: Stearic acid TG; **Bottom graph:** Stearic acid DTA



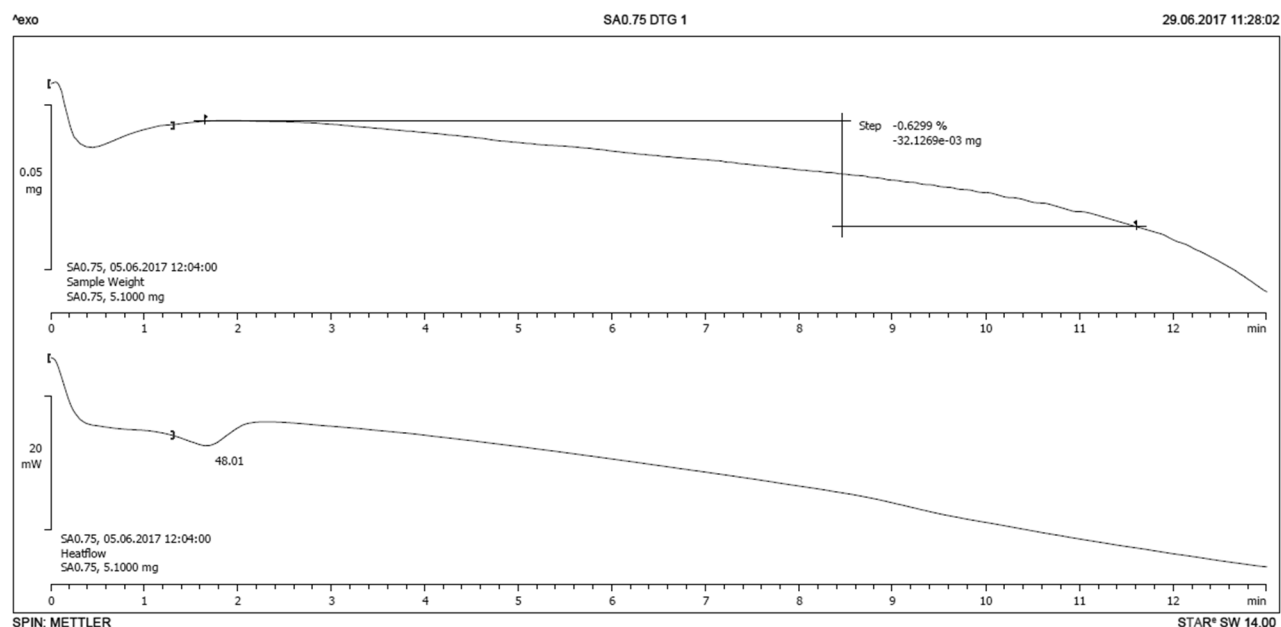
B.9: DTG curve of Stearic Acid 0.5:1 Lipid Dispersion

Top graph: Stearic acid 0.5:1 lipid dispersion TG; **Bottom graph:** Stearic acid 0.5:1 lipid dispersion DTA



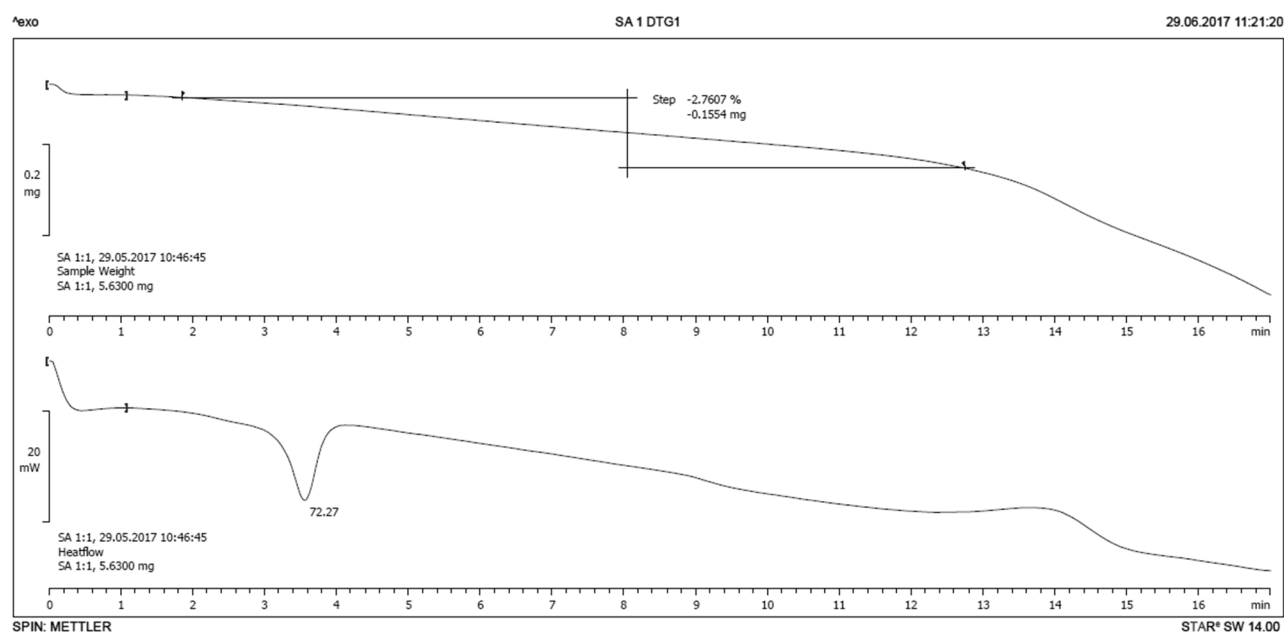
B.10: DTG curve of Stearic Acid 0.75:1 Lipid Dispersion

Top graph: Stearic acid 0.75:1 lipid dispersion TG; **Bottom graph:** Stearic acid 0.75:1 lipid dispersion DTA



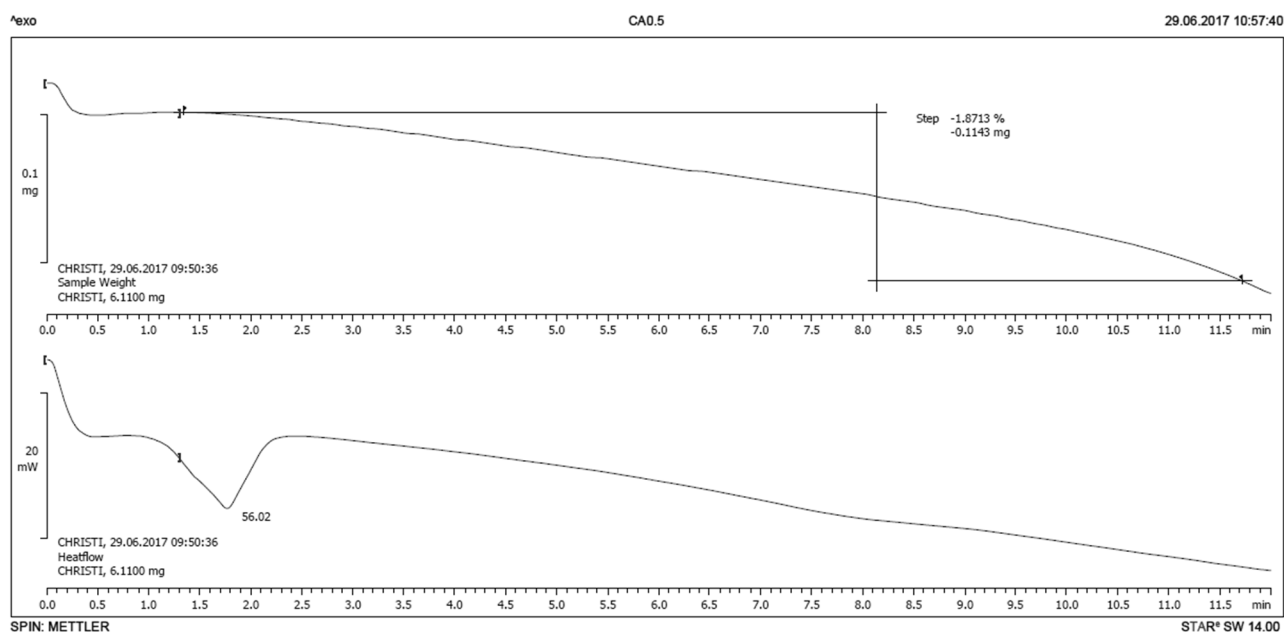
B.11: DTG curve of Stearic Acid 1:1 Lipid Dispersion

Top graph: Stearic acid 1:1 lipid dispersion TG; **Bottom graph:** Stearic acid 1:1 lipid dispersion DTA



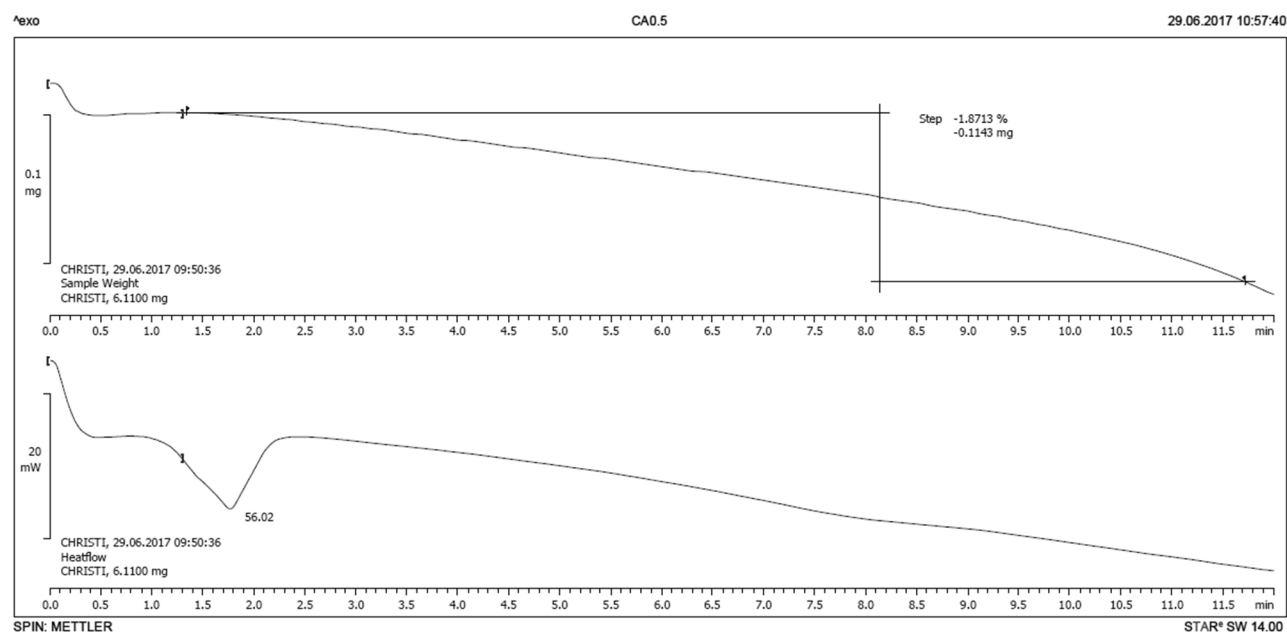
B.12: DTG curve of Cetyl Alcohol Raw Material

Top graph: Cetyl alcohol Pure Lipid TG; **Bottom graph:** Cetyl alcohol Pure Lipid DTA



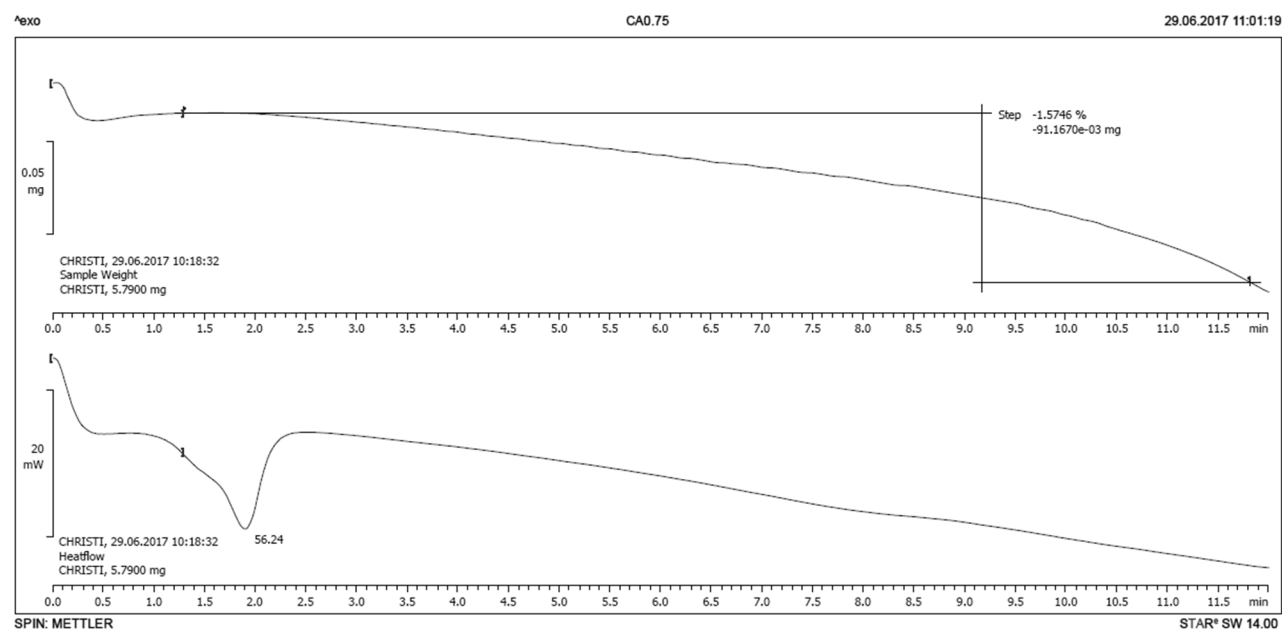
B.13: DTG curve of Cetyl Alcohol 0.5:1 Lipid Dispersion

Top graph: Cetyl alcohol 0.5:1 lipid dispersion TG; **Bottom graph:** Cetyl alcohol 0.5:1 lipid dispersion DTA



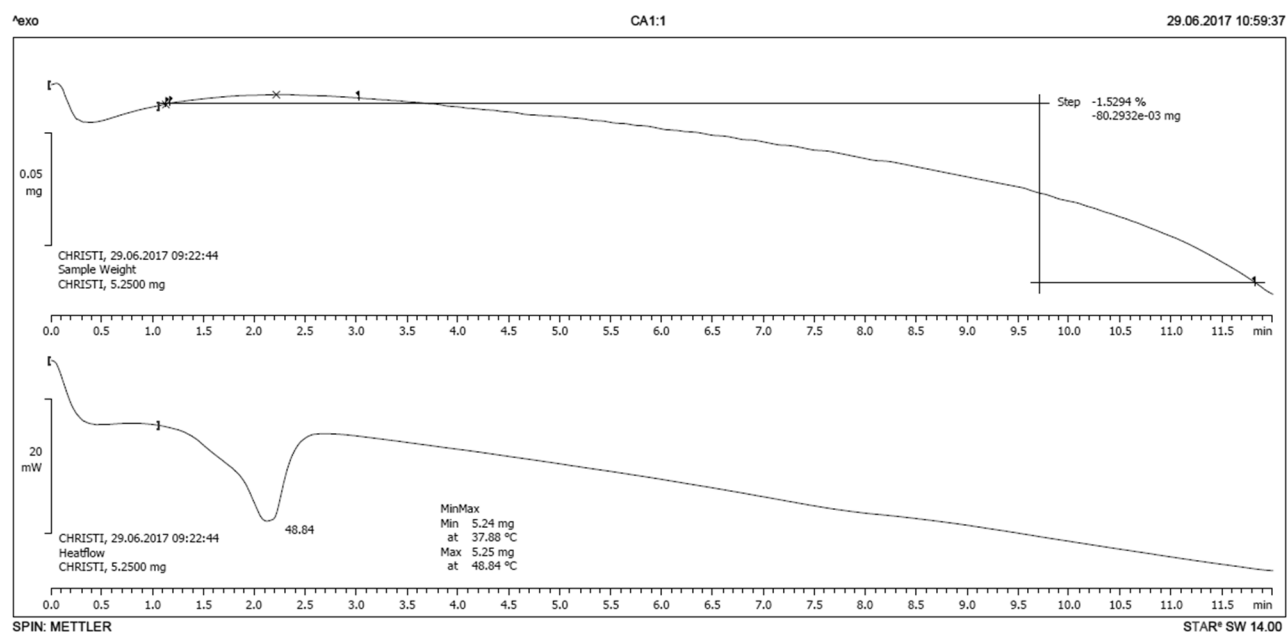
B.14: DTG curve of Cetyl Alcohol 0.75:1 Lipid Dispersion

Top graph: Cetyl alcohol 0.75:1 lipid dispersion TG; **Bottom graph:** Cetyl alcohol 0.75:1 lipid dispersion DTA



B.15: DTG curve of Cetyl Alcohol 1:1 Lipid Dispersion

Top graph: Cetyl alcohol 1:1 lipid dispersion TG; **Bottom graph:** Cetyl alcohol 1:1 lipid dispersion DTA





X-ray Powder Diffraction

Figure C.1: Diffractogram of Artemether	C2
Figure C.2: Diffractogram of Lumefantrine	C2
Figure C.3: Diffractogram of Glycerol Monostearate Raw Material	C3
Figure C.4: Diffractogram of Stearic Acid Raw Material	C3
Figure C.5: Diffractogram of Cetyl Alcohol Raw Material.....	C4
Figure C.6: Diffractogram of Glycerol Monostearate 1:1 Lipid Dispersion	C4
Figure C.7: Diffractogram of Glycerol Monostearate 0.75:1 Lipid Dispersion	C5
Figure C.8: Diffractogram of Glycerol Monostearate 0.5:1 Lipid Dispersion	C5
Figure C.9: Diffractogram of Stearic Acid 1:1 Lipid Dispersion.....	C6
Figure C.10: Diffractogram of Stearic Acid 0.75:1 Lipid Dispersion.....	C6
Figure C.11: Diffractogram of Stearic Acid 0.5:1 Lipid Dispersion	C7
Figure C.12: Diffractogram of Cetyl Alcohol 1:1 Lipid Dispersion.....	C7
Figure C.13: Diffractogram of Cetyl Alcohol 0.75:1 Lipid Dispersion	C8
Figure C.14: Diffractogram of Cetyl Alcohol 0.5:1 Lipid Dispersion	C8

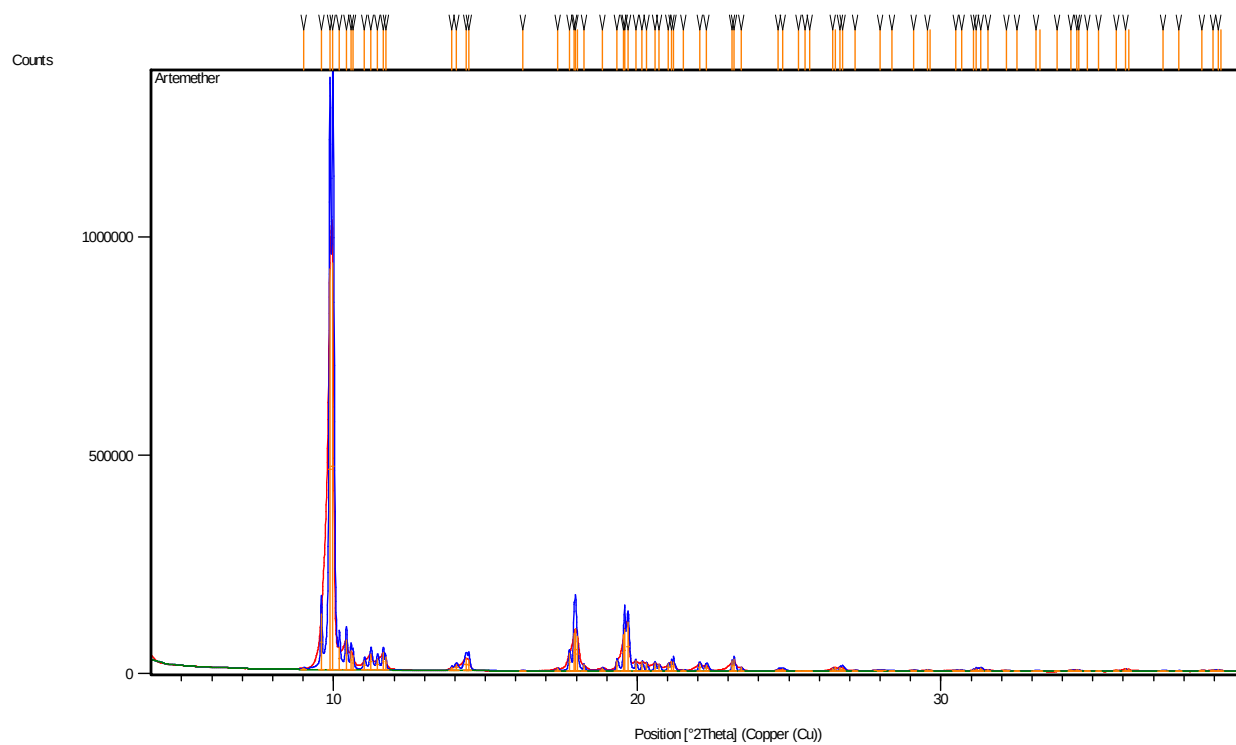


Figure C.1: Diffractogram of Artemether

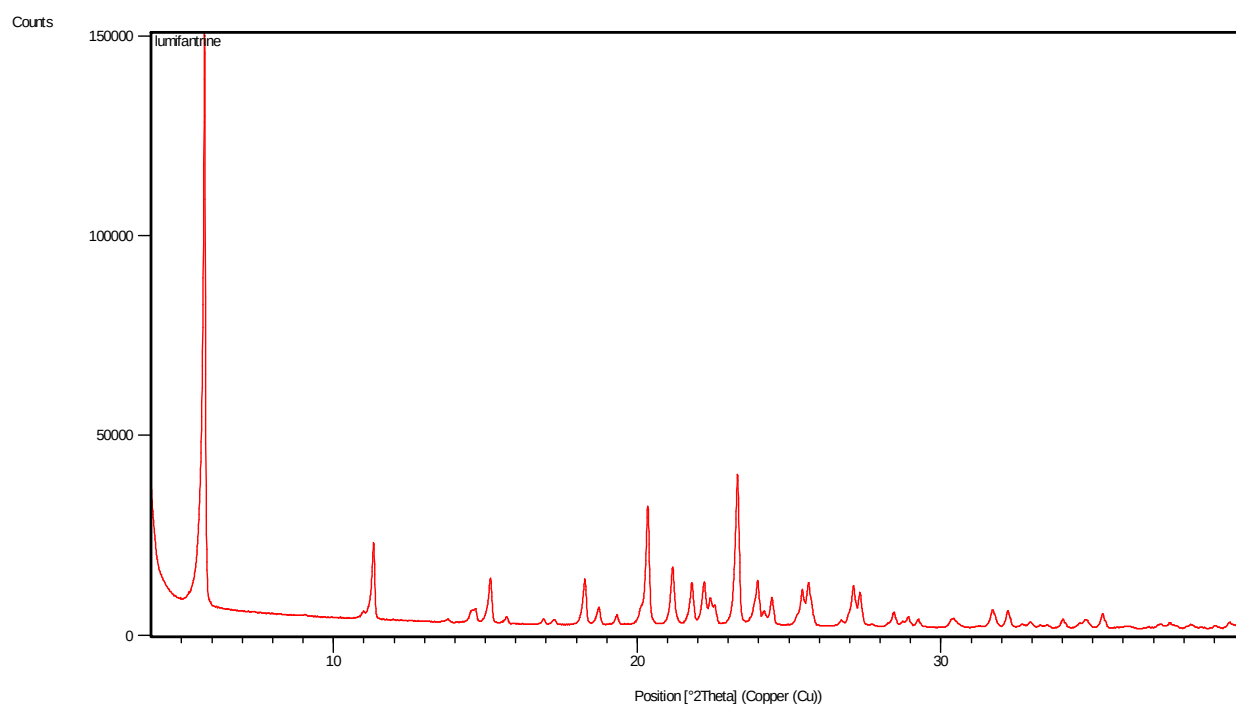


Figure C.2: Diffractogram of Lumefantrine

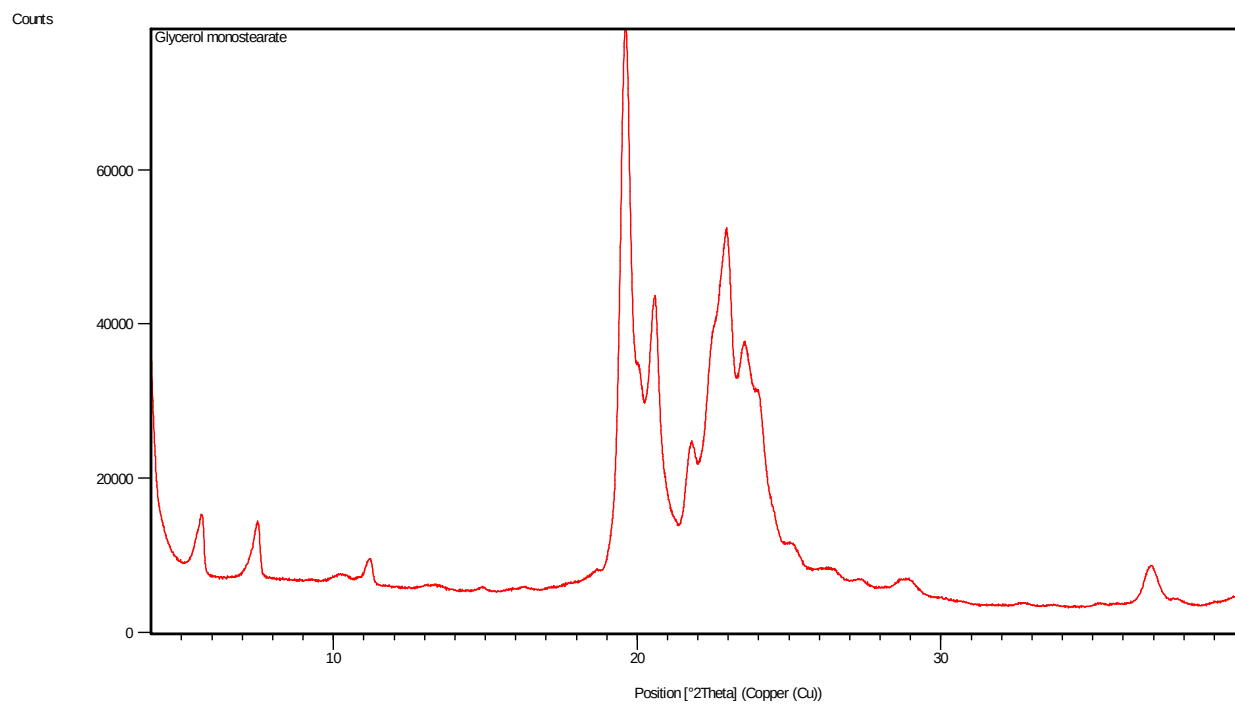


Figure C.3: Diffractogram of Glycerol Monostearate Raw Material

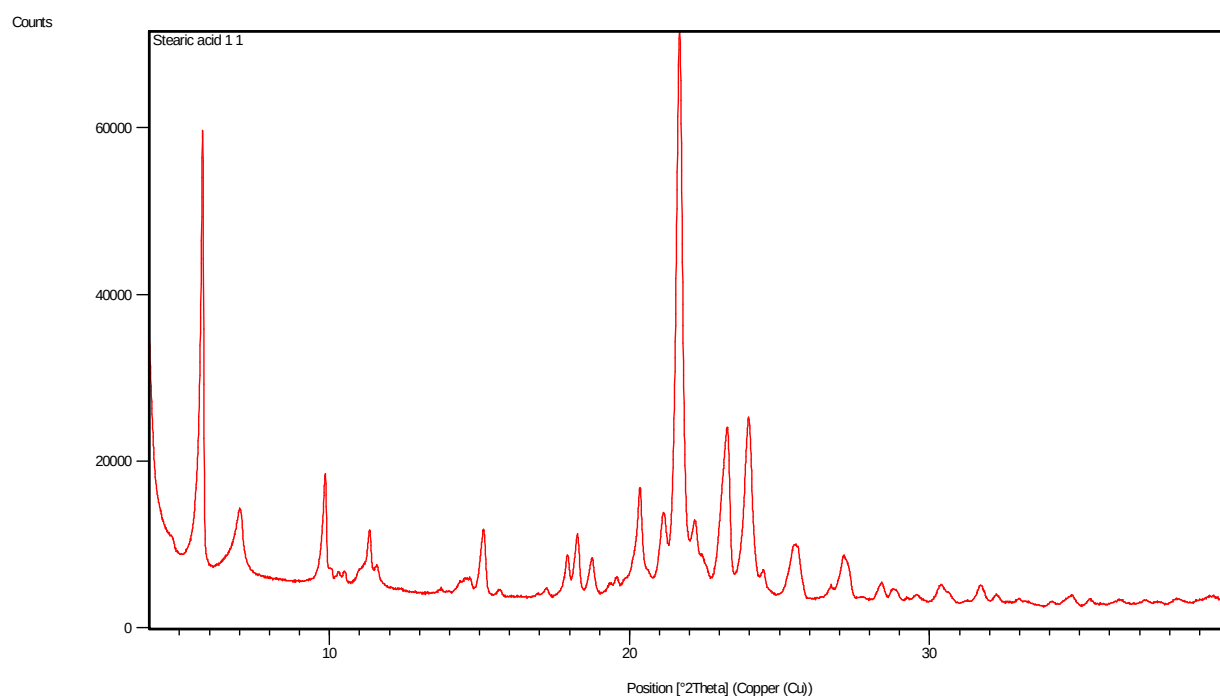


Figure C.4: Diffractogram of Stearic Acid Raw Material

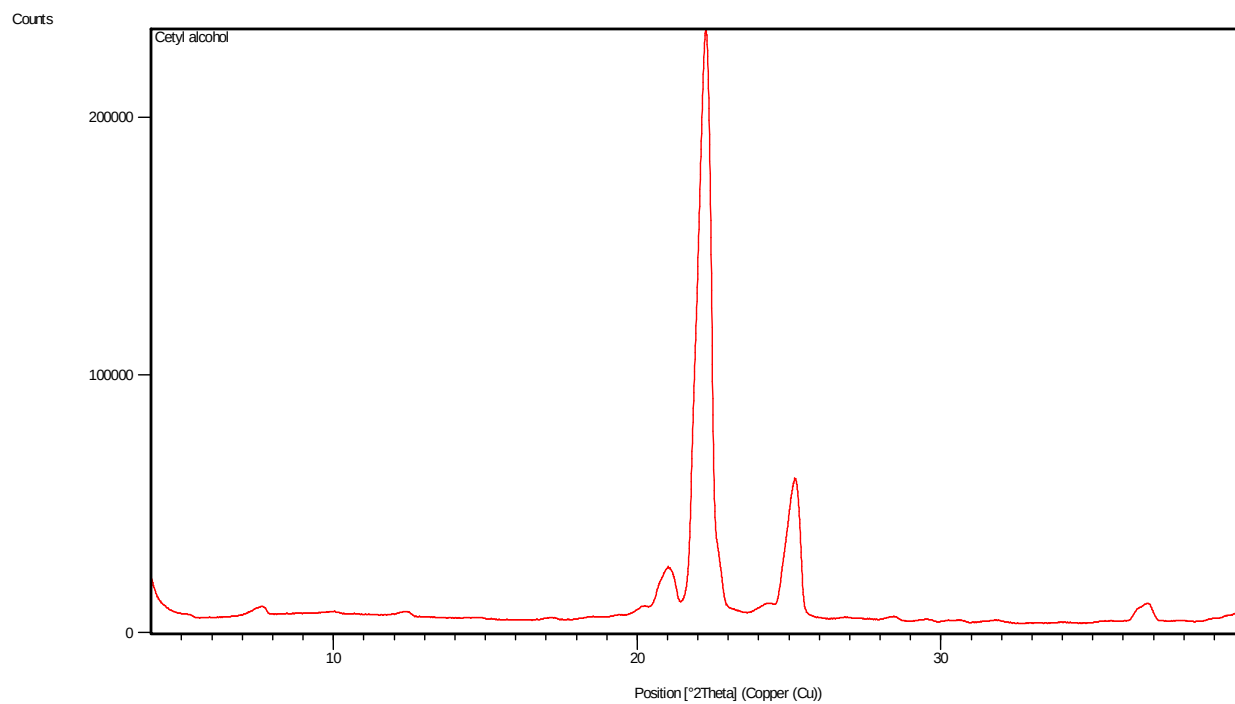


Figure C.5: Diffractogram of Cetyl Alcohol Raw Material

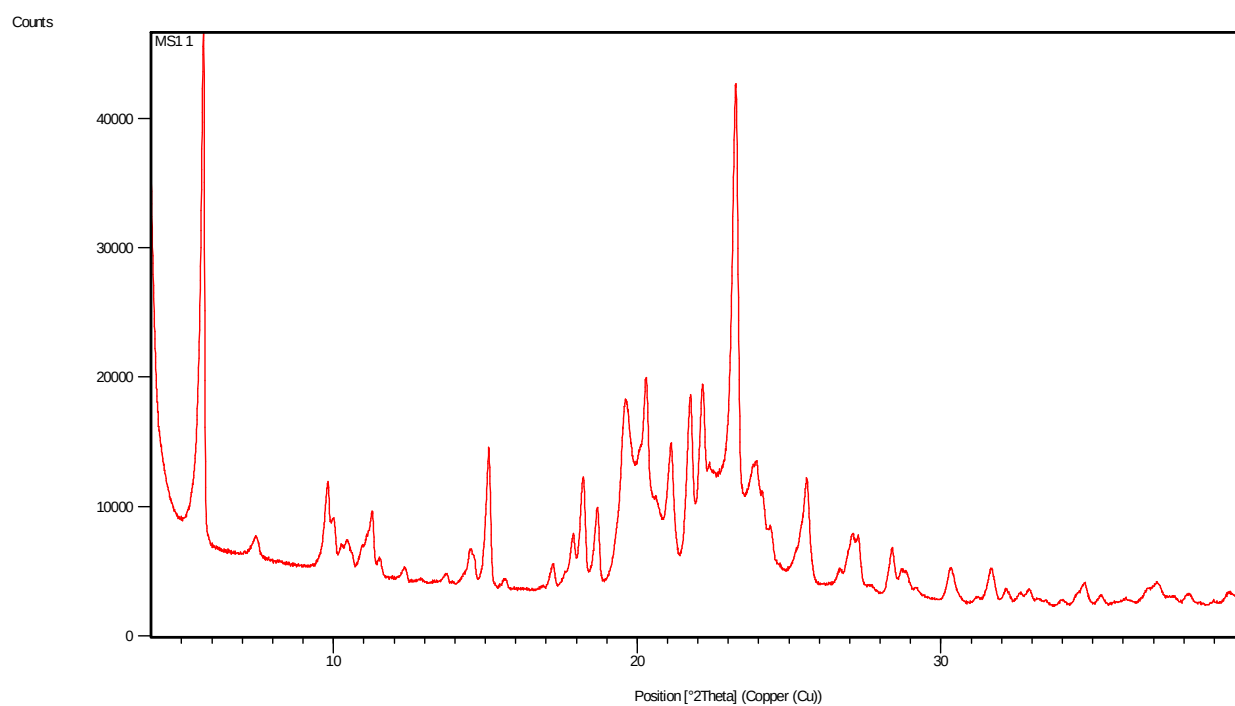


Figure C.6: Diffractogram of Glycerol Monostearate 1:1 Lipid Dispersion

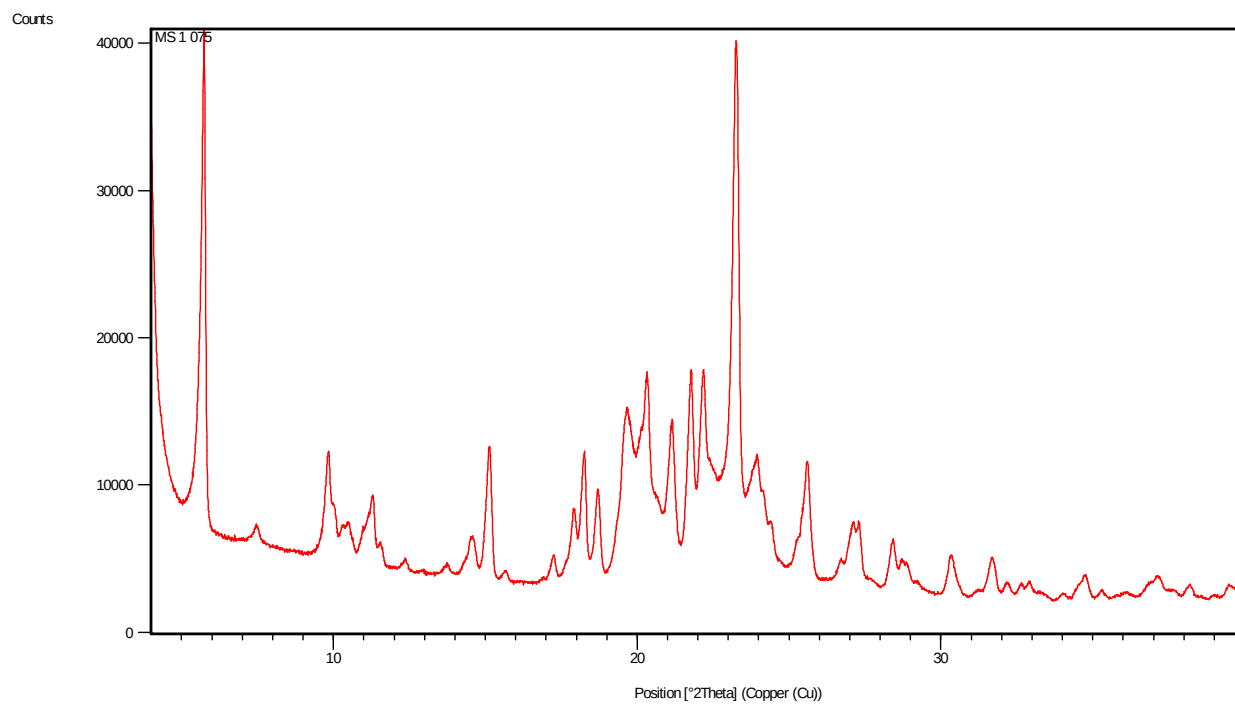


Figure C.7: Diffractogram of Glycerol Monostearate 0.75:1 Lipid Dispersion

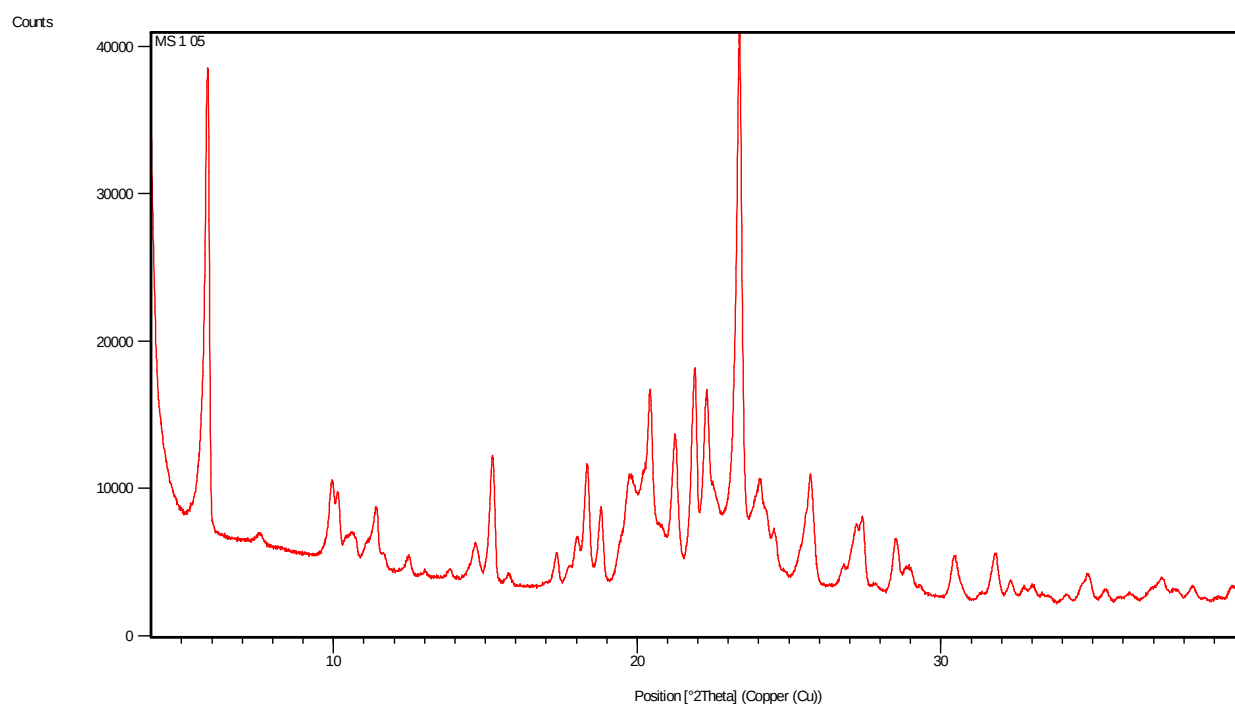


Figure C.8: Diffractogram of Glycerol Monostearate 0.5:1 Lipid Dispersion

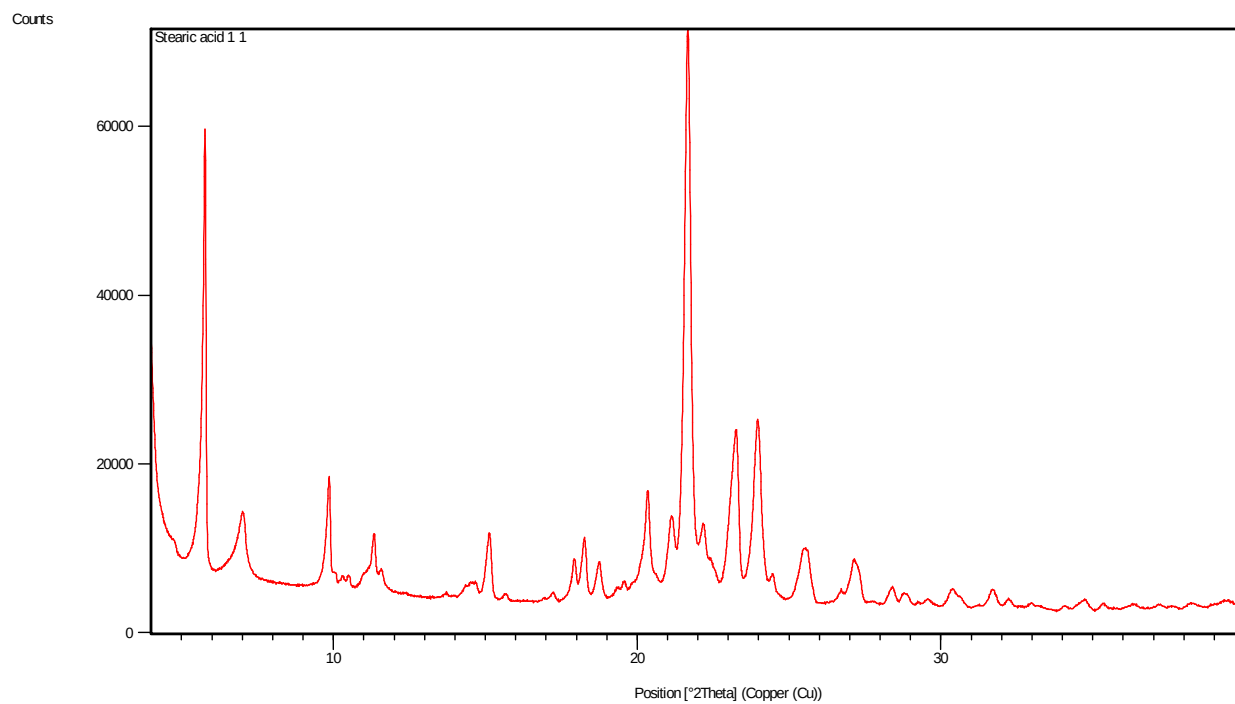


Figure C.9: Diffractogram of Stearic Acid 1:1 Lipid Dispersion

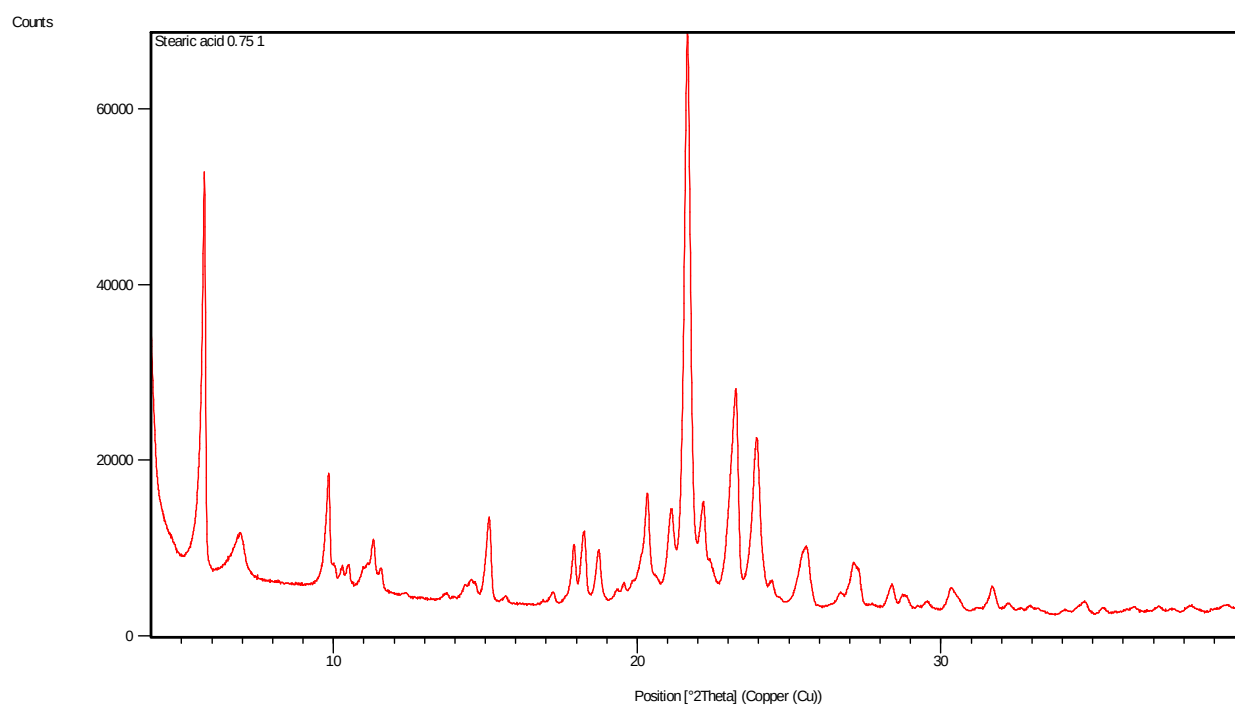


Figure C.10: Diffractogram of Stearic Acid 0.75:1 Lipid Dispersion

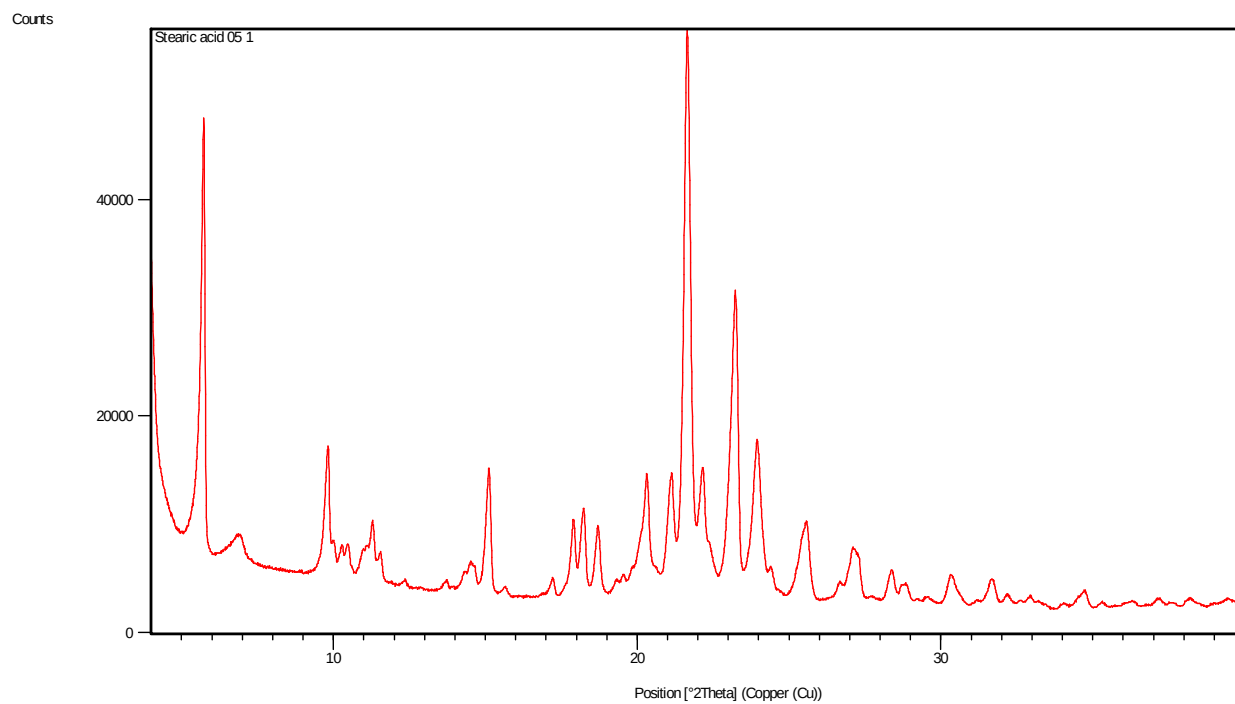


Figure C.11: Diffractogram of Stearic Acid 0.5:1 Lipid Dispersion

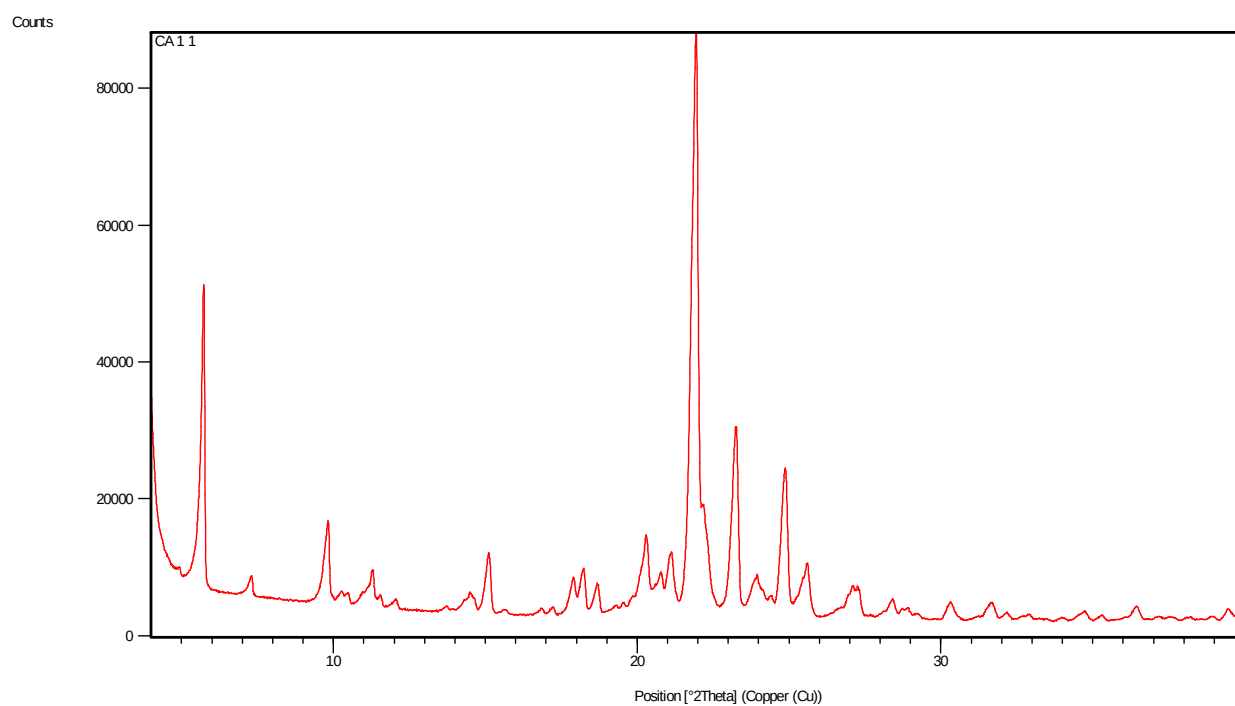


Figure C.12: Diffractogram of Cetyl Alcohol 1:1 Lipid Dispersion

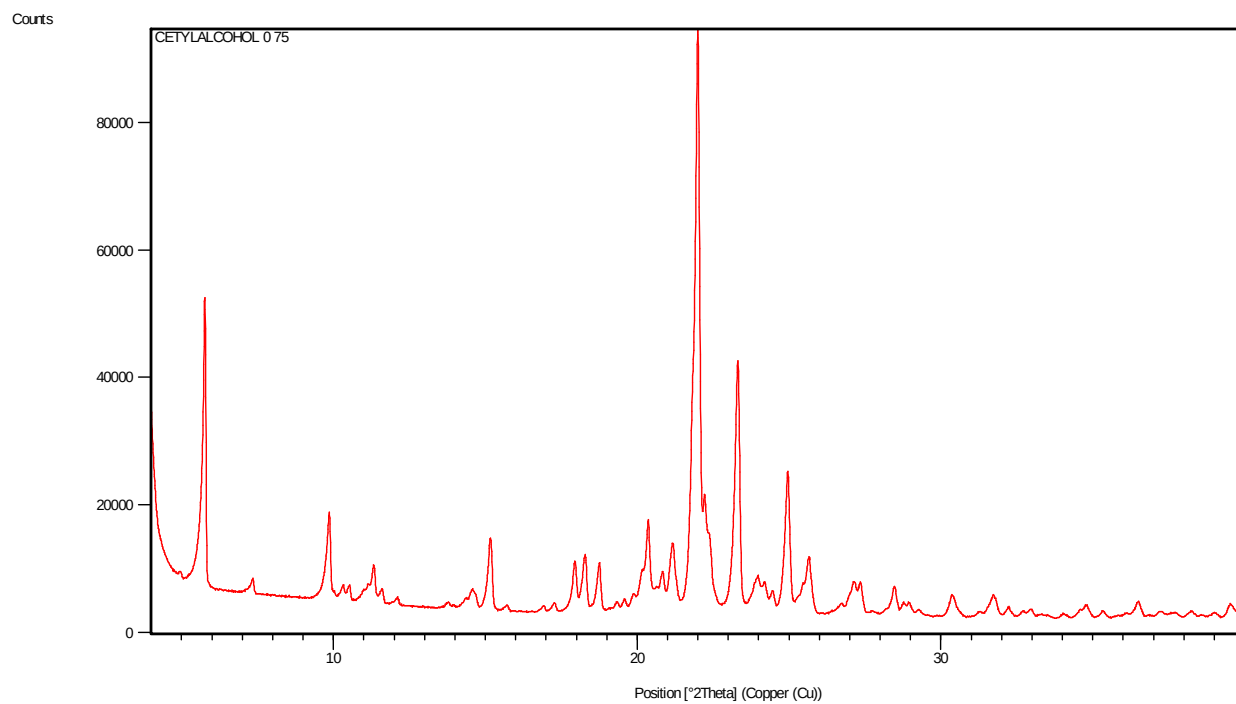


Figure C.13: Diffractogram of Cetyl Alcohol 0.75:1 Lipid Dispersion

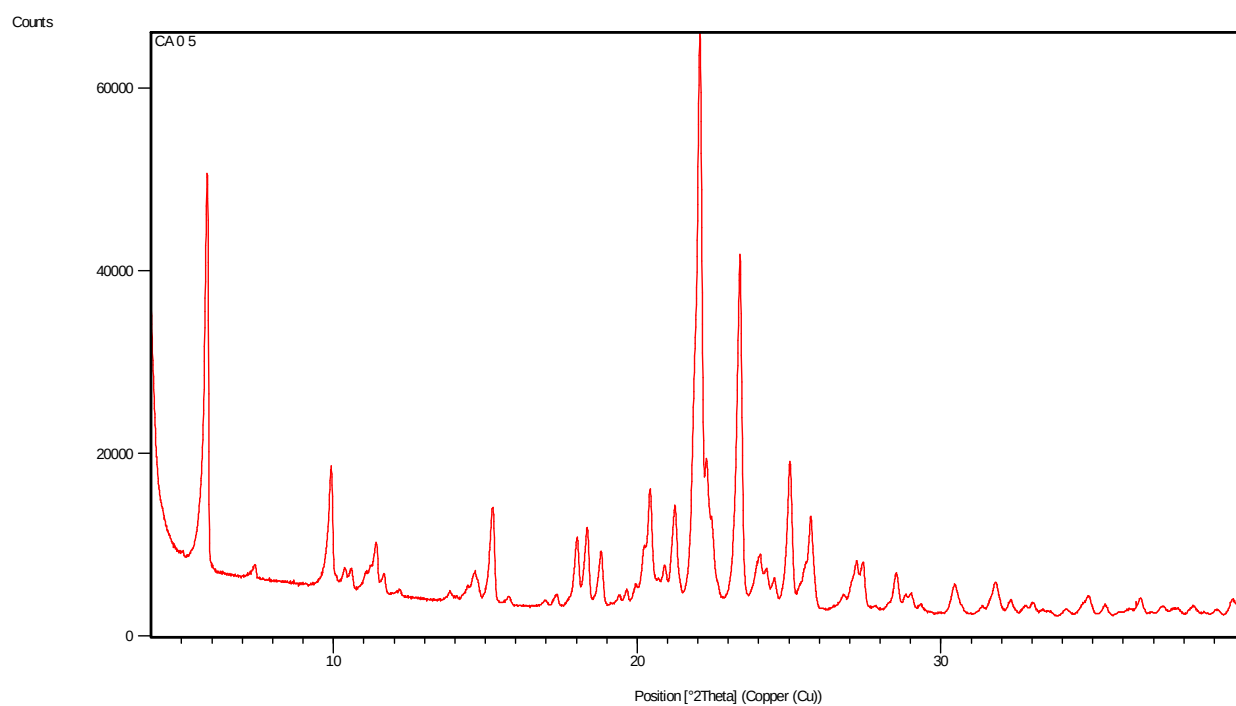


Figure C.14: Diffractogram of Cetyl Alcohol 0.5:1 Lipid Dispersion



Physical Tablet Property Test Results

Table D.1: Summary Table of Mass Variation Results (mg) Recorded.....	D2
Table D.2: Summary Table of the Friability (%) Test Results Recorded	D3
Table D.3: Summary Table of Tensile Strength (N.mm ⁻²) Results Recorded	D4
Table D.4: Summary Table of Disintegration (min) Results Recorded	D5

Table D.1: Summary table of mass variation results (mg) recorded per formulation (%RSD is represented in parenthesis)

			Mass Variation (mg)								
			Glycerol Monostearate			Stearic Acid			Cetyl alcohol		
			0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1
MicroceLac® 100	Magnesium Stearate	1%	492 (1.47)	499.2 (1.94)	514.1 (0.92)	522.8 (0.91)	496 (1.15)	492 (0.85)	489 (1.55)	482 (1.85)	491 (1.89)
		1.25%	497 (1.14)	494.3 (1.73)	485 (1.44)	496.5 (1.15)	495 (0.87)	498 (0.72)	486 (1.96)	488 (2.04)	491 (1.49)
RetaLac®		1%	469.9 (4.11)	486.3 (2.37)	472.1 (6.44)	492.9 (1.07)	487 (1.91)	449.9 (1.67)	NR	NR	NR
		1.25%	482.2 (4.44)	505.8 (1.65)	490.8 (2.88)	504.4 (1.50)	504 (1.30)	507 (3.15)	NR	NR	NR
CombiLac®		1%	520.3 (1.74)	499.5 (1.47)	496.9 (1.06)	513.6 (2.30)	501 (0.98)	501 (0.71)	476 (1.71)	476 (1.61)	486 (1.29)
		1.25%	473.3 (8.28)	505.2 (0.71)	506.3 (1.04)	493.5 (1.02)	492 (0.92)	496 (0.74)	498 (1.83)	475 (1.66)	492 (1.75)
PharmaceLac® 101		1%	507.5 (1.71)	491.2 (3.67)	487.7 (4.18)	488.75 (3.77)	518 (1.22)	502 (2.88)	NR	NR	NR
		1.25%	492.9 (6.03)	481.6 (7.28)	518.3 (1.85)	470.7 (7.66)	490 (2.62)	525 (2.23)	NR	NR	NR

*NR: no result

Table D.2: Summary table of the friability (%) test results recorded per formulation

		Friability (%)									
		Glycerol Monostearate			Stearic Acid			Cetyl Alcohol			
		0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	
MicroceLac® 100	Magnesium Stearate	1%	0.143	0.180	0.136	0.153	0.121	0.122	0.997	0.998	0.999
		1.25%	0.100	0.162	0.226	0.181	0.121	0.101	0.994	0.998	0.995
		1%	12.487	10.322	1.005	3.149	3.149	1.127	NR	NR	NR
		1.25%	76.022	14.653	4.118	10.822	10.822	1.840	NR	NR	NR
		1%	0.114	0.040	0.181	0.234	0.060	0.800	0.998	0.999	0.999
		1.25%	0.313	0.297	0.612	0.283	0.183	0.245	0.998	0.998	0.996
		1%	1.218	0.552	0.521	57.73	3.682	10.722	NR	NR	NR
		1.25%	10.127	2.319	0.269	77.85	0.429	1.869	NR	NR	NR

*NR: no result

Table D.3: Summary table of tensile strength (N.mm⁻²) results recorded per formulation (%RSD is represented in parenthesis)

			Tensile Strength (N.mm ⁻²)								
			Glycerol Monostearate			Stearic Acid			Cetyl Alcohol		
			0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1
Microcelac® 100	Magnesium Stearate	1%	1.856 (0.094)	1.515 (0.631)	1.738 (0.054)	2.393 (0.037)	1.351 (0.090)	1.464 (0.053)	1.863 (0.116)	2.169 (0.123)	2.196 (0.099)
		1.25%	1.785 (0.125)	1.540 (0.150)	1.903 (0.134)	2.859 (0.052)	1.693 (0.081)	1.372 (0.356)	1.992 (0.134)	2.071 (0.195)	1.903 (0.141)
		1%	NR	0.079 (0.717)	0.400 (0.619)	0.333 (0.042)	0.147 (0.153)	0.202 (0.374)	NR	NR	NR
		1.25%	NR	0.093 (0.380)	0.204 (0.557)	0.232 (0.078)	0.173 (0.382)	0.203 (0.082)	NR	NR	NR
		1%	1.563 (0.535)	1.854 (0.096)	1.521 (0.360)	2.886 (0.053)	1.532 (0.072)	1.817 (0.050)	2.073 (0.135)	3.259 (1.187)	1.758 (0.111)
		1.25%	0.928 (0.571)	1.810 (0.065)	1.705 (0.174)	2.874 (0.044)	2.535 (0.092)	1.601 (0.050)	1.931 (0.132)	2.114 (0.038)	1.839 (0.122)
		1%	0.347 (0.145)	0.457 (0.198)	0.628 (0.126)	NR	0.152 (0.193)	0.116 (0.577)	NR	NR	NR
		1.25%	0.206 (0.425)	0.514 (0.555)	0.720 (0.130)	NR	0.374 (0.279)	0.193 (0.365)	NR	NR	NR
RetaLac®		1%	NR	0.079 (0.717)	0.400 (0.619)	0.333 (0.042)	0.147 (0.153)	0.202 (0.374)	NR	NR	NR
CombiLac®		1.25%	NR	0.093 (0.380)	0.204 (0.557)	0.232 (0.078)	0.173 (0.382)	0.203 (0.082)	NR	NR	NR
PharmaceL® 101		1%	1.563 (0.535)	1.854 (0.096)	1.521 (0.360)	2.886 (0.053)	1.532 (0.072)	1.817 (0.050)	2.073 (0.135)	3.259 (1.187)	1.758 (0.111)
		1.25%	0.928 (0.571)	1.810 (0.065)	1.705 (0.174)	2.874 (0.044)	2.535 (0.092)	1.601 (0.050)	1.931 (0.132)	2.114 (0.038)	1.839 (0.122)

*NR: no result

Table D.4: Summary table of disintegration (min) results recorded per formulation

		Disintegration (min)									
		Glycerol Monostearate			Stearic Acid			Cetyl Alcohol			
		0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	0.5:1	0.75:1	1:1	
MicroceLac® 100	Magnesium Stearate	1%	ND	ND	ND	ND	ND	ND	ND	ND	ND
		1.25%	ND	ND	ND	ND	ND	ND	ND	ND	ND
		1%	ND	ND	ND	ND	ND	ND	NR	NR	NR
		1.25%	ND	ND	ND	ND	ND	ND	NR	NR	NR
		1%	ND	ND	ND	ND	ND	ND	ND	ND	ND
		1.25%	ND	ND	ND	ND	ND	ND	ND	ND	ND
		1%	0.592	0.567	2.000	0.361	0.256	0.331	NR	NR	NR
		1.25%	0.553	0.647	1.082	0.336	0.611	0.425	NR	NR	NR
CombiLac®	RetaLac®	MicroceLac® 100									

*ND: non-disintegrating

**NR: no result



Lipid Matrix Tablet Morphology Images

Figure E.1: SA0.5M1 Morphology Images	E2
Figure E.2: SA0.5C1 Morphology Images	E2
Figure E.3: SA0.75M1 Morphology Images	E2
Figure E.4: SA0.75C1 Morphology Images.....	E3
Figure E.5: SA1M1 Morphology Images.....	E3
Figure E.6: SA1C1 Morphology Images	E3
Figure E.7: CA0.5M1 Morphology Images.....	E4
Figure E.8: CA0.5C1 Morphology Images	E4
Figure E.9: CA0.75M1 Morphology Images	E4
Figure E.10: CA0.75C1 Morphology Images.....	E5
Figure E.11: CA1M1 Morphology Images.....	E5
Figure E.12: CA1C1 Morphology Images	E5
Figure E.13: GM0.5M1 Morphology Images	E6
Figure E.14: GM0.5C1 Morphology Images.....	E6
Figure E.15: GM0.75M1 Morphology Images.....	E6
Figure E.16: GM0.75C1 Morphology Images.....	E7
Figure E.17: GM1M1 Morphology Images	E7
Figure E.18: GM1C1 Morphology Images	E7

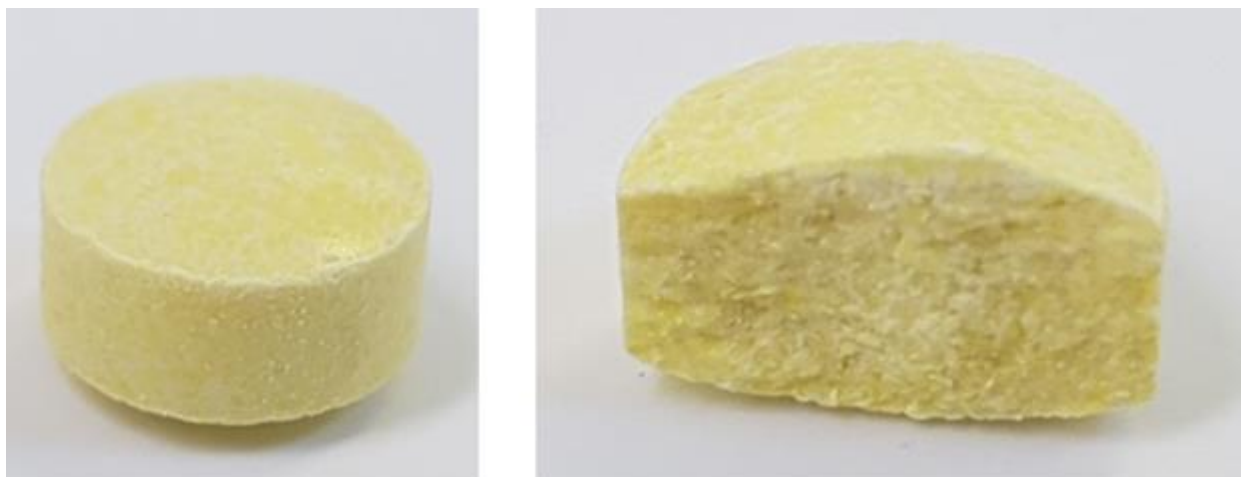


Figure E.1: SA0.5M1 external (left) and internal (right) surface morphology image



Figure E.2: SA0.5C1 external (left) and internal (right) surface morphology image



Figure E.3: SA0.75M1 external (left) and internal (right) surface morphology image



Figure E.4: SA0.75C1 external (left) and internal (right) surface morphology image



Figure E.5: SA1M1 external (left) and internal (right) surface morphology image



Figure E.6: SA1C1 external (left) and internal (right) surface morphology image



Figure E.7: CA0.5M1 external (left) and internal (right) surface morphology image



Figure E.8: CA0.5C1 external (left) and internal (right) surface morphology image



Figure E.9: CA0.75M1 external (left) and internal (right) surface morphology image



Figure E.10: CA0.75C1 external (left) and internal (right) surface morphology image



Figure E.11: CA1M1 external (left) and internal (right) surface morphology image



Figure E.12: CA1C1 external (left) and internal (right) surface morphology image



Figure E.13: GM0.5M1 external (left) and internal (right) surface morphology image



Figure E.14: GM0.5C1 external (left) and internal (right) surface morphology image



Figure E.15: GM0.75M1 external (left) and internal (right) surface morphology image



Figure E.16: GM0.75C1 external (left) and internal (right) surface morphology image



Figure E.17: GM1M1 external (left) and internal (right) surface morphology image



Figure E.18: GM1C1 external (left) and internal (right) surface morphology image



Percentage Swelling Analysis

Figure F.1: Percentage swelling obtained for all viable formulations	F2
Figure F.2: Percentage swelling for the respective stearic acid formulations including error bars.....	F3
Figure F.3: Percentage swelling for the respective glycerol monostearate formulations including error bars	F3
Figure F.4: Percentage swelling for the respective cetyl alcohol formulations including error bars	F4

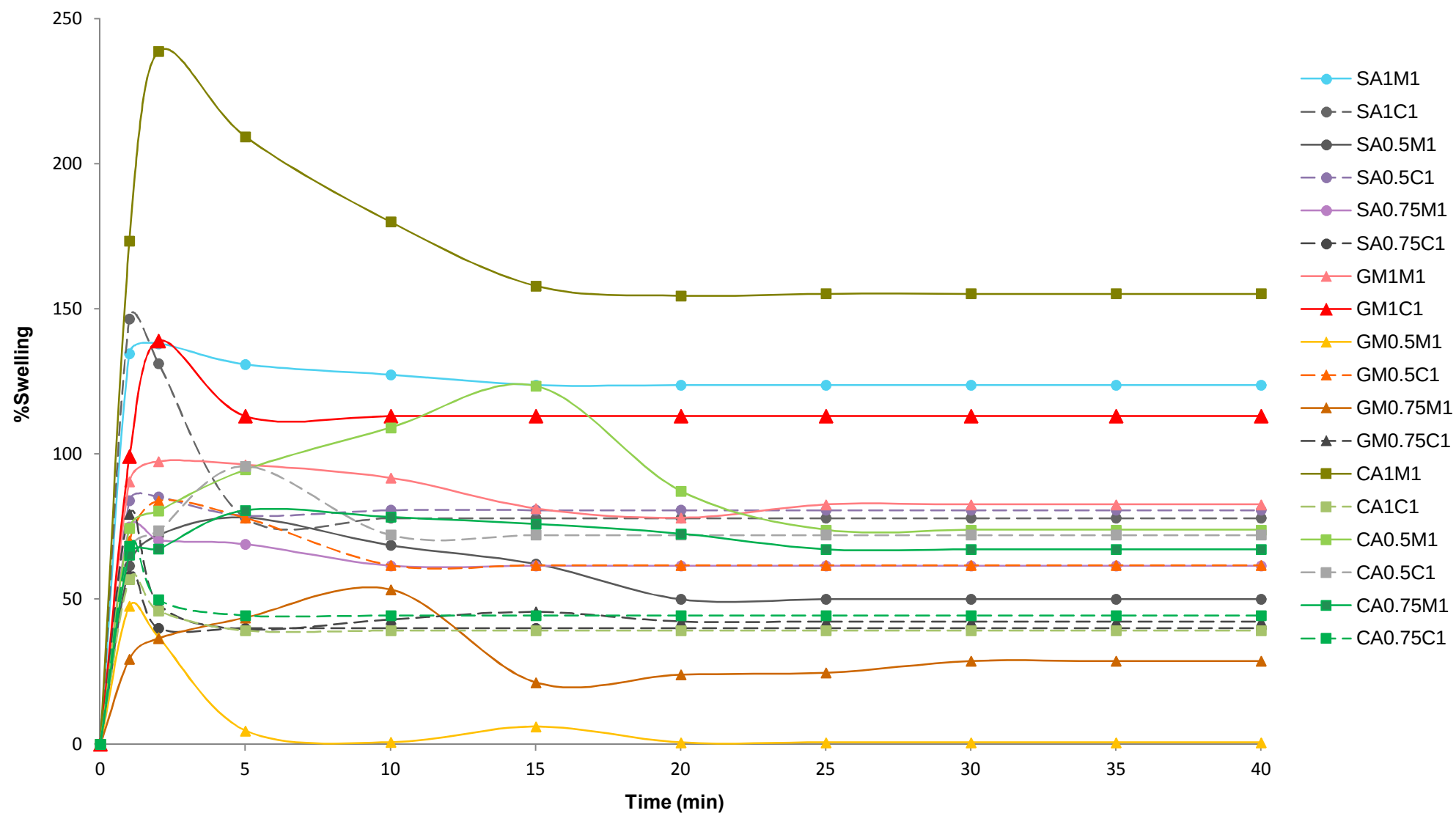


Figure F1: Percentage swelling obtained for all viable formulations

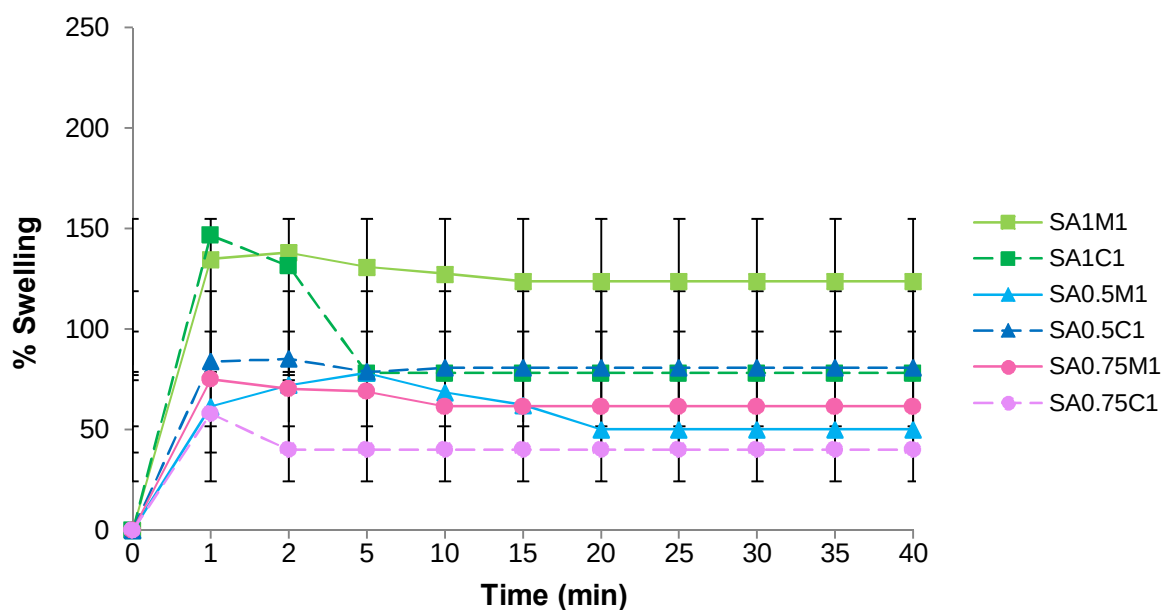


Figure F2: Percentage swelling for the respective stearic acid formulations including error bars

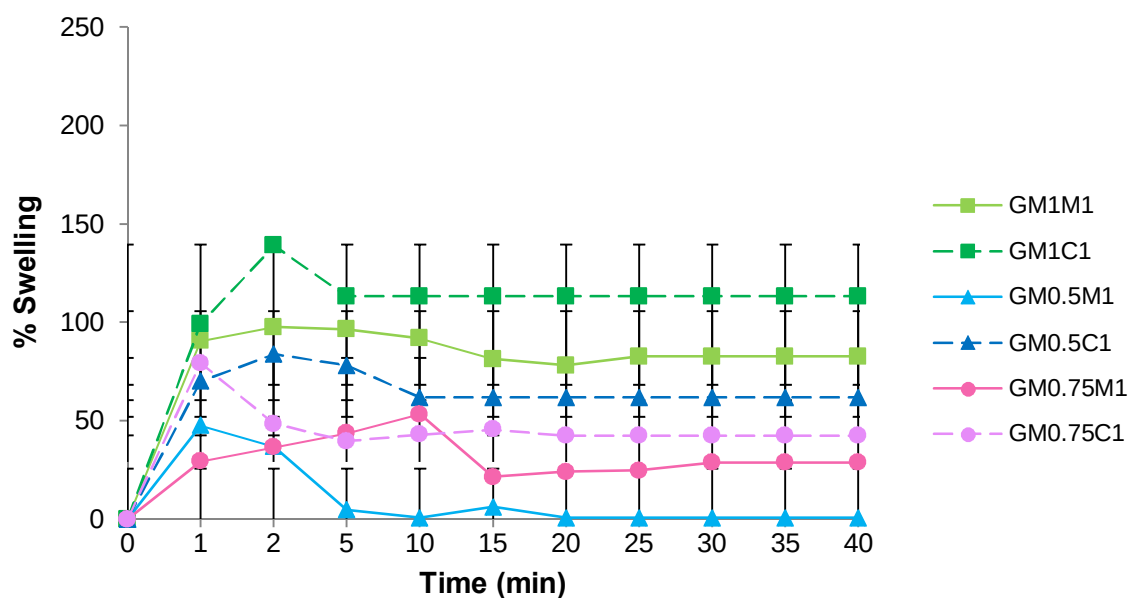


Figure F3: Percentage swelling for the respective glycerol monostearate formulations including error bars

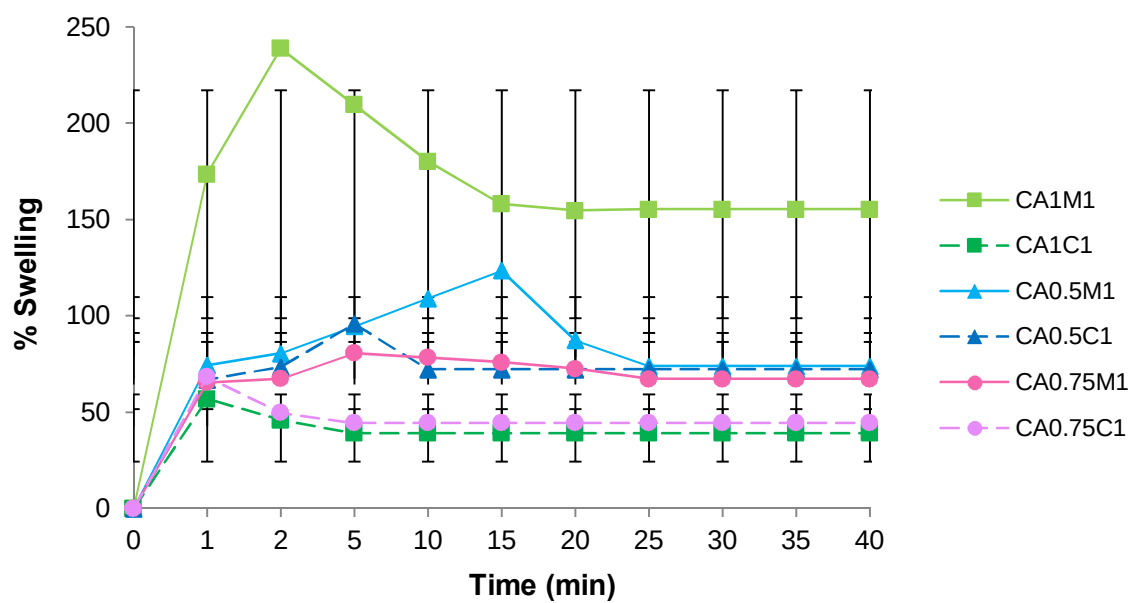


Figure F4: Percentage swelling for the respective cetyl alcohol formulations including error bars



Dissolution Data Analysis

G1:	Fit factors analysed indicating statistically significant differences and similarities	G2
G2:	CA0.5C1 dissolution profile modelling	G3
G3:	CA0.5M1 dissolution profile modelling	G4
G4:	CA0.75C1 dissolution profile modelling	G5
G5:	CA0.75M1 dissolution profile modelling	G6
G6:	CA1C1 dissolution profile modelling	G7
G7:	CA1M1 dissolution profile modelling	G8
G8:	GM0.5C1 dissolution profile modelling	G9
G9:	GM0.5M1 dissolution profile modelling	G10
G10:	GM0.75C1 dissolution profile modelling	G11
G11:	GM0.75M1 dissolution profile modelling	G12
G12:	GM1C1 dissolution profile modelling	G13
G13:	GM1M1 dissolution profile modelling	G14
G14:	SA0.5C1 dissolution profile modelling	G15
G15:	SA0.5M1 dissolution profile modelling	G16
G16:	SA0.75C1 dissolution profile modelling	G17
G17:	SA0.75M1 dissolution profile modelling	G18
G18:	SA1C1 dissolution profile modelling.....	G19
G19:	SA1M1 dissolution profile modelling	G20
G20:	Coartem® dissolution profile modelling	G21

G1: Fit factors analysed indicating statistically significant differences in green and similarities in red

		CA0.5M1	CA0.75M1	CA1M1	CA0.5C1	CA0.75C1	CA1C1	SA0.5M1	SA0.75M1	SA1M1	SA0.5C1	SA0.75C1	SA1C1	GM0.5M1	GM0.75M1	GM1M1	GM0.5C1	GM0.75C1	GM1C1
CA0.5M1	f1		36.91	37.71	36.0														
	f2		38.29	40.29	43.2														
CA0.75M1	f1	36.9		83.38		44.0													
	f2	38.3		29.95		42.3													
CA1M1	f1	37.7	83.4				48.38												
	f2	40.3	30.0				31.08												
CA0.5C1	f1	35.96				4.58	37.26												
	f2	43.20				82.17	40.57												
CA0.75C1	f1		43.98		4.58		34.25												
	f2		42.35		82.17		43.33												
CA1C1	f1			48.38	37.26	34.25													
	f2			31.08	40.57	43.33													
SA0.5M1	f1								13.19	24.76	26.72								
	f2								66.34	50.59	49.39								
SA0.75M1	f1							13.19		37.05		62.38							
	f2							66.34		44.46		35.43							
SA1M1	f1							24.76	37.05				27.07						
	f2							50.59	44.46				46.97						
SA0.5C1	f1							26.72				11.23	35.66						
	f2							49.39				62.67	40.80						
SA0.75C1	f1								62.38		11.23		42.16						
	f2								35.43		62.67		34.89						
SA1C1	f1									27.07	35.66	42.16							
	f2									46.97	40.80	34.89							
GM0.5M1	f1														38.13	122.07	60.89		
	f2														47.77	24.57	40.29		
GM0.75M1	f1													38.13		214.91		191.18	
	f2													47.77		20.97		24.18	
GM1M1	f1													122.07	214.91				47.45
	f2													24.57	20.97				25.67
GM0.5C1	f1													60.89				407.06	554.42
	f2													40.29				19.85	14.08
GM0.75C1	f1														191.18		407.06		41.64
	f2														24.18		19.85		30.94
GM1C1	f1															47.45	554.42	41.64	
	f2															25.67	14.08	30.94	

G2: CA0.5C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

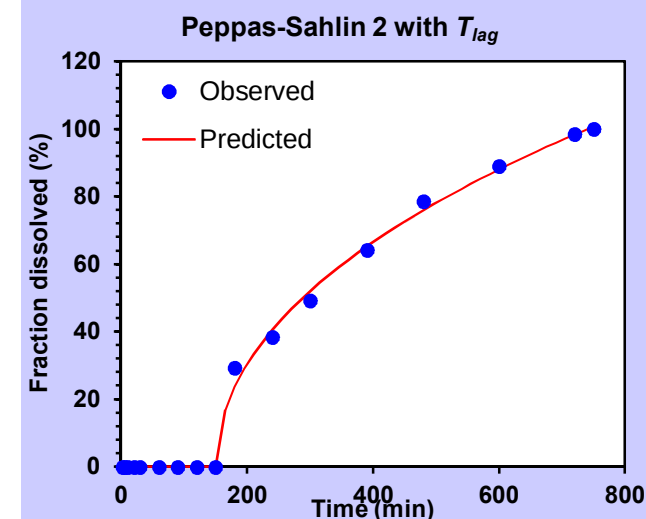
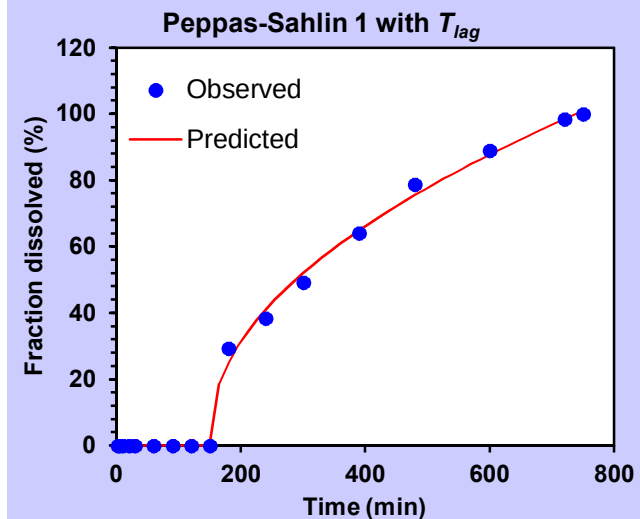
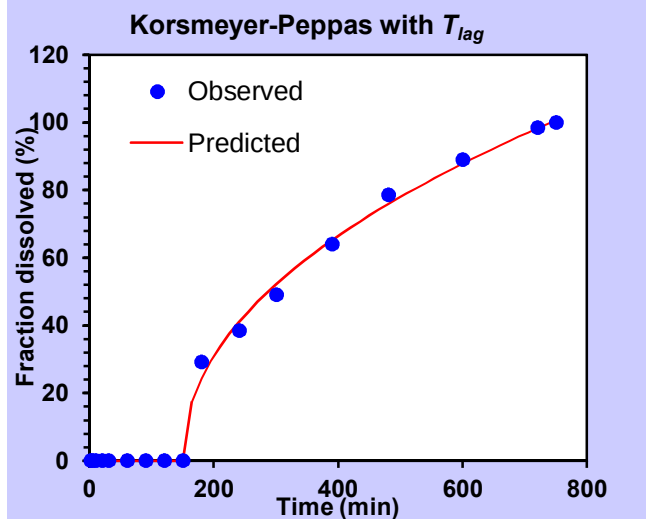
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	4.881	N_observed	17
n	0.473	DF	14
T_{lag}	150.000	R_obs-pre	0.9990
		Rsqr	0.9980
		Rsqr_adj	0.9977
		MSE	3.6260
		MSE_root	1.9042
		Weighting	1
		SS	50.7645
		WSS	50.7645
		AIC	72.7624
		MSC	5.3836

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	4.199	N_observed	17
k2	2.153	DF	13
m	0.279	R_obs-pre	0.9991
T_{lag}	150.000	Rsqr	0.9982
		Rsqr_adj	0.9978
		MSE	3.4605
		MSE_root	1.8603
		Weighting	1
		SS	44.9870
		WSS	44.9870
		AIC	72.7083
		MSC	5.3868

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	4.394	N_observed	17
k2	-0.011	DF	14
T_{lag}	150.000	R_obs-pre	0.9989
		Rsqr	0.9978
		Rsqr_adj	0.9975
		MSE	3.8574
		MSE_root	1.9640
		Weighting	1
		SS	54.0040
		WSS	54.0040
		AIC	73.8140
		MSC	5.3218



G3: CA0.5M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

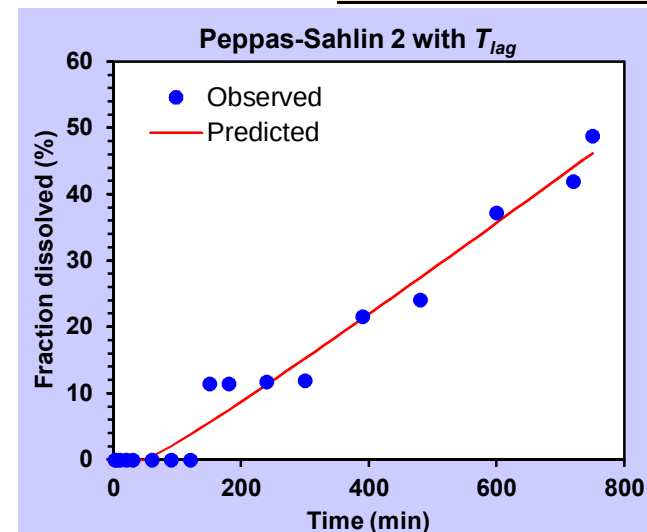
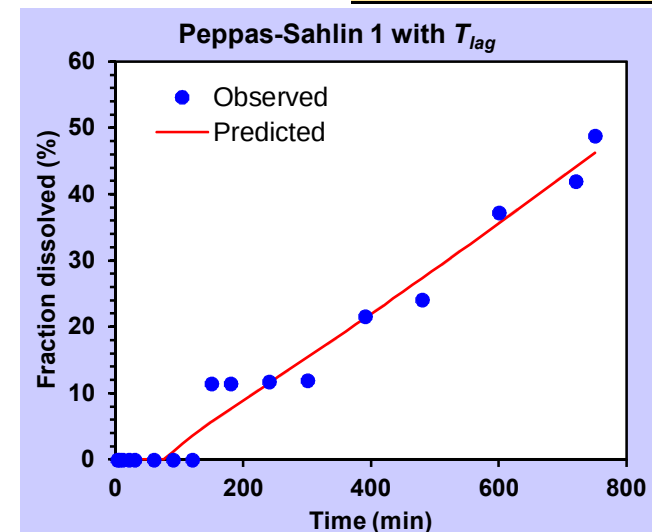
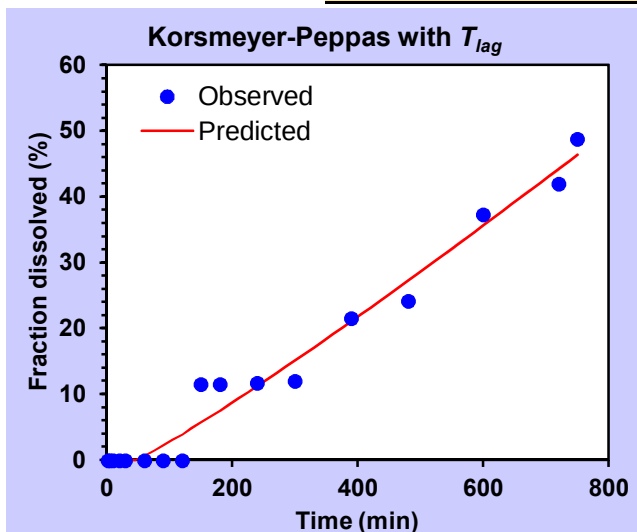
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	0.035	N_observed	17
n	1.098	DF	14
T_{lag}	45.934	R_obs-pre	0.9876
		Rsqr	0.9754
		Rsqr_adj	0.9719
		MSE	7.5258
		MSE_root	2.7433
		Weighting	1
		SS	105.3617
		WSS	105.3617
		AIC	85.1758
		MSC	2.9712

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	0.253	N_observed	17
k2	0.003	DF	13
m	0.684	R_obs-pre	0.9882
T_{lag}	80.842	Rsqr	0.9766
		Rsqr_adj	0.9712
		MSE	7.7087
		MSE_root	2.7765
		Weighting	1
		SS	100.2136
		WSS	100.2136
		AIC	86.3242
		MSC	2.9037

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	-0.319	N_observed	17
k2	0.076	DF	14
T_{lag}	30.009	R_obs-pre	0.9874
		Rsqr	0.9751
		Rsqr_adj	0.9715
		MSE	7.6236
		MSE_root	2.7611
		Weighting	1
		SS	106.7305
		WSS	106.7305
		AIC	85.3952
		MSC	2.9583



G4: CA0.75C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

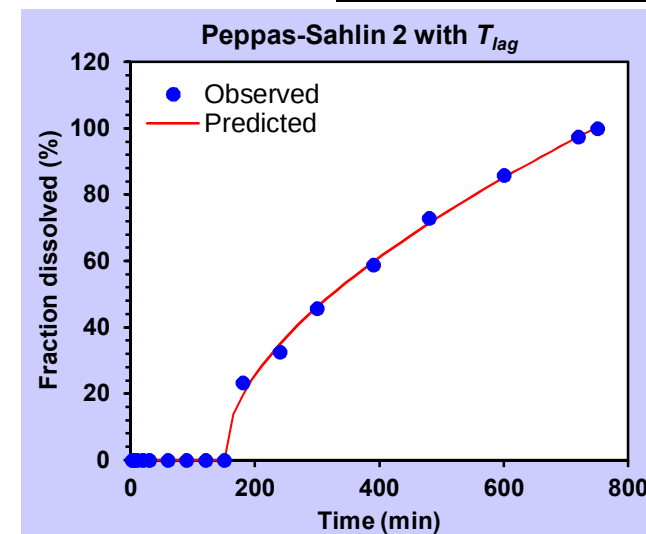
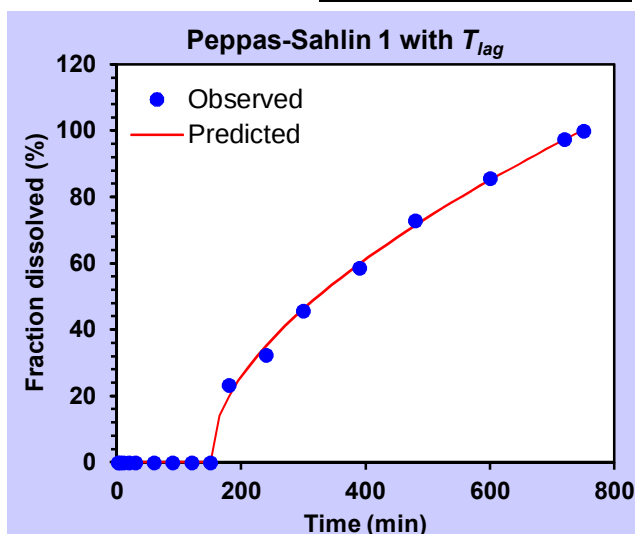
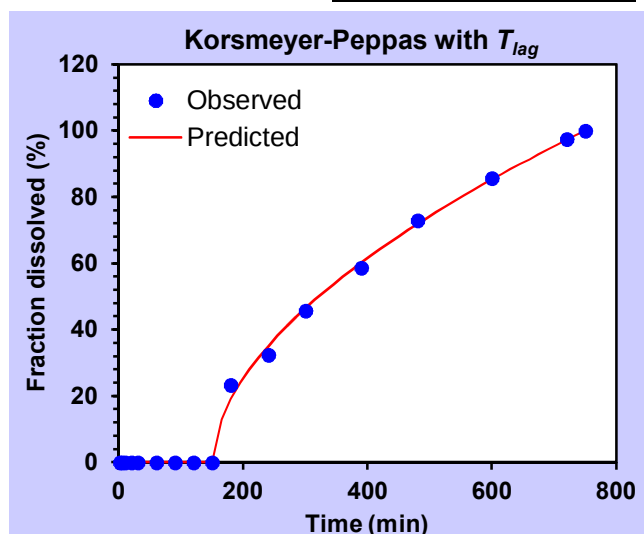
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	2.923	N_observed	17
n	0.552	DF	14
T_{lag}	150.00	R_obs-pre	0.9994
		Rsqr	0.9988
		Rsqr_adj	0.9986
		MSE	2.0943
		MSE_root	1.4472
		Weighting	1
		SS	29.3198
		WSS	29.3198
		AIC	63.4305
		MSC	5.9068

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	2.887	N_observed	17
k2	1.286	DF	13
m	0.321	R_obs-pre	0.9995
T_{lag}	150.0	Rsqr	0.9990
		Rsqr_adj	0.9988
		MSE	1.8205
		MSE_root	1.3493
		Weighting	1
		SS	23.6668
		WSS	23.6668
		AIC	61.7893
		MSC	6.0034

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	3.456	N_observed	17
k2	0.026	DF	14
T_{lag}	150.00	R_obs-pre	0.9995
		Rsqr	0.9989
		Rsqr_adj	0.9988
		MSE	1.7867
		MSE_root	1.3367
		Weighting	1
		SS	25.0134
		WSS	25.0134
		AIC	60.7300
		MSC	6.0657



G5: CA0.75M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

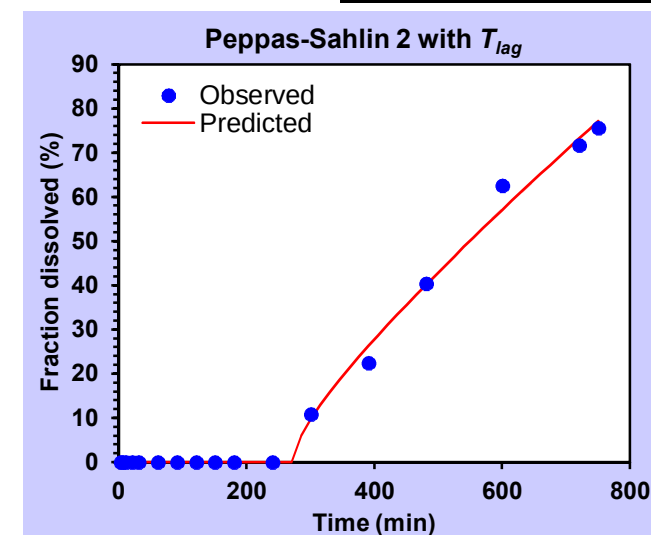
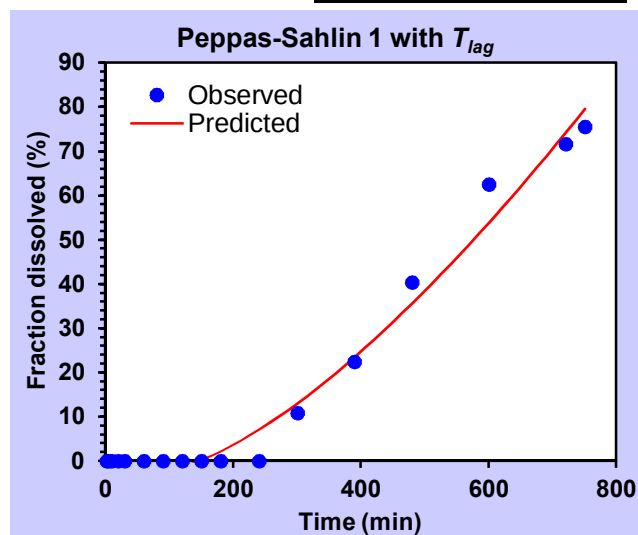
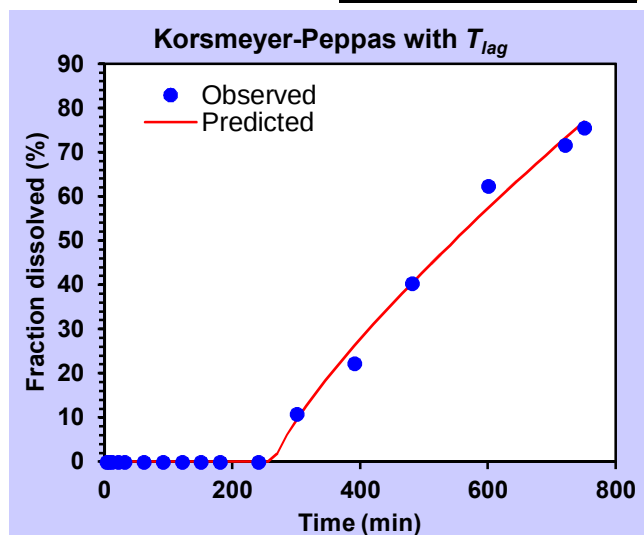
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	0.570	N_observed	17
n	0.793	DF	14
T_{lag}	265.246	R_obs-pre	0.9981
		Rsqr	0.9962
		Rsqr_adj	0.9957
		MSE	3.3337
		MSE_root	1.8259
		Weighting	1
		SS	46.6724
		WSS	46.6724
		AIC	71.3336
		MSC	4.9351

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	-0.384	N_observed	17
k2	0.013	DF	13
m	0.686	R_obs-pre	0.9919
T_{lag}	12.655	Rsqr	0.9837
		Rsqr_adj	0.9799
		MSE	15.4289
		MSE_root	3.9280
		Weighting	1
		SS	200.5760
		WSS	200.5760
		AIC	98.1203
		MSC	3.3595

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	1.349	N_observed	17
k2	0.100	DF	14
T_{lag}	272.821	R_obs-pre	0.9979
		Rsqr	0.9959
		Rsqr_adj	0.9953
		MSE	3.6084
		MSE_root	1.8996
		Weighting	1
		SS	50.5172
		WSS	50.5172
		AIC	72.6793
		MSC	4.8560



G6: CA1C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

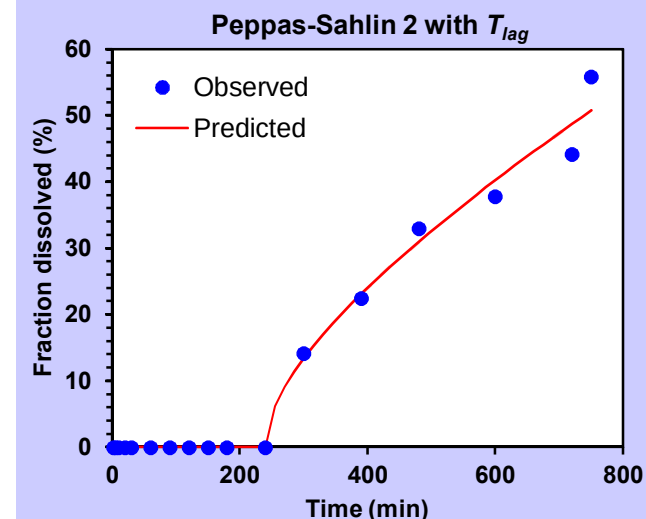
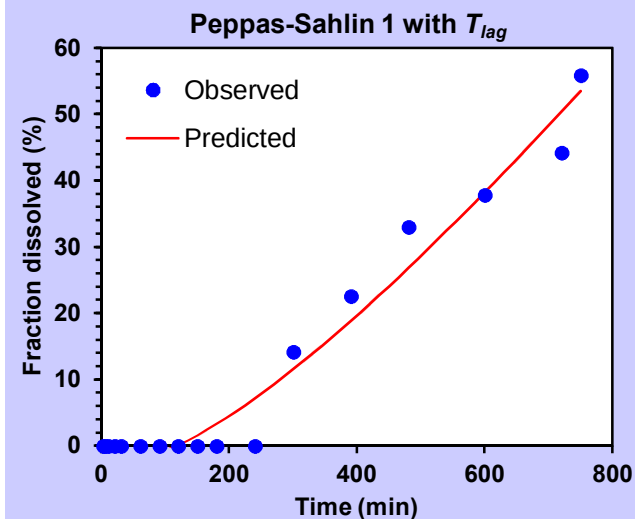
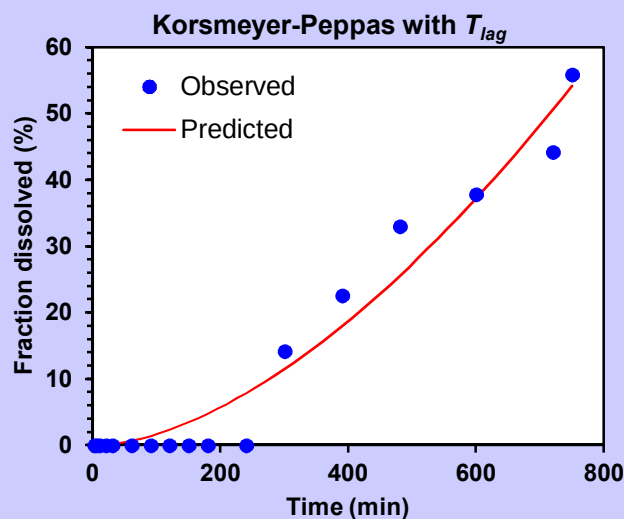
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	0.001	N_observed	17
n	1.667	DF	14
T_{lag}	6.647	R_obs-pre	0.9803
		Rsqr	0.9595
		Rsqr_adj	0.9537
		MSE	16.7076
		MSE_root	4.0875
		Weighting	1
		SS	233.9071
		WSS	233.9071
		AIC	98.7337
		MSC	2.5189

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	-0.482	N_observed	17
k2	0.033	DF	13
m	0.589	R_obs-pre	0.9848
T_{lag}	19.768	Rsqr	0.9698
		Rsqr_adj	0.9628
		MSE	13.4298
		MSE_root	3.6647
		Weighting	1
		SS	174.5872
		WSS	174.5872
		AIC	95.7612
		MSC	2.6938

Peppas-Sahlin 2 with T_{lag}

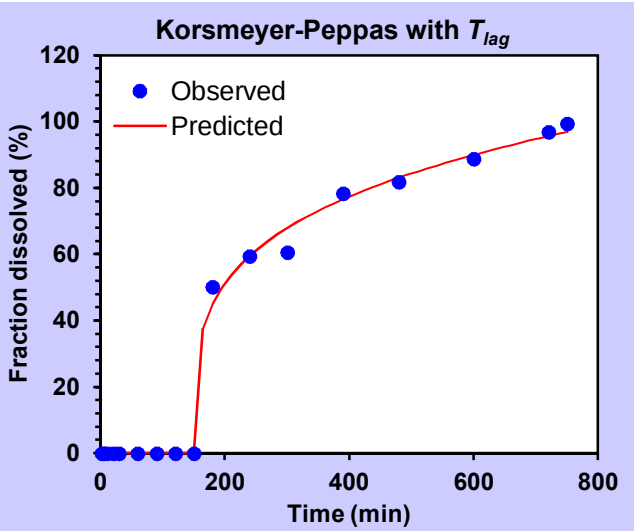
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	1.469	N_observed	17
k2	0.034	DF	14
T_{lag}	240.000	R_obs-pre	0.9950
		Rsqr	0.9900
		Rsqr_adj	0.9885
		MSE	4.1461
		MSE_root	2.0362
		Weighting	1
		SS	58.0456
		WSS	58.0456
		AIC	75.0409
		MSC	3.9126



G7: CA1M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

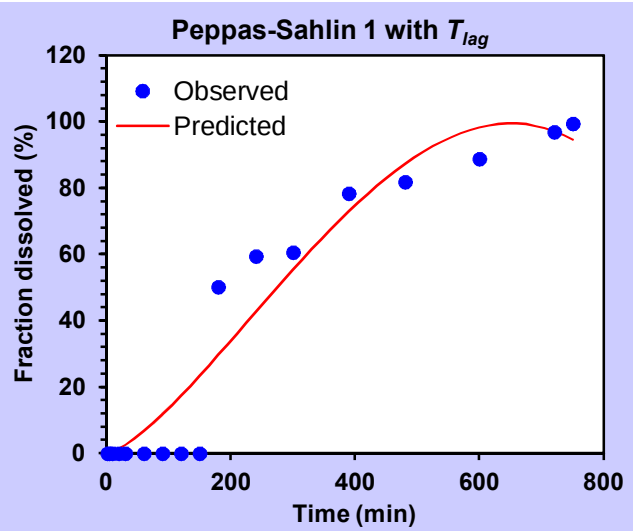
Korsmeyer-Peppas with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	18.868	N_observed	17
n	0.256	DF	14
T_{lag}	150.000	R_obs-pre	0.9983
		Rsqr	0.9966
		Rsqr_adj	0.9961
		MSE	6.7486
		MSE_root	2.5978
		Weighting	1
		SS	94.4802
		WSS	94.4802
		AIC	83.3226
		MSC	4.7571



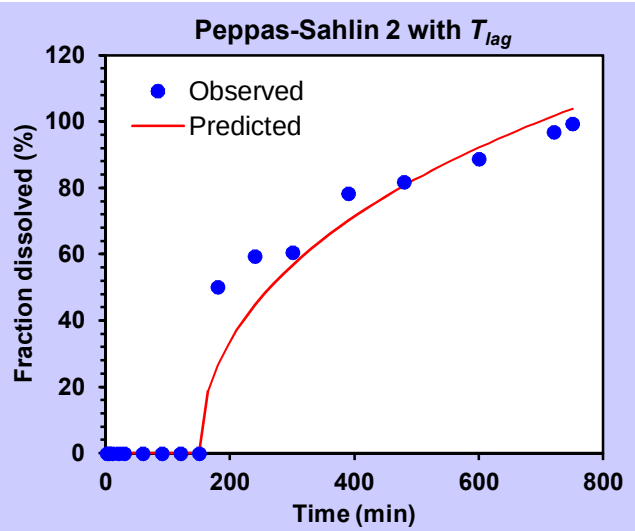
Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	0.021	N_observed	17
k2	0.000	DF	13
m	1.415	R_obs-pre	0.9656
T_{lag}	-0.436	Rsqr	0.9292
		Rsqr_adj	0.9129
		MSE	149.5708
		MSE_root	12.2299
		Weighting	1
		SS	1944.4206
		WSS	1944.4206
		AIC	136.7362
		MSC	1.6151



Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	5.038	N_observed	17
k2	-0.032	DF	14
T_{lag}	150.000	R_obs-pre	0.9851
		Rsqr	0.9671
		Rsqr_adj	0.9624
		MSE	64.4842
		MSE_root	8.0302
		Weighting	1
		SS	902.7794
		WSS	902.7794
		AIC	121.6931
		MSC	2.5000



G8: GM0.5C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

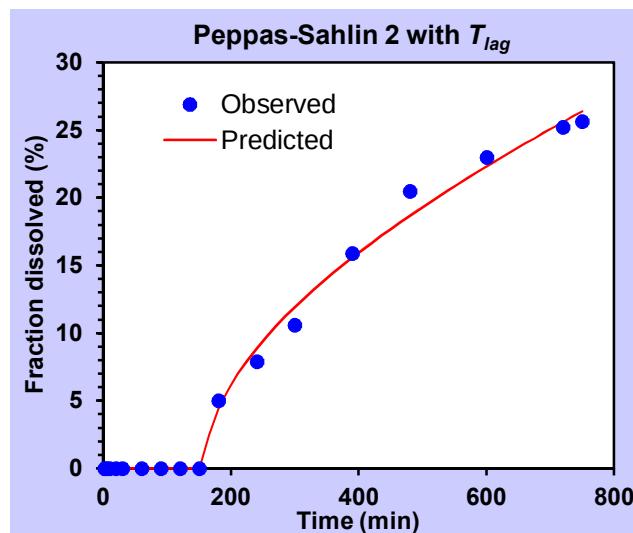
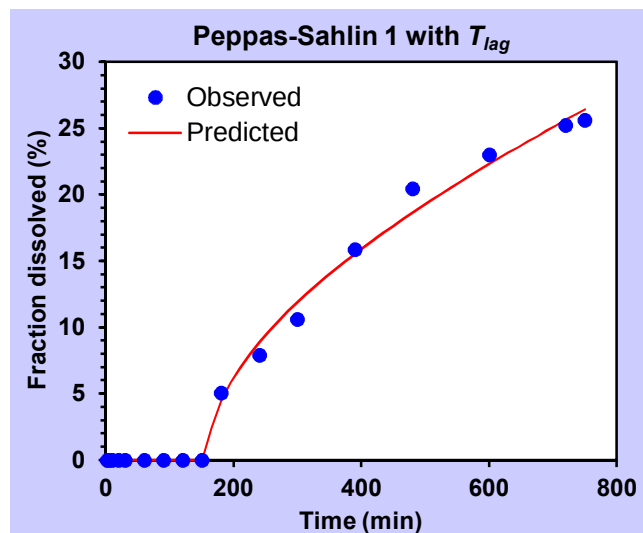
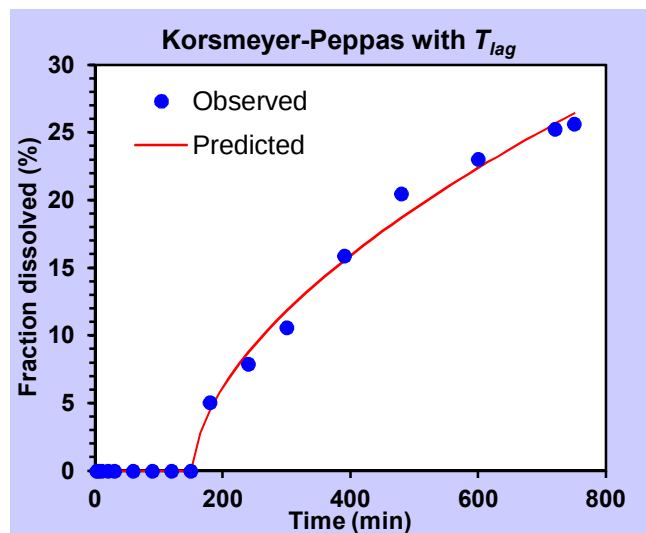
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	0.677	N_observed	17
n	0.573	DF	14
T_{lag}	152.871	R_obs-pre	0.9979
		Rsqr	0.9957
		Rsqr_adj	0.9951
		MSE	0.5026
		MSE_root	0.7090
		Weighting	1
		SS	7.0369
		WSS	7.0369
		AIC	39.1700
		MSC	4.6852

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	1.028	N_observed	17
k2	0.020	DF	13
m	0.460	R_obs-pre	0.9976
T_{lag}	159.109	Rsqr	0.9953
		Rsqr_adj	0.9942
		MSE	0.5972
		MSE_root	0.7728
		Weighting	1
		SS	7.7630
		WSS	7.7630
		AIC	42.8392
		MSC	4.4694

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	0.923	N_observed	17
k2	0.007	DF	14
T_{lag}	158.236	R_obs-pre	0.9977
		Rsqr	0.9954
		Rsqr_adj	0.9947
		MSE	0.5414
		MSE_root	0.7358
		Weighting	1
		SS	7.5796
		WSS	7.5796
		AIC	40.4328
		MSC	4.6109



G9: GM0.5M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

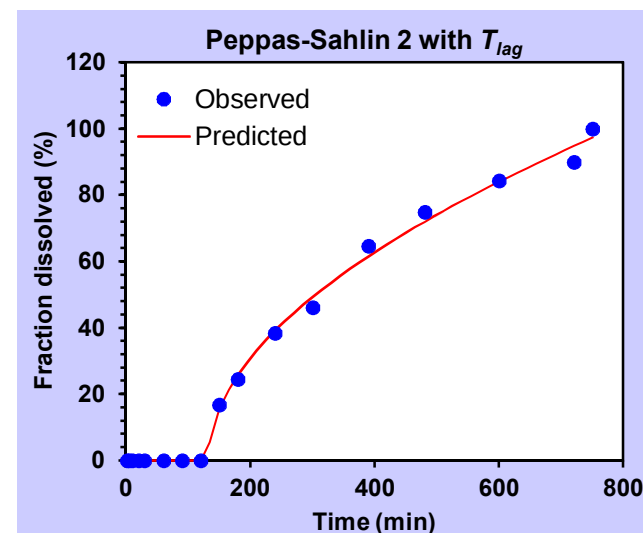
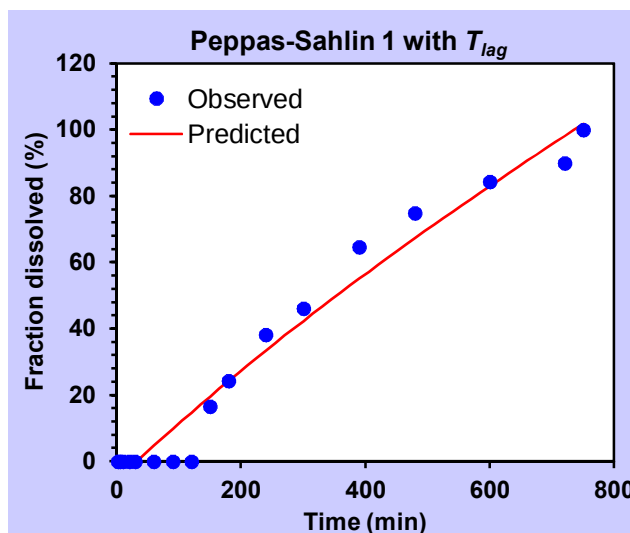
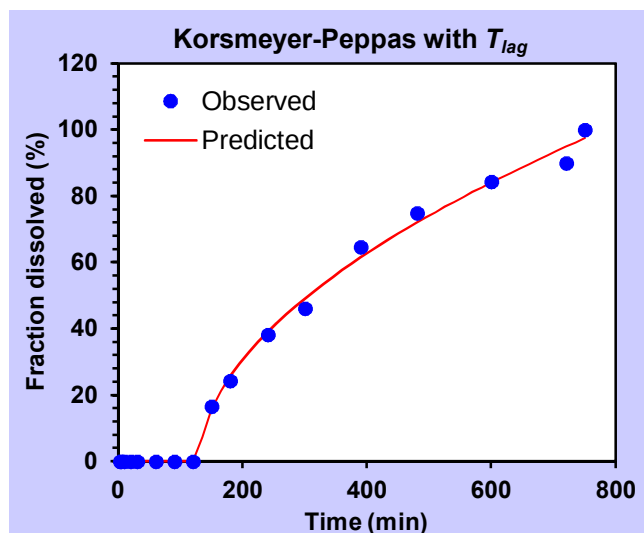
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	3.264	N_observed	17
n	0.528	DF	14
T_{lag}	130.442	R_obs-pre	0.9986
		Rsqr	0.9972
		Rsqr_adj	0.9968
		MSE	4.4032
		MSE_root	2.0984
		Weighting	1
		SS	61.6443
		WSS	61.6443
		AIC	76.0635
		MSC	5.0939

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	-13.591	N_observed	17
k2	3.045	DF	13
m	0.317	R_obs-pre	0.9850
T_{lag}	-79.897	Rsqr	0.9702
		Rsqr_adj	0.9633
		MSE	51.3382
		MSE_root	7.1651
		Weighting	1
		SS	667.3960
		WSS	667.3960
		AIC	118.5575
		MSC	2.5942

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	3.702	N_observed	17
k2	0.009	DF	14
T_{lag}	132.782	R_obs-pre	0.9986
		Rsqr	0.9972
		Rsqr_adj	0.9968
		MSE	4.5236
		MSE_root	2.1269
		Weighting	1
		SS	63.3301
		WSS	63.3301
		AIC	76.5221
		MSC	5.0669



G10: GM0.75C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

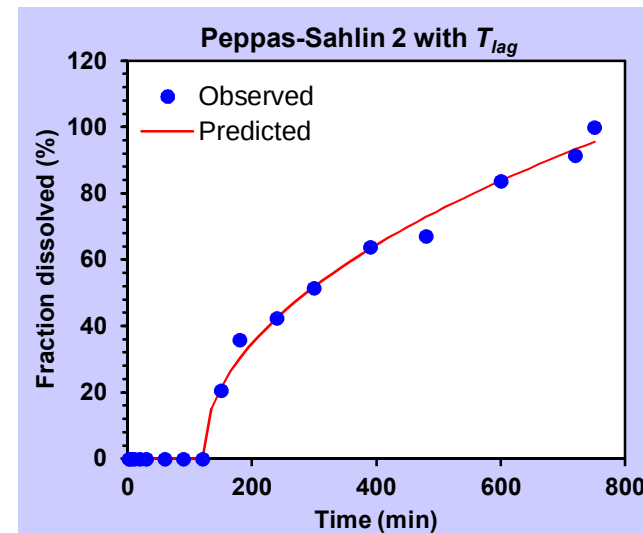
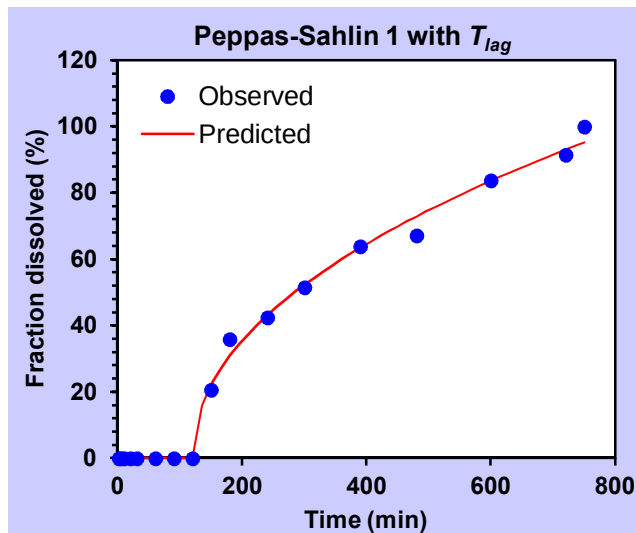
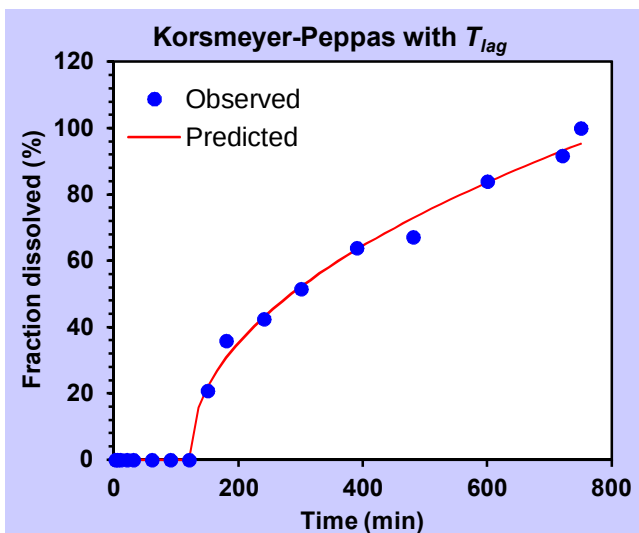
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	4.305	N_observed	17
n	0.481	DF	14
T_{lag}	120.000	R_obs-pre	0.9981
		Rsqr	0.9962
		Rsqr_adj	0.9956
		MSE	5.9842
		MSE_root	2.4463
		Weighting	1
		SS	83.7791
		WSS	83.7791
		AIC	81.2791
		MSC	4.7179

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	-0.041	N_observed	17
k2	4.385	DF	13
m	0.239	R_obs-pre	0.9981
T_{lag}	120.000	Rsqr	0.9962
		Rsqr_adj	0.9953
		MSE	6.4539
		MSE_root	2.5404
		Weighting	1
		SS	83.9002
		WSS	83.9002
		AIC	83.3037
		MSC	4.5988

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	3.951	N_observed	17
k2	-0.006	DF	14
T_{lag}	120.000	R_obs-pre	0.9980
		Rsqr	0.9960
		Rsqr_adj	0.9955
		MSE	6.2257
		MSE_root	2.4951
		Weighting	1
		SS	87.1597
		WSS	87.1597
		AIC	81.9516
		MSC	4.6784



G11: GM0.75M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

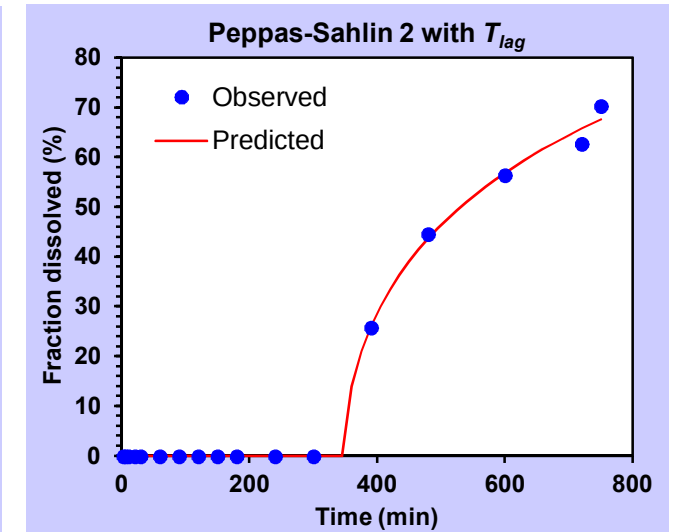
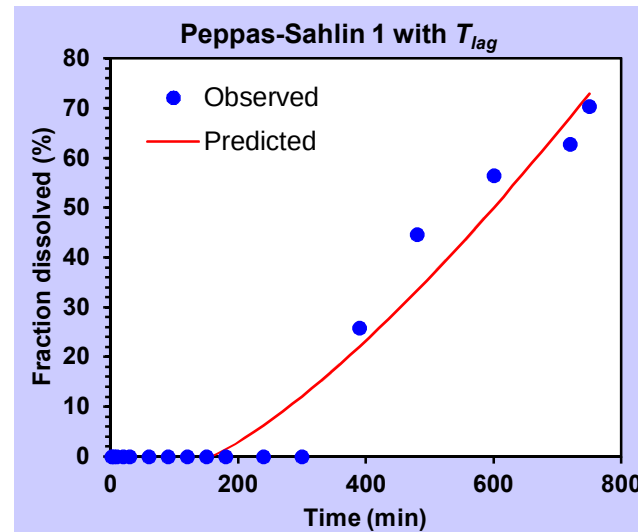
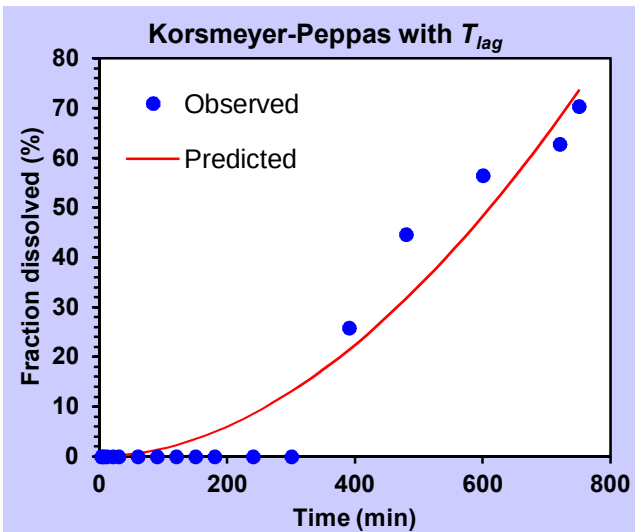
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	0.000	N_observed	17
n	1.876	DF	14
T_{lag}	1.803	R_obs-pre	0.9741
		Rsqr	0.9462
		Rsqr_adj	0.9385
		MSE	41.3279
		MSE_root	6.4287
		Weighting	1
		SS	578.5913
		WSS	578.5913
		AIC	114.1301
		MSC	2.2677

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	-0.609	N_observed	17
k2	0.029	DF	13
m	0.626	R_obs-pre	0.9800
T_{lag}	30.013	Rsqr	0.9603
		Rsqr_adj	0.9512
		MSE	32.8327
		MSE_root	5.7300
		Weighting	1
		SS	426.8247
		WSS	426.8247
		AIC	110.9583
		MSC	2.4542

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	4.403	N_observed	17
k2	-0.051	DF	14
T_{lag}	349.090	R_obs-pre	0.9992
		Rsqr	0.9984
		Rsqr_adj	0.9981
		MSE	1.2628
		MSE_root	1.1237
		Weighting	1
		SS	17.6793
		WSS	17.6793
		AIC	54.8308
		MSC	5.7559



G12: GM1C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

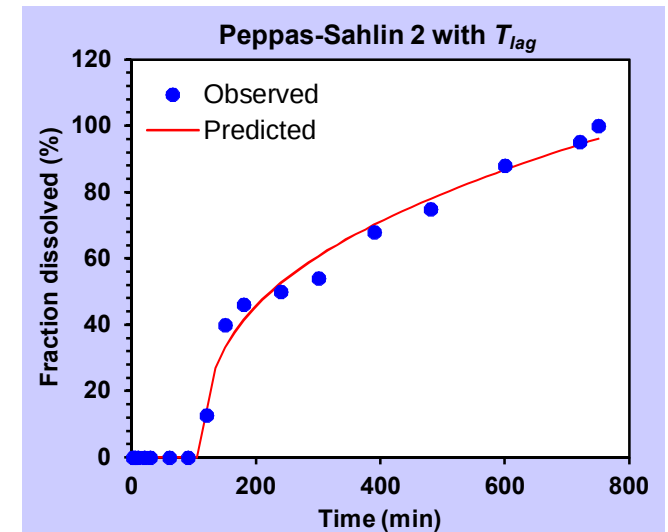
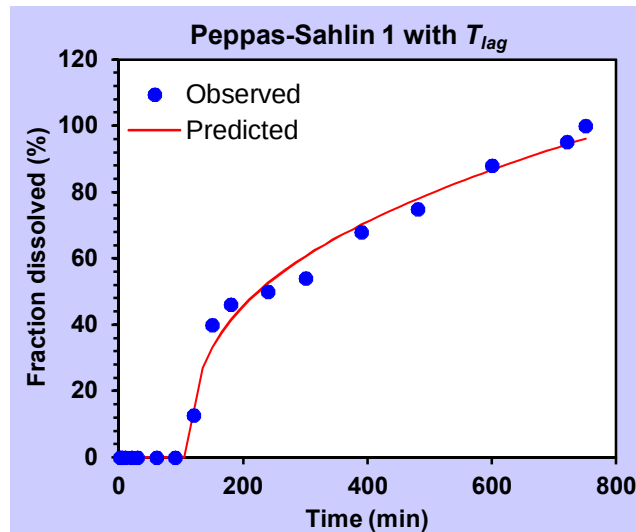
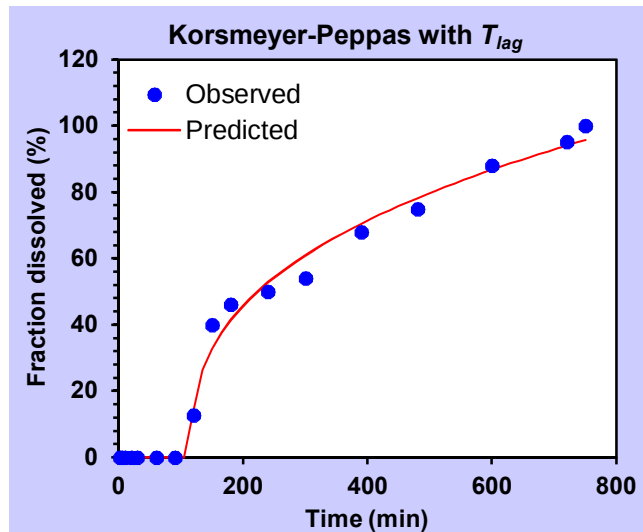
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	9.173	N_observed	17
n	0.364	DF	14
T_{lag}	116.616	R_obs-pre	0.9963
		Rsqr	0.9926
		Rsqr_adj	0.9916
		MSE	12.1775
		MSE_root	3.4896
		Weighting	1
		SS	170.4856
		WSS	170.4856
		AIC	93.3571
		MSC	4.0179

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	10.179	N_observed	17
k2	0.823	DF	13
m	0.285	R_obs-pre	0.9967
T_{lag}	117.893	Rsqr	0.9935
		Rsqr_adj	0.9920
		MSE	11.5644
		MSE_root	3.4006
		Weighting	1
		SS	150.3372
		WSS	150.3372
		AIC	93.2190
		MSC	4.0260

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	4.787	N_observed	17
k2	-0.038	DF	14
T_{lag}	103.196	R_obs-pre	0.9950
		Rsqr	0.9900
		Rsqr_adj	0.9886
		MSE	16.4052
		MSE_root	4.0503
		Weighting	1
		SS	229.6732
		WSS	229.6732
		AIC	98.4232
		MSC	3.7198

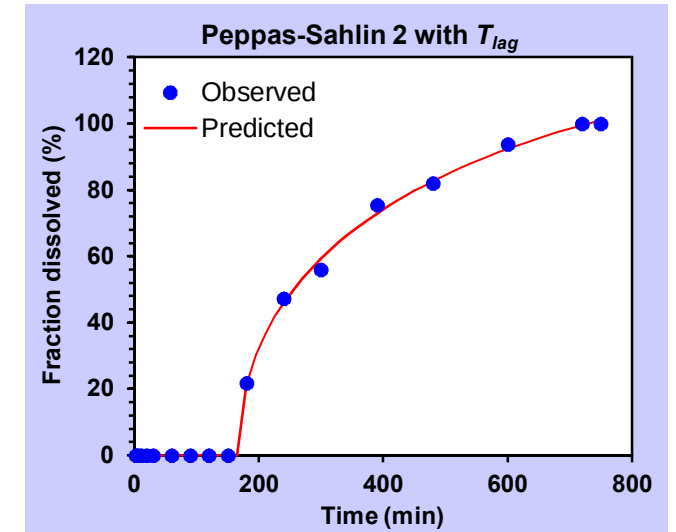
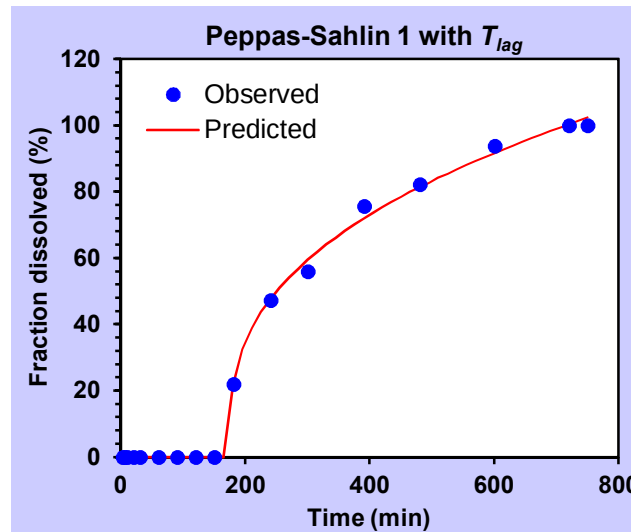
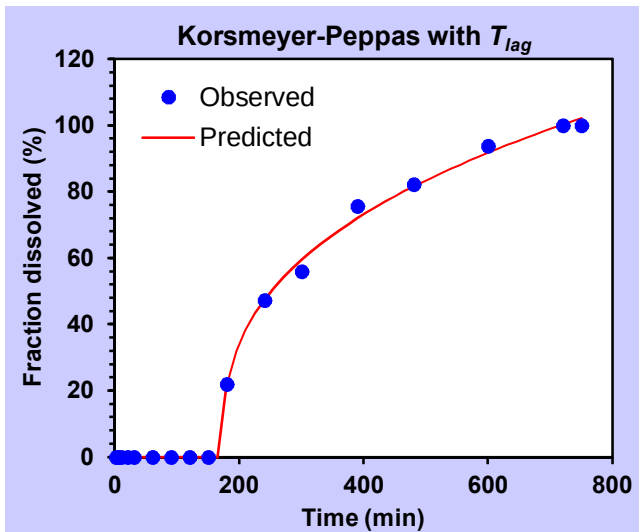


G13: GM1M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}			
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	10.493	N_observed	17
n	0.358	DF	14
T_{lag}	172.444	R_obs-pre	0.9994
		Rsqr	0.9987
		Rsqr_adj	0.9986
		MSE	2.4616
		MSE_root	1.5689
		Weighting	1
		SS	34.4621
		WSS	34.4621
		AIC	66.1776
		MSC	5.8594

Peppas-Sahlin 1 with T_{lag}			
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	8.950	N_observed	17
k2	3.857	DF	13
m	0.223	R_obs-pre	0.9993
T_{lag}	174.227	Rsqr	0.9987
		Rsqr_adj	0.9984
		MSE	2.8302
		MSE_root	1.6823
		Weighting	1
		SS	36.7931
		WSS	36.7931
		AIC	69.2903
		MSC	5.6763

Peppas-Sahlin 2 with T_{lag}			
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	5.997	N_observed	17
k2	-0.075	DF	14
T_{lag}	165.506	R_obs-pre	0.9996
		Rsqr	0.9991
		Rsqr_adj	0.9990
		MSE	1.6835
		MSE_root	1.2975
		Weighting	1
		SS	23.5689
		WSS	23.5689
		AIC	59.7188
		MSC	6.2394



G14

G14: SA0.5C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

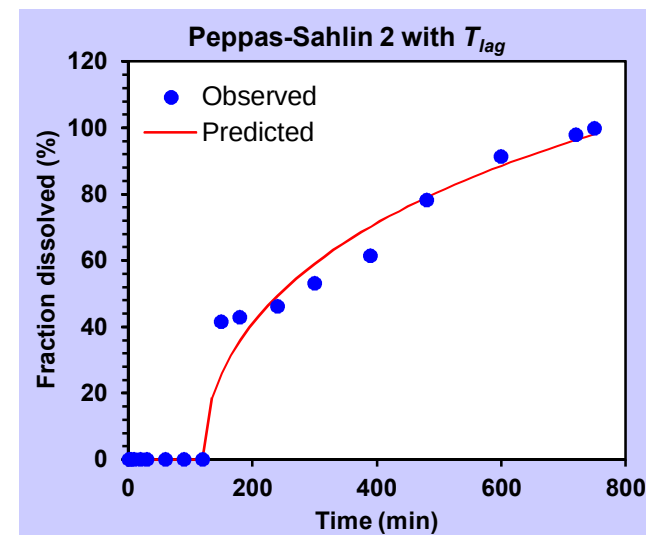
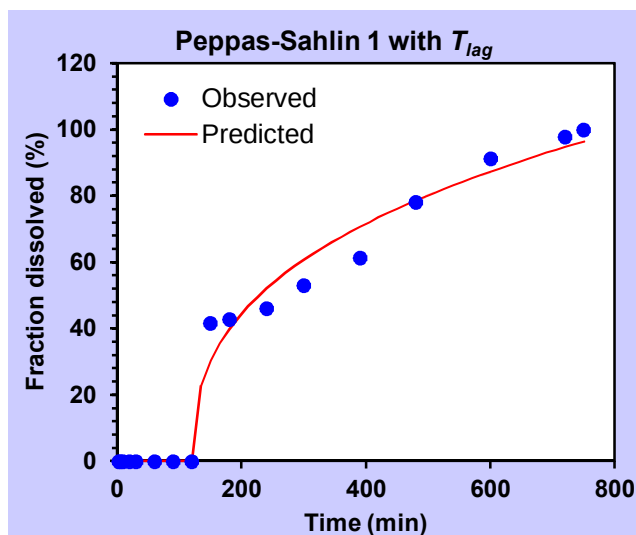
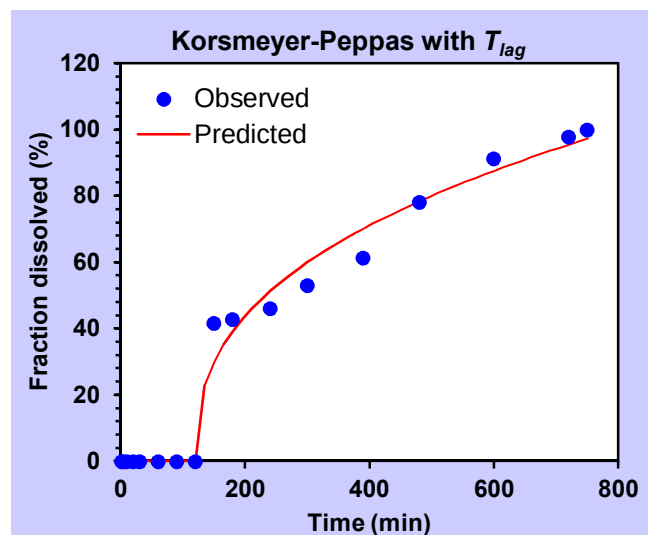
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	8.114	N_observed	17
n	0.385	DF	14
T_{lag}	120.000	R_obs-pre	0.9933
		Rsqr	0.9866
		Rsqr_adj	0.9847
		MSE	23.2034
		MSE_root	4.8170
		Weighting	1
		SS	324.8478
		WSS	324.8478
		AIC	104.3171
		MSC	3.4020

Peppas-Sahlin 1 with T_{lag}

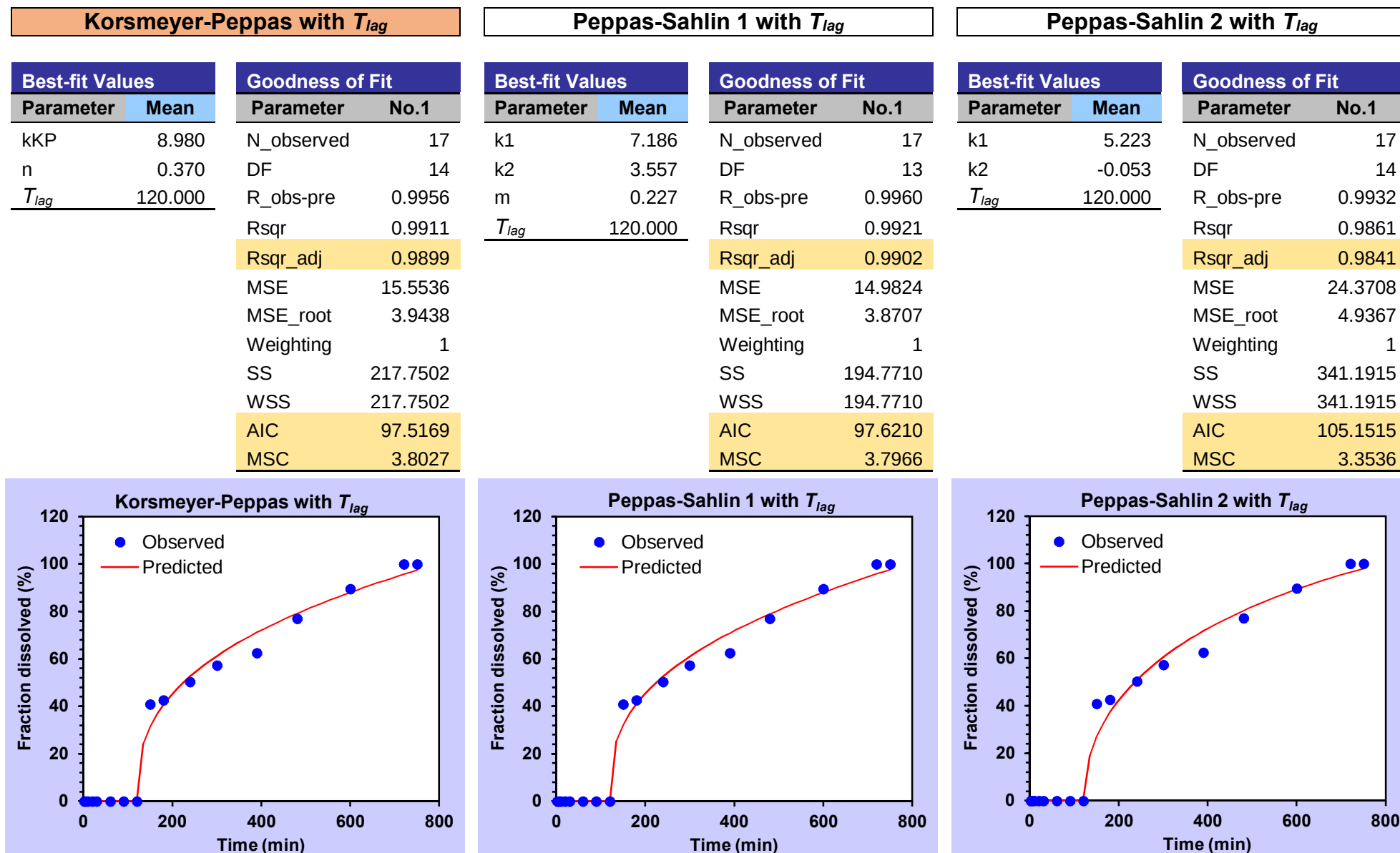
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	-9.869	N_observed	17
k2	16.076	DF	13
m	0.158	R_obs-pre	0.9926
T_{lag}	119.945	Rsqr	0.9853
		Rsqr_adj	0.9819
		MSE	27.5105
		MSE_root	5.2450
		Weighting	1
		SS	357.6362
		WSS	357.6362
		AIC	107.9518
		MSC	3.1882

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	4.979	N_observed	17
k2	-0.043	DF	14
T_{lag}	120.000	R_obs-pre	0.9913
		Rsqr	0.9822
		Rsqr_adj	0.9797
		MSE	30.8554
		MSE_root	5.5548
		Weighting	1
		SS	431.9757
		WSS	431.9757
		AIC	109.1623
		MSC	3.1170



G15: SA0.5M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow



G16: SA0.75C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

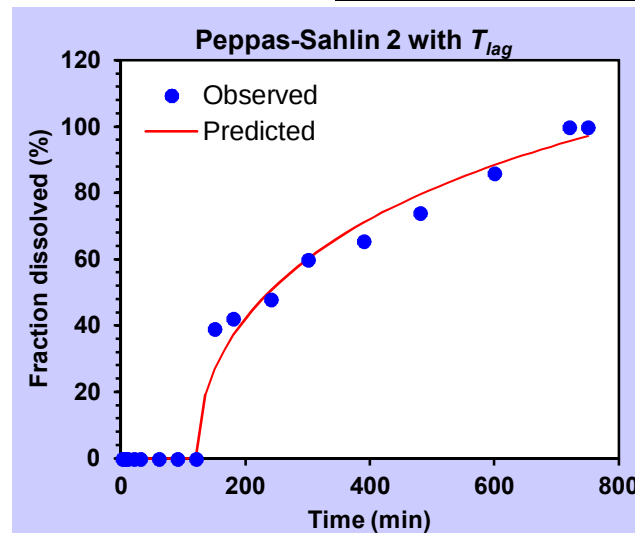
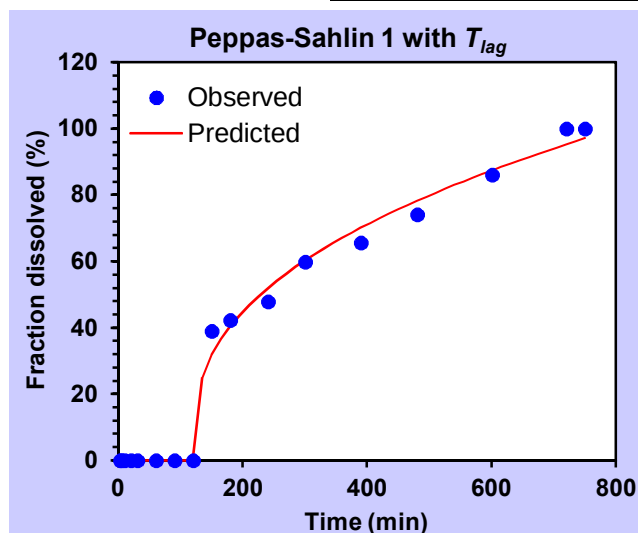
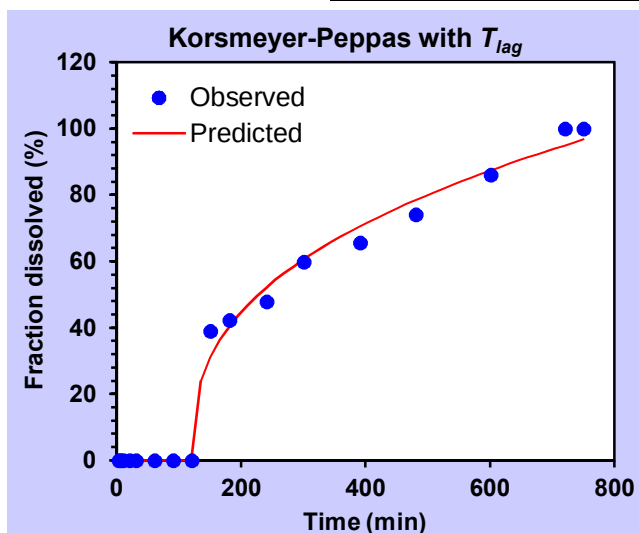
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	8.766	N_observed	17
n	0.373	DF	14
T_{lag}	120.000	R_obs-pre	0.9966
		Rsqr	0.9931
		Rsqr_adj	0.9921
		MSE	11.8976
		MSE_root	3.4493
		Weighting	1
		SS	166.5663
		WSS	166.5663
		AIC	92.9617
		MSC	4.0556

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	8.131	N_observed	17
k2	2.486	DF	13
m	0.244	R_obs-pre	0.9970
T_{lag}	120.000	Rsqr	0.9941
		Rsqr_adj	0.9927
		MSE	10.9933
		MSE_root	3.3156
		Weighting	1
		SS	142.9124
		WSS	142.9124
		AIC	92.3579
		MSC	4.0911

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	5.198	N_observed	17
k2	-0.053	DF	14
T_{lag}	120.000	R_obs-pre	0.9944
		Rsqr	0.9886
		Rsqr_adj	0.9869
		MSE	19.7076
		MSE_root	4.4393
		Weighting	1
		SS	275.9068
		WSS	275.9068
		AIC	101.5411
		MSC	3.5509



G17: SA0.75M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

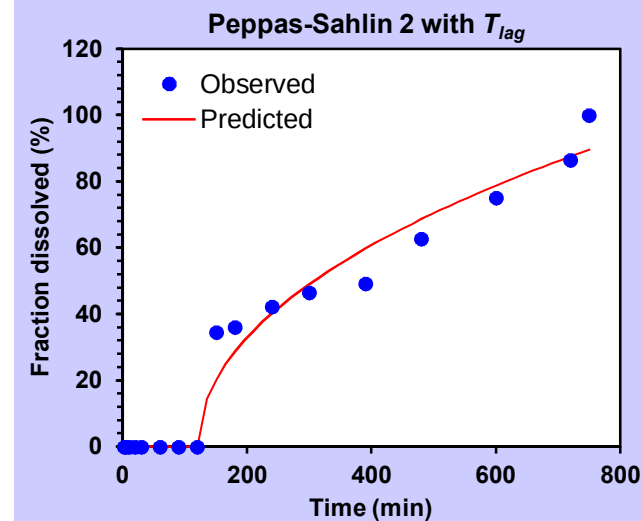
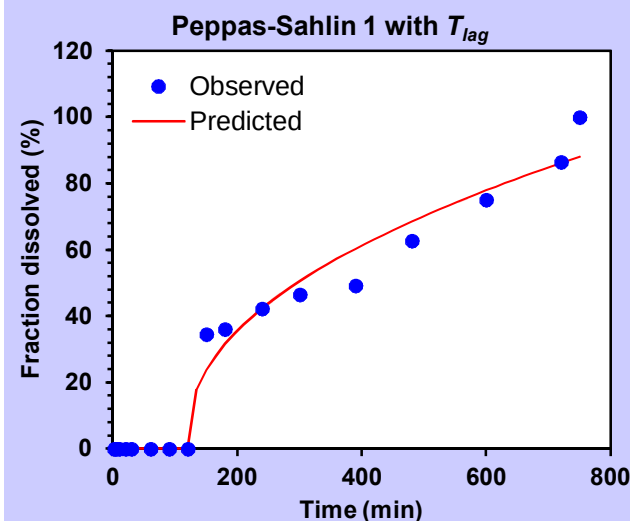
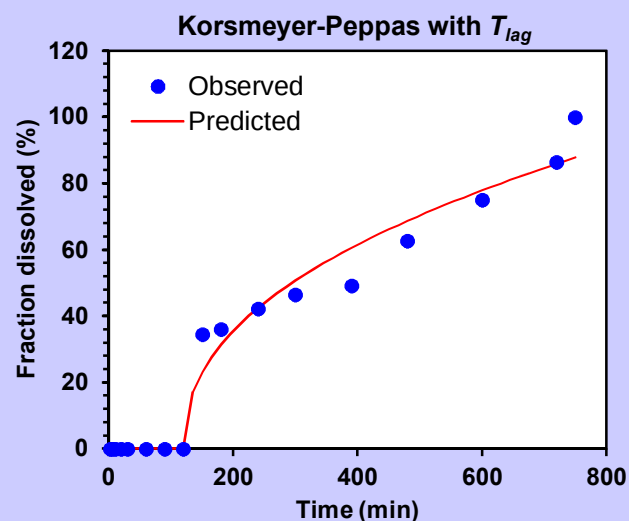
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	5.269	N_observed	17
n	0.436	DF	14
T_{lag}	120.000	R_obs-pre	0.9872
		Rsqr	0.9745
		Rsqr_adj	0.9709
		MSE	35.0721
		MSE_root	5.9222
		Weighting	1
		SS	491.0101
		WSS	491.0101
		AIC	111.3399
		MSC	2.7894

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	4.398	N_observed	17
k2	2.216	DF	13
m	0.261	R_obs-pre	0.9881
T_{lag}	120.000	Rsqr	0.9763
		Rsqr_adj	0.9712
		MSE	35.1581
		MSE_root	5.9294
		Weighting	1
		SS	457.0553
		WSS	457.0553
		AIC	111.1217
		MSC	2.7434

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	3.767	N_observed	17
k2	-0.008	DF	14
T_{lag}	120.000	R_obs-pre	0.9864
		Rsqr	0.9723
		Rsqr_adj	0.9683
		MSE	38.1578
		MSE_root	6.1772
		Weighting	1
		SS	534.2097
		WSS	534.2097
		AIC	112.7734
		MSC	2.7051



G18: SA1C1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

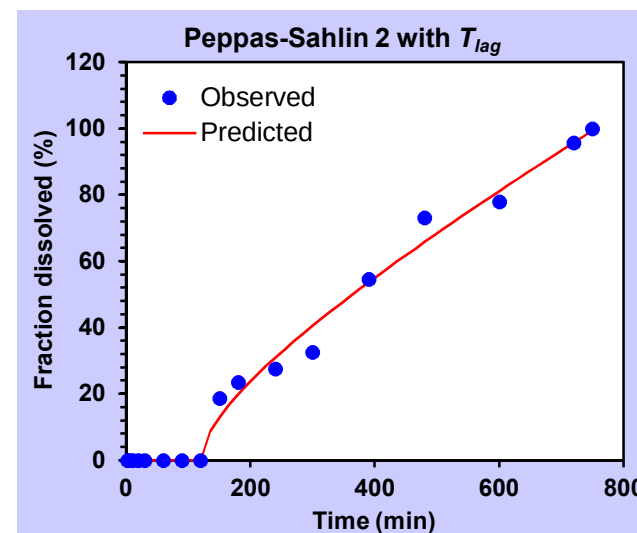
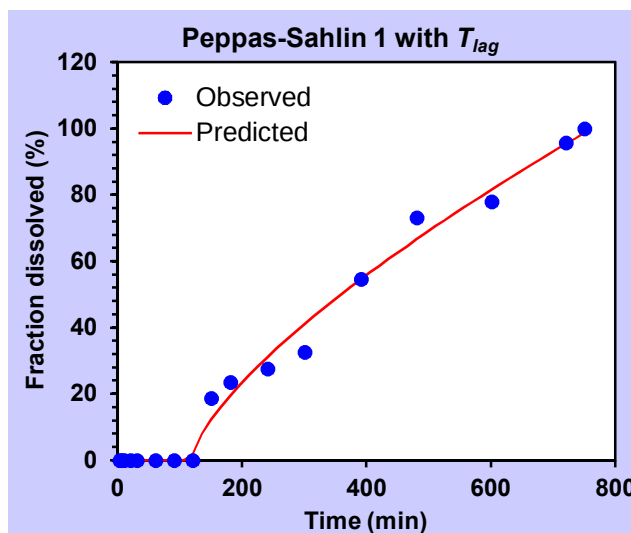
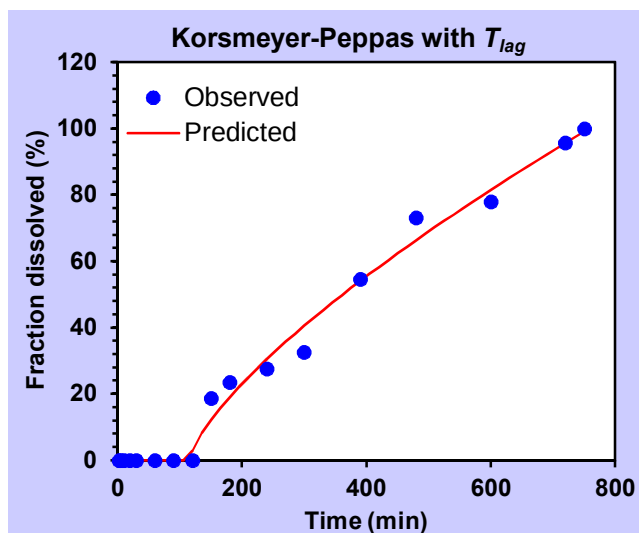
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kH	3.741	N_observed	17
T_{lag}	138.971	DF	15
		R_obs-pre	0.9885
		Rsqr	0.9767
		Rsqr_adj	0.9752
		MSE	33.1519
		MSE_root	5.7578
		Weighting	1
		SS	497.2779
		WSS	497.2779
		AIC	109.5555
		MSC	3.1276

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	0.433	N_observed	17
k2	0.893	DF	13
m	0.361	R_obs-pre	0.9952
T_{lag}	118.427	Rsqr	0.9904
		Rsqr_adj	0.9882
		MSE	15.7659
		MSE_root	3.9706
		Weighting	1
		SS	204.9561
		WSS	204.9561
		AIC	98.4875
		MSC	3.7786

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	1.956	N_observed	17
k2	0.080	DF	14
T_{lag}	120.000	R_obs-pre	0.9956
		Rsqr	0.9912
		Rsqr_adj	0.9900
		MSE	13.3800
		MSE_root	3.6579
		Weighting	1
		SS	187.3203
		WSS	187.3203
		AIC	94.9579
		MSC	3.9862



G19: SA1M1 dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}

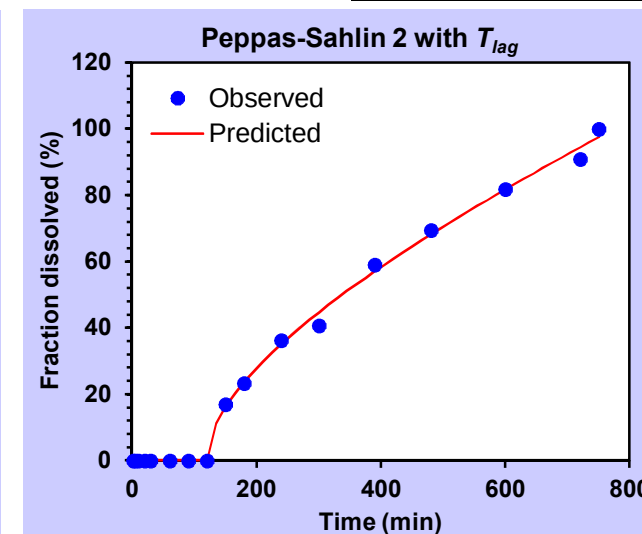
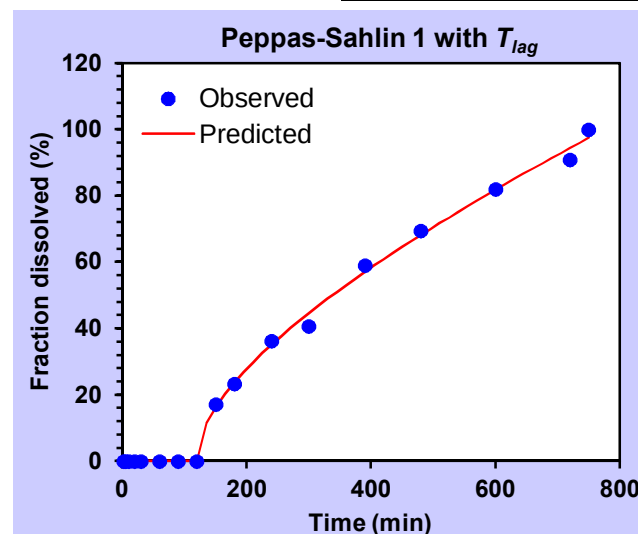
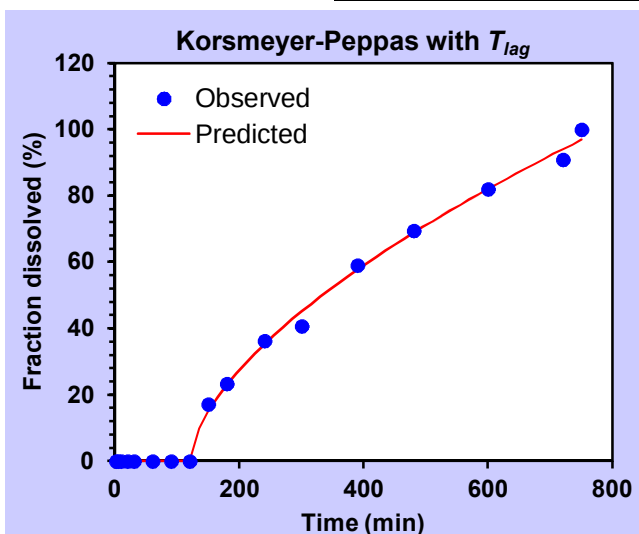
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	1.903	N_observed	17
n	0.610	DF	14
T_{lag}	119.999	R_obs-pre	0.9989
		Rsqr	0.9979
		Rsqr_adj	0.9976
		MSE	3.2118
		MSE_root	1.7922
		Weighting	1
		SS	44.9657
		WSS	44.9657
		AIC	70.7003
		MSC	5.3757

Peppas-Sahlin 1 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	2.889	N_observed	17
k2	0.296	DF	13
m	0.408	R_obs-pre	0.9990
T_{lag}	120.000	Rsqr	0.9981
		Rsqr_adj	0.9976
		MSE	3.1311
		MSE_root	1.7695
		Weighting	1
		SS	40.7047
		WSS	40.7047
		AIC	71.0078
		MSC	5.3576

Peppas-Sahlin 2 with T_{lag}

Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	2.704	N_observed	17
k2	0.047	DF	14
T_{lag}	120.000	R_obs-pre	0.9990
		Rsqr	0.9981
		Rsqr_adj	0.9978
		MSE	2.9393
		MSE_root	1.7145
		Weighting	1
		SS	41.1508
		WSS	41.1508
		AIC	69.1931
		MSC	5.4643



G20: Coartem® dissolution profile modelling. Best fit model highlighted in orange, three criteria values indicated in yellow

Korsmeyer-Peppas with T_{lag}			
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
kKP	11.109	N_observed	17
n	0.315	DF	14
T_{lag}	85.261	R_obs-pre	0.9946
		Rsqr	0.9892
		Rsqr_adj	0.9876
		MSE	14.3480
		MSE_root	3.7879
		Weighting	1
		SS	200.8720
		WSS	200.8720
		AIC	96.1454
		MSC	3.5446

Peppas-Sahlin 1 with T_{lag}			
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	6.550	N_observed	17
k2	-0.117	DF	13
m	0.461	R_obs-pre	0.9952
T_{lag}	80.187	Rsqr	0.9904
		Rsqr_adj	0.9882
		MSE	13.6479
		MSE_root	3.6943
		Weighting	1
		SS	177.4233
		WSS	177.4233
		AIC	96.0352
		MSC	3.5511

Peppas-Sahlin 2 with T_{lag}			
Best-fit Values		Goodness of Fit	
Parameter	Mean	Parameter	No.1
k1	5.421	N_observed	17
k2	-0.084	DF	14
T_{lag}	77.553	R_obs-pre	0.9951
		Rsqr	0.9903
		Rsqr_adj	0.9889
		MSE	12.8478
		MSE_root	3.5844
		Weighting	1
		SS	179.8698
		WSS	179.8698
		AIC	94.2680
		MSC	3.6551

