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

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ABSTRACT

Malaria is one of the most important parasitic diseases as well as one of the most life-threatening diseases known to man. This vector-borne disease caused by *Plasmodium spp*., is responsible for over 438 000 deaths globally, of which 90% of these deaths occur in Africa (WHO, 2017; Sokhna *et al*., 2013; Rosenthal, 2012). The intricate life cycle of the malaria parasite offers numerous attack-points for antimalarial drugs. Rapidly spreading resistance against antimalarial drugs, especially chloroquine, mefloquine, and pyrimethamine-sulphadoxine, emphasises the necessity for new alternatives or alteration of existing antimalarial drugs (Nosten & Brasseur, 2002).

Artemisone, an artemisinin derivative, signifies a new class of antimalarial drugs that is an effective blood schizontocide against strains of drug-resistant *Plasmodium falciparum* malaria. On the other hand, lumefantrine is an antimalarial drug active against the asexual stages of the parasitic reproduction (Nostem *et al.,* 2012). Artemisinin-based combination therapies (ACTs) have been recommended as first-line treatment for uncomplicated *P. falciparum* in countries where malaria is endemic. ACT allows for simultaneous administration of longer- and shorter-acting drugs that have different mechanisms of action, subsequently preventing, or delaying the development of resistance (Basu & Sahi, 2017; Nosten & Brasseur, 2002). By incorporating 80 mg artemisone and 120 mg lumefantrine in a fixed-dose combination, a new ACT may be formulated.

Lumefantrine was chosen as the long acting drug that has poor aqueous solubility, is highly lipophilic and depicts erratic absorption, leading to poor bioavailability (Garg *et al.,* 2017). Artemisone, the short acting drug in this study, also depicts insufficient aqueous solubility as well as poor bioavailability (Pawar *et al.,* 2016; White, 2008). To overcome these drawbacks, lipid-matrix tablets were formulated by means of the hot-melt method.

Lipid matrix formulations attracted significant attention over the past years, especially in cases where drugs with high lipophilicity are to be incorporated into various dosage forms. Lipid based formulations have proven useful in increasing the absorption, and consequently enhancing the bioavailability (Xia *et al.,* 2014). Modified release of the drugs from the lipid-matrices is another advantage of incorporating drugs into a lipid-matrix (Nisha *et al.,* 2012). Drug releases from lipid-matrices occur by means of erosion and/or pore diffusion – in this study pore diffusion is more prevalent. The lipid forms a coating around the drug particles

and subsequently, pores are formed. These pores aid in the modified release of the drugs included in lipid-matrices (Abd-Elbary *et al.,* 2013; Nisha *et al.,* 2012). Modified release drugs will reduce the blood concentration fluctuations and subsequently frequent dosing (Rajabi-Siahboomi *et al.,* 2013; Abdul *et al.,* 2010; Ishida *et al.,* 2008). In this study, lipid-matrices were formed by utilising hot-melt to incorporate a fixed-dose combination of artemisone and lumefantrine into two selected lipids, i.e. stearic acid (SA) and glycerol monostearate (GM), respectively. Hot-melt can be described as a process where a polymer is melted with continuous stirring in a porcelain mortar on heated water. The chosen drugs are homogenously mixed into the melted polymer and allowed to cool. After the mass solidifies, it is grounded and sieved until the appropriate particle size is achieved (Nikghalb *et al.,* 2012; Kalaiselvan *et al.,* 2006; Obaidat & Obaidat, 2001).

The various formulations were developed in three stages: basic formulation development, employing a factorial design to procure optimised formulations, and assessing the optimised formulations. First, differential weight loss thermograms (DTG) and thermal activity monitor (TAM) analysis were conducted to identify any possible interactions between the active pharmaceutical ingredients (drugs); and the drugs' and excipients. Following, the flow properties of the two drugs, the selected fillers (MicroceLac® 100, RetaLac® and CombiLac®), along with the lipid dispersions were characterised by means of bulk- and tapped density; critical orifice diameter (COD); angle of repose; and flow rate. Furthermore, all formulations were tableted by means of direct compression. The lipid-matrix tablets were assessed and characterised in terms of friability, crushing strength, weight variation and disintegration. A full factorial design was utilised to identify the optimal formulations in terms of their physical properties. Dissolution tests, as well as swelling and erosion experiments, were performed on the optimised formulations and analysed by means of high performance liquid chromatography (HPLC).

Results indicated that the type of lipid, the drug:lipid ratio, the type of filler as well as the concentration (%w/w) in which the lubricant was incorporated into the formulations, influenced the friability, weight variation, crushing strength and disintegration of the lipid-matrix tablets. CombiLac® produced the hardest tablets, followed by MicroceLac® 100, then RetaLac®. MicroceLac® 100 formulations, conversely, depicted the most ideal friability results but illustrated a relatively high average weight variation. RetaLac® displayed the least weight variation, indicating the formulations comprising RetaLac® are the most uniform concerning average weight. The formulations that incorporated stearic acid (1:0.5 ratio) and CombiLac®, was the only formulation that disintegrated in 15 min, thus did not portray modified release, Formulations containing 0.5% w/w magnesium stearate proofed more

acceptable compared to formulations that included 1% w/w lubricant, however, the 0.5% w/w lubricant formulations were aesthetically undesirable. Thus, only the formulations comprising 1% w/w magnesium stearate were included in the optimised formulations.

Artemisone exhibited a delayed release profile from all of the lipid matrix tablet formulations. CombiLac® was deemed unacceptable as artemisone did not display any dissolution from the formulations S1C1, G0.5C1 and G1C1. The formulation S0.5C1 disintegrated in 15 min, thus did not depict modified release. Lumefantrine, conversely, displayed burst release profiles from all of the optimised formulations. Stearic acid illustrated slightly higher percentage dissolution for lumefantrine in comparison with glycerol monostearate, but unfortunately no modified release, whilst the fillers did not play a significant role in the different formulations.

In conclusion, the formulations need further work to be perfected. Formulations comprised MicroceLac® 100 and stearic acid displayed the most delayed release for artremisone and release lumefantrine to a slightly higher extent comparative to the other formulations.

Keywords: malaria, artemisone, lumefantrine, artemisinin-based combination therapy, double fixed-dose combination therapy, lipid-matrix tablets, hot-melt, modified release

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"What gives me the most hope every day is God's grace; knowing that His grace is going to give me the strength for whatever I face, knowing that nothing is a surprise to God."

-Rick Warren

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LIST OF ABBREVIATIONS

ACT: Artemisinin-based Combination Therapy

AIDS: Acquired Immune Deficiency Syndrome

ART: Artemisinin

BP: British Pharmacopoeia

BSC: Biopharmaceutical Classification System

CDC: Centre of Disease Control

COD: Critical Orifice Diameter

CRT: Chloroquine Resistance Transporter

CYP: Cytochrome

DHA: Dihydroartemisinin

DHFR: Dihydrofolate Reductase

DHPS: Dihydropteroate Synthase

DSC: Differential Scanning Calorimetry

DTG: Differential Weight loss Thermograms

FDC: Fixed Dose Combination

GI: Gastrointestinal

GM: Glycerol Monostearate

GMO: Genetically Modified Organism

HME: Hot-melt Extrusion

HMF: Hot-melt Fusion

HPLC: High Performance Liquid Chromatography

HPMC: Hydroxymethylpropylcellulose

IPTp: Intermitted Preventative Treatment during Pregnancy

MDR: Multi-drug Resistant

MDT: Mean dissolution time

NV: No Value

PMNS: Post Malaria Neurological Symptoms

PCR: Polymerase-chain Reaction

RSD: Relative Standard Deviation

SA: Stearic Acid

SD: Standard Deviation

TGA: Thermogravimetry Analysis

TAM: Thermal Activity Monitor

WHO: World Health Organisation

XRPD: X-Ray Powder Diffraction

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUD

1.1.1 Malaria

Malaria is a disease that has been known to man from about 2 700 BC. This disease is caused by a protozoan parasite that belongs to the genus called *Plasmodium. Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale* are the four different types of known human malaria. The most recurrent and the most dangerous of the above-mentioned species is *Plasmodium falciparum*, which is carried by the female *Anopheles* mosquito who is able to transmit the parasite to people through a bite (Cox, 2010). The intensity of transmission is influenced by the length of the lifespan of the mosquito. As the mosquito ages, the parasite develops inside the mosquito and if fully developed, the mosquito will choose to bite humans instead of animals (WHO, 2015b; CDC, 2016a Breman *et al*., 2006).

Malaria is most habitually found in the tropical areas of the world such as Asia, sub-Saharan Africa and parts of South-Africa, India, Central- and South-America. Presently, due to people who travel, malaria as well as infected mosquitos are now also observed in non-malaria areas (Bloland *et al.,* 2000). Africa is the continent with the highest percentage of people exposed to malaria transmission and the highest rate of morbidity and mortality is detected here (Kabaghe *et al.,* 2017; WHO, 2017; Snow *et al.,* 1999).

According to the World Health Organization (WHO), the cases of malaria reported worldwide, fell from 262 million in 2000 to an estimate of 214 million cases in 2015 (WHO, 2015b). Even with the decrease, the number of deaths caused by this parasite is still a great burden for the WHO. Malaria can vary from an uncomplicated disease to a significantly complicated and life-threatening disease. Diagnosing people early can stop uncomplicated malaria from progressing into a more severe state. Symptoms of uncomplicated malaria include fever, chills, sweats, loss of appetite, malaise, arthralgia, nausea and vomiting, body aches and headaches (Ashley *et al.,* 2006). Severe malaria has more serious symptoms which include severe anaemia, haemoglobinuria, acute respiratory distress syndrome, metabolic

acidosis, hyperparasitemia, coma, circulatory collapse, impaired consciousness, jaundice and acute kidney failure (CDC, 2016a; Ashley *et al.,* 2006). Due to developing resistance against antimalarial drugs that had been used to treat malaria as well as insecticides used as vector control, an increase in transmission has been detected (Lewison & Srivastava, 2008; Ashley et al., 2006).

There are several reasons for the remaining high percentage of mortality caused by this febrile illness. The first reason being the fact that antimalarial drugs are very expensive; and Africa is a third world continent that does not have the necessary funding for these drugs. Other reasons include that patients are not informed on how to take the medication correctly, the public health care system is not functioning optimally, healthcare workers do not have the expected training to conduct laboratory diagnosis, and a number of people living in rural areas do not have proper access to any antimalarial drugs (Bloland *et al.,* 2000; WHO, 2015b). Furthermore, the antimalarial drugs that have been used to treat malaria in the past are not as effective anymore due to increasing resistance (WHO, 2017; Hanboonkunupakarn & White, 2016; Vivas *et al.,* 2007). Owing to the above mentioned reasons, there is a definite need for an effective product which is also easy to use to ensure patient compliance. In completed field trials, the double effect (prevention of resistance and interruption of the spread of malaria) of artemisinin-combination therapy was shown (Nosten & Brasseur, 2002). For an anti-malarial drug to be considered successful, certain requirements need to be met, namely: the drug has to have a fast onset, it should display effective therapeutic concentrations and have as little as possible side-effects (Jelinek, 2013).

Currently, artemisinin-based combination therapy (ACT) is seen as the general treatment for uncomplicated malaria in more than 80 countries worldwide (Adjei *et al.,* 2016; WHO, 2015a; Nosten & White*.,* 2007). ACTs are fixed dose combination (FDC) products and are imperative to improve patient compliance and therefore therapeutic outcomes (WHO, 2015b). ACTs are potent and quick acting antimalarial drugs that act on the asexual stages of the malaria parasite and reduce the biomass of the parasite in each cycle, which in turn provides adequate relief of the various symptoms (Cheng *et al.,* 2012). Coartem® (artemether/lumefantrine combination) is currently the most widely used therapy for uncomplicated malaria recommended by the WHO. This treatment regime has been adopted in approximately 20 African countries (Sirima *et al.,* 2016; WHO, 2015a). However, various cases of treatment failure were reported due to low *in vivo* lumefantrine concentrations (Mizuno *et al.,* 2009). Owing to these reasons stated, this study focused on an antimalarial combination that circumvents these shortcomings.

1.1.2 Artemisone

Artemisone is a derivative of the anti-malarial drug class called artemisinins (Figure 1.1). This class of anti-malarial drugs is known as the most effective class; and has the most rapid onset compared to all the other anti-malarial dugs. Artemisone is a compound that is thermally stable, and it is lipophilic with a log P value of 2.49 at a pH of 7.4 (Haynes *et al.,* 2006). The elimination half-life after a single dose of 80 mg is 2.79 h (Nagelschmitz *et al.,* 2008).

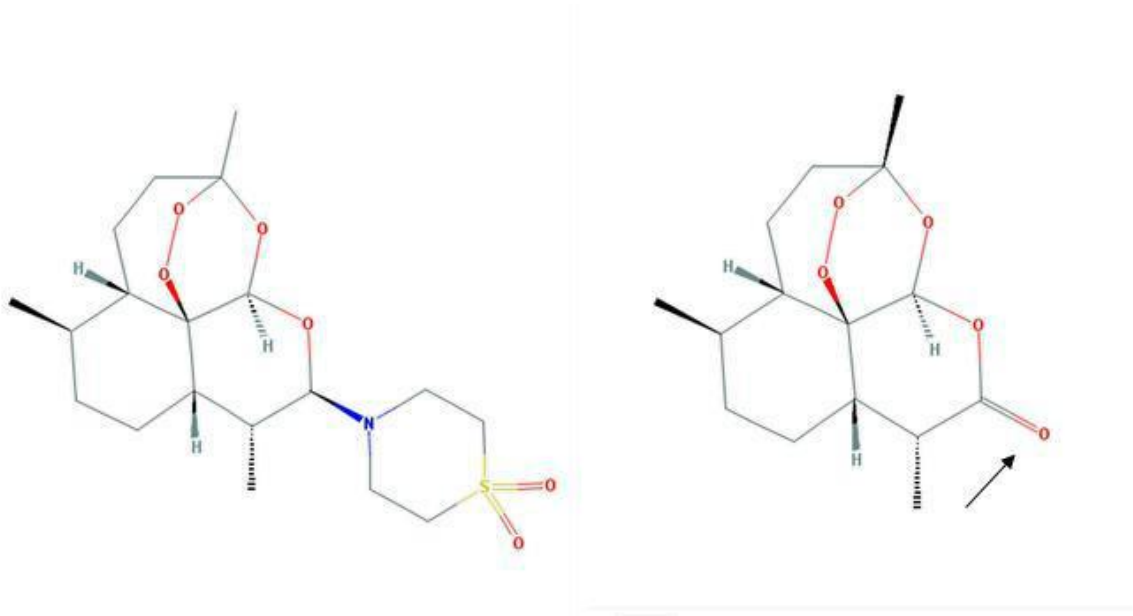


Figure 1.1: *Molecular structure of artemisone (left) compared to artemisinin and other derivatives (right). Difference shown by arrow.* (adapted from PubChem, 2017).

In an *in vivo* study done to compare the efficacy of artemisone against standard drugs used to treat malaria; artemisone was equally effective against resistant and drug-sensitive *Plasmodium*, but depicted the lowest IC_{50}-values. The effective dosage of artemisone is approximately one-third of the effective dose of the "golden standard" artesunate (Grobler *et al.,* 2014). Artemisinins eliminate the malaria parasite by inhibiting its metabolism; and it accomplishes this faster than any other drug used against malaria (Jelinek, 2013). It is well tolerated and has little adverse effects, except for patients with hypersensitivity reactions. The side-effects that patients experience during the use of an antimalarial drug that contains a combination of artemisone and another compound (for example, lumefantrine) are normally due to the drug that is combined with artemisone and not the artemisone itself (Nosten *et al.,* 2007).

1.1.3 Lumefantrine

Lumefantrine used in combination with the artemisinin derivative, artemether, is one of the most prescribed anti-malarial treatments used today. This combination is manufactured by Novartis as Coartem® where 20 mg artemether is combined with 120 mg lumefantrine. The exact anti-malarial action of lumefantrine is not yet known, however, available data on this drug suggests that it interacts with the haem of the malaria parasite; and this inhibits β-haematin from forming. β-haematin is needed in the synthesis of nucleic acid as well as in protein synthesis (Wishart *et al.,* 2006).

Lumefantrine is not administered alone due to its intrinsic value (approximate ability of the drug-receptor complex to produce a functioning response) that is lower than other anti-malarial drugs. However, given in combination due to the long half-life (96–120 h) of this drug, it can be used against all the malaria parasites (Nosten *et al.,* 2007). It is important that the mentioned combination provides a sufficient lumefantrine concentration to extinguish the remaining parasites (Ezzet *et al.,* 2000).

One of the problems with lumefantrine, however, is the fact that the oral bioavailability varies between dosages given, as well as between patients. Lumefantrine bioavailability is furthermore lowered in the acute phase of malaria. Variable bioavailability may be caused through changes in intestinal absorption due to the disease and the fact that lumefantrine is poorly water-soluble (lipophilic); or it may be due to the lack of sufficient intake of fats before taking the drug (Ezzet *et al.,* 2000).

The bioavailability of lumefantrine is highly influenced by food intake and therefore dependents on the intake of a meal that is rich in fats prior to administration to increase the bioavailability of the dose. As stated previously, symptoms of malaria include loss of appetite, nausea, vomiting, diarrhoea and fever. This is important to keep in mind because these particular symptoms can prevent a patient from not eating, which consequently will drastically affect the oral bioavailability of this drug (Jelinek, 2013). It furthermore has a molecular structure that is similar to halofantrine. Figure 1.2 displays the similar molecular structure of halofantrine and lumefantrine.

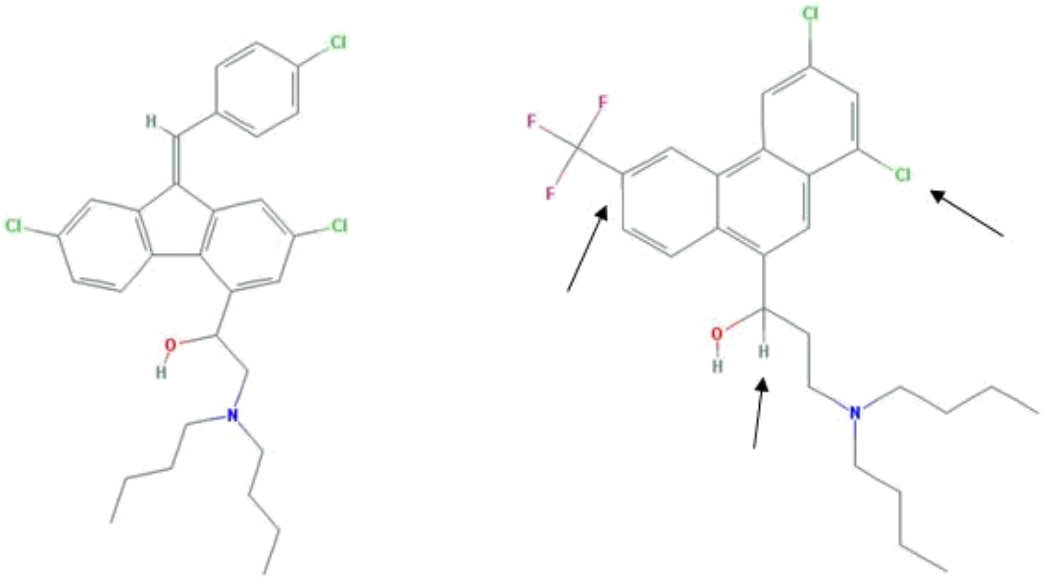


Figure 1.2: *Molecular structure of halofantrine (left) and lumefantrine (right). Some differences are highlighted with arrows.* (adapted from PubChem, 2017).

For an anti-malarial artemisinin-based combination to be an effective and successful 3-day regimen, the drug that is used with the artemisinin derivative must possess a minimum half-life of 24 h. This renders lumefantrine perfect to combine with an artemisinin derivate such as artemisone (Nosten *et al.,* 2007).

1.1.4 Direct compression

Direct compression is a simple and time-saving method to produce tablets. For powder blends to be tableted, certain characteristics such as good flowability, minimum moisture sensitivity and compressibility are imperative (Haware *et al*., 2015). To produce tablets of good quality, manipulation of the characteristics is sometimes needed. Normally, the fillers used determine the properties of the tablets; therefore, it is of utmost importance to choose the correct filler. Co-processed excipients such as MicroceLac® 100, CombiLac® and RetaLac® were developed to adhere to the characteristics needed for direct compression (Gohel & Jogani, 2005). Major advantages of the final product – directly compressed tablets, are accurate dosing, acceptable stability, easy transport and an aesthetic appearance (Dokala & Pallavi, 2013; de Kock, 2005).

1.1.5 Double fixed dose in lipid matrix

A double fixed dose of artemisone and lumefantrine in a lipid matrix tablet is a simple method of preparation; and direct compression is an efficient way to produce tablets on a large scale. The lipid matrix plays a role in modifying the release of the drug, which will assist in ensuring certain parasite death and prevent the occurrence of dose dumping (Abd-Elbary *et al.,* 2013). By incorporating the drugs into inert lipophilic matrices, the rate of drug release is controlled through the pores formed in the matrix. The dissolution rates of the drugs in the stomach are slower due to the slower diffusion from the lipid matrix, thus, the absorption rate into the bloodstream is slower and occurs over a longer period of time. The fact that the dissolution rate is slowed assists in controlling drug diffusion over the small intestinal lining into the bloodstream. This in turn increases the bioavailability of the drugs (Abd-Elbary *et al.,* 2013).

1.2 RESEARCH PROBLEM

Malaria is one of the leading causes of deaths in sub-Saharan Africa (Hanboonkunupakarn & White 2016; WHO, 2015a). Due to resistance against numerous traditional drugs *Plasmodium falciparum* needs to be treated with a combination of drugs. By creating a combination regimen that displays modified release and adheres to the necessary goals set, a decrease in morbidity and mortality can become a reality. Artemisinin based combination therapies (ACT's) combine compounds with different mechanisms of action, thus reducing the risk of malaria parasites forming resistance. The current WHO guidelines for the treatment of uncomplicated malaria includes; artemether + lumefantrine; artesunate + amodiaquine; artesunate + mefloquine; dihydroartemisinin + piperaquine; and artesunate + sulphadoxine-pyrimethamine (Nambozi *et al.,* 2017; WHO, 2015a).

The drugs used in this study have not previously been used in combination to treat malaria. Artemisone has a rapid onset mechanism of action which eliminates all the malaria parasites in the blood, whereas lumefantrine has a longer half-life compared to artemisone. The longer half-life of lumefantrine assists in extinguishing the parasites in the liver cells that were released into the bloodstream (Prabhu *et al.,* 2016; Jelinek, 2013; Omari *et al.,* 2005). A strategy to minimise the development of drug resistance is to synthesise hybrid molecules (Burgess *et al.,* 2006). Hybrid drugs are formed by incorporating two or more chemical substances that differs in pharmacological activities and structural domains with the purpose of exerting dual drug-action (Hulsman *et al.,* 2007). Due to the ability of the malaria parasite to build resistance against drugs, it is of utmost importance to use methods such as hybrid drugs, to prevent this from happening.

Lipid-matrix tablets are classified as monolithic tablets, meaning the plasma concentration of the drug may be improved due to the controlled release of the drug from the dosage form (Feeneye *et al.*, 2016; Nisha *et al*., 2012; Pouton & Porter, 2008). The modified release profiles that lipid matrixes can provide, will ensure that higher concentrations of the drugs are available for absorption due to sustained release of the drug over a longer period (Abd-Elbary *et al*., 2013). No known study has been done on the development of ACT's by means of hot-melt to formulate lipid-matrix tablets. Furthermore, no known studies were found on the development of an antimalarial comprising lumefantrine and artemisone.

1.3 AIMS AND OBJECTIVES

The aim of this study is to develop a fixed dose modified release matrix tablet containing artemisone and lumefantrine utilising different fillers to produce a dosage form for the treatment of uncomplicated malaria. Lipid matrix tablets were prepared utilising the hot-melt process as well as direct compression.

Therefore, the objectives set for this study, were to:

- Prepare fixed dose/lipid solid dispersions using either stearic acid or glycerol monostearate by means of the hot melt method.
- Characterise the fixed dose/lipid solid dispersions by means of differential scanning calorimetry (DSC) and X-ray diffraction studies.
- Formulate various fixed dose/lipid matrix tablet formulations containing MicroceLac® 100, RetaLac® or CombiLac® as fillers by using a full factorial design of experiments.
- Determine the flow properties of the fillers and lipid dispersions including the flow rate, angle of repose, critical orifice diameter (COD), Hausner ratio and Carr's index.
- Directly compress the lipid dispersions into fixed dose/lipid matrix tablets.
- Asses the physical characteristics (tablet hardness, diameter, thickness, tensile strength, friability, disintegration, mass variation, and drug content) and drug release properties of the fixed dose/lipid matrix tablets after direct compression.
- Evaluate swelling and erosion of the chosen tablets.
- Evaluate and compare the physical and dissolution properties of the various fixed dose/lipid matrix tablets produced from the formulations with each other as well as with a commercial product, Coartem®.

CHAPTER 2

MALARIA, ANTIMALARIALS & DOSAGE FORM DESIGN

2.1 INTRODUCTION

A good understanding of diseases and the science associated with pharmaceuticals exists today. As a result, numerous possibilities of advanced drug delivery systems have been developed, irrespective of the administration route. Nonetheless, the purpose of drug delivery systems has remained unchanged. Consideration must be kept in mind in the development of dosage forms, namely that therapeutically efficient dosages of drugs must be targeted for release at a specific time and/or site in the human body, which should transpire at pre-determined drug release rates (McConnell & Basit, 2013; Rajabi-Siahboomi *et al.*, 2013).

The most frequently and popularly used route for administering drugs for a systemic effect is the oral route. It is presumed that no less than 90% of all drugs on the market are taken orally (Kalpana *et al*., 2010). This is the most simple and convenient, as well as the safest route through which to administer most drugs. Capsules and tablets are the preferred oral dosage forms for utilising this drug delivery route, as patients fully control administration, as well as the possibilities of regimen flexibility (Bhattarai & Gupta, 2015; Sakr & Alanzi, 2013).

A drug, also called active pharmaceutical ingredient (API), is never administered alone, but is always accompanied by excipients to form preparations, or medicines. Drugs are formulated with specific excipients, due to the numerous pharmaceutical functions that excipients provide. Tablets are formed through compression of various excipients, together with the active ingredient(s), either through wet granulation, dry granulation, or direct compression (Thoorens *et al*., 2014; York, 2011).

A lipid matrix dosage form is produced when a drug(s) is homogeneously mixed into an inert lipophilic polymer (Abd-Elbary *et al*., 2013). This modified release dosage form may contain a single API, or a combination of APIs, which can increase patient compliance, as it requires less frequent administration (Riss *et al*., 2007). Modified release drugs reduce blood

concentration fluctuations and subsequently require less frequent dosing (Rajabi-Siahboomi *et al*., 2013; Abdul *et al*., 2010; Ishida *et al*., 2008).

During this study, the hot-melt method was used, in an attemp to formulate an effective modified/sustained release lipid matrix dosage form, containing a fixed-dose combination of lumefantrine and artemisone, for the possible treatment of uncomplicated malaria. This chapter focuses on malaria as a life-threatening disease that has become resistant to most antimalarial medications. This chapter further discusses antimalarial treatment regimens, the prevention of resistance towards antimalarial treatment regimens by utilising fixed-dose combination therapies, as well as the advantages of using the hot-melt method for the preparation of lipid matrix tablets. To date, no antimalarial dosage forms, according to the available literature, have been formulated by employing the hot-melt method, nor through the production of lipid matrices.

2.2 TRANSMISSION AND DISTRIBUTION OF MALARIA

The transmission and distribution of malaria occur as a result of various environmental factors. Temperature and humidity affect both the cycle of transmission and the duration of the sporogonic cycle of the mosquito species mainly responsible for the disease, i.e. *Plasmodium falciparum* (*P. falciparum*). Rainfall is another environmental factor that plays a significant role in is spreading, as the breeding sites of mosquitos are dependent upon rainfall and water masses. Rain increases humidity that increases the chances of mosquito larvae to develop into mature mosquito insects. The optimal conditions for the transmission of malaria are temperatures between 20°C and 30°C, as well as high humidity levels. Although the malaria epidemiology is complex and may differ substantially within small geographical areas, low altitude areas are preferred breeding locations (White, 2009; Breman *et al*., 2007). Transmission intensities of malaria vary from low, or no malaria incidences, to exceptionally high, as depicted by Figures 2.1 and 2.2.

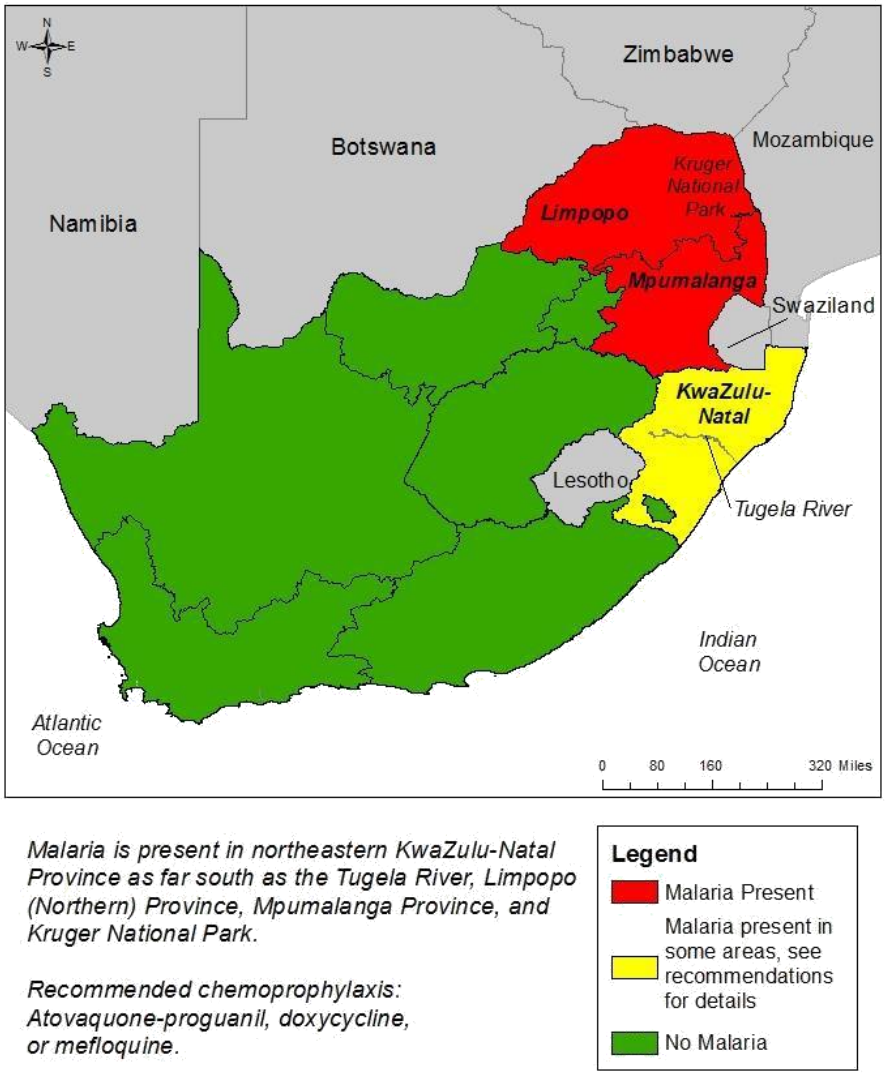


Figure 2.1: *Areas in South Africa that have suitable climates for malaria transmission* (adapted from CDC, 2017a).

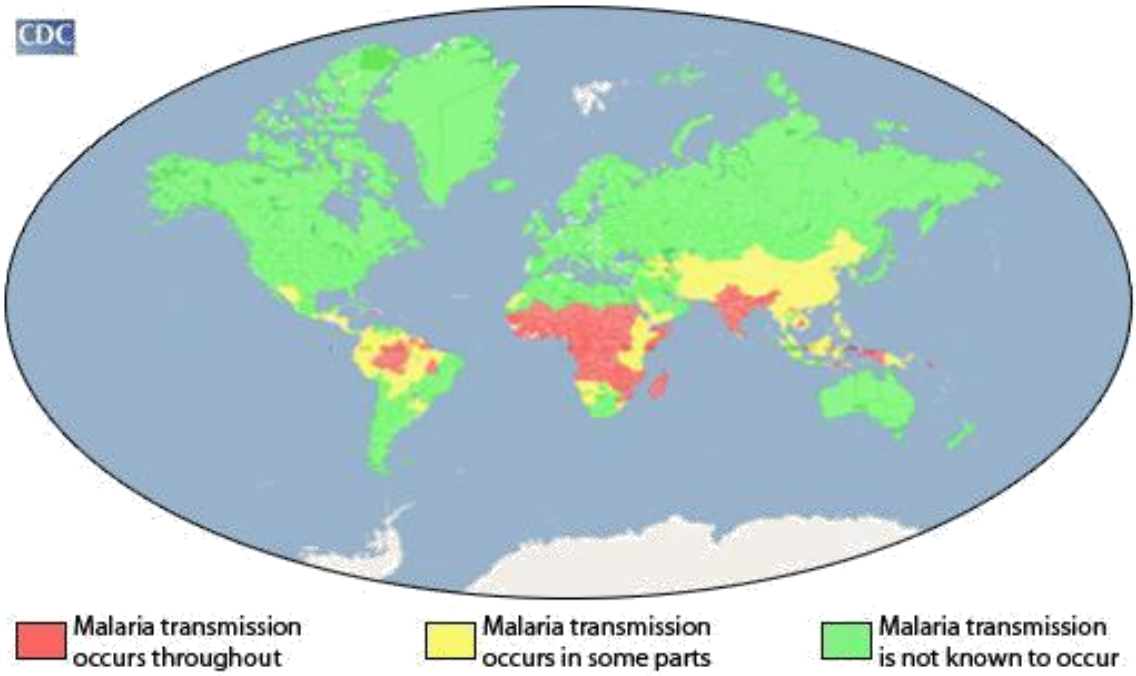


Figure 2.2: *Areas in the world that have a suitable climate for malaria transmission* (adapted from CDC, 2017b).

Malaria is endemic to 41% of the world (109 countries), with an estimated 50% of the world's population being at risk of contracting this disease. Approximately 80% of the mortality numbers represent children less than 5 years old (Achieng *et al*., 2017; Murray *et al*., 2012).

Post malaria neurological syndrome (PMNS) is a clinical manifestation that can occurs after the treatment of malaria, when there is severe inflammation of the brainstem and spinal cord (Pace *et al*., 2013). It was found that the treatment of severe malaria with mefloquine had increased the risk of PMNS (Ashley *et al*., 2006: Thi Hoang Mai *et al*., 1996). Symptoms of PMNS include:

- Confusion,
- Seizures,
- Tremors,
- Impaired consciousness,
- Myoclonus,
- Headaches,
- Cerebellar ataxia,
- Acalculia,

- Agraphia, and
- Aphasia (Chiabi *et al*., 2017; Ashley *et al*., 2006).

The inflammation and symptoms caused by PMNS, however, disappear within 10 days without any treatment (Thi Hoang Mai *et al.,* 1996).

2.3 LIFE-CYCLE OF THE MALARIA PARASITE

During the life cycle of the malaria parasite, it infects two different hosts namely, the female *Anopheles* mosquito and humans. During a blood meal, the *Anopheles* mosquito carrying the parasite injects the sporozoites (the parasite form at that stage) into the bloodstream of the human host. The sporozoites travel to the liver and invade the cells where they grow and divide into thousands of merozoites, which then leave the liver cells to enter the bloodstream, where the parasites invade the erythrocytes. During this stage, asexual replication occurs during which the merozoites evolve into mature schizonts. At this stage, the schizonts and the red blood cells rupture and release newly developed merozoites that then re-occupy other erythrocytes. The release of new merozoites that invade new red blood cells transpires every 1–3 days, which means that in a matter of a few weeks, thousands of infected erythrocytes are present in the human bloodstream, causing illness as well as complications, if not treated properly. During this stage, some of the merozoites do not replicate asexually, but instead develop into male and female parasites that are known as gametocytes. The mature gametocytes circulate in the bloodstream of the human host. It is found that in some of the malaria species, the young gametocytes seclude themselves in some organs and inside the bone marrow that can cause relapses of the illness later. The gametocytes that are present in the bloodstream are ingested if an *Anopheles* mosquito bites an infected host. The infected erythrocytes then burst inside the mid-gut of the *Anopheles* mosquito, where the parasites mature into a sexual (male or female) gamete. Fusing together, the male and female gamete form a diploid zygote that develops into ookinetes, which tunnels through the mid-gut wall to form oocytes on the outside. Oocytes grow, divide and produce thousands of sporozoites, which upon bursting release these sporozoites into the mosquitos' body cavity. The sporozoites penetrate the salivary glands of the mosquito to be again injected into a human host during a blood meal (Achieng *et al*., 2017; Ashley *et al*., 2006). All of these stages are illustrated in Figure 2.3.

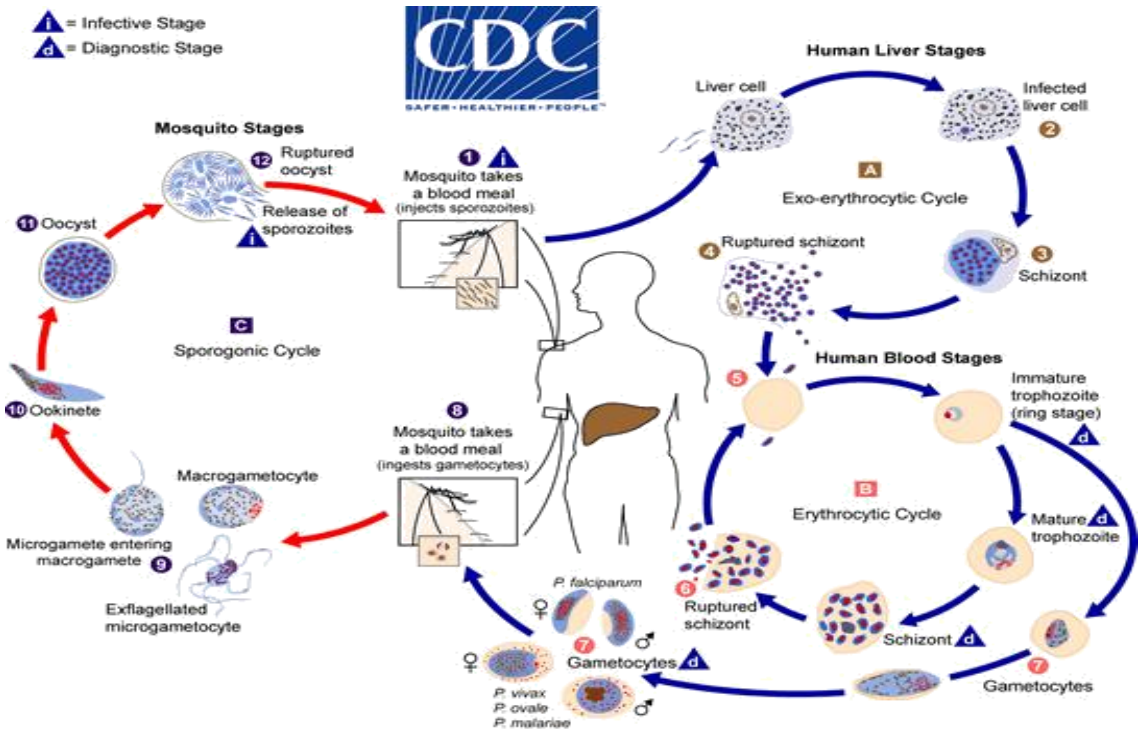


Figure 2.3: *The life cycle of the malaria parasite. This diagram illustrates the different stages of the malaria parasite within the human body and in the mosquito body* (adapted from CDC, 2016a).

2.4 DIAGNOSIS OF MALARIA

The diagnosis of malaria continues to be challenging in most of the countries where malaria is prevalent. Approximately 300 to 500 million clinical cases are reported annually (WHO, 2015b; Bloland, 2001). The earlier the diagnosis is confirmed, the higher the chances of full recovery before severe symptoms manifest (Moody, 2002). Early detection also reduces the mortality and morbidity being caused by this parasite (Chandramohan *et al*., 2001). In most of the countries where malaria is prevalent, cost-effectiveness of the diagnosis and the personnel who are trained to perform diagnostic tests, play important roles (Jani & Peter, 2013; Uzochukwa *et al*., 2009; Amexo *et al*., 2004).

2.4.1 Clinical diagnosis

The fact that the first symptoms of malaria also manifest in other diseases, for example, influenza and viral infections, means that the physical findings during a clinical observation cannot be regarded as a reliable diagnosis (CDC, 2016b). In previous studies done throughout the world, it has been found that no universally applicable criteria exist that can be used during diagnosis, due to different factors, such as the usage of an antimalarial drug before seeking medical attention, the endemicity level of malaria, and other illnesses present that can affect the diagnostic criteria (Chandramohan *et al*., 2001). In more severe malaria

cases, clinical observations are easier, because of the more obvious symptoms. Where it is possible, laboratory tests should follow clinical findings to confirm malaria. It is usually in the malaria endemic countries, where there is a shortage in financial resources and qualified personnel, that the most realistic option is to diagnose malaria through clinical observation (Bloland, 2001; WHO, 2015a).

2.4.2 Antigen detection tests

This approach is based upon detecting the histidine rich protein 2 (HRP-II) of the malaria parasite. The HRP-II is an antigen that can be detected by means of immunochromatographic techniques. This sensitive way of diagnosing malaria is commercially available and only requires a dipstick device and blood from a finger prick, to have the results in approximately 15 minutes (Bell *et al*., 2006). Both advantages, as well as disadvantages are evident when using antigen detection tests. Advantages are that no electricity is necessary to perform the test, no qualified person is needed, minimum training is required, and the reagents used in the test equipment are stable at various temperatures, making it viable when travelling to tropical areas. The disadvantages include that the test cannot measure the density of the malaria infection, the cost per single test is high, while the test cannot differentiate between failing treatment and an infection that is resolving (WHO, 2015b; Bloland, 2001).

2.4.3 Microscopic observations

Blood taken from a patient is stained with either Giemsa, or Field's, or Wright's stain, and is the sample examined under a light microscope (Moody, 2002). This technique can distinguish between the different species of *Plasmodium*, the density of the infection can be quantified, and it is possible to distinguish between the different developmental stages of the parasite. These are all advantages that can assist health workers to manage the disease and track the patients' responses to the provided treatment. Unfortunately, this method is time consuming and require well trained personnel, as well as the correct equipment (WHO, 2015a; Bloland, 2001).

2.4.4 Molecular tests

Diagnosis through molecular tests is becoming more popular. This diagnosis is based upon the use of a polymerase chain reaction (PCR) to detect the genetic material of the parasite. Molecular tests are more accurate than microscopic tests, but are also more expensive and require specialised equipment. Through this method, mixed infections can be detected, and can this method be used when microscopic results of the species are inconclusive (WHO, 2015b; Bloland 2001).

2.4.5 Serology

Antibodies against *Plasmodium* (*P.*) parasites can be detected through serology. Antibodies remain in the patients' blood long after the malaria infection has cleared up (WHO, 2010; Bloland 2001). This means that serology does not necessarily identify that an infection is present in the patient, but rather indicates that the patient had been exposed to malaria. As the serology test method is quite expensive, it is not very often used (Bloland 2001).

2.5 DRUGS AVAILABLE FOR THE TREATMENT OF MALARIA

In the past, effective treatments for malaria included chloroquine, a pyrimethamine-sulfadoxine combination, mefloquine, as well as a combination of atovaquone and proguanil. Malaria has over time developed resistance towards these drugs, which subsequently resulted in an increase in transmission and even in epidemics in certain areas of the world (Cheng *et al*., 2012).

Drugs that are available for the prevention and treatment of malaria have in recent years therefore become limited. Antimalarial drugs can be divided into five classes, namely:

- Antibiotics,
- Hydroxyl-napthoquinones,
- Quinolones and arylaminoalcohols,
- Antifolate combination drugs, and
- Artemisinin compounds.

Quinine and its derivatives, as well as the antifolate combination antimalarials have been the most used drugs for many years (WHO, 2010; Bloland 2001).

2.5.1 Antibiotics

Tetracycline and its derivative, doxycycline, are employed as prophylaxis as well as antimalarial treatments. In areas where quinine sensitivity to the parasite has declined, these two antibiotics can be combined with quinine to improve recovery rates (CDC, 2016b). These combinations should be taken for 7 days. Tetracycline and doxycycline should not be given to any child under the age of 8 years, unless no other treatment options are available, or when no other treatment is tolerated by the child and the benefits of providing a child with these antibiotics outweigh the risks (CDC, 2016b). In cases where tetracycline, or doxycycline is taken as a chemoprophylaxis, the drug should be taken daily with food, starting 2 days before traveling starts, and should it be continued for 4 weeks after the traveller has left the malarious area (Tan *et al*., 2011).

Clindamycin has reportedly been used in the past, but the response to it was slow and the relapse rates high (WHO, 2010; Bloland 2001).

2.5.2 Hydroxyl-naphthoquinones

Halofantrene, a phenanthrene-methanol compound, is recommended as the drug of choice in areas that are known for multiple drug-resistant malaria. This drug has activity against malaria parasites in the erythrocytic stage. Halofantrene has high activity, but can cause potentially fatal arrhythmogenic abnormalities (Nosten *et al.*, 1993). Due to this side-effect, the use of halofantrine is limited. A hydroxynaphthoquinone, called atovaquone, may be used in patients with chloroquine-resistant falciparum. Atovaquone is not used alone, due to rapidly forming resistance, but is preferably used in a fixed combination with proguanil. Another fixed-dose combination being used is lumefantrine (a hydroxyl-naphthoquinone compound) and artemether (WHO, 2010; Bloland 2001). The combinations, atovaquone-proguanil and lumefantrine-artemether can be safely used for paediatric and non-pregnant patients (CDC, 2016b).

2.5.3 Quinolones and arylaminoquinones

The last resorts for treating severe malaria are quinine and dextroisomer quinidine. Other derivatives of quinine are chloroquine, amodiaquine, primaquine and mefloquine. Chloroquine is a synthetic compound of quinine that has been the first choice of treatment for patients with uncomplicated malaria, as well as for chemoprophylaxis. Resistance of falciparum against chloroquine has, however, rapidly decreased its effectiveness and use (WHO, 2010; Bloland 2001).

Primaquine is utilised in the treatment of *P. vivax* and *P. ovale* infections to eliminate exoerythrocytic forms of the parasite and to eliminate the chances of relapses from occurring (Deen *et al*., 2008). Alternatively, mefloquine is a quinolone-methanol compound that can be used as prophylaxis and chemotherapy against *P. falciparum* (Steffen *et al*., 1993). Mefloquine, in combination with pyrimethamine-sulfadoxine, can furthermore be employed to treat chloroquine-resistant malaria. This combination assists in preventing resistance against the individual drugs (White, 1999).

2.5.4 Antifolate combination drugs

The effectiveness of antifolates is due to the fact that these drugs are able to interfere with the metabolism of folate production in the parasite, which in turn plays a role in DNA synthesis (Müller & Hyde, 2013). Antifolate combinations consist of dihydrofolate-reductase inhibitors and sulfa drugs. Dyhidrofolate-reductase inhibitors include proguanil, chlorproguanil, pyrimethamine and trimethoprim, and the drugs classified as sulfas include

dapsone, sulfadoxine, sulfamethoxazole and sulfalene (WHO, 2010; Bloland 2001). Combinations are used for synergistic attacks on malaria and decrease the chances of resistance forming against either of the drugs incorporated in the combination. Antifolate combinations include:

- Co-trimoxazole,
- Sulfadoxine and pyrimethamine,
- Chlorproguanile and dapsone, and
- Sulfalene and pyrimethamine (WHO, 2010; Bloland 2001).

2.5.5 Artemisinin compounds

Artesunate, artemether, arteether, and artemisone are sesquiterpine lactone compounds, synthesised from a plant called *Artemisia annua.* These compounds have rapid clearance times and are widely used in the treatment of severe malaria infections (WHO, 2010; Bloland 2001).

Artemisone's metabolic profile differs from the other artemisinins' (Haynes *et al*., 2006). The display of favourable physicochemical properties with inconsequential cyto- and neurotoxicities, as well the increased antimalarial activity of artemisone are discussed in more detail in section 2.9.1 of this chapter.

2.5.6 Miscellaneous compounds

Two drugs that were originally manufactured in China and that are currently undergoing field trials are:

- Pyronaridine: Although allegedly found 100% effective during a trial study performed in Cameroon (Ringwald *et al*., 1996), it was only found effective between 63% and 88% during another trial in Thailand (Looareensuwan *et al*., 1996).
- Lumefantrine is a fluoromethanol compound drug. Currently, artemether and lumefantrine are manufactured as a fixed-combination tablet. Lumefantrine will be discussed in more detail in section 2.9.2 of this chapter.

2.6 RESISTANCE

Resistance to antimalarial medication is the ability of the malaria parasite to survive and/or multiply, despite the presence of antimalarial drugs in blood concentrations, deemed adequate to kill or control the multiplication of the parasites. Resistance arises due to a selection of genetic changes that occur in the parasites that reduce the susceptibility of the parasite to the drug (WHO, 2015a). This is a major problem in developing countries, where incidences of infectious diseases are higher, and where patients with a resistant infection

have limited access to, or insufficient money to afford second-line treatments. Such second-line treatment regimens are more complex and in most cases more expensive, compared to first-line treatments (Laxminarayan *et al*., 2006).

Several factors contribute towards the development of drug resistances, including the increased usage of antimalarial drugs and antibiotics, poor control over drug prescriptions, insufficient patient compliance to prescribed treatments, the prescription of sub-therapeutic dosages, the traveling speed of the infection, and the inadequate control of the infection. These factors all contribute towards the swift spreading of these drug-resistant organisms (Laxminarayan *et al*., 2006). Resistance in parasites occur spontaneously through gene mutations that reduce susceptibility to drugs and develop through transmission and multiplication (White, 1999). With a general exception of most artemisinin derivates, the *P. falciparum* parasite has developed resistance to all currently available antimalarial drugs (White, 2009). *P. falciparum* portrays multi-drug resistance towards pyrimethamine-sulphadoxine, chloroquine, as well as mefloquine mono-therapies, whereas chloroquine's potency is decreasing (Ashley *et al*., 2006). A more recent study alarmingly found that there is even a decline in susceptibility to artesunate (an artemisinin derivative) in patients living in Cambodia and on the Thailand-Myanmar border (Phyo *et al*., 2016; Dondorp *et al*., 2009).

2.6.1 Resistance to sulphonamides and folate biosynthesis inhibitors

Within a few years after the introduction of mono-therapies with proguanil and pyrimethamine, resistance formed against these drugs (White, 2009). The use of the pyrimethamine-sulfadoxine combination therapy is furthermore decreasing, due to resistance. Although proven resistance to both these drugs occur, if used as mono-therapies, the clinical failure of the combination drug is not yet known. Proguanil (an antifolate drug), which is quickly eliminated, most likely experiences less resistance. The *P. falciparum* parasite remains sensitive towards other dihydrofolate reductase (DHFR) inhibitors, while it has shown resistance to pyrimethamine (Amukoye *et al*., 1997).

Single point mutations in the genes of *P. falciparum* encode the target enzymes so that antifolate drugs are no longer effective against the parasite. A mutation at position 108 in the encoding of the genes, leads to the resistance of DHFR inhibitors (White, 1999; Peterson *et al*., 1988). There are also mutations at different positions (51, 59 and 108) that exert resistance to the pyrimethamine-sulfadoxine combination drug. Parasites with resistant genes to pyrimethamine, however, do not necessarily display resistance to cycloguanil, and vice versa (White, 1999; Watkins *et al*., 1997). Proguanil generally is more clinically effective than pyrimethamine against resistant malaria parasites, but futile against parasites that have the 164 mutation (White, 2009).

Mutations in the gene encoding the dihydropteroate synthase (DHPS) target enzyme develop progressive resistance towards the sulponamides and sulphones. Parasites that possess the DHPS mutation generally have the DHFR mutation as well. Tropical countries present increasing failure rates with the pyrimethamine-sulphadoxine combination treatments, while parasite susceptibility to the artesunate-sulphadoxine-pyrimethamine combination is decreasing also (White, 2009).

Resistance against chloroquine was found to have arisen, due to decreased chloroquine concentrations in the malaria parasite's food vacuole. Although both a reduction in influx and an increase in efflux have been reported, a decrease in the accumulation of the drug in the parasite has been the most common factor that has led to drug resistance (Andriole, 2005; White, 1999).

Mefloquine is used as treatment to uncomplicated multi-drug-resistant (MDR) malaria. Resistance to mefloquine has developed at a faster rate in comparison with chloroquine. Amplifications, or mutations of the adenosine tri-phosphate (ATP) requiring P-glycoprotein pump, which is encoded by the MDR genes, are generally associated with mefloquine resistance, whereas mutations in the chloroquine resistance transporter (CRT), a vacuolar membrane protein associated with transport, plays a role in resistance towards chloroquine, and may also play a part in resistance towards amodiaquine and quinine (White, 2009).

2.6.3 Atovaquone

By interfering with the cytochrome electron transport chain of the parasite, atovaquone kills the parasite by blocking its cellular respiration. Proguanil is used in combination with atovaquone in a fixed-dose, for their synergistic effects. Unfortunately, there has been a reduction in susceptibility to atovaquone, because of single point mutations taking place in the *P. falciparum* cytochrome *b* genes. The combination with proguanil lowers the survival rate of these mutants, but due to proguanils' weak antimalarial activity, protection against these mutants is limited (White, 2009).

2.6.4 Artemisinin and derivatives

Of all the known antimalarial drugs, artemisinin (ART) and its derivatives are the swiftest acting antimalarial medication in all stages of the erythrocytic parasites, as well as the gametocytes. Because these drugs have very short half-lives (±1–3 hours), a long-acting drug must be administered together with the ARTs. The antiplasmodial activities of ARTs are accredited to their endoperoxide rings that instigate oxidative stress upon the interaction with haeme. This promotes the release of free radicals, as well as the alkylation of proteins, resulting in irreparable damage to the *Plasmodium* parasite (Achieng *et al*., 2017).

2.6.5 Prevention of resistance by means of fixed dose combinations

The development of resistance may be unavoidable, but the promptness at which it develops, and spreads can be controlled. It is of utmost importance to treat an infection adequately and to diminish the selective pressures (Hanboonkunupakarn & White, 2016; Bloland, 2001; White, 1999). If two different drugs, with unrelated sites of action, are used concurrently, the chances that a mutant parasite will survive, is the product of the respective mutation prevalence that causes resistance to these drugs (Okell *et al*., 2014; White, 1998). The concept that fixed-dose combinations will delay the development of resistance has been proven (Nosten & Brasseur, 2002; Bloland *et al*., 2000; Peters & Robinson, 1984). The ideal circumstance is that antimalarial drugs should only be used to treat malaria in the endemic areas, and that a full course should be taken by the patients. Drugs with short half-lives, such as artemisinin and its derivatives, need to be prescribed for therapeutic concentrations to be present for a minimum of four asexual parasite cycles (at least 7 days) to eradicate all parasites. This would also contribute towards the prevention of formed resistance. Education of both the dispensers and patients will furthermore donate to successful treatment regimens and less resistance formation (White, 2009).

2.7 FIXED-DOSE COMBINATION THERAPY

There is an ongoing need for new improved treatment regimens against *P. falciparum* malaria (WHO, 2015a). Drug treatments that are effective and efficient contribute towards decreased transmission rates, as well as prevent uncomplicated malaria from developing into a more life-threatening illness (Breman *et al*., 2006; Bukirwa *et al*., 2004). The early diagnosis and correct treatment of malaria play crucial roles in preventing and reducing deaths caused by this parasite (WHO, 2017). It is ideal for the drugs in combination to have similar pharmacokinetic properties to ensure that the drugs are protected by each other. Generally, in combinations with an artemisinin derivative, the partner drug's elimination is relatively slower and does the mechanism of action of each drug differ. This is important to prevent resistance from emerging and to eliminate the residual parasites (White, 2009; Aweeka & German, 2008). The ways in which the different drugs are metabolised in the human body additionally play an imperative role in the selection of drugs that are used in fixed-dose combinations. Most of the antimalarial drugs are either metabolised and/or induce, or inhibit the CYP450 (cytochrome P450) enzymes, and can contribute towards drug-interactions with various other drugs, such as antiretroviral, antifungal, or antituberculosis regimes. Such interactions can compromise the treatment, lead to inefficacy of one/both treatments, or induce drug toxicity (Aweeka & German, 2008).

Current fixed-dose combination antimalarial therapies that are available include artemether and lumefantrine, artesunate and mefloquine, artesunate and amodiaquine, artesunate and pyrimethamine-sulohadoxine, artesunate and tetracycline/doxycycline/clindamycin, and dihydroartemisinin and piperaquine (WHO, 2015a). In South-East Asia, *P. falciparum* has shown resistance towards multiple antimalarial drugs, including the artesunate-mefloquine and pyrimethamine-sulohadoxine combinations (Rogers *et al*., 2009). Resistance towards artesunate-amodiaquine is increasing rapidly in Africa (Mutabingwa, 2005). The current first-line treatment in Cambodia, dihydroartemisinin-piperaquine, is also showing signs of treatment failure (Amaratunga *et al*., 2016). Resistance to the current fixed-dose combination therapies available portrays the need for new combination antimalarial drugs. Drug resistance, poor patient adherence and inadequate, sub-optimal drug exposure generally lead to the recurrence of malaria infections. The choice of an artemisinin derivative for an artemisinin-based combination therapy (ACT) depends upon the level of resistance of the other drug in question. Although it is more expensive, ACTs are more effective in killing the parasites and in lowering recurrence rates (Davis *et al*., 2011).

2.8 INTERMITTED PREVANTATIVE TREATMENT

The estimated number of malaria deaths in 2015 were 438 000, of which more than 90% occurred in Africa, and mostly in pregnant women and children under 5 years of age (WHO, 2015b). The limited treatment regimens that are available for pregnant women and for children under 5 years is an immense concern, especially in malaria endemic areas and high transmission areas, such as in sub-Saharan Africa. Pregnant woman, and woman attempting to get pregnant, should be advised and discouraged to visit these areas. Surviving a malaria episode during pregnancy can have permanent adverse effects on the infant and will most likely impact on the infant's antimalarial immunity. The effects of a malaria infection during pregnancy include stillbirth, spontaneous abortion, low birth weight, pre-term delivery, congenital infection and maternal death. Implementation of intermitted preventative treatment during pregnancy (IPTp) was first implemented only in 2004. Such treatment consists of administrating a single therapeutic dose of an effective antimalarial drug no less than twice during the pregnancy, irrespective of the presence of infection. Currently, the pyrimethamine-sulphadoxine combination, which is considered the safest and the most effective, is being used in areas with stable *P. falciparum* transmission and low pyrimethamine-sulphadoxine resistance. In chloroquine-sensitive areas, it is preferred to use chloroquine in uncomplicated cases, but where chloroquine resistance is present, the use of the pyrmethamine-sulphadoxine combination is preferred (WHO, 2015a; Briand *et al*., 2007).

2.9 THE IMPORTANCE OF ARTEMISONE AND LUMEFANTRINE IN MALARIA TREATMENT

2.9.1 Artemisone, a synthetic derivative of artemisinin

Artemisinin is a sesquiterpene lactone compound that is isolated form the *Artemisia annua* plant (Brossi *et al*., 1988). The *Artemisia annua* is an herb that has been used historically by the Chinese for the treatment of malaria. This herb is a rapid and effective working treatment against malaria, by acting as a blood schizontocide. Artemisinin is effective against chloroquine-resistant *P falciparum,* chloroquine-sensitive *P. falciparum,* as well as *P. vivax* (Baker & Burgin, 1996). Artemisinin and its derivatives mark a cutting-edge class of antimalarial drugs that show efficacy against *Plasmodium* strains that are drug-resistant. This finding was of pronounced significance in the present-day battle against malaria (WHO, 2015; Balint, 2001). The World Health Organisation (WHO) recommended the use of ACTs in 2001, but deployment has been slow. This can be due to factors, such as higher costs, curbed knowledge of combination therapies, insufficient patient compliance, inaccessibility to the needy, and a lack of suitable and effective formulations (Mutabingwa, 2005). ACTs reduce treatment duration, the dose of artemisinin in the combination, and the transmission of resistant malaria parasites (Vivas *et al*., 2007). Recently, artemisinin resistance has been detected in several countries in South-East Asia, especially on the Combodia-Thailand border. This means that the *Plasmodium falciparum* parasite is becoming resistant to most antimalarial drugs on the market (Baragaňa *et al*., 2016).

There currently is no ACT on the market that includes artemisone as one of the drugs in combination. Artemisone is a semi-synthetic 10-alkylaminoartemisinin and can be synthesised through a single step process, using dihydroartemisinin (DHA), as illustrated in Figure 2.4.

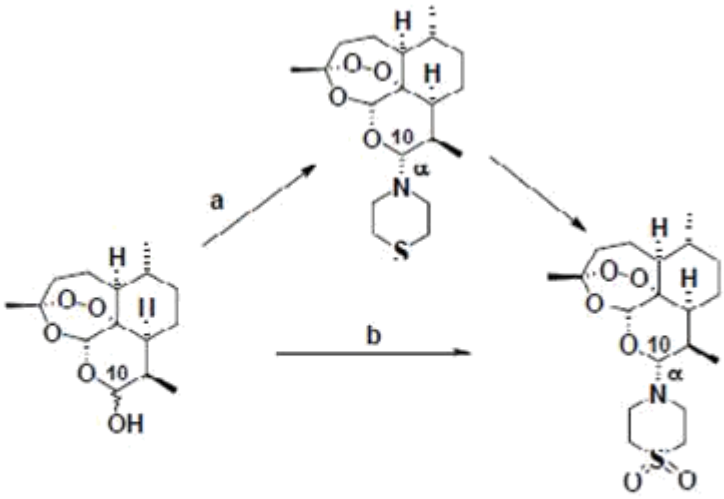


Figure 2.4:

Diagrammatic representation of the preparation of artemisone from dihydroartemisinin.

It is a highly crystalline compound that possesses increased oral bioavailability, inconsequential neuro- and cytotoxicity, increased metabolic stability, and antiplasmodial activity in comparison with other artemisinin derivatives (Guiguembe *et al*., 2014; Vivas *et al*., 2007; Haynes *et al*., 2006). Although artemisone shows increased bioavailability, its aqueous solubility remains low and erratic, leading to its poor bioavailability (Pawar *et al*., 2016; White, 2008). A study done by Vivas *et al.* (2007) showed synergistic interaction between artemisone and lumefantrine. All artemisinin derivatives on the market are metabolised through the liver into DHA, towards which some *P. falciparum* parasites already show resistance. Since artemisone does not convert into DHA, no known resistance occurs. In comparison with DHA, artemisone has improved pharmacokinetics, such as a shorter elimination half-life (±3 – 4 hours), a lower plasma clearance and a larger visible distribution volume (Haynes, 2010; Nagelschmitz *et al*., 2008). This renders artemisone a more suitable candidate for use in combination therapies.

2.9.2 Lumefantrine

Lumefantrine (Figure 2.5) is a compound that belongs to the arylamine alcohols, along with mefloquine, quinine, and halofantrine. This compound (lumefantrine) is administered with artemether as a combination therapy in treating uncomplicated *P. falciparum* malaria worldwide (Padberg, 2015; WHO, 2015a).

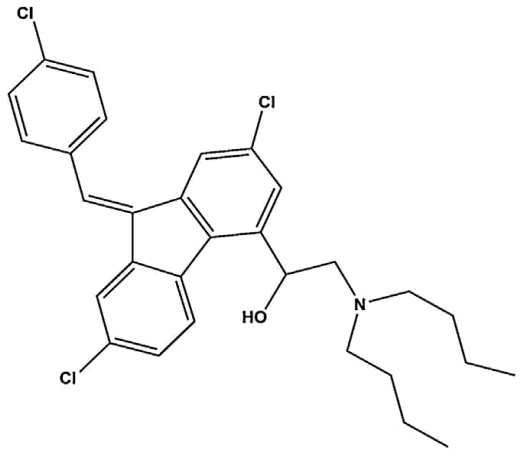


Figure 2.5: *Chemical structure of lumefantrine* (adapted from Song *et al*., 2016).

The advantage that lumefantrine holds, is that it has only been used as mono-therapy in the 1980's for a short period of time, and is it hence less likely that parasites had developed resistance against this compound. Since lumefantrine is only available in combination therapy, data regarding lumefantrine is limited. Although the exact mechanism of action is still unknown, lumefantrine most likely inhibits the formation of β-hematin by forming a complex with hemin. Lumefantrine is a blood schizontocide but displays no antimalarial activity against the pre-erythrocytic liver stage, and is lumefantrine thus not active against the hypnozoite, nor gametocyte stages of malaria (Garg *et al*., 2017; Nosten *et al*., 2002).

Lumefantrine has a half-life of 3–6 days, thus supplying a more effective long-term cure rate after administration of a short course of treatment (Aliyu, 2012; Kester *et al*., 2012; Makanga & Krusdood, 2009). Its oral bioavailability can be increased by 108% when a fatty meal is taken close to administration of the drug. After administration, an initial lag time of up to 2 hours occurs before the absorption of lumefantrine, before achieving peak plasma concentrations around 6–8 hours afterwards (Khuda *et al*., 2014). CYP3A4 metabolises lumefantrine into desbutyl-lumefantrine, but it is mostly eliminated as the parent drug (Nosten *et al*., 2002). Oral bioavailability of lumefantrine is limited, due to its poor aqueous solubility, which should be kept in mind when formulating an antimalarial dosage form, as well as when lumefantrine is provided to patients in areas where malnutrition is prevalent (Garg *et al*., 2017; Ezzet *et al*., 2000). Furthermore, patients diagnosed with malaria frequently suffer from nausea and anorexia, making it difficult to comply with dietary advice (Ashley *et al*., 2007). Sub-therapeutic plasma concentrations are of serious concern when

lipophilic drugs, such as lumefantrine, are administered without food (Kalepu *et al*., 2013; Borrmann *et al*., 2010).

When ingesting a meal rich in fat, invokes the “food-effect” and affects the gastrointestinal (GI) physiology, which in turn maximises the absorption of lipophilic drugs into the systemic circulation. The lipid component of fatty foods is vital for the absorption of lipophilic drugs and thus for increasing their bioavailability (Kalepu *et al*., 2013; Borrmann *et al*., 2010). This is as a result of the ability of a fatty meal to stimulate pancreatic and biliary secretions that subsequently increase the permeability of the intestinal wall, which leads to improved transport through the lymphatic system. A meal real in fat also elevates the triglyceride rich lipoproteins that associate with the drug molecules to enhance their lymphatic transport. Bioavailability that depends upon the co-administration of food can be significantly reduced when formulating drugs as lipid-based formulations. When incorporating lumefantrine into a lipid, the solubility and bioavailability of lumefantrine are improved, without the need for a meal rich in fat (Kalepu *et al*., 2013).

2.9.3 Combination treatment with lumefantrine and artemisone

Due to the resistance that *P. falciparum* parasites have built against conventional antimalarial drugs, it is crucial to develop new antimalarials to overcome this obstacle. The development of a fixed-dose combination therapy, consisting of drugs that differ in their mechanisms of action, as well as in their half-lives, would reduce the chances of resistance from forming against the individual drugs and would it furthermore interrupt the transmission of *P. falciparum* (Basu & Sahi, 2017; Nosten & Brasseur, 2002).

Artemisinins are the group of antimalarial drugs that reduce parasitaemia per asexual cycle the most effectively. Resistance towards the artemisinin derivatives that have already been used in antimalarial treatments, was recognised on the Western Cambodian border, as well as on the border of Thailand-Myanmar. The fact that artemisone had not been used as antimalarial previously, and the fact that lumefantrine had only been used in combination therapies, is advantageous (Phyo *et al*., 2016; Fisher *et al*., 2007). Because artemisone, with its short half-life, and lumefantrine, with its longer half-life, do not show any interactions, they can be formulated into a single, fixed-dose antimalarial to simplify treatment regimens and boost patient compliance. By incorporating the fixed-dose into a lipid dispersion, the problematic bioavailability of lumefantrine would be improved (Fule *et al*., 2013). As described in section 2.11.1, lipid-based formulations have proven valuable in enhancing the absorption, and subsequently the bioavailability of lipophilic drugs, such as artemisone and lumefantrine (Pouton, 2006).

According to the WHO (2015a), pregnant women in their first trimester are treated with quinine and clindamycin, and in their second- or third trimester of pregnancy (if they suffer from uncomplicated malaria), they can be treated with ACTs, such as artemether in combination with lumefantrine. Although evidence with regards to the safety of artemisinin administration during pregnancy is scarce, there is no evidence of mutagenicity, nor teratogenicity (McGready *et al*., 2001). A study done by Moore *et al.* (2016) showed that there is an increased risk of miscarriage in the first trimester when infected with *P. falciparum* malaria. Nonetheless, no evidence supports the association that treatment with artemisinin derivatives in the first trimester has increased the risk of congenital malformations, nor miscarriage (Moore *et al*., 2016).

It was found that the anti-angiogenic, anti-etrythropoietic and antivasculogenic effects that are linked to artemisinin, when given to pregnant women in their first trimester, have been due to the aftermath of the metabolite, DHA embriotoxicity. Therefore, the postulation can be made that artemisone may be safe in the first trimester of pregnancy, since artemisone does not convert into DHA (Li & Weina, 2009). With this in mind, it is possible to say that the combination of artemisone and lumefantrine would be safe for all to use as treatment for malaria.

2.10 CLASSIFICATION OF MATRIX SYSTEMS

Modified release drug delivery systems have become a promising tactic to deliver drugs that are taken orally, but have several factors that can contribute towards sub-optimal therapeutic levels, i.e. short half-lives, frequent administration, specific drug targets and local side effects. In these circumstances, matrix systems that offer modified drug release, have made a breakthrough. A matrix system is composed of drug(s) being dispersed uniformly throughout a polymer matrix (Patel *et al*., 2011; Özyazici *et al*., 2006).

Matrix systems are recommended due to their simplicity, their ability to target specific organs or tissue, as well as their ability to improve the bioavailability of the incorporated drugs (Reddy *et al*., 2017; Patel *et al*., 2011). There are different types of matrices, based upon what polymer is used in the production.

2.10.1 Hydrophilic matrix system

Hydrophilic matrices are mostly used in oral drug delivery systems, with controlled release as the objective. This is due to their flexibility to acquire the desirable release profile, as well as the fact that this matrix can be tableted through direct compression and wet granulation. A gelling agent is used to form this type of matrix system. To trigger the release mechanism,

water is needed for the gel diffusion barrier to form. This diffusion barrier controls drug release. Polymers, such as hydroxyethyl cellulose, hydroxypropyl methylcellulose (HPMC), chitosan and modified starches can be used in the formulation of hydrophilic matrices (Reddy *et al*., 2017; Patel *et al*., 2011).

2.10.2 Hydrophobic matrix system

To form a hydrophobic matrix, a hydrophobic or inert polymer is mixed with a drug and tableted by means of direct compression. This matrix type is used when the goal is to formulate a tablet for sustained drug release. The dissolving drug must first diffuse through a web of channels that formed between the compressed polymer particles, thus providing the patient with sustained drug release. The rate at which a liquid can penetrate the matrix, acts as the rate controlling step. Hydrophobic matrices become inert upon contact with water and GI fluid. Examples of hydrophobic polymers that are used to formulate these types of matrices are polyvinyl chloride, ethyl cellulose, stearic acid, cetostearyl alcohol and beeswax (Reddy *et al*., 2017; Patel *et al*., 2011).

2.10.3 Biodegradable matrix systems

When formulating a biodegradable matrix, it is important to bear in mind that these matrices are biologically degradable and/or erodible by either enzymes that are produced by adjacent living cells, or by non-enzymatic processes that degrade the polymers into monomers and oligomers so that the body can metabolise and excrete them. Biodegradable matrices are prepared by utilising polymers, such as proteins, polysaccharides, aliphatic polyesters and –poly-anhydrides. These polymers consist of monomers that are linked through various functional groups (Reddy *et al*., 2017; Kalepu *et al*., 2013; Patel *et al*., 2011).

2.10.4 Lipid matrix systems

To formulate drugs with poor solubility and permeability in an efficacious and safe form, a balance between toxicity and bioavailability should be maintained. One of the techniques used to overcome some of these issues, is solid dispersions. Lipid matrices are formed by utilising lipid waxes and/or related materials. Moreover, lipids as carriers of poorly aqueous soluble drug delivery, have gained interest in the last decade. Upon contact with a liquid, drug release occurs through erosion, as well as pore diffusion. Sensitivity to GI digestive fluids is more apparent with lipid matrices, compared to insoluble polymers. Examples of polymers used in lipid matrices include a combination of carnauba wax and stearic acid or

stearyl alcohol, semisynthetic triglycerides, natural fats and natural oils (Reddy *et al*., 2017; Kalepu *et al*., 2013; Patel *et al*., 2011).

2.10.5 Mineral matrix systems

According to Reddy *et al.* (2017), the polymeric materials being utilised in this type of matrices are hydrophilic carbohydrates. Hydrophilic carbohydrates are found in *Phaephyceae* (species of brown seaweed).

2.11 THE ADVANTAGES OF USING HOT-MELT TO PREPARE THE LIPID DISPERSIONS

2.11.1 Lipid-matrix tablets

Lipid matrix particles have attracted significant attention over the past years, especially in cases where drugs with high lipophilicity need to be incorporated into various dosage forms (Xia *et al*., 2014). The use of lipid-based formulations holds several advantages when used correctly. A wide variety of either natural or synthetic lipids can be employed as a multi-purpose excipient, to improve absorption and to play a crucial role, especially in the release of lipophilic drugs (Feeney *et al*., 2016). The triggering of gall bladder contractions, as well as the increase of the secretions of the gallbladder and the pancreas are two ways in which lipid-based drug dispersions seemingly improve the GI absorption of lipophilic drugs, such as artemisone and lumefantrine (Elgart *et al*., 2012). The incorporation of lipids can increase their bioavailability, by enhancing lymphatic transport that hence avoids first-pass hepatic metabolism (Xia *et al.*, 2014).

Drug release can occur in one of two ways, i.e. through erosion and/or pore diffusion. In this study for example, the mechanism of drug release action that was used comprised of pore diffusion, since a matrix is formed when the lipid (stearic acid or glycerol mono-stearate) is melted and the drugs are added. When the dispersion is cooled, the lipids form a coating around the drug particles and are pores formed in the matrix. These pores aid in the sustained release of the drugs incorporated within the lipid matrix (Abd-Elbary *et al*., 2013; Nisha *et al*., 2012).

Many pharmaceutical excipients are suitable for preparing drug delivery systems by means of the hot-melt method. In this study, the lipids used were stearic acid (SA) and glycerol mono-stearate (GM). Glycerol mono-stearate is an ester of stearic acid, with a hydrophobic tail and a hydrophilic head. It has a low hydrophilic-lipophilic balance and has non-iconic surfactant properties. Due to these properties, GM is an ideal excipient to be utilised as a lubricant, emulsifier, emollient, or as a dispersant in numerous applications (Han & Wang,

2016). GM has a melting point of 77–79°C with a log *P* of 7.4 (NCBI, 2015), indicative of its high lipophilicity. SA (stearic acid) on the other hand has a higher log *P* of 8.23, and a melting point of 66–69°C (Lohan *et al*., 2016; NCBI, 2014). SA is furthermore relatively inexpensive, rendering it suitable for this study (Gonzalez *et al*., 2014).

Malaria undermines the economy in various ways, thus by reducing the expenses of excipients, the cost of the product is also reduced, rendering the tablets more affordable for malaria prone third world countries (Davis *et al*., 2011; Gallup & Sachs, 2001). SA shows erodibility that can lead to higher release rates in dissolution studies (Özyazici *et al*., 2006). These lipophilic materials (GM and SA) offer good stability at various pH values and are safe for use in humans (Obaidat & Obaidat, 2001).

2.11.2 Hot-melt lipid dispersions

Taking drugs orally is the most suitable and preferred choice when it comes to administering medication (York, 2011). It is also the administration method of choice for malaria treatments, due to several factors, including the fact that malaria is prevalent in poor and secluded areas, where the necessary equipment needed to administer drugs intramuscularly or intravenously are not available, where there are no health care workers with the necessary knowledge or skill on how to administer these drugs, and where the patients are poor and cannot afford the treatment (Worrall *et al*., 2005; Bloland, 2001).

The problem with more than 60% of drugs, such as lumefantrine and artemisone (class II drugs), is the fact that they portray low aqueous solubility, and therefore, poor bioavailability. Poor aqueous solubility can be defined as the requirement of a longer period to dissolve in the GI fluid than the period it takes to be absorbed in the GI tract. This can lead to promising products not reaching patients. The usage of poorly soluble drugs in oral formulations presents a significant challenge to formulators. Two factors that play a critical role in controlling drug dissolution and the dissolution rate from the dosage form are the physicochemical properties and the water solubility of the API in question. The dissolution of the drug(s) is essential for its absorption from the GI tract, therefore it is essential to understand the behaviours (in dissolution and absorption) of the drugs to profitably formulate them into solid dosage forms that are adequately bioavailable (Kalpana *et al*., 2010).

Solid dosage forms are always prepared in conjunction with excipients that can influence the properties of the API(s). The Biopharmaceutical Classification System (BCS) accentuate dissolution rate as the restrictive factor of class II (high permeability, low solubility) and class IV (low permeability, low solubility) drugs (Kalpana *et al*., 2010). In trying to improve a compound’s aqueous solubility, the absorption in the GI tract can be impaired. There are,

however, various methods to increase drug bioavailability by improving the dissolution rate, such as through salt formation, pH modification, micronisation and emulsification (Pawar *et al*., 2016; Fule *et al*., 2013; Steyn *et al*., 2011).

Lately, hot-melt has gained popularity as a method for producing solid dispersions. Solid dispersions can be used to describe a group of dosage forms where the drug(s) is dispersed within an inert matrix with the purpose of enhancing its bioavailability (Kalpana *et al*., 2010). Hot-melt can be used for the preparation of various delivery systems, including pellets, granules, sustained release tablets, transmucosal and transdermal delivery systems, as well as implants (Repka *et al*., 2007; Özyazici *et al*., 2006). There are two approaches to hot-melt, i.e. hot-melt fusion and hot-melt extrusion.

Hot-melt extrusion (HME) is a process that comprises of a feeding system, an extruder (with conveying, melting and mixing sections), a die, and processing units. A polymer, the drugs and other excipients are forced though a rotating screw whilst applying heat. The process is set up in such a way that the formulation heats up and distorts inside of the extruder system. It then solidifies at the exit where the die regulates the form and size of the extrudate (Roblegg *et al*., 2011). This continuous process is well known to produce products of uniform density and shape (Crowley *et al*., 2007). HME is a cost effective, solvent free, environmentally friendly and easy technique, and can be used in the industry. It is thus a practical method for developing dosage forms (Pina *et al*., 2014; Fule *et al*., 2013; Roblegg *et al*., 2011).

Hot-melt fusion (HMF), on the other hand, can be described as a process where a polymer is melted with unceasing stirring in a porcelain mortar on heated water. The chosen drugs are homogenously mixed into the melted polymer and allowed to cool. After the mass solidifies, it is ground and sieved until the appropriate particle size is achieved (Nikghalb *et al*., 2012; Kalaiselvan *et al*., 2006; Obaidat & Obaidat, 2001). The vigorous mixing during the melting step de-aggregates the particles and improves the homogeneousness of the content. Content uniformity plays an important part in dosage repeatability to secure optimal drug levels in the blood of the patient (Özyazici *et al*., 2006).

Hot-melt improves the saturation solubility of drugs, as well as offers a cost-effective, inert, simple, and stable method at different pH values (Abd-Elbary *et al*., 2013). Another advantage that hot-melt presents are the fact that it is able to convert the crystalline form of most drugs from crystalline into the stable, amorphous state (Fule *et al*., 2013; Takeuchi & Nagira, 2004). The amorphous form of a drug can improve dissolution behaviour, as well as the bioavailability of a drug with low aqueous solubility. By means of hot-melt, the chemical

and physical properties of drugs, such as their solubility, stability, density and bioavailability can be altered (Kulkarni *et al*., 2013). It is critical to keep in mind that the mechanical properties of drugs and excipients can also be altered through hot-melt, thus interfering with processes, such as tableting, may be apparent (Grymonpré *et al*., 2016). The mechanical strengths of these lipid matrix dosage forms should be tested to establish whether GI motility would not cause an unexpected burst effect that would lead to dose dumping, or toxic drug levels (Özyazici *et al*., 2006). In this study, the hot-melt method was utilised in the formulation of a fixed-dose antimalarial dosage forms.

2.12 SUMMARY

Malaria, together with acquired immunodeficiency syndrome (AIDS) and tuberculosis are major life-threatening diseases in Africa. Although malaria is found throughout the world, it is prevalent and more severe in tropical climates, but to a lesser degree in South-Africa. Factors, such as poverty, poor health infrastructure, malnutrition and poor patient compliance aggravate malaria control (Worrall *et al*., 2005; Bloland, 2001).

ACTs are recommended by the WHO (2015) as the first-line treatment of malaria, due to the resistance by malarial parasites towards several single component antimalarial drugs. Combination drugs have proven to be more effective and to prevent the appearance of resistance towards antimalarials when used in combinations. For a combination drug to be successful, the APIs must have different mechanisms of action, have different half-lives, while appropriate dosages must be used (White, 2009; Aweeka & German, 2008).

The artemisinin derivative, artemisone, is a very potent antimalarial drug that eliminates the largest fraction of the parasites, thus, leaving a smaller fraction of the malaria parasites for the partner drug to abolish (Basu & Sahi, 2017; Nosten & Brasseur, 2002). The combination of artemisone and lumefantrine is a newly proposed combination drug that may be more effective when compared to an artemether and lumefantrine combination.

CHAPTER 3

METHODS & MATERIALS

3.1 INTRODUCTION

The tempo and the extent of drug dissolution is subjected to the solubility properties of the drugs utilised and the characteristics of the dosage form in question. It is critical to produce a dosage form comprising the correct formulation to release the drug at the precise moment, at the site of absorption (Weston & Yeboah, 2013). Bearing in mind that a drug administered orally should be in solution to have a therapeutic effect; consequently, only drugs in solution could be absorbed in the stomach or gastrointestinal tract to reach systematic circulation. The quantities of the dosage as well as the dosage form furthermore play a vital part in the rate of absorption and the degree of the therapeutic effect (Perioli *et al.,* 2012).

To develop an effective new ACT (artemisinin combination therapy), a selected variety of excipients were used and tested in this study. Different lipids (i.e. glycerol monostearate and stearic acid) were respectively combined with the artemisone and lumefantrine combination by means of hot-melting. Designated fillers, i.e. MicroceLac® 100, RetaLac® and CombiLac®, were subsequently individually mixed with the lipid dispersions and tableted by means of direct compression.

This chapter deals with the various materials used and formulations tested in this study; as well as the different experimental methods employed to develop and evaluate a fixed-dose drug delivery system. Methods discussed in this chapter were performed to evaluate the flow properties of the different fillers; to assess physical properties of the different formulations; and analyse and compare the dissolution profiles of the various selected formulations.

3.2 MATERIALS

The drugs and excipients used in this study are presented in Table 3.1.

Table 3.1: *Information on the excipients and drugs used to conduct this study*

Ingredient	Batch number	Manufacturer
Lumefantrine	20161225	Kindly donated by the MRC Flagship Programme
Artemisone	N/A	Kindly donated by the MRC Flagship Programme
Chloroform	29393	Associated Chemical Enterprise (Pty) Ltd, Johannesburg, South Africa
Magnesium Stearate	21203	Warren Chem, Cape Town South Africa
Stearic Acid	32192	Associated Chemical Enterprise (Pty) Ltd, Johannesburg, South Africa
Glycerol Monostearate	J05W007	Associated Chemical Enterprise (Pty) Ltd, Johannesburg, South Africa
Hydrochloric acid 32%	33080 32385	Associated Chemical Enterprise (Pty) Ltd, Johannesburg, South Africa
Tri-Sodium orthophosphate	31841	Associated Chemical Enterprise (Pty) Ltd, Johannesburg, South Africa
Sodium lauryl sulphate	1043002	Merck (Pty) Ltd, Germany
Sodium hydroxide pearls	19898	Merck (Pty) Ltd, Germany
Orthophosphoric acid	338306	Merck (Pty) Ltd, Germany
Acetonitirle	I089093722	Merck (Pty) Ltd, Germany
Methanol	I880807711	Merck (Pty) Ltd, Germany
Octane sulphonic acid sodium salt	K48694707707	Merck (Pty) Ltd, Germany
CombiLac®	L1434	Meggle Group, Wasserburg, BG Excipients & Technology
MicroceLac 100®	L96887	Meggle Group, Wasserburg, BG Excipients & Technology
RetaLac®	L416300	Meggle Group, Wasserburg, BG Excipients & Technology

3.3 PREPARATION OF FIXED-DOSE/LIPID DISPERSIONS

Fixed dose/lipid dispersions containing 80 mg artemisone and 120 mg lumefantrine were prepared by means of the hot fusion method. First, 20 g of the lipids (stearic acid or cetostearyl alcohol) were individually melted in a porcelain dish on top of a water bath at a temperature of 75°C (±2°C). Both the accurately weighed artimisone and lumefantrine were added to the melted lipids whilst the stirring continued until a homogenous mixture (drug-

loaded melt dispersion) was obtained. Different ratios of the drug-loaded melt dispersions were used namely, 1:0.5 (i.e. 200 mg artimisone/lumefantrine combination and 100 mg lipid) and 1:1 (i.e. 200 mg of both the artimisone/lumefantrine combination and the lipid). These drug-loaded melt dispersions were allowed to cool and solidify at 25°C (±2°C). Consequently, the hardened mass was grounded, pulverised and passed through a 60 mesh or <300 µm sieve. All of the resulting powder formulations were stored at 25 ± 0.5°C in sealed glass containers, covered with Parafilm®, until utilised (Abd-Elbary *et al.,* 2013).

3.4 CHARACTERISATION OF FIXED-DOSE/LIPID DISPERSTIONS

3.4.1 Differential Weight Loss Thermogram (DTG)

Differential weight loss thermogram (DTG) integrates both thermogravimetry analysis (TGA) and differential scanning calorimetry (DSC) concurrently. DSC is a renowned thermoanalytical method that is utilised to establish information such as the melting point, enthalpies of conformational change, heat of fusion, purity of samples, potential polymorphs, thermal stability as well as decomposition of the sample being analysed (Sedov *et al.,* 2016; Brown, 2001). This method was utilised to determine the variance in the heat flow rate of the tested samples under controlled conditions. Occurrence of any exothermic and/or endothermic reactions indicates an interaction between the components the samples are comprised of (Klančnik *et al.,* 2010; Höhne *et al.,* 2003; Clas *et al.,* 1999). TGA is utilised to explore chemical reactions in solid and liquid states at raised temperatures. Structural changes and other properties can be studied under controlled heating conditions. TGA monitors sample mass loss typically associated with dehydration, decomposition and/or oxidation, whilst exposed to increasing temperatures (Klančnik *et al.,* 2010).

A Shimadzu DSC-60A (Shimadzu Instruments, Kyoto, Japan) was used to obtain DSC- and TGA-thermograms of the different samples (i.e. artemisone/lumefantrine combination; lipid; artemisone/lumefantrine/lipid physical mixture in different ratios; artemisone/ lumefantrine/lipid dispersions; and combinations of artemisone/lumefantrine/lipid dispersions and one of the fillers). The sample weighed approximately 2 mg and were placed in aluminium samples holders (100 µl). The gas flow of the nitrogen was set to 30 $cm^3.min^{-1}$ (Lemmer, 2012; de Kock, 2005). The temperature was predetermined conferring with the melting points of the active ingredients and excipients used in this study.

3.4.2 Thermal Activity Monitor analysis

Isothermal microcalorimetry was utilised to determine whether interactions occurred with the components of the lipid matrix tablets. In this study, a Thermal Activity Monitor (TAMIII) apparatus (TA Instruments, New Castle, Delaware, United States of America) equipped with

an oil bath with a stability of approximately 100 µK was used over a 24 h period. The temperature of 50°C was maintained, as this is below each component's melting point. A mass of approximately 100 mg of each sample was used throughout the duration of the experiment. To identify any possible reactions between the various components, a baseline for each individual component had to be established. The calorimetric output by the separate components was summarised as a theoretical response, which is the anticipated output should the components not show any interaction. If an interaction was detected, the calorimetric output of the components would vary from the theoretical response.

3.4.3 X-ray diffraction

X-Ray Powder Diffraction studies are utilised to identify the crystalline structure of a solid-state sample to confirm if amorphous forms exist and to distinguish between them. X-ray powder diffraction (XRPD) was used to differentiate between the various crystalline forms and to confirm whether the hot-melt method formed amorphous solid dispersions (Bhattacharya *et al.,* 2009).

The X-ray spectra of each of the powdered samples weighing approximately 20 mg (i.e. either artemisone/lumefantrine combination; lipid; artemisone/lumefantrine/lipid physical mixture in different ratios; artemisone/lumefantrine/lipid dispersions; or combinations of artemisone/lumefantrine/lipid dispersions and one of the fillers) was recorded on an X-ray diffractometer (PANalytical, Almelo, The Netherlands) at room temperature. The experimental conditions were: Ni-filtered CuK_{α} radiation at a wave of 1.542 Å; tube current at 40 mA; voltage at 45 kV; divergence slit, 2 mm; antiscatter slit at 2 mm, 0.6 mm and 0.2 mm; and scanning speed at 2°/min over a range up to 4°2θ.

3.5 POWDER FLOW PROPERTIES

Characterisation of the flow properties was conducted on the active ingredients, selected fillers used in this study, as well as on the fixed dose lipid dispersions (preparation described in section 3.6.2). Characterisation was executed according to the British Pharmacopoeia (BP, 2017) to compare, evaluate and identify the most suitable powders and powder mixtures for the study. The following sections will converse the parameters for the characterisation of the flow properties.

3.5.1 Powder densities

The flow of a powder and its densities are linked. If a powder has a higher bulk density, it is most likely to have a poor flowability, whereas a lower density powder will have more improved flow properties. Density is the ratio between weight and volume of a material

indicated as $g.cm^{-3}$. The parameters that were used to obtain the necessary powder density information include bulk density and tapped density (Jallo *et al*., 2012; de Kock, 2005).

Bulk density is defined as the mass per unit volume including the inter-particular and inter-particular spaces in between the powder particles. Tapped density, on the other hand, is defined as the mass per unit volume in the cylinder after it has been tapped for a specified period of time, thus, minimising the amount of inter-particular spaces between the particles (Amidon *et al.,* 2009).

Both the bulk and tapped densities of the drugs, selected fillers, as well as the different ratios of drug-loaded melt dispersions were determined by an Erweka® Tapped Density Tester (SVM 121/221, Germany). Samples of each powder weighing approximately 50 g were transferred to a 250 ml cylinder. The initial volume was noted as the bulk volume for each formulation. The Erweka® Tapped Density Tester was set at an amplitude of 5 A for 4 min and turned on. During this time, a constant volume was obtained and recorded as the tapped volume. The densities were subsequently calculated, where Equation 3.1 was employed to determine the bulk density and Equation 3.2 was utilised to calculate the tapped density.

$$\rho_b = \frac{m}{v_b} \qquad \textbf{[3.1]}$$

Where P_b is the bulk density, m the mass and V_b the initial volume of the powder (BP, 2016).

$$\rho_t = \frac{m}{v_t} \qquad \textbf{[3.2]}$$

Where P_t is the tapped density, m the mass and V_t the tapped volume of the powder (BP, 2017).

3.5.2 Compressibility (Hausner ratio and Carr's index)

The bulk and tapped densities of the different samples were utilised in calculating the Hausner ratio together with the percentage compressibility (Carr's index) for each sample (BP, 2017). Compressibility provides a finer understanding of the flowability of powders. The following equations were used:

$$\text{Hausner ratio} = \frac{T}{B} \qquad \textbf{[3.3]}$$

$$\%\text{Compressibility} = \frac{\rho_T - \rho_B}{\rho_T} \quad 100 \qquad \textbf{[3.4]}$$

3.5.3 Critical orifice diameter

Critical orifice diameter (COD) can be defined as the smallest diameter through which a powder can freely and continuously flow (Buys, 2006). The method and apparatus first described by Buys *et al.* (2006) were employed. Copper disks with different sized openings through their centres were placed on top of each other to form a smooth funnel with the largest opening facing upwards. The assembly was equipped with a shutter and raised approximately 10 cm above the horizontal surface. A cylinder containing the powder sample (100 g) to be tested were placed on top of the stack of disks. The shutter was opened, and the interchangeable copper disks were removed one by one from the stack starting from the top. Once the powder started to flow through the bottom most disk still in the assembly, the diameter of that disk's orifice was noted as the COD. This experiment was conducted in triplicate.

3.5.4 Flow rate

The flow rate was calculated by measuring the time it takes 100 g of each powder sample to flow through an orifice with a 10 mm diameter at a set height of 100 mm from the horizontal surface. This experiment was conducted in triplicate for each powder sample that was tested. A shutter was placed over the outlet of the funnel and the funnel was filled with 100 g of a specific powder sample. The shutter was removed, and the powder could freely flow through the orifice while the time it took the entire powder sample to flow through, was recorded. The experiment was conducted in triplicate. Subsequently the flow rate was calculated by means of Equation 3.5.

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

Where the flow rate (F) is measured in $g.s^{-1}$; M is the mass (g) and t is time (sec) taken.

3.5.5 Angle of repose

The angle of repose was measured in triplicate as stated in the BP (2017) in Appendix XVII N. The apparatus consists of a stainless-steel funnel with an orifice diameter of 10 mm fitted with a shutter. The funnel was secured at a height of 100 mm above the surface. This ensured that the diameter and height were set standards and that consistent results were achieved (Lavoie *et al*., 2002). A powder sample (100 g) was weighed and placed in the funnel; where after the shutter was opened. A powder cone formed as the powder flowed through the orifice unto the horizontal surface; and the height of the cone as well as the length of the base were measured and noted. To calculate the angle of repose, the following equation was used:

$$\tan \theta = \frac{h}{r} \qquad \textbf{[3.6]}$$

Where θ is the angle of repose; h, is the height of the heap in mm, and r, is the radius of the heap also in mm.

3.6 PREPARATION OF FIXED-DOSE LIPID MATRIX TABLETS

3.6.1 Full factorial design

A full factorial design was employed to study the effects of the different formulation factors on the properties of the fixed-dose/lipid dispersions. The motivation behind using a factorial design is to determine interactions and effects of numerous combinations of a variety of factors as well as the levels thereof. In formulation, a factorial design provides an objective, systematic guideline to evaluate the different excipients and their concentrations to find the optimum formulation. The use of this statistical method removes the guess work and trial-and-error method from experimentation. A factorial design condenses the numerous experiments that should be executed by studying several factors simultaneously (de Kock, 2005).

In this study, formulation variables were assessed, namely the two types of lipid (i.e., glycerol monostearate and stearic acid); the ratio of drugs to lipid (1:05 and 1:1); type of filler (MicroceLac® 100, RetaLac® and CombiLac®); and magnesium stearate concentration (lubricant included in either 0.5 or 1% w/w). In Table 3.2 the abbreviations assigned to each factor are given. These factors were assessed at different levels, namely the two types of lipid (glycerol monostearate and stearic acid); the drugs-lipid ratio at two levels (1:0.5 and 1:1); three types of fillers (MicroceLac® 100, RetaLac® and CombiLac®); and the magnesium stearate concentration at two levels (0.5 and 1% w/w), respectively.

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

Factors	Variables	Levels
Type of lipid	Glycerol monostearate (G)	2
	Stearic acid (S)	
Filler type	MicroceLac® 100 (M)	3
	RetaLac® (R)	
	CombiLac® (C)	
drug:lipid ratio	1:0.5	2
	1:1	
Magnesium Stearate (lubricant)	0.5% w/w	2
	1% w/w	

Table 3.3 shows the full factorial design with the different formulations that were prepared for tableting by means of direct compressed. For example, G1R0.5 represents glycerol monostearate as the lipid, 1:1 drugs:lipid ratio, RetaLac® as the filler, and 0.5% w/w magnesium stearate used to formulate the tablets. Similarly, S0.5M1 signifies tablets containing stearic acid as lipid in a 1:0.5 drugs:lipid ratio, with MicroceLac® 100 as filler, and 1% w/w magnesium stearate.

Table 3.3*: Full factorial design of this study*

				Filler type:					
				MicroceLac®100		RetaLac®		CombiLac®	
				Lubricant concentration (% w/w)					
				0.5	1.0	0.5	1.0	0.5	1.0
Lipid type:	Stearic acid	API:lipid concentration	1:1	SA1:1M0.5	SA1:1M1	SA1:1R0.5	SA1:1R1	SA1:1C0.5	SA1:1C1
			1:0.5	SA1:0.5M0.5	SA1:0.5M1	SA1:0.5R0.5	SA1:0.5R1	SA1:0.5C0.5	SA1:0.5C1
	Glycerol monostearae		1:1	GM1:1M0.5	GM1:1M1	GM1:1R0.5	GM1:1R1	GM1:1C0.5	GM1:1C1
			1:0.5	GM1:0.5M0.5	GM1:0.5M1	GM1:0.5R0.5	GM1:0.5R1	GM1:0.5C0.5	GM1:0.5C1

3.6.2 Preparation of powder mixtures and solid dispersions

To formulate the lipid matrix tablets, glycerol monostearate and stearic acid were used individually. A porcelain bowl was heated over water (±75°C) and the lipid (20 g) placed into the bowl. Whilst stirring, the drugs (artimisone/lumefantrine : 80/120 mg) were added to the melted lipid in a drugs:lipid ratio of either 1:1 (i.e. 200 mg of both the artimisone/lumefantrine combination and the lipid) or 1:0.5 (i.e. 200 mg artimisone/lumefantrine combination and 100 mg lipid). After the selected lipid and the drugs were melted and thoroughly mixed, this lipid dispersion was left to cool and solidify at room temperature (25 ± 2°C). Pulverising and grounding the solid dispersion was the next step before passing the pulverised dispersion through a sieve (60 mesh or <300 µm) to acquire the desired particle size (Abd-Elbary *et al.,* 2013) and finally producing a drug-melted lipid dispersion.

The artemisone/lumefantrine lipid solid dispersions were mixed with one of the selected fillers as predetermined by the full factorial design using a Turbula® mixer (T2C, W.A. Bachofen AG Maschinenfabrik, Bastle, Switzerland). These formulation ingredients were mixed in sealed glass containers at 69 rpm for 7 min. Post mixing, the glass containers were sealed with Parafilm® until utilised for direct compression.

3.6.3 Direct compression

Each powder mixture, as predetermined by the full factorial design; and containing an artemisone/lumefantrine lipid solid dispersion, one of the selected fillers, and a predetermined concentration magnesium stearate was compressed into 10 mm tablets of approximately 500 mg in weight (adjusting the lower punch for consistency) using concave faced punches and an eccentric single tablet press (Cadmach®, India). An upper punch setting that determined the applied load, was adjusted with each formulation so that the tablets would be hard enough to remain intact. The upper load setting varied between 20 and 30.

With each formulation, the first twenty tablets compressed were discarded. Afterwards the tablets were placed in clean 250 cm^2 glass bottles and sealed off with Parafilm® before screwing on the bottle caps. Tablets were stored at room temperature (25 ± 2°C) in dark cabinets for no less than 24 h prior to analysis.

3.7 PHYSICAL EVALUATION OF FIXED-DOSE LIPID MATRIX TABLET FORMULATIONS

3.7.1 Assay

Twenty tablets from a specific formulated batch were placed in a mortar and crushed with a pestle. The subsequent powder was weighed to an equivalent of half a tablet (250 mg) per sample. Each sample was transferred into a 100 ml volumetric flask. This was repeated 9 times. Each sample was dissolved in 100 ml HPLC-grade methanol (Graves *et al.,* 2015) and ultrasonicated for 15 min in a Labotec Ecobath® (model 103, Labotec, South-Africa) for complete dissolution. Afterwards, the solution was filtered through a 0.45 µm membrane filter attached to a syringe; and the filtrate was diluted in order to determine drug content. The filtrate was analysed by means of High Performance Liquid Chromatography (HPLC) as described in section 3.9. The experiment was conducted in triplicate.

3.7.2 Mass variation

According to the British Pharmacopoeia (2016) criteria, weight variation was conducted on 20 tablets that were selected at random from each formulation. Excess powder was dusted from each tablet with an art brush and weighed individually on a Precisa® analytical balance (Precisa®, Zurich, Switzerland). Each of the 20 tablets' weight was noted and the average weight (mg), standard deviation (SD) and percentage relative standard deviation (%RSD) were computed and evaluated (BP, 2017; Viljoen *et al.,* 2014).

3.7.3 Crushing strength, diameter, thickness and tensile strength

Utilising the Pharma Test® (model PTB 311, Switzerland) apparatus, ten randomly selected, 10 mm concave tablets from each formulation were crushed at a rate of 0.1 $cm.min^{-1}$, where the resistance that the tablets had shown were measured in Newton (N). The machine also took measurements of the diameter and thickness of the tablets.

The average crushing strength (N), standard deviation (SD) and percentage relative standard deviation (%RSD) were calculated; and the tensile strength for concave tablets was calculated utilising Equation 3.7.

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

Where, σ, is the tensile strength ($N.mm^{-1}$); 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 (Viljoen *et al.,* 2014).

3.7.4 Friability

To measure the friability, 10 arbitrarily selected tablets from each formulation were dusted with an art brush and weighed; where the measurement was noted as the initial weight (W_1). The 10 tablets were placed in the friabilator (ERWEKA® GmbH, Heusenstamm, Germany) that ran at 25 rpm for 4 min. After rotation, the sample was taken from the friabilator, dusted, weighed again, and the measurement was recorded as the end weight (W_2) (Mallipeddi *et al.,* 2010). Percentage mass lost or friability (%F) was computed using the following equation (BP, 2017; Viljoen *et al.,* 2014):

$$\%F = \frac{W_1 - W_2}{W_1} 100 \qquad \textbf{[3.8]}$$

3.7.5 Disintegration

Disintegration experiments were conducted on all of the different tablet batches formulated. The BP (Appendix XII A) states that for conventional tablet batches to be deemed acceptable, 6 randomly selected tablets of the specific batch should fully disintegrate within 15°min in a disintegration bath filled with water and maintained at a temperature of 37 ± 0.5°C (BP, 2017). As this study attempted to produce delayed drug release tablets, it was decided that the tablets had to remain intact for at least 15°min in order to possibly accomplish modified/delayed release. Therefore, 6 tablets were randomly selected from each formulation and individually placed into the baskets of a basket-rack assembly disintegration type II apparatus (Erweka® model D-63150, Heusenstamm, Germany). The baskets were mechanically raised and lowered in a water bath containing distilled water that was maintained at 37°C (± 2°C). Each tablet's disintegration time was noted once complete disintegration occurred, or the apparatus was stopped after 15 min and the mean disintegration time calculated.

3.7.6 Swelling and erosion

The method that was used to determine the swelling and erosion of the prepared tablets was published by Singh *et al.* (2009). Ten randomly selected tablets were individually weighed and placed into a type II dissolution Distek 2500 system basket (2501049, North Brunswick, New Jersey, USA). The conditions in the dissolution system were standard (37 ±0.5°C) with distilled water used as media. All the swelling and erosion tests were done in tenfold. The initial mass (T) of each of the baskets containing the individual samples was recorded. At specified time intervals (5, 10, 15, 30, 45, 60, 75, 90, 120 and 150 min), the dissolution system was stopped or until the tablets were fully disintegrated. Each basket was removed, dried with filter paper and weighed. This weight was recorded as the swollen weight (S).

Following the swelling experiments, the swollen samples were individually placed in an EcoTherm Labotec oven at 40°C to dry until no further loss in tablet mass could be measured at specified time intervals (5, 10, 15, 30, 45, 60, 75, 90, 120 and 150 min). This weight was recorded as the final mass (R) and was used to measure if any erosion occurred. By using Equations 3.9 and 3.10, the percentage swelling and erosion were respectively calculated, (Singh *et al.,* 2013):

$$\% \text{ Swelling} = \frac{S}{T} \quad 100 \qquad \textbf{[3.9]}$$

$$\% \text{ Erosion} = \frac{T - R}{T} \quad 100 \qquad \textbf{[3.10]}$$

Where, T, is the initial mass (g); S, is the swollen weight (g); and, R, is the mass after erosion (g).

3.8 DRUG RELEASE PROPERTIES OF SELECTED FIXED-DOSE LIPID MATRIX TABLET FORMULATIONS

The Distek® dissolution system (model 2500, Distek® Inc, North Brunswick, New Jersey) was utilised to conduct drug release studies for 12 h; and was connected to the Distek® Evolution 4300 auto sampler and a Distek syringe pump (SP02716). The test was conducted in 6-fold. Dissolution media (900 ml) at a set temperature of 37°C (± 0.5ºC) was used in each test and the paddle speed was set to 50 rpm. For the first 2 h of the experiment, 600 ml hydrochloric acid (HCl) with a concentration of 0.1 M was utilised as the dissolution media. Sodium lauryl sulphate, a surfactant, was added to the HCl at 1% w/v of the total volume (900 ml) of each chamber. After the first 2 h, 300 ml of phosphate buffer was added (pH 6.8) and used for the next 10 h (Singh *et al.,* 2009). Samples that consisted of 5 ml each were withdrawn at intervals of 5, 10, 15, 30, 60, 90, 120, 150, 180, 240, 300, 390, 480, 600 and 720 min and the 5 ml extracted, was replaced with fresh pre-heated dissolution media.

For the first 120 minutes of dissolution, 600 ml of a 0.1 M HCl (pH of 1.2) was poured in the dissolution chambers and heated to a temperature of 37 ± 0.5°C. Sampling at the predetermined times (5, 10, 15, 30, 60, 90 and 120 min) followed for the first 120 min. After the 120 minute sample, 300 ml of trisodium phosphate dodecahydrate buffer was added to each chamber. A following 5 min were allocated to adjust the pH from 1.2 to 6.8 using either a 2 M HCl or a 2 M NaOH solution. In the buffer phase, sampling followed the time schedule of 150, 180, 240, 300, 390, 480, 600, and 720 min where the infinity sample was withdrawn (paddle speed adjusted from 50 rpm to 150 rpm for 30 min). All samples were withdrawn

through a membrane filter of 10 µm (053112-SSK, PINFIL10S-DK, Cannula filters, Telford, Pennsylvania, USA) by means of a syringe and injected into a clean test tube. The samples were replaced with 5 ml fresh dissolution media. The concentration of the compounds in the samples was measured utilising a validated HPLC method at the Analytical Technology Laboratory, NWU Potchefstroom (Section 3.9).

3.9 ANALYSIS OF DRUG CONCENTRATION BY MEANS OF HIGHPERFORMANCE LIQUID CHROMATOGRAPHY

A high performance liquid chromatography (HPLC) method was used to quantify and identify analytes from the dissolution samples. Methods for both artemisone and lumefantrine are available from the Flagship Programme; however, these methods first needed to be validated. The validation of the HPLC method included evaluation of linearity, specificity, range, repeatability, accuracy, limit of detection, and limit of quantification based on the ICH guidelines (ICH, 2005).

Concentration assays were conducted utilising an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA). The system was equipped with a Venusil C_{18}-column, 150 x 4.6 mm, 5 µm, (Agela Technologies, Newark, DE) and the following parameters were set upon analysis of the artemisone concentration: the mobile phase was acetonitrile with 0.05 M octane sulphonic acid in a 75:25 ratio; the injection volume was set to 20 µl; the flow rate to 1.0 ml/min; and the detection wavelength to 205 nm. For lumefantrine, the mobile phase consisted of 0.05 M octane sulfonic acid (1.74 g/l) and acetonitrile in a 75:25 ratio with a pH of 3.5. The injection volume was set to 20 µl; the flow rate to 1.0 ml/min; and the detection wavelength to 238 nm.

3.9.1 Linearity and range

Linearity was determined for both drugs (lumefantrine and artemisone) using linear regression analysis on an axis. To prepare the standard solutions, approximately 20 mg of each drug was individually weighed and dissolved in 100 ml methanol. The standard solution was subsequently diluted with methanol to obtain a concentration range from 0.002 mg/ml to 0.2 mg/ml for each drug.

3.9.2 Precision and accuracy

Repeatability (precision) was determined analysing samples with low, medium and high concentrations for both drugs. Fifteen tablets of any formula in the factorial design (G0.5R1 was selected) were crushed using a pestle and mortar. This powder was used to obtain three samples of 200 mg; 250 mg and 300 mg powder of the crushed tablets and each of

these nine samples were filled to a volume of 100 ml with methanol. Next, 10 ml were diluted to 50 ml and the diluted samples analysed by means of HPLC.

The closer the value is that was obtained by means of HPLC analysis to the true value, the more accurate the method is. Accuracy was established by an analysis done on 3 concentrations at 90, 100 and 110%. This was done in triplicate (ICH, 2005).

3.9.3 Specificity

To determine the specificity, three solutions containing approximately 20 mg lumefantrine (sample 1), 20 mg artemisone (sample 2) or a combination of lumefantrine and artemisone, in a 3:2 ratio, (sample 3) were prepared. A sample containing the placebo (all the excipients used, *i.e.* the fillers, the lipids, and the lubricant), mobile phase, solvent (methanol) and the dissolution media was also injected into the HPLC. These samples were analysed using the conditions stated in section 3.7. The analysis of sample 1, 2 and 3 identified the time of retention for lumefantrine and artemisone. Analyses of the sample containing the mobile phase, solvent, placebo and the dissolution media showed the retention time of these components. For the method to be classified as specific all peaks should be identified and a clear distinction between the other components and the drugs should exist (ICH, 2005).

3.10 STATISTICAL DATA ANALYSIS

The data collected through this study were statistically analysed by means of one way analysis of variance (ANOVA) at the 5% significance level to show significant differences and variations ($p < 0.05$). From the dissolution data obtained, the parameters, described in the following sections, were calculated.

3.10.1 Mean dissolution time

The mean dissolution time (MDT) shows the average time it will take the entire drug dose to be released from the dosage form into solution. MDT was calculated for both artemisone and lumefantrine using Equation 3.11 (Rinaki *et al.,* 2003):

$$MDT = \frac{\sum_{j=1}^{n} t_{mid} x_d}{\sum_{j=1} x_d} \qquad \textbf{[3.11]}$$

Where j is the sample number; n is the total number of samples; t_{mid} is midpoint time between j and j -1; and x_d, is the additional mass of drug dissolved between j and j –1 (Costa & Lobo, 2001).

3.10.2 Fit factors

By using fit factors, a comparison can be drawn between the dissolution profiles of a test and reference formulation; as well as the different formulations prepared in this study. There are two fit factors namely, f_1 and f_2. Fit factor, f_1, was used to measure the percentage error between the two curves. A value of 0 represents indistinguishable curves and as the value increases, the variation between the two curves also increases. To show that the percentage drug that dissolved in a certain amount of time is similar for both the test and the reference values, the value of f_1 must be ≤ 15. For fit factor, f_2, the value of the dissolution profiles must be ≥ 50 to be similar. A fit factor (f_2) that equals 100 shows that the two samples are identical (Moore & Flanner, 1996). The following equations (3.12 and 3.13) represent the fit factors, f_1 and f_2, respectively:

$$f_1 = \frac{\sum_{j=1}^{n} |R_j - T_j|}{\sum_{j=1}^{n} (R_j - T_j) / 2} \quad 100 \qquad \textbf{[3.12]}$$

$$f_2 = 50 \log \left\{ 1 + \left(\frac{1}{n}\right) \sum_{j=1}^{n} |R_j - T_j|^2 \right\}^{-0.5} 100 \qquad \textbf{[3.13]}$$

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).

CHAPTER 4

RESULTS & DISCUSSION

4.1 INTRODUCTION

The aim of this study was to develop lipid matrix tablets containing a double fixed-dose of lumefantrine and artemisone. Formulation of double fixed-dose combinations are challenging due to various complex factors, including the possibility of incompatibilities between the different drugs (Okwelogu *et al.,* 2010).

The selected fillers and lipids were evaluated in terms of their morphology and flow properties, *i.e.* densities, Hausner ratio, Carr's index, flow rate, angle of repose and critical orifice diameter (COD), in accordance to the British Pharmacopoeia (BP) standards of 2016. Characterisation of the fixed-does lipid dispersions (differential scanning calorimetry, thermal activity monitor and X-ray diffraction studies) were also assessed. Subsequently, in order to be able to determine the most acceptable formulation(s) for lipid matrix tablets containing a double fixed-dose of lumefantrine and artemisone, a full factorial design was implemented to prepare the lipid matrix tablets. Post manufacture of these tablets, the physical properties of the tablets (mass variation, friability, hardness, thickness, diameter, tensile strength and disintegration) were evaluated. The formulations that fulfilled the standards set by the BP for the above-mentioned tests were further evaluated regarding dissolution, swelling and erosion, and tested in agreement with the BP criteria (BP, 2017).

4.2 CHARACTERISATION OF FIXED-DOSE/LIPID DISPERSIONS

For a formulator, the pre-formulation studies can be a valuable guideline to assess the formulations and to establish whether any changes are deemed necessary. The flow properties of the APIs (lumefantrine and artemisone), excipients (RetaLac®, CombiLac®, and MicroceLac®100) and the lipid dispersions (glycerol monostearate and stearic acid) were characterised and discussed in section 4.3. Utilising DSC, TGA, TAM and X-ray diffraction studies, the thermal analysis profile of the active ingredients, fillers and dispersions were analysed.

4.2.1 Differential Weight Loss Thermogram (DTG)

DTG was used to simultaneously investigate the heat flow (DSC) as well as the percentage mass loss (TGA). Mechanical treatment, such as milling and pulverising, of drugs may bring forth the phenomenon of crystal and/or amorphous changes (Guinot & Leveiller, 1999). In Figure 4.2 the different transitions in the DSC thermogram can be seen. A small shift in the baseline (a) indicates a glass transition. With an endothermic change (b), a trough is seen on the thermogram which indicates the point where melting occurred. An exothermic peak on the thermogram (c) shows that crystallisation or decomposition occurred. The mass loss associated with TGA transpires when a component contains a volatile substance (Vo, C.L. *et al.,* 2013; Guinot & Leveiller, 1999).

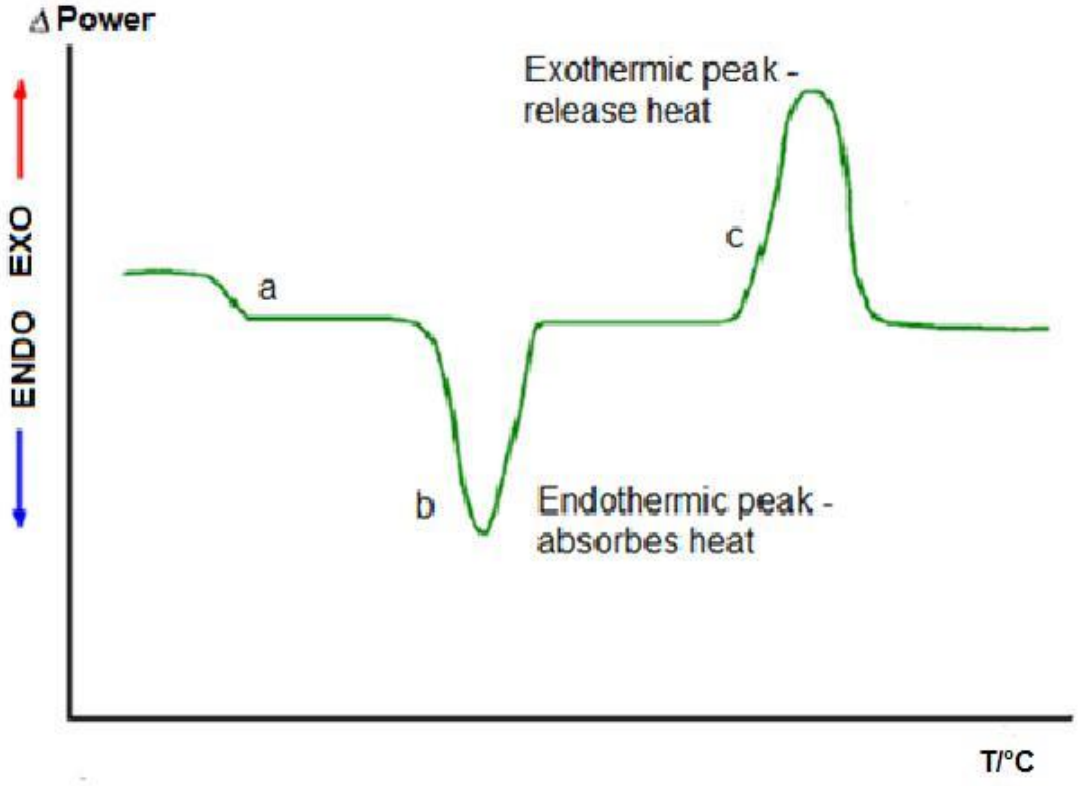


Figure 4.1: *Interpretation of various DSC transitions* (adapted from Kodre *et al.,* 2014).

Artemisone (Figure 4.2) depicted an exothermic reaction starting at 152.90°C and peaked at 166.22°C due to degradation, corresponding with the melting point of artemisinins of 151-153°C (Guo, 2016). The weight loss of 0.6166 mg (11.7%) seen in Figure 4.3 may be due to decomposition of artemisone due to melting.

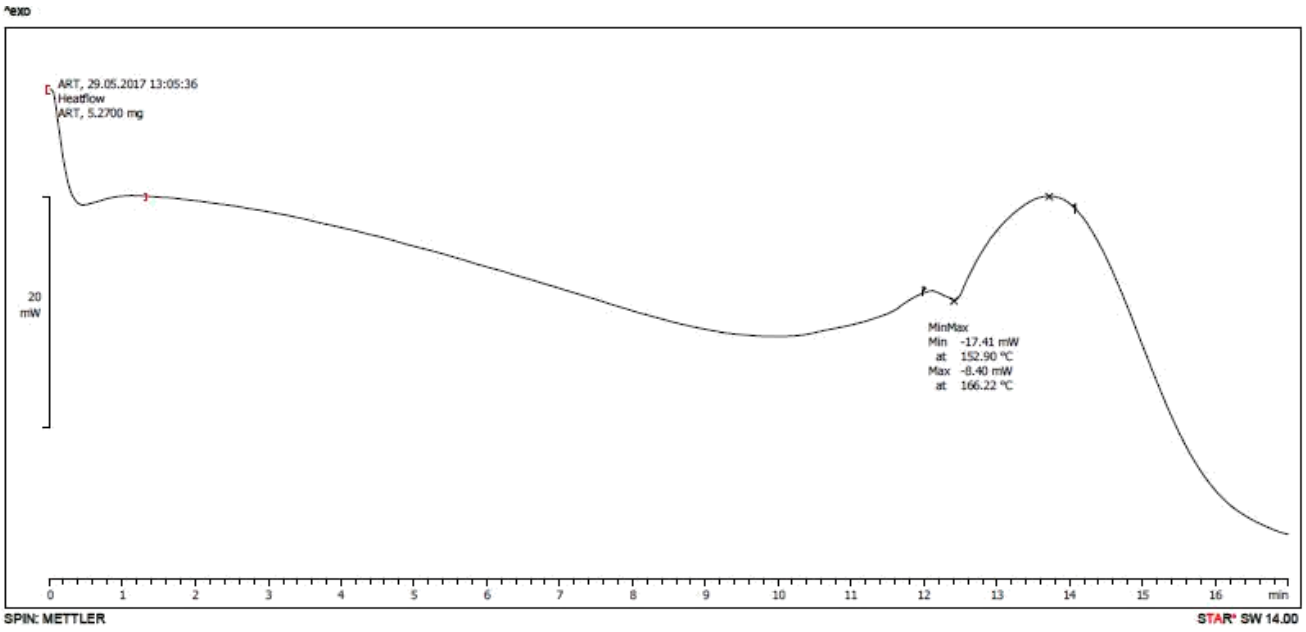


Figure 4.2: *DSC thermogram of artemisone.*

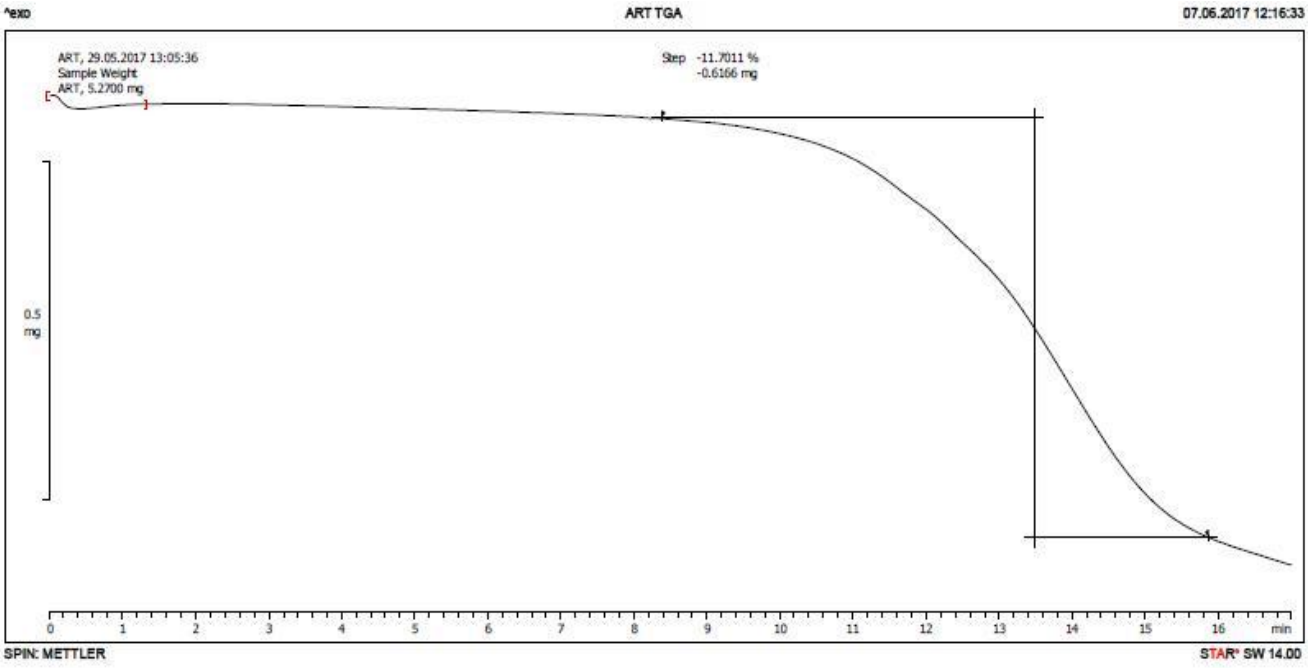


Figure 4.3: *TGA thermogram of artemisone.*

Lumefantrine (Figure 4.4) depicted an endothermic melting peak at 132.55˚C, that correlates with the literature's statements that lumefantrine melts in the range of 128–132˚C (Kotila *et al.,* 2013). The TGA of lumefantrine (Figure 4.5) showed a mass loss of 0.589%, depicting a small amount of moisture evaporated during the heating process.

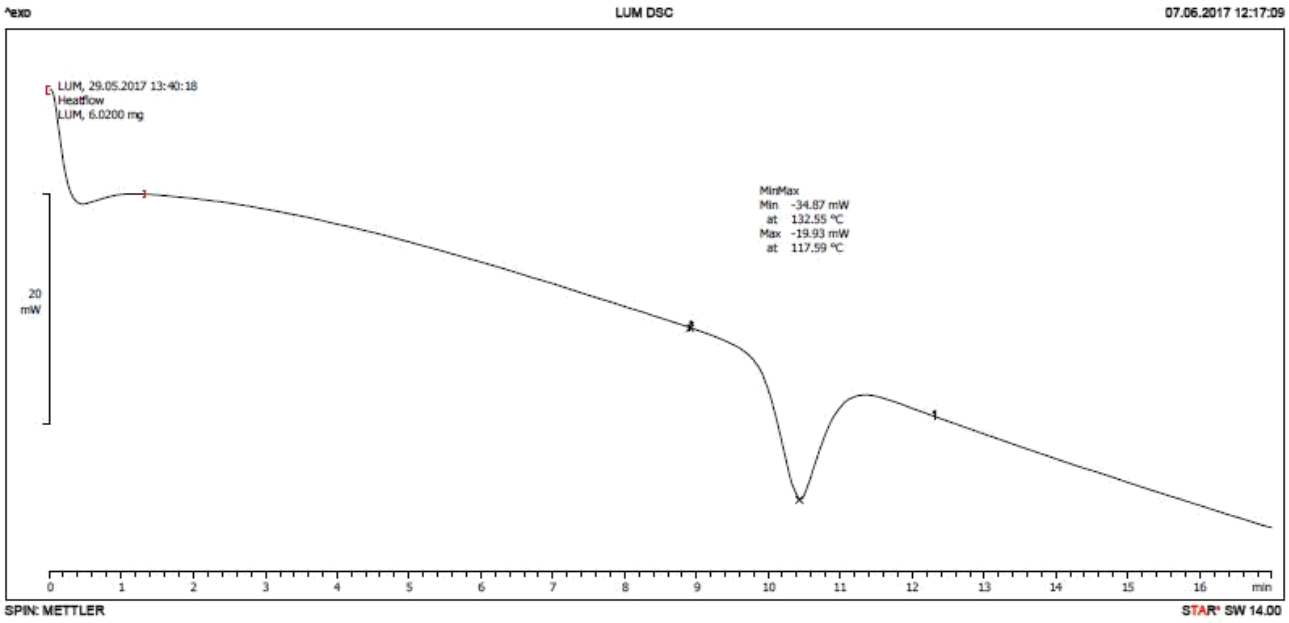


Figure 4.4: *DSC thermogram of lumefantrine.*

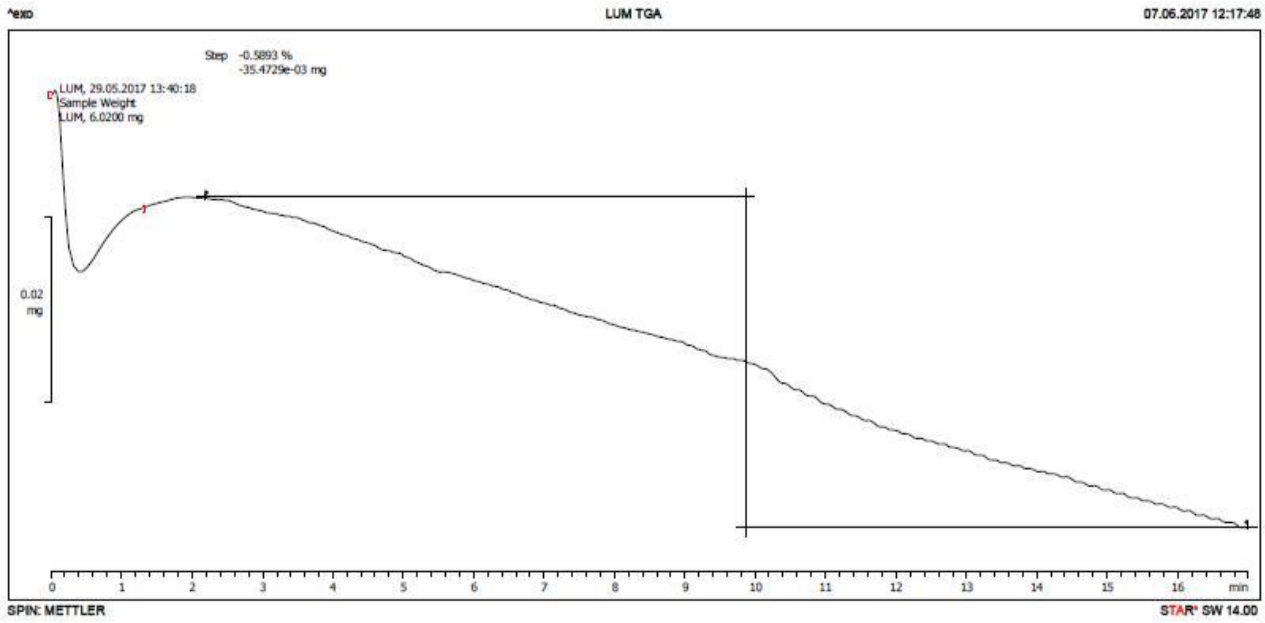


Figure 4.5: *TGA thermogram of lumefantrine.*

In Annexure B, the thermograms regarding lipid dispersions containing stearic acid in a 1:0.5 and 1:1 ratio as well as glycerol monostearate in 1:0.5 and 1:1 ratios are depicted. No conclusions could be formed regarding the thermograms as these are multi-complex systems. The melting point of artemisone is easily distinguishable, however the melting points of lumefantrine and both lipids are similar, thus it is difficult to differentiate between them. The melting points of both drugs shifted after the incorporation of the lipids but no conclusions concerning compatibilities could be made.

4.2.2 Thermal Activity Monitor (TAM)

This compatibility study was performed by means of microcalorimetry. Firstly, to explain, *calorimetry* refers to measuring techniques that are used for direct determination of rate of heat production, heat and heat capacity as a function of temperature and time.

Microcalorimetry is a valuable tool for detecting incompatibilities and instabilities between active pharmaceutical ingredients (APIs) and/or excipients. The method of microcalorimetry is a reliable way of discovering incompatibilities being that nearly all physical and chemical processes are accompanied by heat exchange. Consequently, microcalorimetry is sensitive to all physical and chemical processes associated with heat flow. The high sensitivity of this method makes it possible to perform measurements at temperatures close to real conditions and to identify very slow reactions. It should be stated that heat flow data will comprise contributions from either one process or several processes. To be able to differentiate precise contributions careful experimental preparation will be essential as well as adequate background relating to the samples being analysed. Figure 4.6 and Figure 4.7 depicts the heat flow *versus* time graphs obtained for the various samples.

Figure 4.6, showed that lumefantrine, artemisone, stearic acid and magnesium stearate are compatible with one another. An average heat flow of 45.06 ± 50.46 µW/g was calculated for this combination.

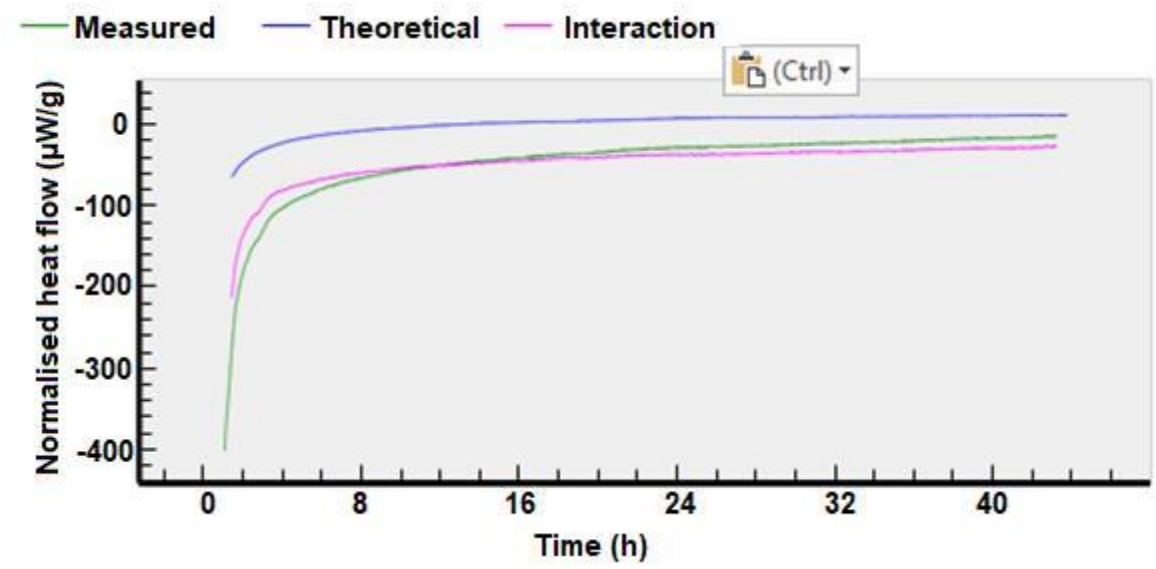


Figure 4.6: *Heat flow versus time graph obtained for lumefantrine, artemisone, stearic acid, magnesium stearate at 50°C.*

Figure 4.7 shows the heat flow *versus* time data obtained for the combination of artemisone,

lumefantrine, stearic acid and magnesium stearate. The average heat flow was calculated to be 21.15 ± 30.64 µW/g. This is an indication that all the compounds in this mixture are compatible with one another.

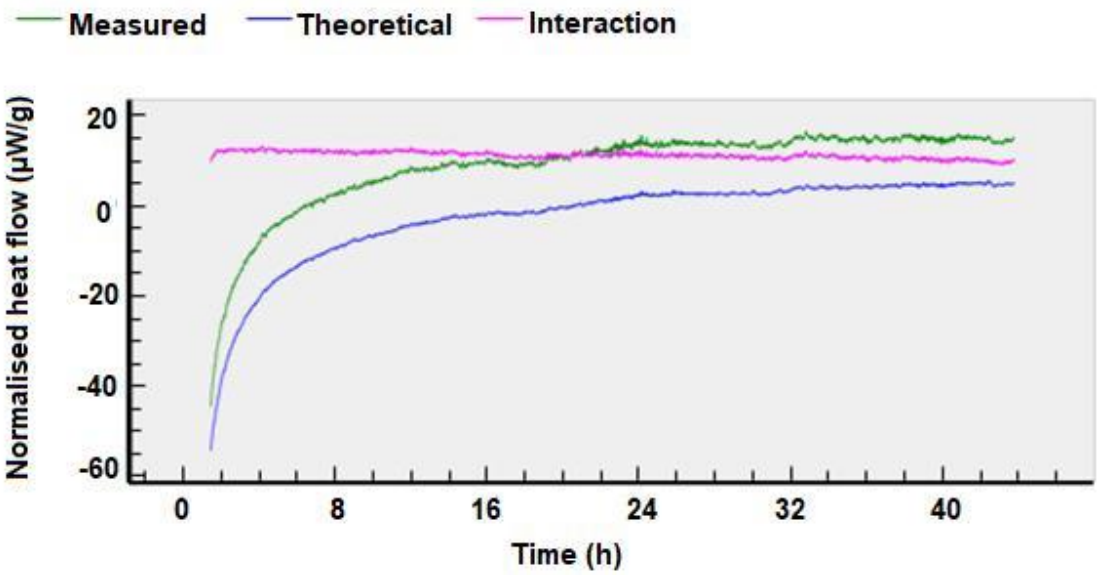


Figure 4.7: *Heat flow versus time graph obtained for a combination of artemisone, lumefantrine, glycerol monostearate and magnesium stearate at 50°C.*

4.2.3 X-Ray Powder Diffraction Studies (XRPD)

X-Ray powder diffraction (XRPD) studies were performed to see whether the crystalline form of the drugs (lumefantrine and artemisone) were influenced whilst producing the formulations by means of hot-melt. Distinct XRPD peaks are an indication of the crystallinity of the sample tested whereas the presence of a “halo” pattern is an indication of amorphous material (Vo *et al.,* 2013; Bates *et al.,* 2006). These peaks only occur when atoms are arranged in crystalline form, subsequently scattering the light and forming diffraction patterns (Speakman, 2016).

Figure 4.8 portrays the diffractogram of artemisone where the intensity count was more than 60000. In Figure 4.9, the diffractogram of lumefantrine depicts in intensity count of more than 150000. Both drugs’ intensity counts are indicative of a highly crystalline raw material.

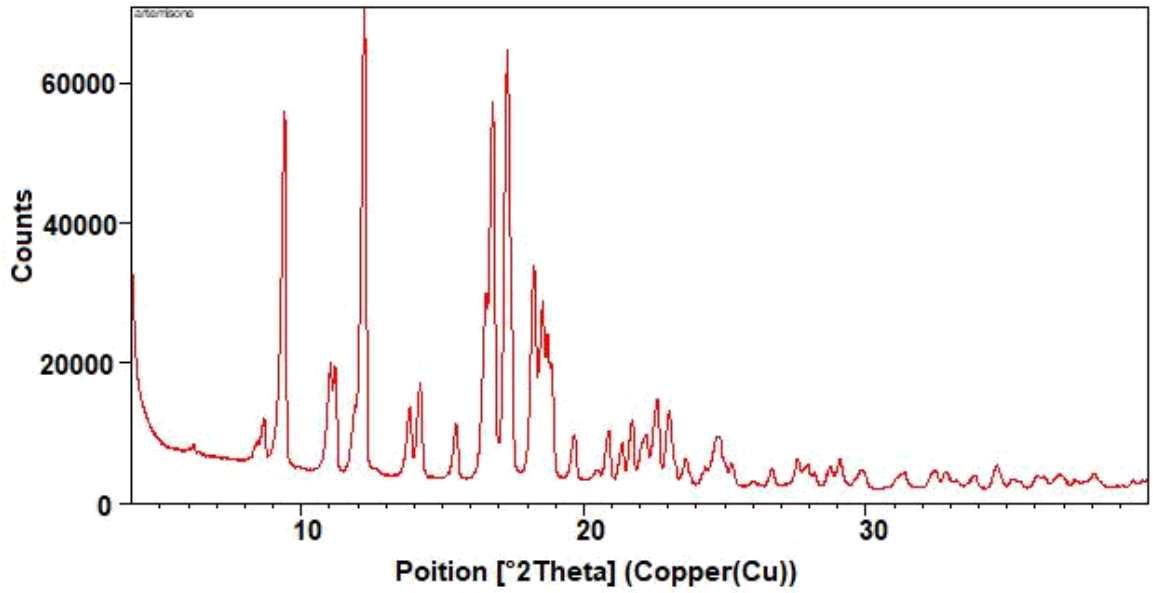


Figure 4.8: *Diffractogram of artemisone.*

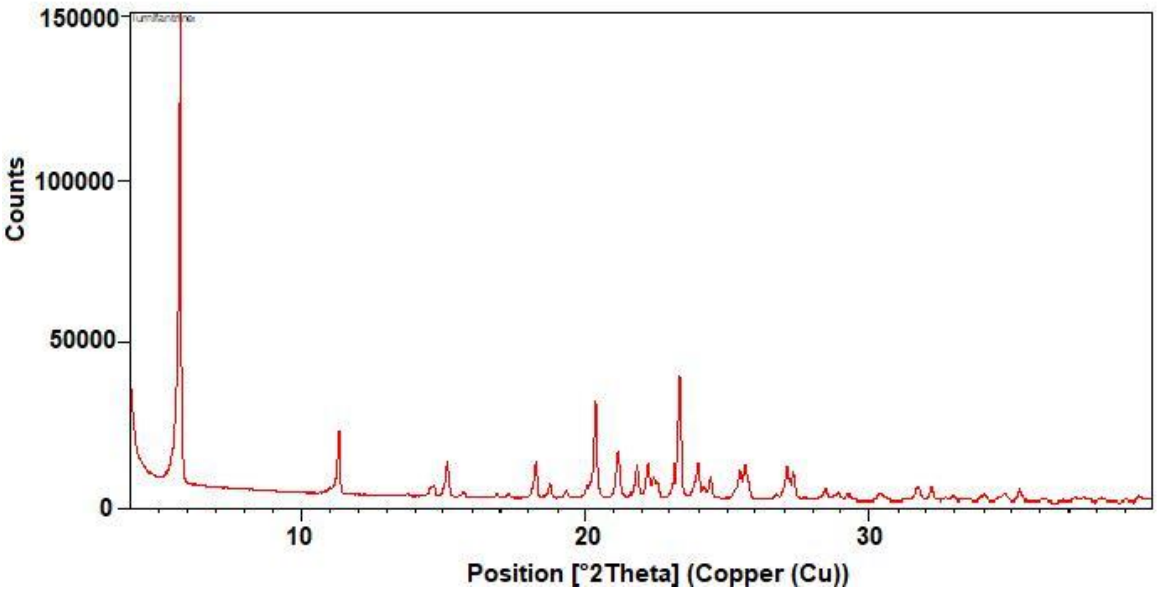


Figure 4.9: *Diffractogram of lumefantrine.*

Formulations comprising of both stearic acid and glycerol monostearate, respectively were also evaluated by means of XRPD. The diffractograms of the tested formulations can be found in Annexure E.

Studying the various diffractograms a conclusion can be made that all the intensity counts are over 30 000, thus all mixtures were crystalline (Vo *et al.,* 2013; Bates *et al.,* 2006). Although there is a decrease in intensity count from the samples containing the drugs, individually (>150 000 for lumefantrine and >60 000 for artemisone) to the various lipid dispersion samples, no amorphous content was obtained. This study revealed the permanence of the drugs in its original crystalline form in addition to proving that the hot-melt process is non-detrimental towards lumefantrine and artemisone (; van der Watt, 2014Abd-Elbary *et al.,* 2013). Crystalline form of drugs is physically and chemically more stable and have a higher melting point than the amorphous form (Vasconcelos *et al.,* 2007; Hancock *et al.,* 2002). These properties will be advantageous when the lipid-matrix tablets are transported and stored in tropical areas where malaria is prevalent.

4.2.4 Morphology of the filler powders

The filler powders used in this study were RetaLac®, CombiLac® and MicroceLac® 100. Scanning electron microscopy (SEM) images of the lipid dispersions could not be taken due to the possibility of the lipids melting subsequently contaminating the apparatus. SEM images were adapted from the Meggle's technical brochures (consent form from Meggle in Annexure C). With SEM images, the evidence composed on a microscopic level can improve our understanding of the macroscopic occurrences (De Kock, 2005). Studying the SEM images in Figure 4.10, it was apparent that CombiLac® would most likely depict the best flow properties due to the particles' highly spherical shape in comparison with MicroceLac® 100 that displays a nearly spherical shape or the RetaLac® particles that are irregular structured (Meggle, 2014a; Meggle, 2014b; Meggle, 2014c).

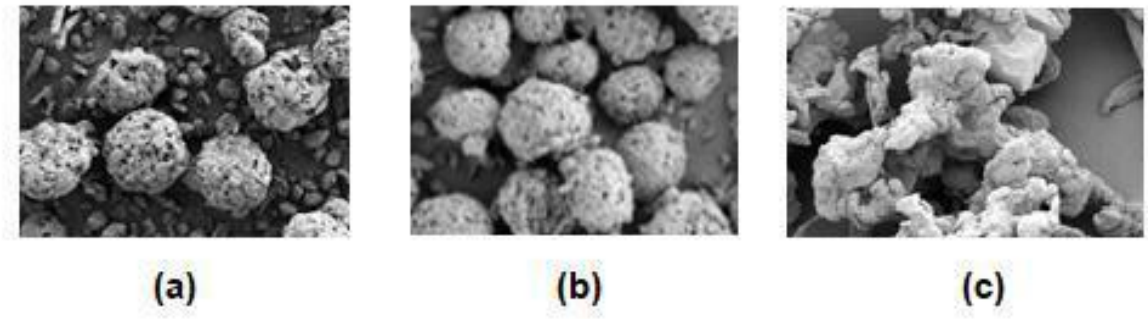


Figure 4.10: *SEM images of the powder particles of the fillers used in this study: (a) MicroceLac® 100; (b) CombiLac®; and (c) RetaLac® at 800 µm each* (adapted from Meggle*).*

The highly spherical particles of CombiLac® are made up of 10% genetically modified organism (GMO) free corn starch, 20% microcrystalline cellulose (MCC) and 70% alpha-lactose monohydrate that are spray-dried together to form an integrated system (Meggle,

2014b). MicroceLac® 100 on the other hand, is a combination of 75% alpha-lactose monohydrate and 25% MCC, which are co-spray-dried to form spherical particles (Meggle, 2014a), whereas RetaLac® consists of a 1:1 ratio crystalline alpha-lactose monohydrate and hypromellose, forming a co-processed excipient with an irregular structure (Meggle, 2014c).

4.2.5 Powder flow properties

The characterisation of the active ingredients, fillers and the lipid dispersions were done according to the BP standards (2016) and the methods described in section 3.5. The following parameters were utilised: density, Carr's index, Hausner ratio, critical orifice diameter, angle of repose and flow rate. Adapted from the BP (2016), Table 4.1 displays that excellent flowability of the fillers will be attained if the material has a Carr's index of 1 to 10%, a Hausner ratio less than 1─1.11 and an angle of repose smaller than 30˚.

Table 4.1: *Powder flow scale prerequisites*

Flow property	Angle of repose (°)	Carr's index (%)	Hausner ratio
Excellent	25-30	1-10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1.26-1.34
Poor	46-50	26-31	1.35-1.45
Very poor	56-60	32-37	1.46-1.59
Extremely poor	>66	>38	>1.60

The flow properties tested in this study for the selected fillers (MicroceLac® 100, CombiLac® and RetaLac®,) are summarised in Table 4.1. The values given in the parenthesis specifies the percentage relative standard deviation (%RSD). Furthermore, the results obtained in this study were compared with the results published by Meggle – the manufacturers of these specific fillers.

Table 4.2: *Results of powder flow property: Fillers (%RSD indicated in parentheses)*

	MicroceLac® 100	RetaLac®	CombiLac®
Bulk Density (g/cm^3)	**0.498** (0.574)	**0.323** (1.448)	**0.476** (0.000)
Tapped Density (g/cm^3)	**0.588** (0.000)	**0.416** (1.264)	**0.575** (1.150)
Hausner Ratio	**1.180** (0.575)	**1.286** (0.193)	**1.207** (1.150)
Carr's Index (%)	**15.281** (3.180)	**22.280** (0.674)	**17.143** (5.556)
COD (mm)	3–8	3–8	3–8
Flow Rate (g/s^{-1})	**74.454** (1.560)	**53.172** (7.020)	**56.648** (6.207)
Angle of Repose (°)	**19.298** (3.927)	**27.308** (0.595)	**19.308** (2.064)

Meggle conducted flowability tests (2014a) that showed MicroceLac® 100 attained a Carr's index of 20.69%; a 34˚ angle of repose; and a Hausner ratio of 1.26. Overall, this indicates good to passable flowability (Table 4.1). CombiLac® exhibits flow properties that can be classified fair to excellent when looking at a Hausner ratio of 1.19; an angle of repose of 30˚; and a Carr's index of 16 (Meggle 2014b). Conversely, RetaLac® depicts fair to poor flowability, having a Hausner ratio of 1.35; an angle of repose that is 36˚; and a 26.09% Carr's index (Meggle, 2014c). The summary of the results obtained in this study (Table 4.2) also depicted enhanced/increased flowability for MicroceLac® 100 and CombiLac® in comparison with RetaLac®. The flow rate of MicroceLac® 100 and CombiLac® (74.454 ± 0.016 g/s^{-1} and 56.648 ± 0.062 g/s^{-1}, respectively) were superior to the flow rate of RetaLac® (53.172 ± 0.070 g/s^{-1}). As seen in this study, the particle shape and size influence the flowability of a powder (Li *et al.,* 2008). Larger, more spherical particles will naturally exhibit a better flow than that of a smaller particle with an uneven surface (Fu *et al.,* 2012). Particles with an irregular surface are more inclined to experience interlocking that portrays a negative impact the flowability of the powder. Spherical particles have a smaller surface to weight relation, thus the cohesive force between the particles are lower in comparison with non-spherical particles (Alderborn, 2013). Poor flowability can influence the tableting process and sequentially the results from the mass variation of the formulation containing an excipient with poor flow properties.

The flowability of the active ingredients was evaluated individually and the results obtained are noted in Table 4.3. This was done to recognise the influence of the active ingredients on the formulations. The different lipid dispersions' (glycerol monostearate and stearic acid) flow properties were also assessed and tabulated in Table 4.4.

Table 4 3: *Results of powder flow property: Active ingredients (%RSD indicated in parentheses)*

	Artemisone	**Lumefantrine**
Bulk Density (g/cm^3)	**0.711** (2.028)	**0.590** (1.643)
Tapped Density (g/cm^3)	**0.904** (0.523)	**0.639** (1.897)
Hausner Ratio	***NV**	**1.490** (0.817)
Carr's Index (%)	***NV**	**33.009** (1.731)
COD (mm)	****NF**	16–20
Flow Rate (g/s^{-1})	****NF**	**2.323** (3.120)
Angle of Repose (°)	***NV**	**38.989** (0.129)

**NV = No value **NF = No flow*

Artemisone demonstrated no flow according to the standards set in the BP (2016). With the flow rate test, artemisone did not flow when the shutter opened, thus no results could be obtained. Compared to artemisone, lumefantrine depicted better flowability, but still exhibited poor flow with a Carr's index of 33.009 ± 1.731%; an angle of 38.989 ± 0.129˚; and a Hausner ratio of 1.490.

Table 4.4: *Results of powder flow property: Lipid dispersions (%RSD indicated in parentheses)*

	Glycerol Monostearate		Stearic acid	
	1:1	**1:0.5**	**1:1**	**1:0.5**
Bulk Density (g/cm^3)	**0.475** (1.094)	**0.487** (1.067)	**0.482** (35.935)	**0.514** (1.236)
Tapped Density (g/cm^3)	**0.551** (2.618)	**0.610** (0.000)	**0.568** (0.000)	**0.615** (3.818)
Hausner ratio	**1.161** (1.515)	**1.252** (1.045)	**1.178** (0.359)	**1.198** (4.261)
Carr's index (%)	**13.823** (9.362)	**20.151** (4.116)	**15.141** (2.014)	**16.426** (21.163)
COD (mm)	**8-12**	**8-12**	**12-16**	**12-16**
Flow rate (g/s^{-1})	**61.490** (1.867)	**13.102** (1.066)	**48.190** (6.047)	**9.610** (12.637)
Angle of repose (˚)	**38.337** (5.335)	**42.153** (8.497)	**20.167** (2.929)	**23.580** (2.202)

Regardless of the type of lipid, or the ratio of active ingredient: lipid base, the flowability improved in comparison with the flow properties of the individual active ingredients. Overall, lipid dispersion containing stearic acid achieved more acceptable results in comparison with lipid dispersions that comprised glycerol monostearate in terms of Hausner ratio, Carr's index; angle of repose; and flow rate. The 1:1 ratio of stearic acid depicted good to excellent flow when characterised regarding the Hausner ratio, angle of repose and Carr's index (1.178 ± 0.359; 15.141 ± 2.014˚; and 20.167 ± 2.929%, respectively), and increased the flow rate of artemisone and lumefantrine (no flow; and 2.323 ± 3.120 g/s^{-1}, correspondingly) to 48.190 ± 6.047 g/s^{-1}. Stearic acid 1:0.5 ratio had the least extensive increase on the flow rate (9.610 ± 12.637g/s^{-1}) in comparison to lumefantrine alone (2.323 ± 0.039 g/s^{-1}) whereas lipid dispersions containing glycerol monostearate increased the flow rate to 61.490 ± 1.867 g/s^{-1}.

Considering the results from Table 4.4, it can be concluded that the incorporation of the active ingredients into a lipid dispersion lead to an overall enhancement in the powder flow properties. It can be concluded that there is a correlation between the amount of lipid base in the formulation, and the flowability of the dispersion – the 1:1 ratio of both glycerol monostearate and stearic acid depicted improved flowability compared to the 1:0.5 ratio.

4.3 EVALUATION OF TABLETS

4.3.1 Preparation of tablets

Every formulation consists of not only the active ingredient, but several other excipients with specific purpose as well. Drug(s), filler and a lubricant are the foundation of a basic formulation (York, 2011). In this study, the drugs are artemisone and lumefantrine that were incorporated into a selected melted lipid (either glycerol monostearate or stearic acid, respectively) by means of the hot-melt method. It was decided to add one of the selected fillers (RetaLac®, CombiLac® and MicroceLac® 100); and a lubricant (magnesium stearate) in different concentrations into the subsequent formulations. The composition of the various formulations is shown in Table 4.5.

Table 4.5: *Composition of the different formulations displayed in mg*

	Active ingredients:lipid (Lubricant concentration)			
	1:1 (1% w/w)	**1:1 (0.5% w/w)**	**1:0.5 (1% w/w)**	**1:0.5 (0.5% w/w)**
Artemisone (mg)	80	80	80	80
Lumefantrine (mg)	120	120	120	120
Lipid (mg)	200	200	100	100
Filler (mg)	95	97.5	195	197.5
Magnesium stearate (mg)	5	2.5	5	2.5
Total (mg)	±500	±500	±500	±500

During the formulation development, it was decided that the lubricant, magnesium stearate, needed to be added. Thus, order to establish what concentration would suffice, it was included in two different concentrations to aid in the direct compressionn of the tablet formulations. Magnesium stearate is a hydrophobic lubricant that repels water from entering the lipid matrix during disintegration, thus, extending the disintegration time of the tablets which is normally a negative property of this lubricant (Uzunović & Vranić, 2007). However, in this study, an extended disintegration time is needed for sustained release as stated in the

objectives and therefore the addition of this lubricant and its properties would not compromise the objectives set for the study. The different concentrations were furthermore compared to establish which formulation produced the more accepted tablet properties.

4.3.2 Full factorial design of experiments

As stated formerly, a full factorial design was implemented to investigate the effect of numerous formulation variables on the physical characteristics of matrix tablets compressed from fixed-dose/lipid dispersion formulations that consisted of a fixed-dose of 80 mg artemisone and 120 mg lumefantrine; one of the selected filler powders (RetaLac®, CombiLac® and MicroceLac® 100); two different lipids (i.e. glycerol monostearate and stearic acid) included in two different ratios (1:1 and 1:0.5); and two concentrations of magnesium stearate (1% w/w and 0.5% w/w). During pre-formulation studies, no viable tablets could be produced from formulations comprising 0% w/w magnesium stearate, thus, this level was excluded from the full factorial design.

These formulations were subsequently tableted by means of direct compression and are displayed in Table 4.6. The acronyms that were allocated to the factors measured are, as previously described, presented in Table 3.2. Formulations that complied with all of the set criteria concerning the physical properties are printed in bold and highlighted with a green background on the table. Consequently, these formulations were further assessed in terms of swelling, erosion, the average concentrations artemisone and lumefantrine included in the formulations (i.e. assay analysis), as well as the drug release profiles of these formulations (dissolution studies).

Throughout this analysis process to obtain the most acceptable fixed-dose matrix tablet formulation which displayed optimum physical and drug release properties, several formulations were eliminated as they were unable to produce feasible tablets for evaluation. For example, the G1M1 formulation (indicated with a red background on Table 4.6) comprising a ratio of 1:1 fixed-dose combination:glycerol monostearate; MicroceLac® 100 as filler and 1% w/w magnesium stearate, could be tableted, however, upon ejection from the tablet press it capped to such an extent that the tablets were deemed unacceptable.

Table 4.6: *Full factorial design employed to identify formulations for further evaluation.*

				Filler type:					
				MicroceLac®100		RetaLac®		CombiLac®	
				Lubricant concentration (% w/w)					
				0.5	1.0	0.5	1.0	0.5	1.0
Lipid type:	Stearic acid	API:lipid concentration	1:0.5	S0.5M0.5	**S0.5M1**	S0.5R0.5	**S0.5R1**	S0.5C0.5	**S0.5C1**
			1:1	S1M0.5	**S1M1**	S1R0.5	**S1R1**	S1C0.5	**S1C1**
	Glycerol monostearate		1:0.5	G0.5M0.5	**G0.5M1**	G0.5R0.5	**G0.5R1**	G0.5C0.5	**G0.5C1**
			1:1	G1M0.5	G1M1	G1R0.5	**G1:R1**	G1C0.5	**G1C1**

4.3.3 Analysis of the physical properties of the tablets

For this study, all of the formulations in the factorial design were directly compressed and assessed in accordance with the criteria set by the BP (2016). Post direct compression, the different tablets were stored for at least 24 h in glass containers that were sealed with Parafilm® and closed with screw caps prior to tablet testing. The storage period and conditions were constant for all of the formulations in order to ensure consistency during analysis. Including the BP (2016) criteria; and for this study, the formulation that depicted the least mass variation; highest tensile strength; lowest % friability; and an average disintegration time longer than 15 min, was considered the most ideal as one of the objectives was to obtain modified release tablets. All of the individual results obtained for mass variation, friability, tensile strength, and disintegration time are recorded in Annexure A and the average values calculated are presented in Tables 4.7–4.10.

From Table 4.7 it is clear that all of the double fixed dose lipid matrix tablets containing glycerol monostearate in a 1:0.5 ratio could be directly tableted. Overall, none of the formulations displayed any disintegration during the 15 min testing period, indicating that all of these tablets will probably present modified release properties, which is considered ideal for this study. Additionally, tablets comprising CombiLac® as filler have a relatively lower average mass, whereas tablets that included RetaLac® depicted the highest overall tablet weight. Interestingly, an increase in magnesium stearate relatively increased the tablet weight, regardless the filler utilised, however, this increase is not as notable with tablets containing CombiLac®. This increase in lubricant concentration furthermore decreased the average weight variation as specified by the percentage relative standard deviations indicated in parentheses in Table 4.7. Generally, double fixed dose lipid matrix tablets containing glycerol monostearate (1:0.5) and MicroceLac® 100 as filler portrayed the highest average weight variation regardless the lubricant concentration, whereas formulations consisting of RetaLac® showed the least weight variation indicating that these tablets produced are the most uniform in terms of average weight.

Table 4.7: *Values obtained for physical properties analysed for the lipid matrix tablets containing the double fixed dose combination and glycerol monostearate in a 1:0.5 ratio (%RSD indicated in parentheses)*

Formulation	Average mass (mg)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (sec)
G0.5M0.5	**486.128** (6.601)	**1.527** (34.748)	**0.199**	**>900**
G0.5M1	**511.026** (3.668)	**1.284** (13.017)	**0.000**	**>900**
G0.5R0.5	**467.357** (2.710)	**1.456** (6.577)	**0.215**	**>900**
G0.5R1	**543.651** (1.113)	**1.299** (5.464)	**2.518**	**>900**
G0.5C0.5	**482.332** (4.019)	**1.913** (11.819)	**0.000**	**>900**
G0.5C1	**483.024** (3.988)	**1.535** (28.673)	**1.029**	**>900**

Considering the tensile strength results it could be established that in general formulations containing CombiLac® produced tablets that are harder, followed by formulations containing MicroceLac® 100. Formulations that comprised RetaLac® are only marginally softer compared to the MicroceLac® 100 formulations. An increase in magnesium stearate concentration rendered an overall decrease in the average tablet tensile strength, which is expected as this lubricant is known to decrease tablet strength due to its hydrophobic nature that hinders interparticle interactions (Uzunović & Vranić, 2007). Although an anticipated indirect correlation was observed between the average tensile strength and friability values for both the formulations comprising CombiLac® and RetaLac® as fillers, this relationship could not be established for the formulations containing MicroceLac® 100. Formulations that consisted of MicroceLac® 100 did, however, display the most ideal friability values. Additionally, in general it could be concluded that as the magnesium stearate concentration increased, the friability of the tablets increased, which again, was expected. Thus, according to the set values of the BP (2016), the formulations, G0.5R1 and G0.5C1, did not adhere to the criteria as their percentage friability was more than the allowed maximum of 1%.

In Table 4.8 and as stated previously, the formulation, G1M1, indicated with a red background could not be tableted; though, overall double fixed dose lipid matrix tablets containing a double fixed dose:glycerol monostearate in a 1:1 ratio could also be

successfully directly tableted. Again, all of the formulations could be considered non-disintegrating as none of the formulations disintegrated within the set time period of 15 min. Overall, these formulations showed a higher average weight compared to formulations containing a double fixed dose:glycerol monostearate in a 1:0.5 ratio. No clear trend could be established that indicated which of the selected fillers that were included rendered tablets that displayed the highest or lowest average tablet weight. Thus, here the type of filler did not have such a noteworthy effect in terms of tablet weight, granting that the formulations comprising RetaLac® again depicted a slightly higher overall tablet weight. In terms of weight variation it could be recognised that lipid matrix tablets containing the double fixed dose:glycerol monostearate in a 1:1 ratio displayed relatively lower average weight variation compared to the formulations that included a double fixed dose:glycerol monostearate in a 1:0.5 ratio. Thus, these tablets are considered more uniform in weight. Once more, as the magnesium stearate concentration increased, the tablet weight increased, however, contrary to previous findings, the weight variation also increased with higher lubricant amounts included.

Table 4.8: *Values obtained for physical properties analysed for the lipid matrix tablets containing the double fixed dose combination and glycerol monostearate in a 1:1 ratio (%RSD indicated in parentheses)*

Formulation	Average mass (g)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (sec)
G1M0.5	**0.507** (3.165)	**1.089** (7.687)	**0.198**	**>900**
G1M1	***NV**	***NV**	***NV**	***NV**
G1R0.5	**0.486** (0.818)	**1.294** (7.060)	**0.207**	**>900**
G1R1	**0.560** (1.289)	**0.983** (11.012)	**0.178**	**>900**
G1C0.5	**0.497** (2.329)	**1.095** (11.237)	**0.598**	**>900**
G1C1	**0.507** (4.272)	**0.793** (8.767)	**0.200**	**>900**

**NV= No Value – could not be tableted*

In terms of the tensile strength results obtained, it was interesting to note that the inclusion of a double fixed dose:glycerol monostearate in a 1:1 ratio, produced tablets that display generally lower average tensile strength values compared to lipid matrix tablets containing a

double fixed dose:glycerol monostearate in a 1:0.5 ratio; nevertheless, this was not significant. A remarkable observation is that as the tensile strength increased, the friability of the lipid matrix tablets decreased which is contradictory to the outcome expected. In addition, as the lubricant concentration increased the tensile strength values decreased, which was again expected, but here the friability values also unpredictably decreased with an increase in magnesium stearate concentration, indicating that the tablets are less friable. The formulation, G1R0.5 depicted the lowest %RSD in terms of mass variation, the highest tensile strength and a % friability of only 0.207% demonstrating that this formulation showed the most promise for formulations containing a double fixed dose:glycerol monostearate in a 1:1 ratio.

Results shown in Table 4.9 were achieved from lipid matrix tablets comprising a double fixed dose:stearic acid in a 1:0.5 ratio. None of the formulations, except S0.5C1 disintegrated within 15 min. This formulation is thus less likely to be able to depict modified drug release profiles that are undesirable for this study. Unlike with the lipid matrix tablets that included a double fixed dose:glycerol monostearate in a 1:0.5 ratio, these formulations containing MicroceLac® 100 as filler displayed the highest average tablet weight with the most uniform weight variation (lowest %RSD), whereas formulations that comprised RetaLac® portrayed the lowest overall tablet weight. A trend existed between the increase in magnesium stearate concentration and the increase in tablet weight, with a subsequent decrease in %RSD of the lipid matrix tablets containing either MicroceLac® 100 or RetaLac®. However, this could not be stated for formulations containing CombiLac®. Considering the tablet tensile strength and friability, it could generally be seen that these tablet formulations depicted overall higher tensile strength values and lower friability values compared to the double fixed dose:glycerol monostearate (1:0.5 ratio) formulations. Moreover, formulations comprising MicroceLac® 100 showed higher tensile strength values, followed by formulations that included CombiLac®, and subsequently RetaLac®. This trend was not followed for the friability results as formulations that included RetaLac® displayed the lowest friability values, followed by CombiLac® and MicroceLac® 100, indicating that as the tensile strength increased the tablets became more friable. The increase in magnesium stearate concentration led to a decrease in tensile strength values and increase in friability values for formulations that included MicroceLac® 100 and CombiLac®, but this change in results was not as evident with formulations that comprised RetaLac® as filler. Overall, no final conclusion could be drawn as to which matrix tablet formulation comprising a double fixed dose:stearic acid in a 1:0.5 ratio, displayed the most ideal tablet properties for this study and therefore further investigation was necessary as discussed later.

Table 4.9: *Values obtained for physical properties analysed for the lipid matrix tablets containing the double fixed dose combination and stearic acid in a 1:0.5 ratio (%RSD indicated in parentheses)*

Formulation	Average mass (g)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (sec)
S0.5M0.5	**0.529** (2.142)	**2.225** (3.420)	**0.379**	**>900**
S0.5M1	**0.549** (0.530)	**2.080** (3.983)	**0.545**	**>900**
S0.5R0.5	**0.446** (7.863)	**1.197** (44.873)	**0.219**	**>900**
S0.5R1	**0.523** (1.501)	**1.389** (13.073)	**0.192**	**>900**
S0.5C0.5	**0.518** (1.591)	**2.426** (5.012)	**0.385**	**>900**
S0.5C1	**0.502** (7.792)	**1.884** (31.912)	**0.398**	**585.445** (0.429)

The production of matrix tablets that included a double fixed dose:stearic acid in a 1:1 ratio overall rendered tablets that could be considered non-disintegrating (Table 4.10). The increase in the ratio double fixed dose:stearic acid decreased the overall variation in tablet weight (i.e. lower %RSD), therefore, these tablets could be considered more uniform in terms of tablet weight. No other clear trend could further be established for tablet weight and mass variation results. Likewise, no significant differences could be recognised for tensile strength values with the addition of one of the selected fillers; or with an increase in magnesium stearate concentration. An increase in the lubricant concentration did however have a notable negative effect on the friability values for formulations that contained either MicroceLac® 100 or CombiLac®, whereas the lipid matrix tablets that comprised RetaLac® were not sensitive to the increase in lubricant concentration. Again, no assumption could be made as to which matrix tablet formulation comprising a double fixed dose:stearic acid in a 1:1 ratio, demonstrated the most ideal tablet properties for this study and consequently additional research was required.

Table 4.10: *Values obtained for physical properties analysed for the lipid matrix tablets containing the double fixed dose combination and stearic acid in a 1:1 ratio (%RSD indicated in parentheses)*

Formulation	Average mass (g)	Tensile strength ($N.mm^{-2}$)	Friability (%)	Disintegration (sec)
S1M0.5	**0.518** (1.355)	**1.602** (13.515)	**0.386**	**>900**
S1M1	**0.494** (3.992)	**1.506** (18.092)	**0.619**	**>900**
S1R0.5	**0.465** (5.153)	**1.540** (10.448)	**0.428**	**>900**
S1R1	**0.512** (0.650)	**1.705** (7.419)	**0.393**	**>900**
S1C0.5	**0.506** (1.598)	**1.532** (18.358)	**0.197**	**>900**
S1C1	**0.542** (1.736)	**1.995** (5.344)	**0.555**	**>900**

As specified beforehand, the purpose of a full factorial design is to determine significant interactions as well as effects of various combinations of factors and levels of these factors. It may provide an impartial, systematic guide to evaluation of various excipients and their corresponding concentrations in order to enhance these variables. Briefly, it could be assumed that the inclusion of a factorial design as a statistical instrument eradicates the presumption form research (De Kock, 2005:120; Steyn *et al.*, 2000:526). Thus, a summary of the average values for each level of the different variables of each response in the full factorial design are portrayed Table 4.11.

Table 4.11: *Average responses calculated from the results measured for each variable at each level.*

		Mass variation		%Friability	Tensile strength	
Measured response		**Mass (g)**	**%RSD**		**N.mm^{-2}**	**%RSD**
Filler type	**MicroceLac® 100**	0.508	3.065	0.332	1.616	13.495
	RetaLac®	0.500	2.637	0.544	1.358	13.241
	CombiLac®	0.505	3.415	0.420	1.647	15.140
	Glycerol monostearate	0.503	3.088	0.486	1.297	12.369
	Stearic acid	0.509	2.992	0.391	1.757	14.627
	0.5%	0.492	3.279	0.284	1.575	14.563
	1%	0.521	2.776	0.602	1.492	13.341
Lipid ratio	**1:0.5**	0.503	3.501	0.507	1.685	16.881
	1:1	0.509	2.279	0.360	1.376	10.813

From the values obtained it is clear that an increase in the magnesium stearate concentration played the most noticeable role in terms of mass variation. As the concentration of the lubricant increased, the average mass of the tablets increased with 0.029 g, however, the uniformity in mass increased as well as seen in the decrease in %RSD for mass variation. Furthermore, considering filler type, tablets manufactured from RetaLac® depicted the most uniform tablet weight, followed by MicroceLac® 100 and then CombiLac®. This observation was however not significant. The type of lipid included did not show any significant differences in the mass variation, but as the ratio double fixed dose:lipid increased, the tablets were relatively more uniform in terms of their weight.

Double fixed dose formulations that contained MicroceLac® 100 were harder compared to formulations containing either RetaLac® or CombiLac® in view of friability and tensile strength. These formulations did not crumble easily and remained intact after extreme forces were applied. RetaLac® formulations on the other hand, were considered softer and more friable, though these formulations still adhered to the criteria of the BP (2016). In terms of the type of lipid utilised in the double fixed dose matrix tablets, the formulations that comprised stearic acid performed slightly better as these formulations depicted harder

tablets that were less friable. An increase in magnesium stearate concentration rendered controversial results as a lower concentration produced tablets that were less friable, but depicted lower tensile strength values, whereas the inclusion of a higher concentration lubricant formed tablets that portrayed harder tablets that were more brittle. The same trend was followed with the ratio of double fixed dose:lipid. A 1:0.5 ratio rendered tablets that were harder and less friable, whereas the 1:1 ratio produced tablets that were comparatively softer, but less brittle. Thus, according to the full factorial design, the formulation, S1M0.5, will possibly possess slightly more acceptable tablet properties compared to the other double fixed dose matrix tablets.

After tableting and inspecting all the formulations, a recurring outcome was noticed with most of the tablets that contained 0.5% magnesium stearate. Some of the formulations experienced capping to some extent, whilst other formulations displayed a dent on the top where the tablet adhered to the punch of the tablet press. Although the 0.5% magnesium stearate formulas did not depict sub-standard results, the aesthetics of the tablets were considered unsatisfactory. Figure 4.11 visually illustrates double fixed dose matrix tablets that contained 0.5% lubricant whereas Figure 4.12 demonstrates the aesthetic appearance of the tablets that contained 1% magnesium stearate.

Figure 4.11: *Double fixed dose matrix tablet formulations containing 0.5% lubricant.*

Figure 4.12: *Formulations containing 1% lubricant.*

Therefore, considering all of the above-mentioned results, it was decided to further evaluate all the double fixed dose matrix tablet formulations comprising 1% magnesium stearate and to compare the filler type; two different lipids; and two ratios in terms of swelling and erosion, as well as drug release by means of drug dissolution studies. The formulations chosen for further evaluation are highlighted in in bold and green in Table 4.12.

Table 4.12: *Full factorial design employed to identify formulations for dissolution*

				Filler type:					
				MicroceLac®100		RetaLac®		CombiLac®	
				Lubricant concentration (% w/w)					
				0.5	1.0	0.5	1.0	0.5	1.0
Lipid type:	Stearic acid	API:lipid concentration	1:0.5	S0.5M0.5	**S0.5M1**	S0.5R0.5	**S0.5R1**	S0.5C0.5	**S0.5C1**
			1:1	S1M0.5	**S1M1**	S1R0.5	**S1R1**	S1C0.5	**S1C1**
	Glycerol monostearate		1:0.5	G0.5M0.5	**G0.5M1**	G0.5R0.5	**G0.5R1**	G0.5C0.5	**G0.5C1**
			1:1	G1M0.5	G1M1	G1R0.5	**G1:R1**	G1C0.5	**G1C1**

4.3.4 Swelling and erosion

The swelling and erosion properties of the optimised lipid-matrix tablets were evaluated as described in section 3.5.6. The tables depicting the swelling and erosion values obtained for the 11 formulations (Table 4.12) that were chosen for further evaluation are presented in Annexure D. From the results obtained, Figures 4.13 and 4.14 were constructed that show the swelling characteristics of double fixed dose matrix tablets containing glycerol monostearate or stearic acid, respectively. Viewing these figures, it is clear that swelling of the different tablet formulations commence as the tablets were subjected into the media and the tablet weight and water uptake increased. Once the measured swelling weight of the tablets remained the same for 3 consecutive readings, the swelling test was concluded. Afterwards the tablets were dried until the no loss in tablet weight was recorded for 3 successive readings.

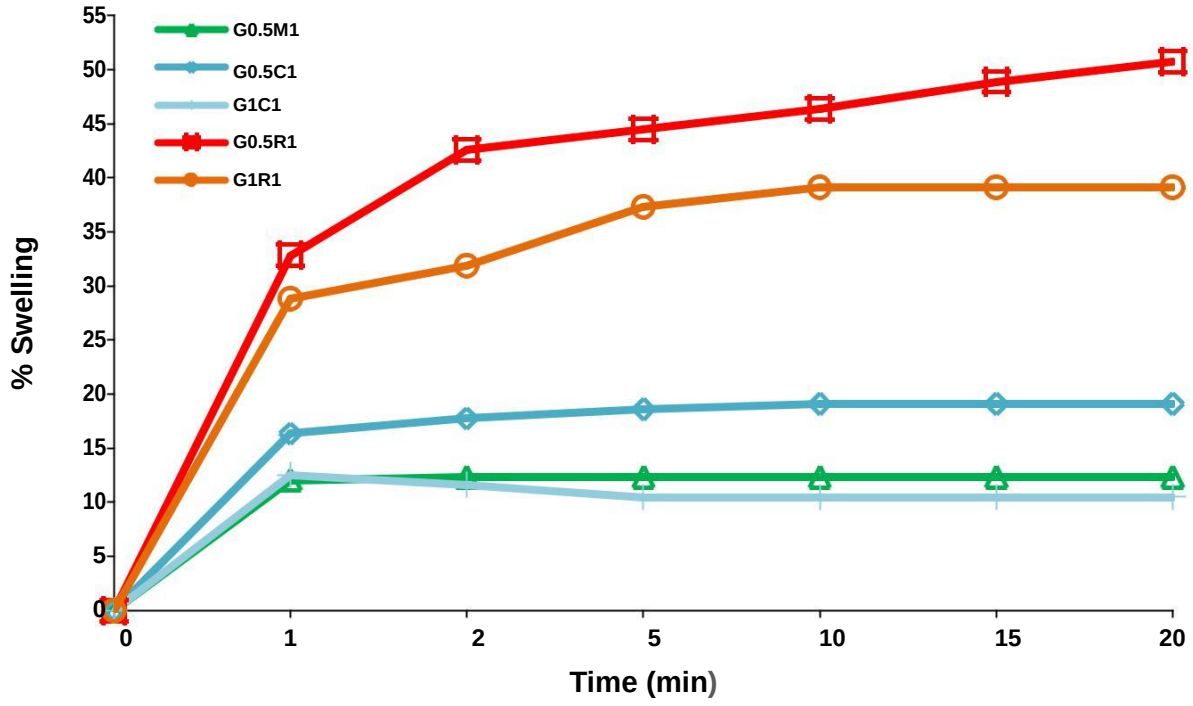


Figure 4.13: *Average percentage swelling of glycerol monostearate formulations*

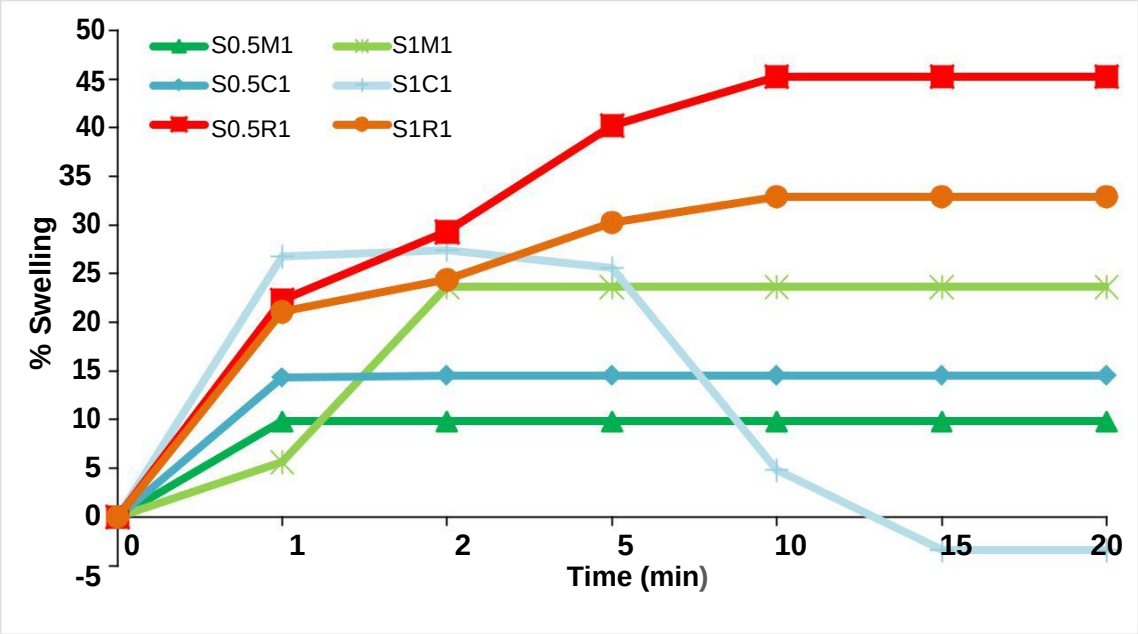


Figure 4.14: *Average percentage swelling of stearic acid formulations*

Overall, the formulations containing MicroceLac® 100, regardless the type of lipid included, depicted minor swelling for no more than 2 min. MicroceLac® 100 comprises 25% microcrystalline cellulose (MCC) and 75% alpha-lactose monohydrate which are co-spray-dried to create a monoparticulate system. This is a non-hydroscopic filler (Meggle, 2014a) meaning MicroceLac® 100 does not readily take up or retain moisture from the atmosphere. MCC is furthermore insoluble with a minimal porosity characteristic that limits the water uptake and the swelling properties of MicroceLac® 100 (Alvarez *et al.* 2003).

RetaLac® formulations conversely, depicted noticeable swelling properties as seen in Figures 4.13 and 4.14 (as well as in Annexure D), irrespective of the lipid that was incorporated into the matrix tablets. RetaLac® depicted the highest % swelling as well as the longest swelling time, compared to the other fillers used in this study. RetaLac® consists of a 1:1 ratio of hypromellose (hydroxypropyl methylcellulose, HPMC) and alpha-lactose monohydrate (Meggle, 2014c). HPMC is commonly used in oral delivery systems to enable sustained drug release since it forms a hydrophobic matrix once in solution (Esterhuizen-Rudolph, 2015; Sriamornsak *et al.,* 2007; Levina & Rajabi-Siahboomi, 2004). Alderborn (2013), as well as Qui (2009) stated that the mechanism of drug release from a hydrophobic matrix mostly occurs through the formation of a viscous gel layer encircling the tablet. This hydrated layer acts as a barrier to produce drug release by both water penetration and movement of the drug particles out of the matrix. Drug release may be influenced by the hydration properties of the polymer (HPMC) as well as the physical properties of the hydrated gel layer. As soon as the polymer matrix tablets are exposed to any liquid (water,

dissolution media or GI-fluid), the dry polymer becomes hydrated, swelling occurs and a gel layer forms around the tablet, thus reducing diffusion of the drug(s) out of the matrix. The higher the polymer hydration is, the more the gel layer is diluted and the closer the system moves to the “disentanglement concentration”; consequently, the polymer chains unravel and separate from the gel matrix (Esterhuizen-Rudolph, 2015; Kavanagh & Corrigan, 2004).

Described in the previous paragraph, the hydrophobic gel layer forms a barrier around the tablet thus delaying water uptake and swelling. Co- hypromellose with lactose increase the wettability of the filler leading to a higher water uptake and subsequently a higher percentage swelling (Meggle, 2014c). From Figures 4.13 and 4.14 it is clear that the RetaLac® matrix tablet formulations that consisted of a 1:0.5 (double fixed dose:lipid.) ratio depicted a higher percentage swelling compared to the 1:1 ratio. The reason being that the 1:0.5 formulations contained more filler per tablet (200 mg artemisone+lumefantrine; 100 mg lipid; 200 mg filler) than the 1:1 formulations (200 mg artemisone+lumefantrine; 200 mg lipid; 100 mg filler) which may indicate that the lipid included expectedly inhibited water absorption due to the fact that it is hydrophobic. G0.5R1 depicted a 50.763% swelling in 20 min, whereas G1R1 portrayed 39.079% swelling within 10 min. The same phenomenon could be observed with the stearic acid formulations where S0.5R1 depicted a maximum swelling of 45.232% in comparison to S1R1 that swelled only 32.915%. CombiLac® formulations depicted a higher initial swelling percentage in comparison with MicroceLac® 100 formulations due to the added corn starch that displays water absorption characteristics and the fact that CombiLac® is partly soluble in water. CombiLac® is a co-processed filler consisting of 70% alpha-lactose monohydrate, 10% white corn starch and 20% MCC. White corn starch is a traditional disintegrant that facilitates quick water uptake into the tablet. (Meggle, 2014b). Water uptake facilitated by the corn starch is, however, inversely proportional to the tablet hardness (Meggle, 2014b). In Annexure D it could be noted that the formulations comprising CombiLac® swelled for 1 min; thereafter the tablets started to erode slightly, but the G1C1 formulation stabilised after 5 min.

Once the swelling weight of the tablets stabilised, the tablets were dried to determine the percentage erosion for each formulation and portrayed in Figures 4.18 and 4.19. Initially the average mass of G1C1 tablets was 507.001 mg. After a drying time of 45 min, the average weight of the tablets was weight at 223.656 mg, thus illustrating a 57.07% erosion - the highest mass loss in this experiment. In contrast, G0.5C1 only eroded 5.827%. S1C1 (Annexure D) eroded completely within 15 min - this formulation was the only formulation that disintegrated within 15 min as well as completely eroded, whereas S0.5C1 showed a percentage erosion of only 6.790%. G1R1 and G0.5R1 depicted a 6.693% and 6.222%

erosion, respectively, whilst S1R1 and S0.5R1 portrayed more erosion – 36.627% and 13.377%, respectively. The following rank order for erosion of formulations containing MicroceLac® 100 could be obtained: G0.5M1 << S0.5M1 << S1M1. It therefore seems that MicroceLac® 100 formulations comprising stearic acid eroded relatively more to the formulations containing glycerol monostearate.

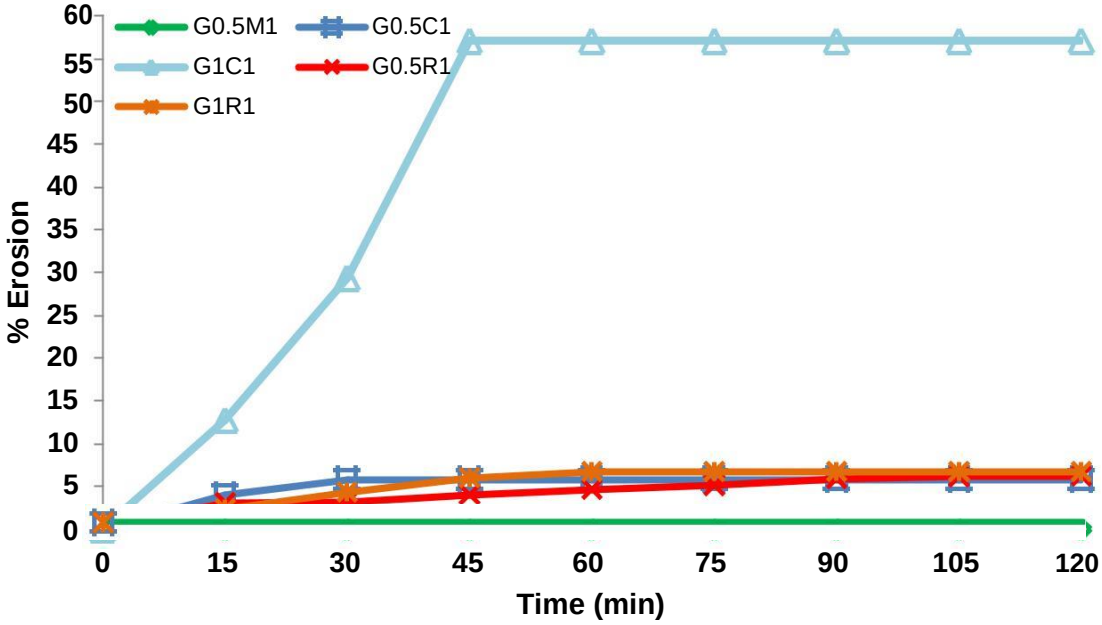


Figure 4.15: *Average percentage erosion of glycerol monostearate formulations*

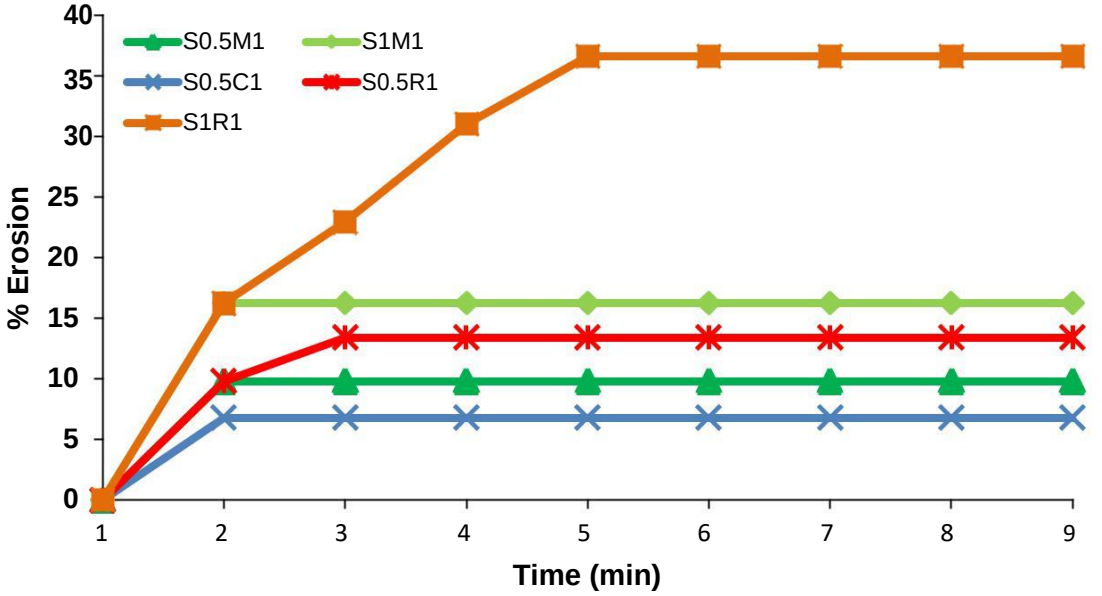


Figure 4.16: *Average percentage erosion of stearic acid formulations*

In conclusion, RetaLac® formulations depicted the highest swelling ability, followed by MicroceLac® 100 and CombiLac® formulations. Considering the type of lipid included in the

different formulations, no significant difference in terms of swelling could be established. On the other hand, the type of lipid played a more pronounced role in the erosion experiments. Clearly, the formulations containing glycerol monostearate did not erode to the same extent as formulations that included stearic acid. The only exception was G1C1 that eroded by approximately 57%. No distinct differences between the types of filler incorporated could be established for the erosion tests.

4.4 HIG H PERFORMANCE LIQUID CHROMATOGRAPHY VALIDATION

The reason behind validating a method is to prove that it is useful and up to standard. This HPLC method was validated to confirm that this method is sensitive, accurate and reliable when determining the concentration of the drugs utilised in this study. This method was developed and validated with the aid of by Prof JL du Preez at the Centre of excellence for Pharmaceutical Sciences, North-West University.

4.4.1 Specificity

Specificity was tested by analysing a blank tablet sample (placebo) to ensure that none of the components interfered with the analyte peaks. A standard solution containing both artemisone and lumefantrine, was also left to stand for one week at room temperature and then analysed. Standard solutions were degraded by adding 200 µl of 0.2 M hydrochloric acid, 0.2 M sodium hydroxide and 0.2 M hydrogen peroxide to 1 ml of standard solution and letting it stand for 2 h before analysis to allow degradation to occur. Figures 4.17–4.23 exhibit chromatograms of the various solutions.

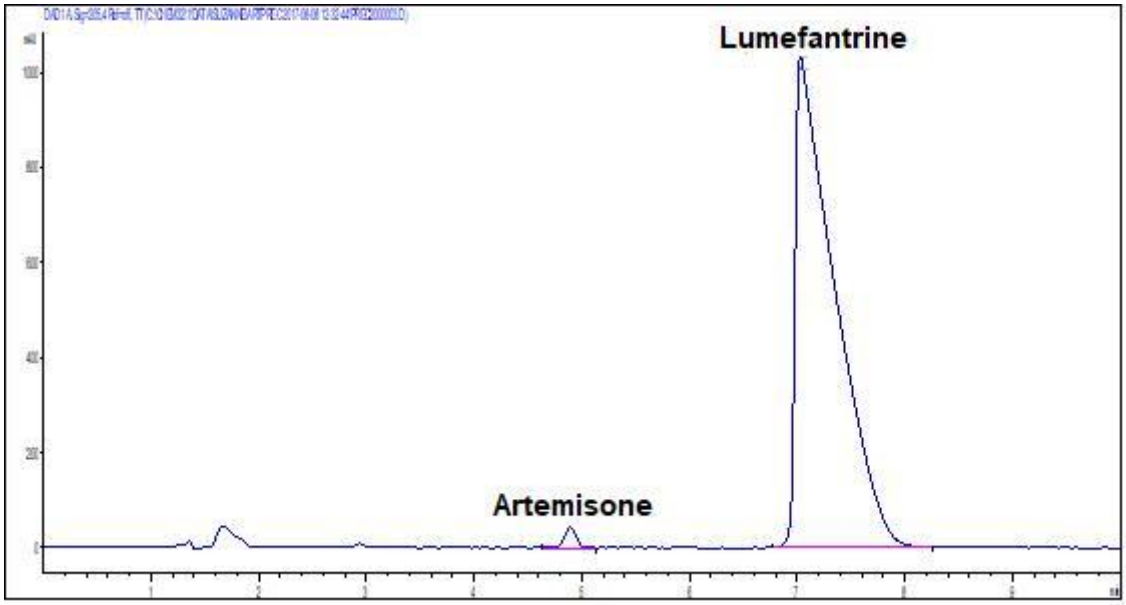


Figure 4.17: *Chromatogram of the standard solution containing artemisone and lumefantrine*

In Figure 4.17 it can be seen that artemisone peak at approximately 4.5 min, whereas lumefantrine peak at around 7 min. The peaks clearly distinguishable and therefore will not be confused,

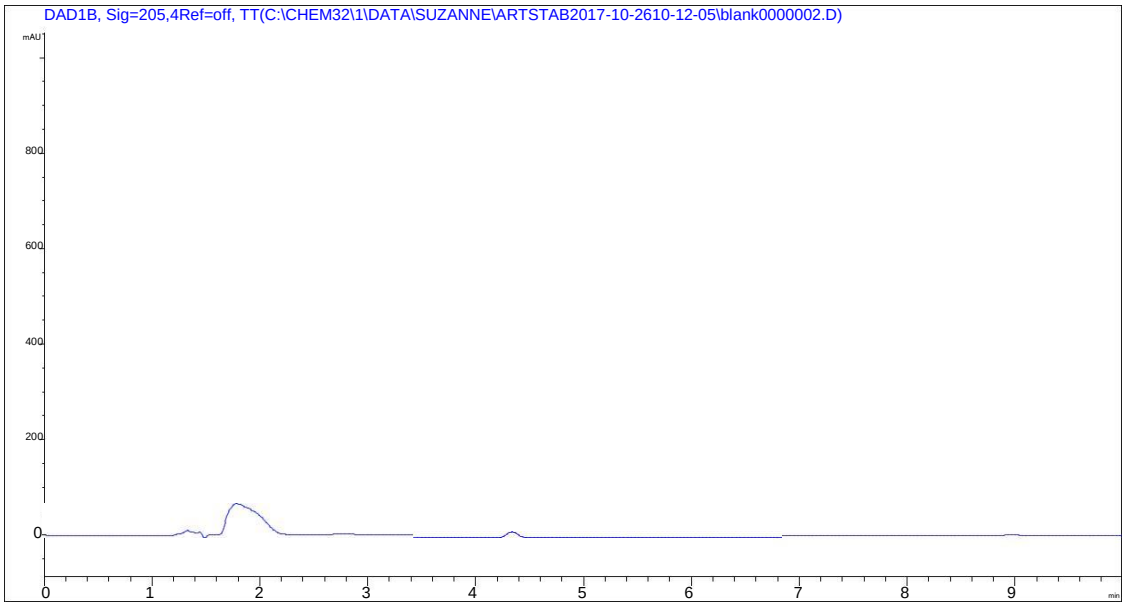


Figure 4.18: *Chromatogram of solvent blank.*

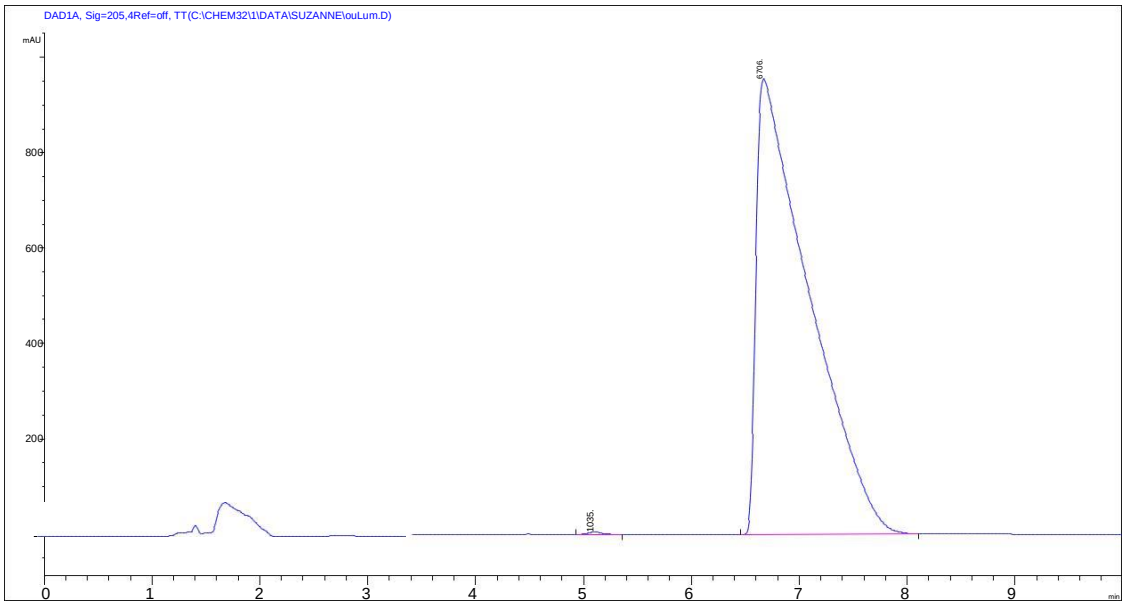


Figure 4.19: *Chromatogram of standard containing artemisone and lumefantrine stressed for one week.*

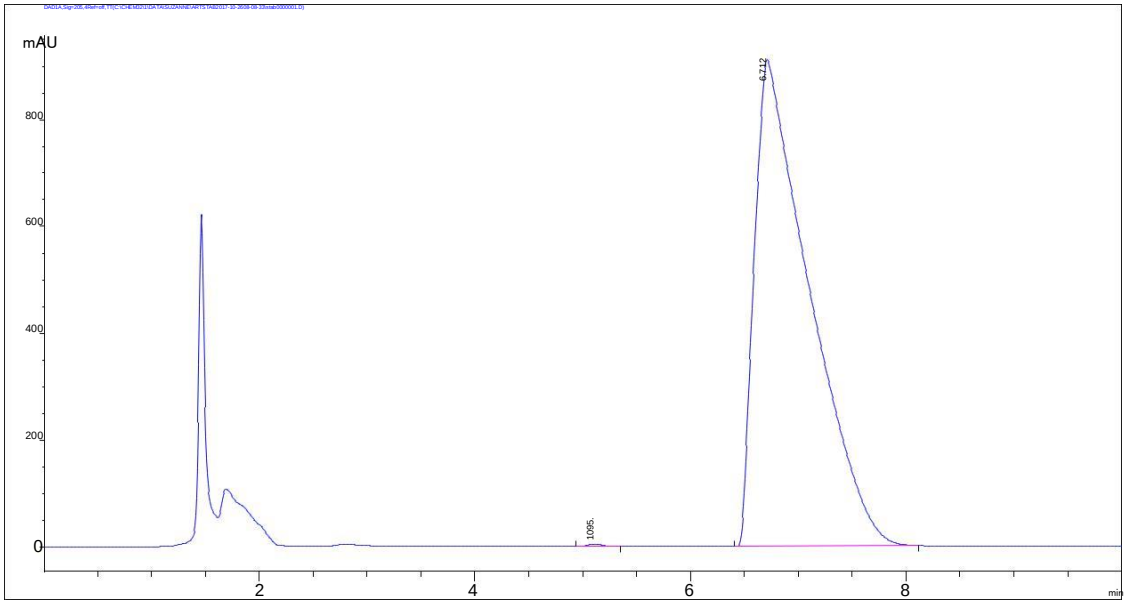


Figure 4.20: *Chromatogram of standard containing artemisone and lumefantrine stressed in HCl*.

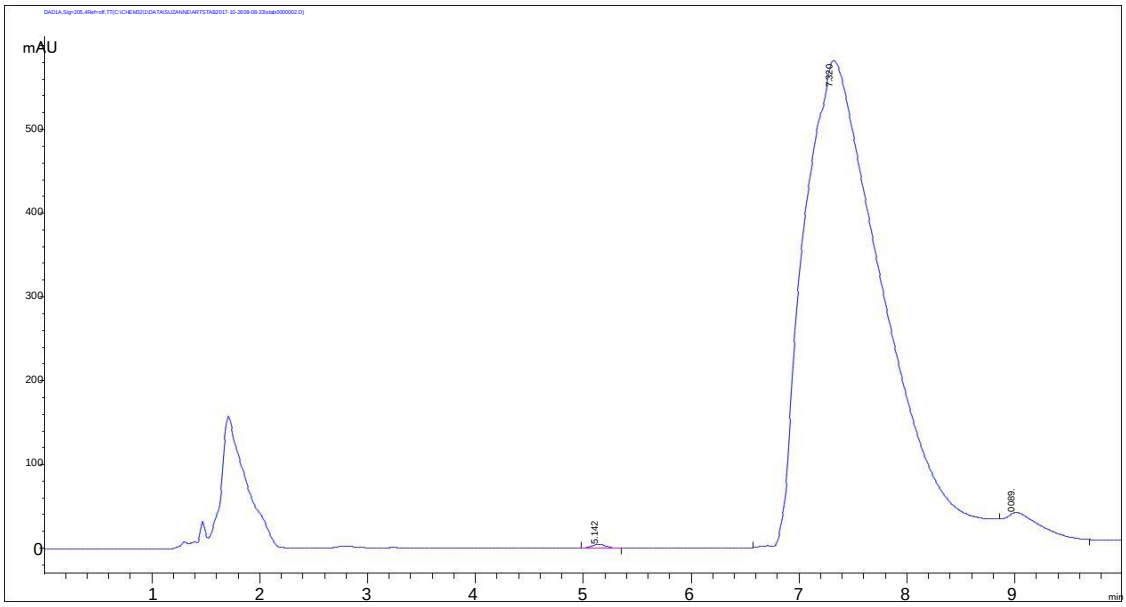


Figure 4.21: *Chromatogram of standard containing artemisone and lumefantrine stressed in NaOH.*

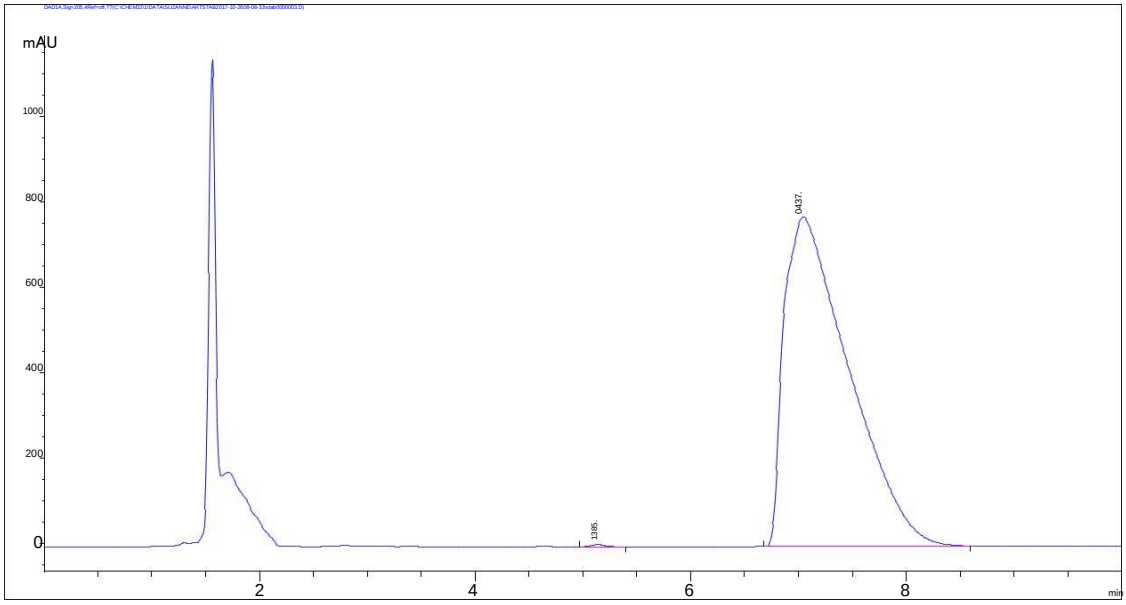


Figure 4.22: *Chromatogram of standard containing artemisone and lumefantrine stressed in H_2O_2.*

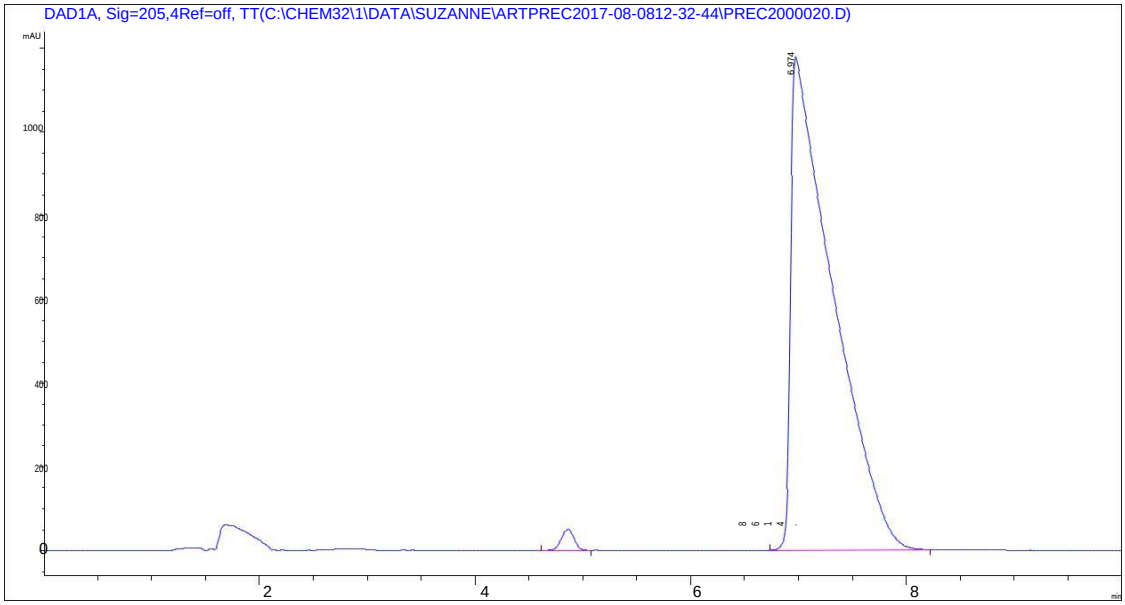


Figure 4.23: *Chromatogram of tablet sample.*

The blank sample did not contain any interfering peaks; and the standard solution did not display any interfering peaks after a week. Although the sample stressed in hydrochloric acid showed degradation, the analyte peaks remained the same and eluded at the same time as initially observed. In sodium hydroxide and hydrogen peroxide extensive degradation was observed and these peaks were clearly contaminated. However, the sodium hydroxide and hydrogen peroxide were not utilised in this study in any way.

4.4.1.1 Linearity and range

Table 4.13 depicts the linearity as well as the range for artemisone and lumefantrine. The regression statistics for both drugs are given in Table 4.14 and the linear regression of artemisone and lumefantrine are portrayed in Figures 4.24 and 4.25, respectively.

Table 4.13: *Linearity and range of artemisone and lumefantrine*

API	µg/ml	Area		Mean
Artemisone	2.002	10.7	12.3	11.5
	5.005	7.4	9.8	8.6
	10.010	16.1	23.1	19.6
	15.015	25.4	28.0	26.7
	20.020	36.7	40.4	38.6
	50.050	86.3	82.5	84.4
	100.100	171.0	168.6	169.8
	150.150	251.7	248.3	250.0
	200.200	334.7	336.8	335.8
Lumefantrine	1.0	31.1	39.4	35.3
	1.5	69.4	70.0	69.7
	2.0	101.4	89.0	95.2
	5.0	258.1	269.1	263.6
	10.0	508.9	466.9	487.9
	14.9	739.0	743.4	741.2
	19.9	1039.3	1029.4	1034.3
	49.8	2741.9	2743.4	2742.6
	99.7	5712.8	5690.2	5701.5
	149.5	8781.8	8685.5	8733.7
	199.3	11728.8	116740.4	64234.6

Table 4.14: *Regression statistics of artemisone and lumefantrine*

Artemisone	**R Squared**	**0.999587**	**Lower 95%**	**Upper 95%**
	Intercept	3.499542	0.740004	6.259078
	Slope	1.653233	1.623184	1.683281
Lumefantrine	**R Squared**	0.999564	Lower 95%	Upper 95%
	Intercept	-73.2368	-132.5292	-13.9444
	Slope	58.41981	57.42477	59.41485

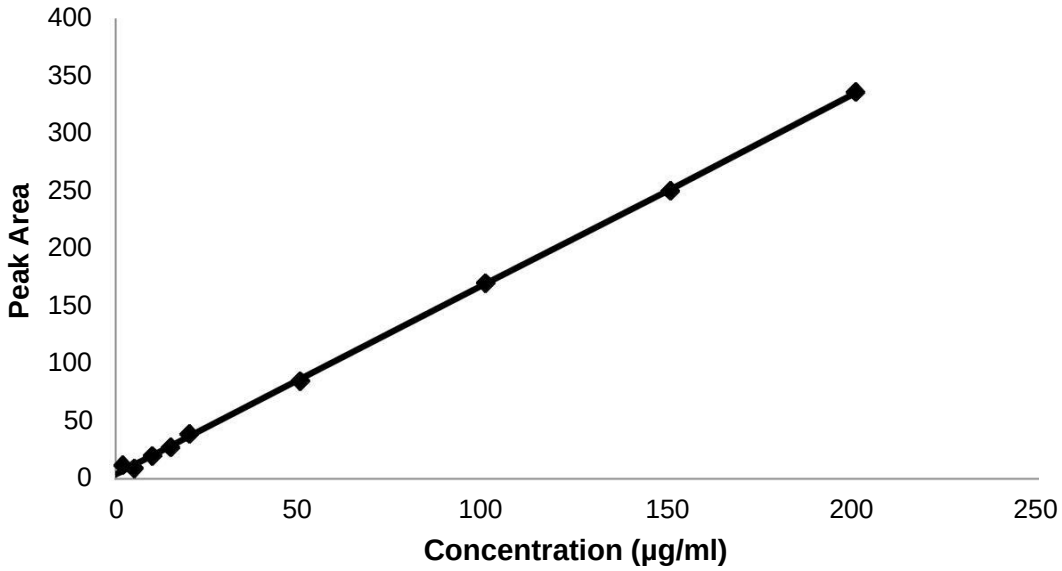


Figure 4.24: *Linear regression graph for artemisone*

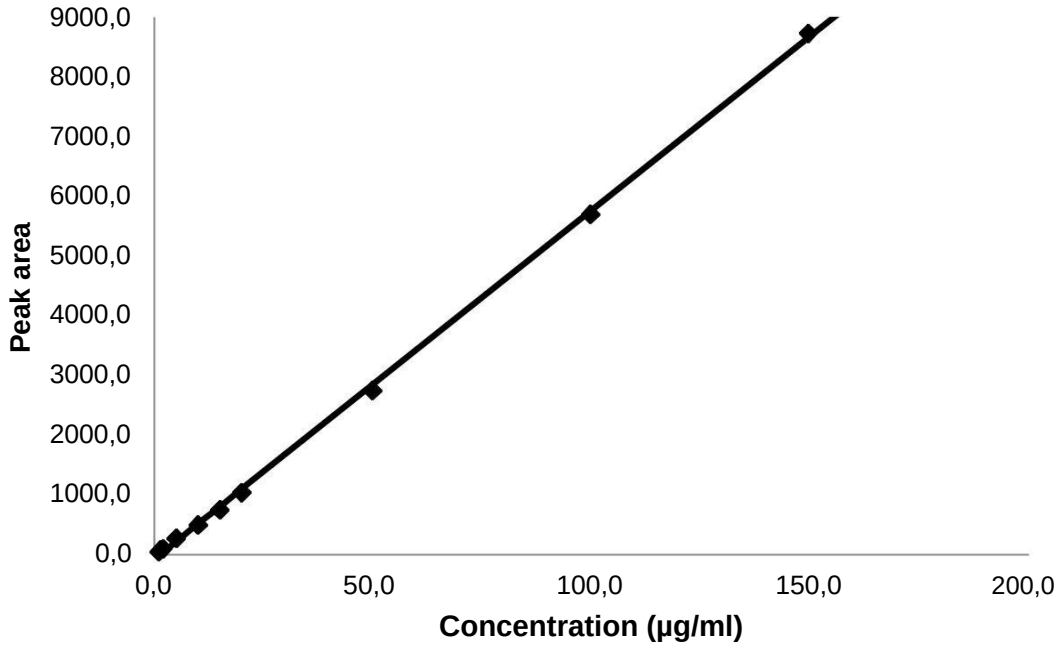


Figure 4.25: *Linear regression graph for lumefantrine*

The HPLC-method was deemed adequate as linearity could be established for both artemisone and lumefantrine over a concentration range of 2–200 µg/ml.

4.4.1.2 Limit of detection and limit of quantification

The limit of detection (LOD) was determined as the smallest concentration that was discernible from baseline noise by a factor of about three (ICH 2005). The limit of quantification (LOQ) is in fact the more important of these parameters as it relates to a concentration at which the method will be able to perform with certain measurable values of quantification. In this case the LOQ was set at the lowest concentration that could be determined repeatedly with an RSD of ≤ 20% (FDA, 2013). For artemisone the LOD was determined at 1.5 µg/ml and the LOQ at 2.0 µg/ml (RSD of six replicates was 15.3%), whereas for lumefantrine the LOD was determined as 0.5 µg/ml and the LOQ was 1.0 µg/ml (RSD of six replicates was 14.6 %).

4.4.1.3 Accuracy of artemisone and lumefantrine

In Table 4.15 the accuracy results obtained for artemisone and lumefantrine are shown, furthermore, the statistical analysis of these drugs is depicted in Table 4.16.

Table 4.15: *Table depicting accuracy of artemisone and lumefantrine*

API	Conc. spiked				Recovery	
	µg/ml	Area 1	Area 2	Mean	µg/ml	%
Artemisone	120.6	267.4	270.4	268.9	124.6	103.3
	120.6	267.6	280.6	274.1	127.1	105.4
	120.6	275.0	264.9	270.0	125.1	103.7
	160.8	341.0	349.0	345.0	160.6	99.9
	160.8	336.5	335.5	336.0	156.3	97.2
	160.8	335.4	336.7	336.1	156.4	97.2
	201.0	429.4	417.7	423.6	197.7	98.4
	201.0	448.6	435.5	442.1	206.5	102.7
	201.0	434.0	442.5	438.3	204.7	101.8
Lumefantrine	188.4	20703.6	20705.5	20704.6	199.7	106.0
	188.4	20273.2	20248.7	20261.0	195.4	103.7
	188.4	20120.5	20209.0	20164.8	194.4	103.2
	251.2	26040.6	26031.1	26035.9	252.1	100.4
	251.2	25140.1	25158.0	25149.1	243.4	96.9
	251.2	25119.5	25475.0	25297.3	244.8	97.5
	314.0	31804.5	31853.8	31829.2	309.0	98.4
	314.0	32357.0	32296.2	32326.6	313.9	100.0
	314.0	32599.3	32592.9	32596.1	316.6	100.8

Table 4.16: *Statistical analysis of accuracy for artemisone and lumefantrine*

Statistical analysis		
	Artemisone	Lumefantrine
Mean	101.1	100.8
SD	2.8	2.9
% RSD	2.8	2.8
95% confidence intervals		
Lower limit	105.35	106.01
Upper limit	97.22	96.89
Estimated median	101.84	100.36
Confidence Level	2.31	2.34

To conclude: over the range, the method for artemisone yielded a mean recovery of 101.1 % and for lumefantrine a mean recovery of 100.8% was generated.

4.4.1.4 Intraday and interday precision

Intraday and interday precision

Intraday and interday precision were performed on both artemisone and lumefantrine. Table 4.17 portrays the intraday precision of these drugs, whilst Table 4.18 shows the data for the interday precision.

Table 4.17:

Intra day precision of artemisone and lumefantrine

API	Mass weighed				Recovery	
	mg	Area 1	Area 2	Mean	µg/ml	%
Artemisone	204.0	122.8	122.6	122.7	159.7	78.3
	202.3	121.4	120.7	121.0	157.5	77.9
	205.0	122.4	122.4	122.4	159.3	77.7
	253.5	146.4	145.3	145.9	190.1	75.0
	251.6	157.6	157.8	157.7	205.6	81.7
	252.0	157.6	157.8	157.7	205.6	81.6
	305.8	185.6	184.4	185.0	241.3	78.9
	303.0	183.8	184.0	183.9	239.9	79.2
	304.5	174.3	173.7	174.0	227.0	74.5
					Mean	**78.8**
					SD	**2.0**
					RSD %	**2.6**
Lumefantrine	**mg**	**Area 1**	**Area 2**	**Mean**	**µg/ml**	**%**
	204.0	10718.0	10676.5	10697.3	247.2	121.2
	202.3	10469.7	10479.3	10474.5	242.0	119.6
	205.0	10572.0	10588.7	10580.4	244.5	119.3
	253.5	12544.4	12536.6	12540.5	289.7	114.3
	251.6	13577.9	13583.5	13580.7	313.7	124.7
	252.0	13577.9	13583.5	13580.7	313.7	124.5
	305.8	15052.4	15815.9	15770.8	364.3	119.1
	303.0	15292.0	15778.2	15758.0	364.0	120.1
	304.5	15400.2	15091.5	14989.7	346.3	113.7
					Mean	**120.3**
					SD	**3.1**
					% RSD	**2.6**

Table 4.18: *Interday precision of artemisone and lumefantrine*

API		Day 1	Day 2	Day 3	Between days
Artemisone		75.0	76.6	76.8	
		81.7	79.8	80.1	
		81.6	80.6	79.2	
	Mean	**79.42**	**79.01**	**78.72**	**79.05**
	SD	**3.14**	**1.71**	**1.39**	**2.24**
	RSD %	**3.96**	**2.17**	**1.77**	**2.83**
Lumefantrine		114.3	120.6	117.5	
		124.7	117.0	124.3	
		124.5	122.4	120.6	
	Mean	**121.16**	**119.98**	**120.80**	**120.65**
	RSD %	**4.01**	**1.89**	**2.30**	**2.92**

From the intraday and interday precision experiments for artemisone it was recognised that the content of the tablets comprising artemisone was lower than expected. Nonetheless, the intraday precision yielded an RSD of 2.6%, which is acceptable for dissolution studies and experimental formulation. The interday precision for artemisone was calculated as 2.83%. Intraday and interday precision for lumefantrine tablets were higher than expected where the intraday precision produced an RSD of 2.6%, which is suitable for dissolution studies and experimental formulations. The interday precision was determined as 2.92% for lumefantrine.

4.4.1.5 Stability of sample solutions

A sample of each drug was left on the autosampler tray and reanalysed over several time intervals to determine the sample stability. Results acquired are shown in Table 4.19.

Table 4.19:

Resu

lts of stability testing for artemisone and lumefantrine

	Artemisone		Lumefantrine	
Time (h)	**Peak Area**	**% Remaining**	**Peak Area**	**% Remaining**
0	440.1	100.0	440.1	100.0
1	439.6	99.9	439.6	99.9
2	440.3	100.1	440.3	100.1
3	440.1	100.0	440.1	100.0
4	440.1	100.0	440.1	100.0
5	440.1	100.0	440.1	100.0
6	440.4	100.1	440.4	100.1
7	440.1	100.0	440.1	100.0
8	439.8	99.9	439.8	99.9
9	441.0	100.2	441.0	100.2
10	440.5	100.1	440.5	100.1
11	440.9	100.2	440.9	100.2
12	440.9	100.2	440.9	100.2
13	441.0	100.2	441.0	100.2
14	440.4	100.1	440.4	100.1
15	440.5	100.1	440.5	100.1
16	440.9	100.2	440.9	100.2
17	440.5	100.1	440.5	100.1
18	440.9	100.2	440.9	100.2
19	440.9	100.2	440.9	100.2
20	439.2	99.8	439.2	99.8
21	439.8	99.9	439.8	99.9
22	439.5	99.9	439.5	99.9
23	439.3	99.8	439.3	99.8
24	440.0	100.0	440.0	100.0
Mean	**440.3**	**100.0**	**440.3**	**100.0**
SD	**0.53**	**0.12**	**0.53**	**0.12**
%RSD	**0.12**	**0.12**	**0.12**	**0.12**

Artemisone and lumefantrine are stable over a period of 24 h under normal laboratory conditions, thus, the method is suitable for sample analysis over an extended period of time.

4.4.1.6 System repeatability of artemisone and lumefantrine

A sample of artemisone as well as lumefantrine, individually, was injected six times in order to test the repeatability of the peak area as well as the retention time. These results are portrayed in Table 4.20.

Table 4.20:

Results of system repeatability of artemisone and lumefantrine.

	Artemisone		Lumefantrine	
Injection	**Area**	**Retention time (min)**	**Area**	**Retention time (min)**
1	416.50	4.86	31907.00	6.97
2	413.45	4.86	31912.00	6.98
3	406.86	4.87	31958.00	6.97
4	410.88	4.86	31981.00	6.97
5	410.45	4.86	31968.00	6.88
6	414.69	4.85	31802.00	6.88
Mean	**412.1**	**4.858**	**31921.3**	**6.942**
SD	**3.15**	**0.006**	**60.02**	**0.043**
RSD %	**0.76**	**0.130**	**0.19**	**0.625**

System performance of both artemisone and lumefantrine proved well within the RSD values of 0.76% (artemisone) and 0.19% (lumefantrine) for peak area; and 0.13% and 0.63% for retention time, respectively.

4.4.1.7 Robustness

The following changes in the chromatographic operating parameters were made as it was deemed more acceptable for analysis:

Column: Luna C18-2 column, 150 x 4.6 mm, 5 µm was found to be suitable.

Mobile phase: Concentrations of 72–78% acetonitrile was still suitable in spite of differences in retention time.

Flow rate: 0.8–1.2 ml/minute.

Wavelength: The wavelength can be altered by ± 3 nm without any ill effect.

In conclusion, the method was able to tolerate small changes in the chromatographic conditions and should perform well under normal use.

4.4.1.8 Chromatographic performance parameters

In Tables 4.21 and 4.22 the chromatographic performance parameters for both artemisone and lumefantrine are depicted. The resolution between the artemisone and lumefantrine peaks are 4.070.

Table 4.21 *Retention time for artemisone and lumefantrine*

API	Retention time (min)
Artemisone	4.86
Lumefantrine	6.94

Table 4.22: *Performance parameters for artemisone and lumefantrine*

	Artemisone	Lumefantrine
Number of theoretical plates (N)	8099	1222
USP Tailing factor (T)	1.003	5.513
Capacity factor (k')	2.200	3.750

Overall, the method performed well and should be suitable to be able to analyse artemisone and lumefantrine content during assay and drug release experiments.

4.4.2 Assay for optimised formulations

The assay for content of active ingredients was performed on all optimised tablets in this study (Table 4.12). The tablets were formulated to each contain 120 mg lumefantrine and 80 mg artemisone. Theoretically, each tablet therefore contained 200 mg of the active ingredients.

In order to correctly determine the concentrations of the model composites in each optimised formula, standard solutions of known concentrations were prepared and evaluated utilising HPLC. The peak values of a sequence of dilutions of each model compound were utilised to

build reference standard curves that were essential to exhibit a linear regression value (R^2). The standard curve obtained for artemisone is provided in Figure 4.26, whereas the standard curve obtained for lumefantrine is given in Figure 4.27.

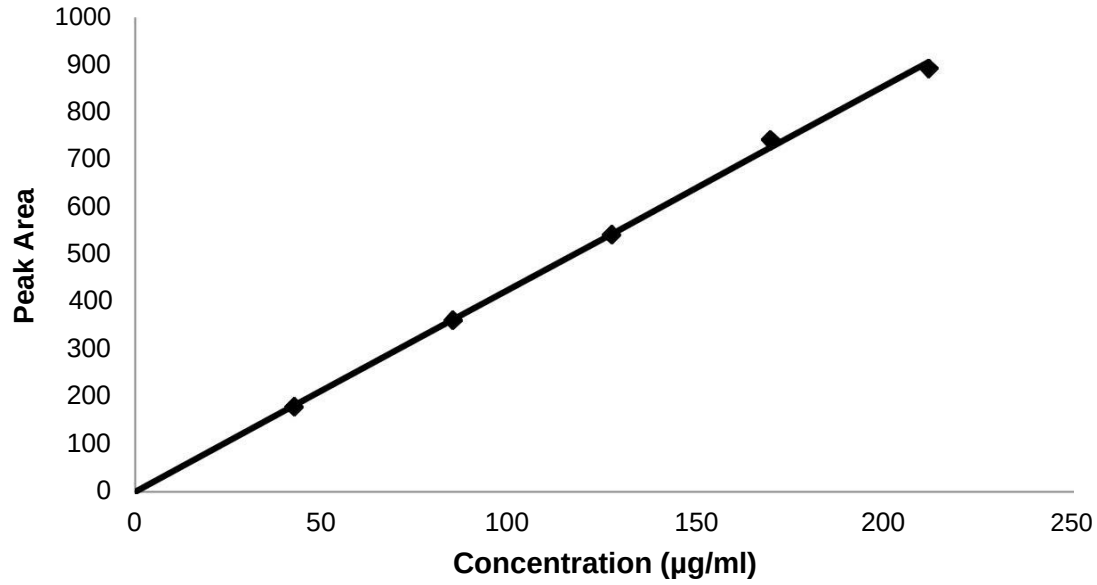


Figure 4.26:

Standard curve of assay for artemisone.

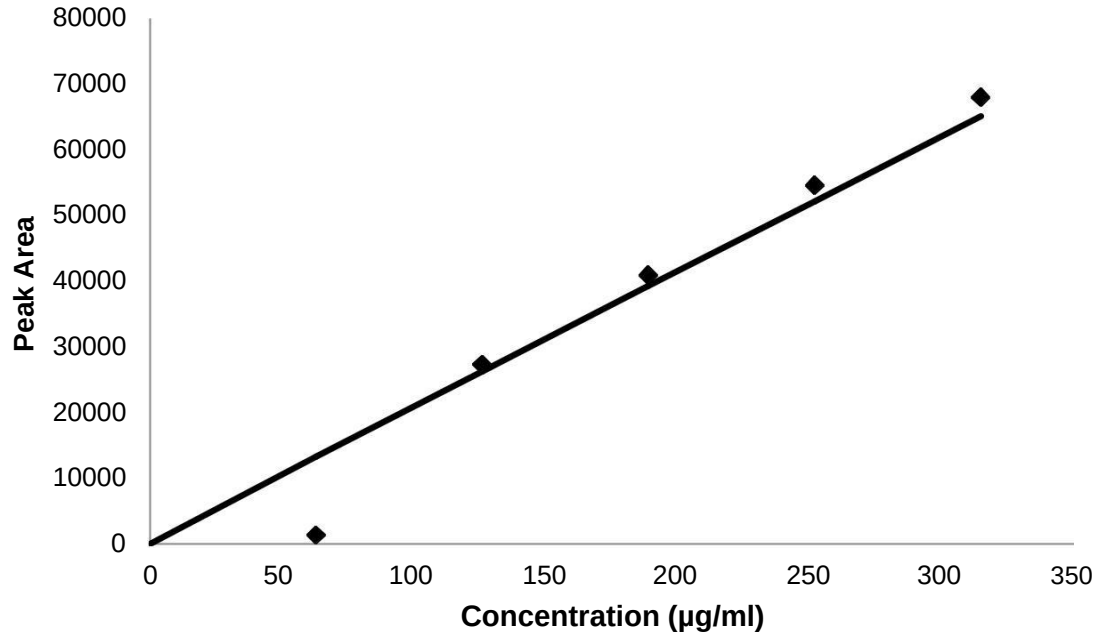


Figure 4.27: *Standard curve of assay for lumefantrine.*

Results acquired for the assay experiments are summarised in Table 4.23 and Table 4.24 for formulations containing one of the selected fillers and either glycerol monostearate or stearic acid as lipid, respectively.

Table 4.23: Assay of lumefantrine and artemisone (mg) included in the optimised formulations containing glycerol monostearate.

	G0.5M1	**G0.5R1**	**G1R1**	**G0.5C1**	**G1C1**
Lumefantrine	129.840	117.360	115.440	135.360	108.960
Artemisone	75.520	63.600	63.360	78.560	61.520

Table 4.24: Assay of lumefantrine and artemisone (mg) included in the optimised formulations containing stearic acid.

	S0.5M1	**S1M1**	**S0.5R1**	**S1R1**	**S0.5C1**	**S1C1**
Lumefantrine	62.520	91.320	29.640	88.080	110.880	123.360
Artemisone	72.080	57.920	17.280	47.920	68.480	78.480

Generally, formulations that included glycerol monostearate depicted considerably higher drug concentrations in the formulations for both artemisone and lumefantrine. No definite trends could further be recognised in terms of the type of filler incorporated. Formulations that comprised glycerol monostearate as lipid and RetaLac® as filler, did however, display concentrations that were more uniform, although the artemisone content was only approximately 79% of what was intended.

4.5 DISSOLUTION STUDIES

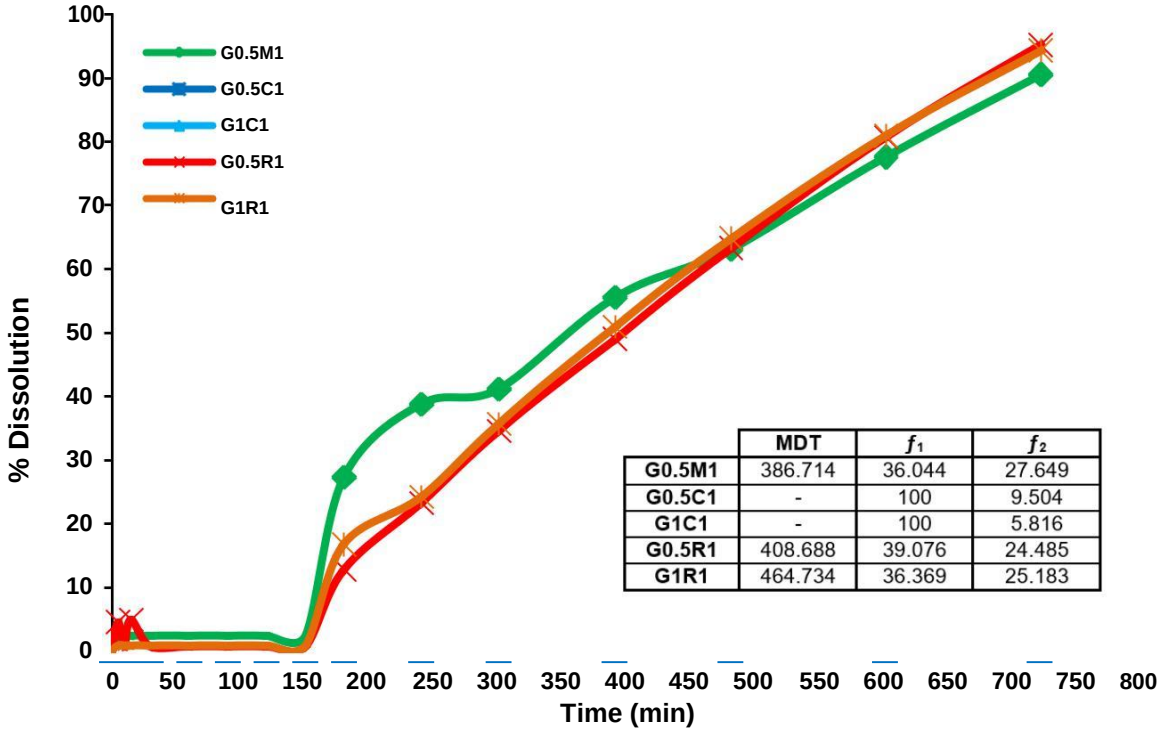
All of the dissolution studies performed in this study were done in six-fold. A 0.1 M HCl solution (pH ± 1.2) were used as dissolution media for the first 120 min, followed by a change in media (combination of the 0.1 M HCl solution and the buffer solution, trisodium orthophosphate) to alter the pH to 6.8 for another 600 min. Every dissolution basket contained a single lipid-matrix tablet of a particular formulation and the dissolution properties were assessed according to the method described in section 3.5.7. A total of 11 formulations were deemed acceptable according to results obtained by the full factorial

design and were subsequently analysed in order to determine the release profiles of artemisone and lumefantrine, respectively, from these formulations. Figures 4.28–4.3 illustrate the different drug release profiles acquired for the respective drugs.

The average dissolution time of the percentage drug released for all points tested during dissolution, is defined as the mean dissolution time (MDT). A higher MDT value is an indication of a slower drug release, whereas low MDT values signify faster drug release properties (Qui, 2009). As stated in Chapter 1; one of the objectives set for this study was to produce a lipid matrix tablet formulation that presented with a modified release profile (i.e. a higher MDT preferably). Fit factors (f_1 and f_2 values) were calculated to determine if any significant differences could be attained between these optimised formulations. The criteria indicate that formulations where $f_1 < 15$ and $f_2 > 50$, are considered similar in their dissolution profiles as explained in section 3.10.2 (Breier *et al.,* 2005). Therefore, MDT and fit factors were calculated and portrayed on the dissolution graphs. The formulation, S0.5C1, was utilised to compare all of the other selected lipid matrix tablet formulations due to the fact that this formulation performed poorly during disintegration experiments as it disintegrated within 15 min and could not be considered non-disintegrating. It was expected that this formulation would show fast and high drug release.

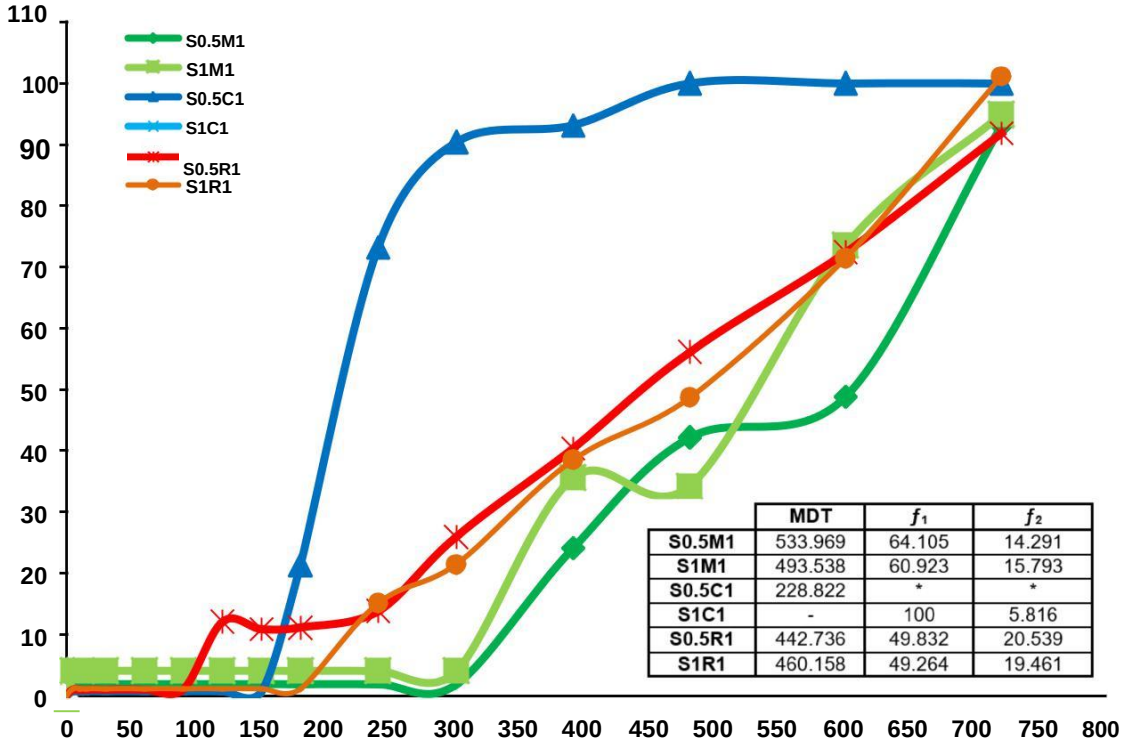
From Figures 4.28 and 4.29, it is clear that artemisone depicted delayed release from all of the different lipid matrix tablet formulations. Drug release occurred (for all of the artemisone formulations) after 120 min as the media was changed to simulate the small intestine surroundings. Regardless the type of lipid incorporated, the formulations that contained CombiLac® did not release artemisone from the tablets, except for S0.5C1. However, this was the only lipid matrix tablet that disintegrated before the set limit for this study. The G0.5M1 formulation illustrated a mean dissolution time (MDT) of 386.714 min, whereas RetaLac®/glycerol monostearate formulations displayed MDT values of 408.688 and 464.734 min for G0.5R1 and G1R1, respectively. Although it seemed as if these dissolution profiles might differ significantly due to the relatively large difference in MDT values, fit-factors calculated between these formulations indicated that none of the formulations differed statistically significantly (f_1=15.975 and 13.725; and f_2=54.267 and 57.970, correspondingly). Lipid matrix tablet formulations comprised stearic acid portrayed more distinct differences in the artemisone release profiles. Formulations that included MicroceLac® 100/stearic acid as filler started releasing artemisone approximately 2.5–3.5 h later relative to the RetaLac®/stearic acid formulations. Although both S0.5M1 and S1M1 followed a similar dissolution profile in comparison to S0.5R1 and S1R1, respectively, the formulations portrayed a significant statistical difference. The fit factors calculated between

the MicroceLac® 100/stearic acid formulations, were f_1=24.999 and f_2=50.354, whilst the fit factor values between the RetaLac®/stearic acid formulations were calculated at f_1=18.553 and f_2=56.600, respectively. Overall, considering modified release properties, the MicroceLac® 100/stearic acid formulations displayed the highest MDT values (approximately 8.6 h) relative to the RetaLac®/stearic acid formulations (approximately 7.5 h); and could therefore be considered most ideal.



	MDT	f_1	f_2
G0.5M1	386.714	36.044	27.649
G0.5C1	-	100	9.504
G1C1	-	100	5.816
G0.5R1	408.688	39.076	24.485
G1R1	464.734	36.369	25.183

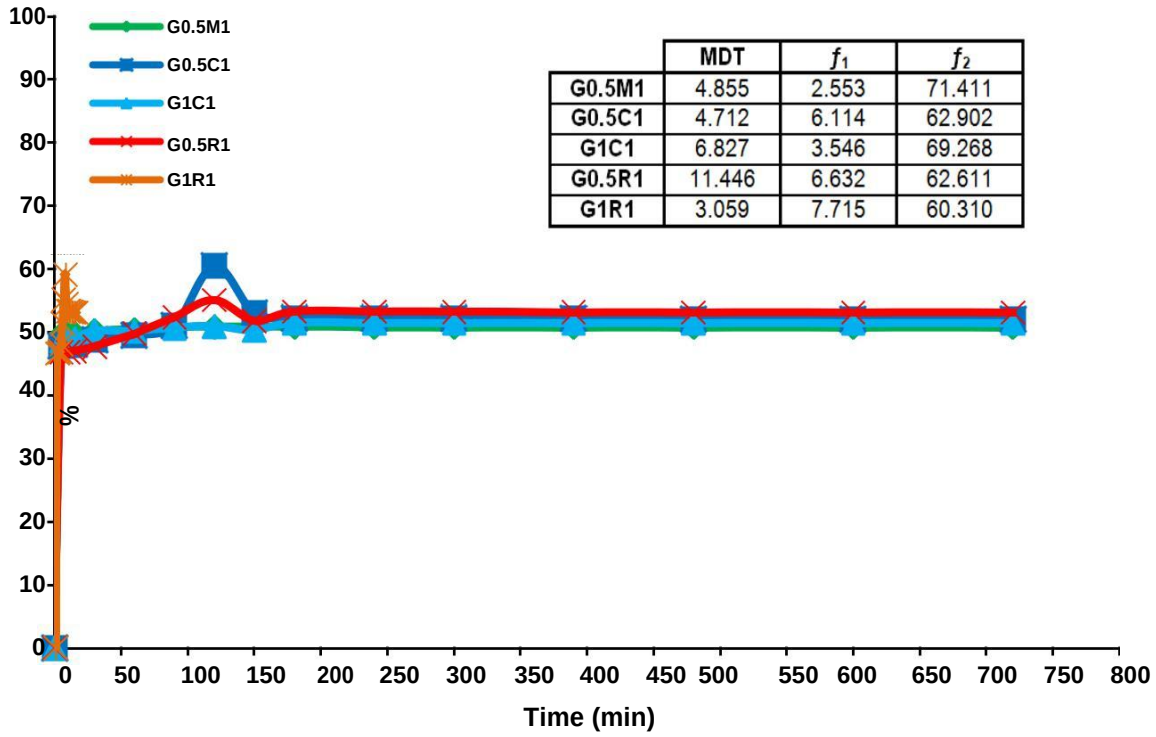
Figure 4.28: *Percentage artemisone dissolution for the different glycerol monostearate formulations prepared as a function of time.*



	MDT	f_1	f_2
S0.5M1	533.969	64.105	14.291
S1M1	493.538	60.923	15.793
S0.5C1	228.822	*	*
S1C1	-	100	5.816
S0.5R1	442.736	49.832	20.539
S1R1	460.158	49.264	19.461

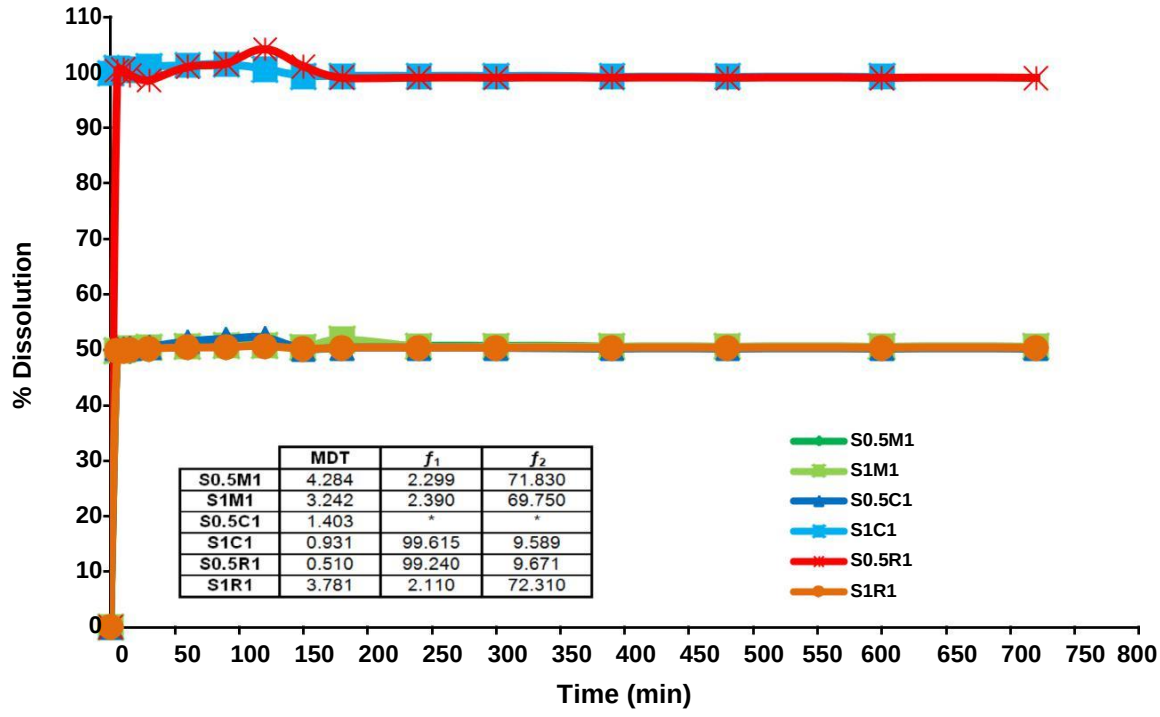
Figure 4.29: *Percentage artemisone dissolution for the different stearic acid formulations prepared as a function of time.*

Lipid matrix formulations comprising lumefantrine clearly depicted burst release profiles for all of the formulations, irrespective of the lipid included (Figures 4.30 and 4.31). All of the formulations where glycerol monostearate was utilised as the lipid, illustrated comparable dissolution profiles, which is confirmed by the fit factors that indicated no statistical significant differences (Figure 4.30). These formulations furthermore depicted relatively similar MDT values confirming the initial burst release of lumefantrine from all of the said formulations. Lipid matrix formulations that included stearic acid showed relatively similar dissolution profiles to those that contained glycerol monostearate, i.e. burst release (Figure 4.31). However, these formulations displayed a similar or higher total percentage lumefantrine released (50% versus 100%) comparatively. From Figure 4.31 it is evident that S1C1 and S0.5R1 formulations released lumefantrine in a more acceptable concentration (f_1=99.615 and f_2=9.589; andf_1=99.240 and f_2=9.671, correspondingly). Nevertheless, no lipid matrix tablet formulations were able to release lumefantrine in a modified manner, rendering these formulations inadequate.



	MDT	f_1	f_2
G0.5M1	4.855	2.553	71.411
G0.5C1	4.712	6.114	62.902
G1C1	6.827	3.546	69.268
G0.5R1	11.446	6.632	62.611
G1R1	3.059	7.715	60.310

Figure 4.30 *Percentage lumefantrine dissolution for the different glycerol monostearate formulations prepared as a function of time.*



	MDT	f_1	f_2
S0.5M1	4.284	2.299	71.830
S1M1	3.242	2.390	69.750
S0.5C1	1.403	*	*
S1C1	0.931	99.615	9.589
S0.5R1	0.510	99.240	9.671
S1R1	3.781	2.110	72.310

Figure 4.31: *Percentage lumefantrine dissolution for the different stearic acid formulations prepared as a function of time.*

In summary, it can be concluded that artemisone and lumefantrine portrayed significantly different release profiles, where the release of artemisone could be described as delayed

and release of lumefantrine is considered a burst effect. Considering the release of artemisone, formulations that comprised CombiLac® were deemed unacceptable for modified release. On the other hand, MicroceLac® 100 formulations illustrated the most delayed release profiles for artemisone, but RetaLac® formulations displayed a more uniform artemisone release profile. No substantial conclusion could be drawn in terms of the type of filler utilised when reviewing the release profiles of lumefantrine. With reference to the type of lipid incorporated as well as with regards to artemisone, formulations that contained stearic acid showed a slightly more delayed release compared to formulations containing glycerol monostearate as seen with the average MDT values for the respective lipids (431.85 min and 420.05 min, respectively). Lipid matrix tablet formulations that included glycerol monostearate overall released lumefantrine to a lesser extent comparative to formulations that contained stearic acid. Although formulations comprising stearic acid displayed slightly faster release rates (MDT ~ 2.36 min) relative to glycerol monostearate formulations (MDT ~ 6.18 min), this was not considered significant. Furthermore, the ratio lipid included rendered no significant difference in neither artemisone's, nor lumefantrine's release rate.

CHAPTER 5

SUMMARY & FUTURE PROSPECTS

SUMMARY

The aim of this study is to develop a fixed dose modified release matrix tablet containing artemisone and lumefantrine utilising different fillers in order to produce a dosage form for the treatment of uncomplicated malaria. Lipid matrix tablets were prepared utilising the hot-melt process as well as direct compression. The following objectives set, were met:

- Lipid matrix tablet formulations were produced (as determined by factorial design) by means of hot-melt through incorporating two different selected lipids (i.e. glycerol monostearate and stearic acid) in two different double fixed dose: lipid ratios (i.e. 1:0.5 and 1:1). Three individually selected fillers, namely MicroceLac® 100, RetaLac® and CombiLac® were incorporated into the various formulations; and magnesium stearate was included into each formulation in either a 0.5% w/w or a 1% w/w concentration.
- Flow properties of the drugs, filler powders, and the lipid dispersions were successfully evaluated. It was evident that all the lipid dispersions, irrespective of the filler or lipid

included, portrayed improved flowability when compared to the flow properties of the drugs alone, due to enhanced particle size.

- The morphology of the various formulations was evaluated by means of DTG, TAM and XRPD. These results depicted no incompatibilities between the drugs and multiple excipients used, as well as the permanence of the crystallinity of the drugs and excipients.
- The various lipid dispersions were successfully directly compressed into lipid matrix tablets after selection using a full factorial design and utilising a Cadmach® eccentric single tablet press.
- All the formulations were effectively assessed in terms of their physical tablet properties. It could be concluded that each factor (type of filler, type of lipid, double fixed dose:lipid ratio, concentration lubricant) did indeed have an undeniable influence on the physical properties of the lipid matrix tablets as seen in Tables 4.7–4.10.
- Swelling and erosion experiments conducted on the optimised lipid matrix tablets showed that the RetaLac® formulations depicted the highest percentage swelling, irrespective of the lipid used or the ratio in which the double fixed dose:lipid was incorporated.
- Dissolution studies were subsequently performed on the optimised double fixed dose lipid matrix tablets. It was noted that glycerol monostearate in a 1:1 ratio depicted a more desirable dissolution profile in comparison with stearic acid. Formulations that comprised RetaLac® portrayed the most desirable drug release results relative to the criteria set for this study. A tendency of sustained/modified release was evident in the dissolution profiles of most of the formulations, which was also an objective set for this study.

In summary, it could be resolved that artemisone and lumefantrine displayed significantly different release profiles. Artemisone was released in a modified/delayed manner, whereas lumefantrine is considered to be released by means of a burst effect, irrespective of the type of filler, lipid, or double fixed dose:lipid ratio incorporated. CombiLac® as filler is believed to be unacceptable for modified release in this study in view of the release of artemisone from the matrix tablet formulations. MicroceLac® 100 formulations, on the other hand, depicted the most delayed release profiles for artemisone, however RetaLac® formulations displayed a more uniform artemisone release profile. The release profiles of lumefantrine could not lead to any substantial conclusions pertaining to the type of filler as neither delayed, nor sustained release could be obtained. With regards to the type of lipid incorporated, formulations that contained stearic acid illustrated slightly more delayed artemisone release profiles. Furthermore, double fixed dose lipid matrix tablet formulations that included

glycerol monostearate, overall, released lumefantrine to a lesser extent; and granting formulations comprising stearic acid portrayed slightly faster release rates relative to glycerol monostearate formulations, this was not considered significant. Furthermore, the ratio double fixed dose:lipid included rendered no significant difference in neither artemisone's, nor lumefantrine's release rates from the different lipid matrix tablet formulations.

Lipid matrix tablets are advanced and innovative dosage forms that unquestionably deserve to be investigated in more detail. This study confirmed that lipid matrices containing a double fixed dose of artemisone and lumefantrine have the potential to be utilised as modified release dosage forms for the treatment of uncomplicated *Plasmodium falciparum* malaria. This combination of artemisone and lumefantrine holds promising potential for a possible new artemisinin-based combination therapy (ACT) alternative.

FUTURE PROSPECTS

The following recommendations for upcoming investigations are suggested:

- Investigate the influence of other lipids (i.e. cetostearyl alcohol, glyceryl behenic acid, and glyceryl palmitostearate) on the compressibility, drug release properties and bioavailability of both lumefantrine and artemisone.
- Conduct a particle size and particle size distribution study on the selected fillers, active ingredients and lipid dispersions. Particle size can significantly influence the flow properties of powders and is therefore an important parameter to measure. Larger, more sphere-shaped particles will naturally exhibit a better flow than that of a smaller particle with an uneven surface (Fu *et al.,* 2012). Spherical particles have a smaller surface to weight relation, therefore the cohesive force amongst the particles are less comparative to non-spherical particles (Alderborn, 2013). Poor flowability can influence the tableting process and consecutively the results from the mass variation of the formulation containing an excipient with poor flow properties.
- Perform accelerated or real-time stability experiments on the double fixed dose lipid matrix tablets over an extended time period (6 months for accelerated stability studies and a 2 year period for real time stability studies) in order to establish whether these formulations are indeed stable in the long run.
- Incorporate bile salts whilst performing dissolution studies to establish the effect of bile salts on the release rate and solubility of the drugs investigated.
- Utilise *in vivo* studies to assess the effect of the sustained drug release on the plasma drug levels over a prolonged period of time.
- Conduct *in-vitro* permeability experiments with selected optimal lipid-matrix formulations across different sections of porcine intestinal tissues, to subsequently compare the

results to known ACT's in order to be able to conclude if this drug therapy will be more effective.

- Examining hot-melt extrusion as another method to prepare the formulations on a larger scale (Maniruzzaman & Nokhodchi, 2017).
- Explore other sustained release matrices such as pellets, cylindrical implants and microspheres (Abd-Elbary *et al.,* 2013).
- Investigate the incorporation of other lubricants such as Pruv®, a hydrophilic lubricant, or talc; and the effect of these lubricants on the dissolution of the studied drugs.
- Incorporate surfactants, such as sucrose stearate (sucrose stearate D-1805® or sucrose stearate D-1811®) in the formulation to evaluate the influence of the surfactants on the dissolution and bioavailability of lumefantrine and artemisone. Sucrose stearate is a non-ionic surfactant that have low toxicity, exceptional biodegradability, and high biocompatibility (Abd-Elbary *et al.*, 2013).
- Fluctuate the double fixed dose:surfactant ratios with the intention of determining the optimal ratio by means of comparing the dissolution data.

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ANNEXURE A

Table A.1:

Summary Table of Mass Variation Results (g)

			Mass Variation (g)			
			Glycerol Monostearate		Stearic Acid	
			1:0.5	1:1	1:0.5	1:1
MicroceLac®100	Magnesium Stearate	0.5%	0.486 (6.601)	0.507 (3.165)	0.529 (2.142)	0.518 (1.355)
		1%	0.511 (3.668)	*NV	0.549 (0.530)	0.494 (3.992)
RetaLac®		0.5%	0.467 (2.710)	0.486 (0.818)	0.446 (7.863)	0.465 (5.153)
		1%	0.543 (1.113)	0.560 (1.289)	0.523 (1.501)	0.512 (0.650)
CombiLac®		0.5%	0.482 (4.019)	0.497 (2.329)	0.518 (1.591)	0.506 (1.598)
		1%	0.483 (3.988)	0.507 (4.272)	0.502 (7.792)	0.542 (1.736)

Table A.2:

Summary Table of the Friability (%) Test Results

			Friability (%)			
			Glycerol Monostearate		Stearic Acid	
			1:0.5	1:1	1:0.5	1:1
MicroceLac®100	Magnesium Stearate	0.5%	0.199	0.195	0.379	0.386
		1%	0.000	*NV	0.545	0.619
RetaLac®		0.5%	0.215	0.207	0.219	0.428
		1%	2.518	0.178	0.192	0.393
CombiLac®		0.5%	0.000	0.589	0.385	0.197
		1%	1.029	0.200	0.398	0.555

Table A.3:

Summary Table of Tensile Strength (N/mm^2) Results

			Tensile Strength (N/mm^2)			
			Glycerol Monostearate		Stearic Acid	
			1:0.5	1:1	1:0.5	1:1
MicroceLac®100	Magnesium Stearate	0.5%	1.527 (34.748)	1.089 (7.687)	2.225 (3.420)	1.602 (13.515)
		1%	1.284 (13.017)	*NV	2.080 (3.983)	1.506 (3.992)
RetaLac®		0.5%	1.456 (6.577)	1.294 (7.060)	1.197 (44.873)	1.540 (10.448)
		1%	1.299 (5.464)	0.983 (11.012)	1.389 (13.073)	1.705 (7.419)
CombiLac®		0.5%	1.913 (11.819)	1.095 (11.237)	2.426 (5.012)	1.532 (18.358)
		1%	1.535 (28.673)	0.793 (8.767)	1.884 (31.912)	1.995 (5.344)

Table A.4:

Summary Table of Disintegration (sec) Results

			Disintegration (sec)			
			Glycerol Monostearate		Stearic Acid	
			1:0.5	1:1	1:0.5	1:1
MicroceLac®100	Magnesium Stearate	0.5%	>900 (0.000)	>900 (0.000)	>900 (0.000)	>900 (0.000)
		1%	>900 (0.000)	*NV	>900 (0.000)	>900 (0.000)
RetaLac®		0.5%	>900 (0.000)	>900 (0.000)	>900 (0.000)	>900 (0.000)
		1%	>900 (0.000)	>900 (0.000)	>900 (0.000)	>900 (0.000)
CombiLac®		0.5%	>900 (0.000)	>900 (0.000)	>900 (0.000)	>900 (0.000)
		1%	>900 (0.000)	>900 (0.000)	>900 (0.000)	1.995 (5.344)

Table A.5: Mass Variation Results (g) of Glycerol Monostearate 1:0.5

GM 1:0,5	**RetaLac 1%**	**RetaLac 0,5%**	**CombiLac 1%**	**CombiLac 0,5%**	**MicroceLac 1%**	**MicroceLac 0,5%**
	Massa (g)	Massa (g)	Massa (g)	Massa (g)	Massa (g)	Massa (g)
1	0,549	0,464	0,508	0,508	0,5	0,52
2	0,541	0,462	0,507	0,507	0,526	0,467
3	0,546	0,462	0,469	0,469	0,48	0,515
4	0,540	0,453	0,510	0,510	0,529	0,455
5	0,545	0,466	0,454	0,454	0,527	0,511
6	0,549	0,500	0,478	0,478	0,512	0,513
7	0,539	0,462	0,511	0,511	0,53	0,457
8	0,537	0,469	0,487	0,487	0,509	0,438
9	0,555	0,467	0,460	0,464	0,494	0,541
10	0,542	0,461	0,509	0,509	0,493	0,45
11	0,552	0,501	0,459	0,459	0,51	0,486
12	0,545	0,465	0,473	0,473	0,476	0,501
13	0,544	0,455	0,465	0,465	0,527	0,498
14	0,551	0,455	0,489	0,489	0,519	0,528
15	0,540	0,462	0,482	0,482	0,477	0,439
16	0,543	0,4700	0,475	0,475	0,527	0,463
17	0,543	0,469	0,499	0,499	0,505	0,459
18	0,536	0,462	0,481	0,481	0,531	0,513
19	0,543	0,462	0,456	0,472	0,526	0,509
20	0,529	0,477	0,492	0,456	0,525	0,462
Average	0,543	0,467	0,483	0,482	0,511	0,486
STD DEV	0,0060	0,0127	0,0194	0,0192	0,0188	0,0321
%RSD	1,113%	2,710%	4,019%	3,988%	3,668%	6,601%

Table A.6: Mass Variation Results (g) of Glycerol Monostearate 1:1

GM 1:1	**RetaLac 1%**	**RetaLac 0,5%**	**CombiLac 1%**	**CombiLac 0,5%**	**MicroceLac 1%**	**MicroceLac 0,5%**
	Massa (g)	Massa (g)	Massa (g)	Massa (g)	Massa (g)	Massa (g)
1	0,558	0,483	0,510	0,490	x	0,504
2	0,560	0,482	0,502	0,459	x	0,520
3	0,564	0,484	0,508	0,503	x	0,543
4	0,563	0,487	0,497	0,498	x	0,494
5	0,558	0,478	0,510	0,515	x	0,499
6	0,558	0,493	0,499	0,493	x	0,505
7	0,564	0,488	0,498	0,488	x	0,49
8	0,561	0,488	0,507	0,502	x	0,491
9	0,570	0,483	0,596	0,499	x	0,493
10	0,558	0,490	0,499	0,488	x	0,508
11	0,562	0,493	0,507	0,487	x	0,496
12	0,562	0,487	0,502	0,509	x	0,495
13	0,565	0,485	0,484	0,503	x	0,500
14	0,557	0,485	0,510	0,508	x	0,507
15	0,567	0,482	0,503	0,490	x	0,483
16	0,532	0,484	0,486	0,503	x	0,494
17	0,561	0,494	0,502	0,501	x	0,549
18	0,560	0,487	0,502	0,505	x	0,514
19	0,561	0,486	0,502	0,503	x	0,503
20	0,563	0,484	0,509	0,493	x	0,544
Average	0,560	0,486	0,507	0,497	x	0,507
STD DEV	0,007	0,004	0,022	0,012	x	0,018
%RSD	1,289%	0,818%	4,272%	2,329%	x	3,615%

Table A.7: Mass Variation Results (g) of Stearic Acid 1:0.5

SA 1:0,5	**RetaLac 1%**	**RetaLac 0,5%**	**CombiLac 1%**	**CombiLac 0,5%**	**MicroceLac 1%**	**MicroceLac 0,5%**
	Massa (g)	Massa (g)	Massa (g)	Massa (g)	Massa (g)	Massa (g)
1	0,528	0,435	0,523	0,510	0,552	0,525
2	0,516	0,506	0,471	0,509	0,552	0,533
3	0,525	0,418	0,527	0,510	0,552	0,538
4	0,526	0,453	0,496	0,528	0,549	0,56
5	0,524	0,409	0,535	0,513	0,546	0,515
6	0,528	0,436	0,486	0,515	0,551	0,543
7	0,529	0,447	0,493	0,512	0,545	0,515
8	0,525	0,43	0,494	0,516	0,552	0,524
9	0,516	0,439	0,524	0,515	0,549	0,522
10	0,516	0,403	0,483	0,521	0,55	0,521
11	0,513	0,429	0,480	0,518	0,542	0,535
12	0,536	0,399	0,428	0,527	0,55	0,536
13	0,528	0,423	0,470	0,514	0,553	0,536
14	0,506	0,509	0,542	0,512	0,547	0,526
15	0,525	0,464	0,509	0,514	0,547	0,52
16	0,514	0,488	0,586	0,526	0,545	0,52
17	0,524	0,409	0,512	0,509	0,549	0,53
18	0,521	0,489	0,540	0,534	0,549	0,515
19	0,538	0,433	0,417	0,536	0,551	0,525
20	0,528	0,498	0,515	0,514	0,55	0,54
Gemiddeld	0,523	0,446	0,502	0,518	0,549	0,529
STD DEV	0,0079	0,0351	0,0391	0,0082	0,0029	0,0113
%RSD	1,501%	7,863%	7,792%	1,591%	0,530%	2,142%

Table A.8: Mass Variation Results (g) of Stearic Acid 1:1

SA 1:1	**RetaLac 1%**	**RetaLac 0,5%**	**CombiLac 1%**	**CombiLac 0,5%**	**MicroceLac 1%**	**MicroceLac 0,5%**
	Massa (g)	Massa (g)	Massa (g)	Massa (g)	Massa (g)	Massa (g)
1	0,512	0,471	0,544	0,511	0,475	0,507
2	0,509	0,512	0,546	0,508	0,499	0,522
3	0,515	0,467	0,523	0,495	0,515	0,519
4	0,508	0,452	0,538	0,510	0,502	0,517
5	0,506	0,496	0,548	0,512	0,492	0,514
6	0,515	0,427	0,536	0,514	0,517	0,522
7	0,514	0,458	0,509	0,498	0,492	0,500
8	0,517	0,453	0,544	0,504	0,488	0,523
9	0,513	0,492	0,546	0,511	0,524	0,505
10	0,512	0,502	0,550	0,498	0,525	0,516
11	0,506	0,458	0,543	0,499	0,485	0,519
12	0,513	0,458	0,544	0,508	0,516	0,521
13	0,514	0,466	0,545	0,512	0,478	0,522
14	0,513	0,434	0,544	0,512	0,483	0,520
15	0,508	0,457	0,548	0,513	0,476	0,524
16	0,513	0,482	0,548	0,483	0,450	0,518
17	0,514	0,437	0,545	0,500	0,477	0,529
18	0,508	0,493	0,545	0,515	0,517	0,523
19	0,517	0,428	0,546	0,514	0,480	0,527
20	0,515	0,46	0,544	0,505	0,479	0,52
Gemiddeld	0,512	0,465	0,542	0,506	0,494	0,518
STD DEV	0,003	0,024	0,009	0,008	0,020	0,007
%RSD	0,650%	5,153%	1,736%	1,598%	3,992%	1,355%

Table A.9: Hardness Results (N) of Glycerol Monostearate 1:0.5

GM 1:0,5	RetaLac 1%	RetaLac 0,5%	CombiLac 1%	CombiLac 0,5%	MicroceLac 1%	MicroceLac 0,5%
1	113,9	103,1	103,4	110,8	123,3	101,8
2	107,8	110,8	99,7	133,3	93,7	113,9
3	121,2	109,1	111,5	127,6	72,9	117,2
4	116,9	99,1	94,7	126,3	99,1	87,7
5	118,6	104,8	98,1	131,7	109,8	117,5
6	106,5	110,5	103,4	130,3	110,8	110,2
7	116,9	110,8	101,1	107,1	104,8	114,5
8	126,9	104,4	84,3	145,4	101,4	96,7
9	116,2	116,9	99,4	112,2	108,5	94,4
10	125,9	93,4	102,1	112,5	94,4	107,1
Average	117,08	106,29	99,77	123,72	101,87	106,10
STD DEV	6,688	6,772	6,985	12,438	13,433	10,460
% RSD	0,057	0,064	0,070	0,101	0,132	0,099

Table A.10: Hardness Results (N) of Glycerol Monostearate 1:1

GM 1:1	RetaLac 1%	RetaLac 0,5%	CombiLac 1%	CombiLac 0,5%	MicroceLac 1%	MicroceLac 0,5%
1	80,1	98,4	62,0	96,7	x	96,1
2	80,6	96,1	67,8	73,9	x	96,4
3	98,1	100,8	65,8	93,0	x	86,0
4	96,4	104,8	63,5	87,3	x	89,0
5	88,0	108,8	67,2	105,1	x	97,7
6	97,3	102,8	55,4	90,7	x	94,0
7	93,2	95,4	57,1	90,3	x	86,6
8	82,5	98,4	70,5	83,6	x	94,4
9	93,1	100,1	69,9	104,8	x	95,7
10	95,8	92,4	62,5	93,4	x	97,4
Average	90,510	99,800	64,170	91,880	x	93,330
STD DEV	7,129	4,805	5,086	9,340	x	4,444
% RSD	0,079	0,048	0,079	0,102	x	0,048

Table A.11: Hardness Results (N) of Stearic Acid 1:0.5

SA 1:0,5	RetaLac 1%	RetaLac 0,5%	CombiLac 1%	CombiLac 0,5%	MicroceLac 1%	MicroceLac 0,5%
1	117,5	55,8	134	192,4	162,2	182,7
2	123,3	96,4	233,4	171,6	176,7	185,1
3	121,9	113,5	197,1	192,4	182,4	184,7
4	103,8	97,4	103,1	196,8	178,7	174
5	124,3	105,1	120,2	186,7	175	178,3
6	119,9	58,8	172,6	181	170,3	192,8
7	78,6	151,1	126,9	178,3	179,3	174,6
8	131,7	55,1	145,4	199,2	168,9	179,7
9	114,9	79,9	128	196,8	191,1	189,4
10	116,5	75,9	114,3	183,4	185,1	189,1
Average	115,24	88,9	147,5	187,86	176,97	183,04
STD DEV	14,773	30,293	41,203	9,151	8,402	6,378
% RSD	0,128	0,341	0,279	0,049	0,047	0,035

Table A.12: Hardness Results (N) of Stearic Acid 1:1

SA 1:1	RetaLac 1%	RetaLac 0,5%	CombiLac 1%	CombiLac 0,5%	MicroceLac 1%	MicroceLac 0,5%
1	166	140,4	172,6	156,5	130,3	121,6
2	140	131,7	176,7	118,2	82,6	144,7
3	135	118,9	173,3	118,9	103,8	137,4
4	145	136,4	177,7	99,1	77,2	140,7
5	157	128,6	144,7	141,7	129,3	107,5
6	148	98,7	175,0	146,1	130,3	108,1
7	149	123,3	164,2	110,5	123,9	122,6
8	143	118,6	174,3	149,5	133,7	139,0
9	142	118,6	179,7	143,1	123,9	114,2
10	138	114,2	171,6	94,4	135,3	149,5
Gemiddeld	146,300	122,94	170,98	127,80	117,03	128,53
STD DEV	9,310	12,078	10,144	22,278	21,488	15,584
% RSD	0,064	0,098	0,059	0,174	0,184	0,121

Table A.13: Flow Properties of Fillers

Flow properties	**MicroceLac®**	**RetaLac®**	**CombiLac®**
Flow rate (s)	1,35	1,74	1,9
	1,32	1,95	1,69
	1,36	1,97	1,72
average	1,343	1,887	1,770
stdev	0,021	0,127	0,114
%RSD	1,550%	6,753%	6,417%
Bulk density (ml)	200	308,8	210
	200	314,4	210
	202	305,5	210
average	200,667	309,567	210
stdev	1,155	4,499	0
%RSD	0,575	1,453	0
Tapped density (ml)	170	240	172
	170	244	174
	170	238	176
average	170	240,667	174
stdev	0	3,055	2
%RSD	0%	1,269%	1,149%
Hausner ratio	0,850	0,777	0,819
	0,850	0,776	0,829
	0,842	0,779	0,838
average	0,847	0,777	0,829
stdev	0,005	0,001	0,010
%RSD	0,574%	0,193%	1,149%
% Compressibility	17,647%	28,667%	22,093%
	17,647%	28,852%	20,690%
	18,824%	28,361%	19,318%
average	18,039%	28,627%	20,700%
stdev	0,007	0,002	0,0139
%RSD	3,765%	0,866%	6,703%

Table A.14: Flow

Properties of Glycerol Monostearate Lipid Dispersions

Flow properties	**(1:1)**	**(1:0,5)**
Flow rate (g/s)	46,729	9,328
	51,546	10,941
	46,296	5,656
average	48,190	8,642
stdev	2,914	2,708
%RSD	6,047%	31,342%
Bulk density (g/ml)	0,481	0,521
	0,481	0,510
	0,484	0,510
average	0,482	0,514
stdev	0,002	0,006
%RSD	35,935%	1,236%
Tapped density (g/ml)	0,568	0,610
	0,568	0,595
	0,568	0,641
average	0,568	0,615
stdev	0	0,023
%RSD	0,000%	3,818%
Hausner ratio	1,181	1,170
	1,181	1,167
	1,174	1,257
average	1,178	1,198
stdev	0,004	0,051
%RSD	0,359%	4,261%
% Compressibility	15,317%	14,555%
	15,317%	14,286%
	14,789%	20,437%
average	15,141%	16,426%
stdev	0,003	0,035
%RSD	2,014%	21,163%
Angle of repose	20,136	23,847
	19,592	23,912
	20,772	22,982
average	20,167	23,580
stdev	0,591	0,519
%RSD	2,929%	2,202%

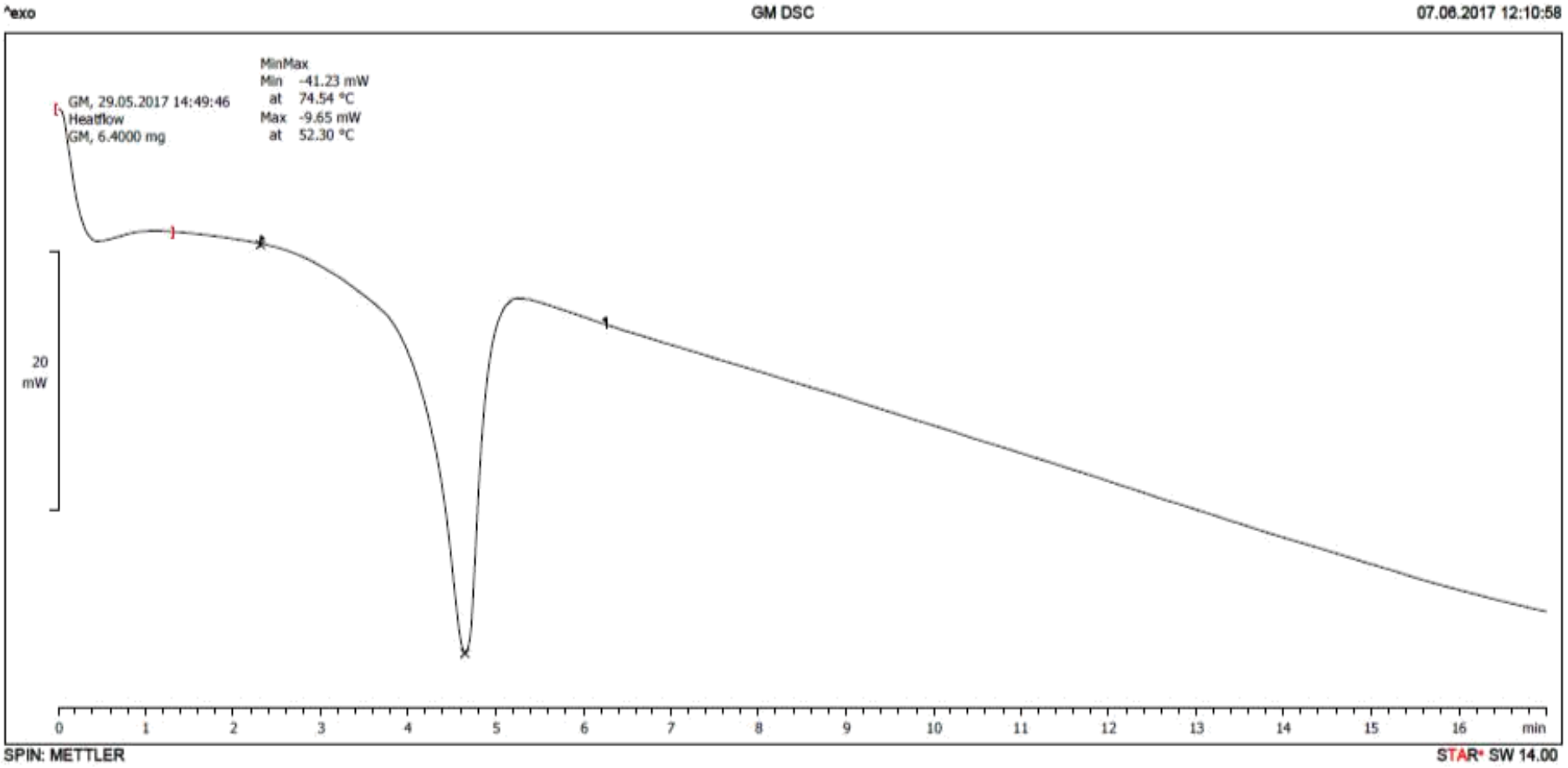
Table A.15: Flow

Properties of Stearic Acid Lipid Dispersions

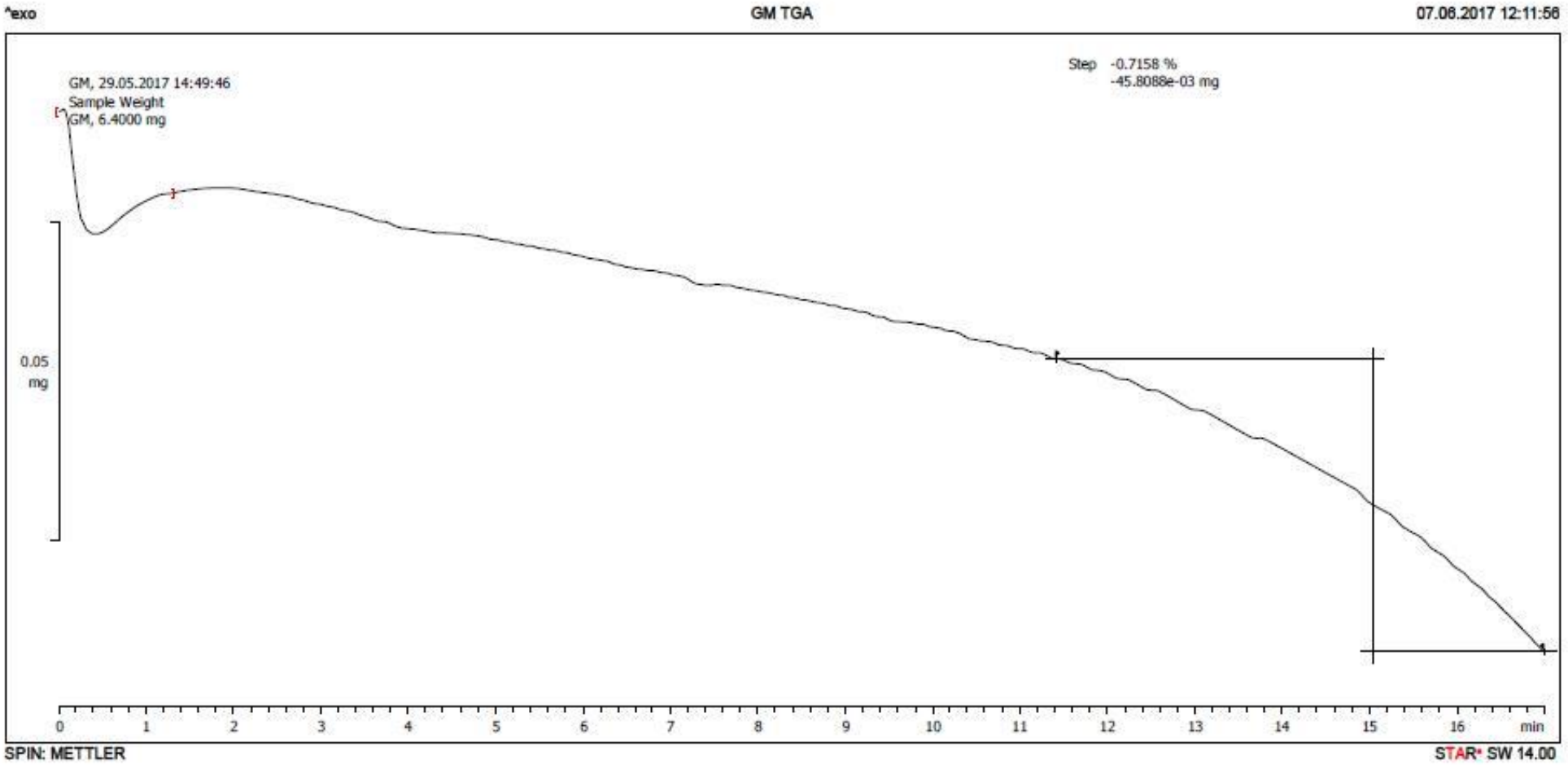
Flow properties	**(1:1)**	**(1:0,5)**
Flow rate (g/s)	61,728	13,263
	60,241	13,021
	62,500	13,021
average	61,490	13,102
stdev	1,148	0,140
%RSD	1,867%	1,066%
Bulk density (g/ml)	0,472	0,481
	0,472	0,490
	0,481	0,490
average	0,475	0,487
stdev	0,005	0,005
%RSD	1,094%	1,067%
Tapped density (ml)	0,543	0,610
	0,543	0,610
	0,568	0,610
average	0,551	0,610
stdev	0,014	0,000
%RSD	2.618%	0,000%
Hausner ratio	1,150	1,268
	1,150	1,245
	1,181	1,245
average	1,161	1,252
stdev	0,018	0,013
%RSD	1,515%	1,045%
% Compressibility	13,076%	21,109%
	13,076%	19,672%
	15,317%	19,672%
average	13,823%	20,151%
stdev	0,013	0,008
%RSD	9,362%	4,116%
Angle of repose	38,314	42,103
	37,998	42,151
	38,700	42,205
average	38,337	42,153
stdev	0,352	0,0510
%RSD	5,335%	8,497%

ANNEXURE B

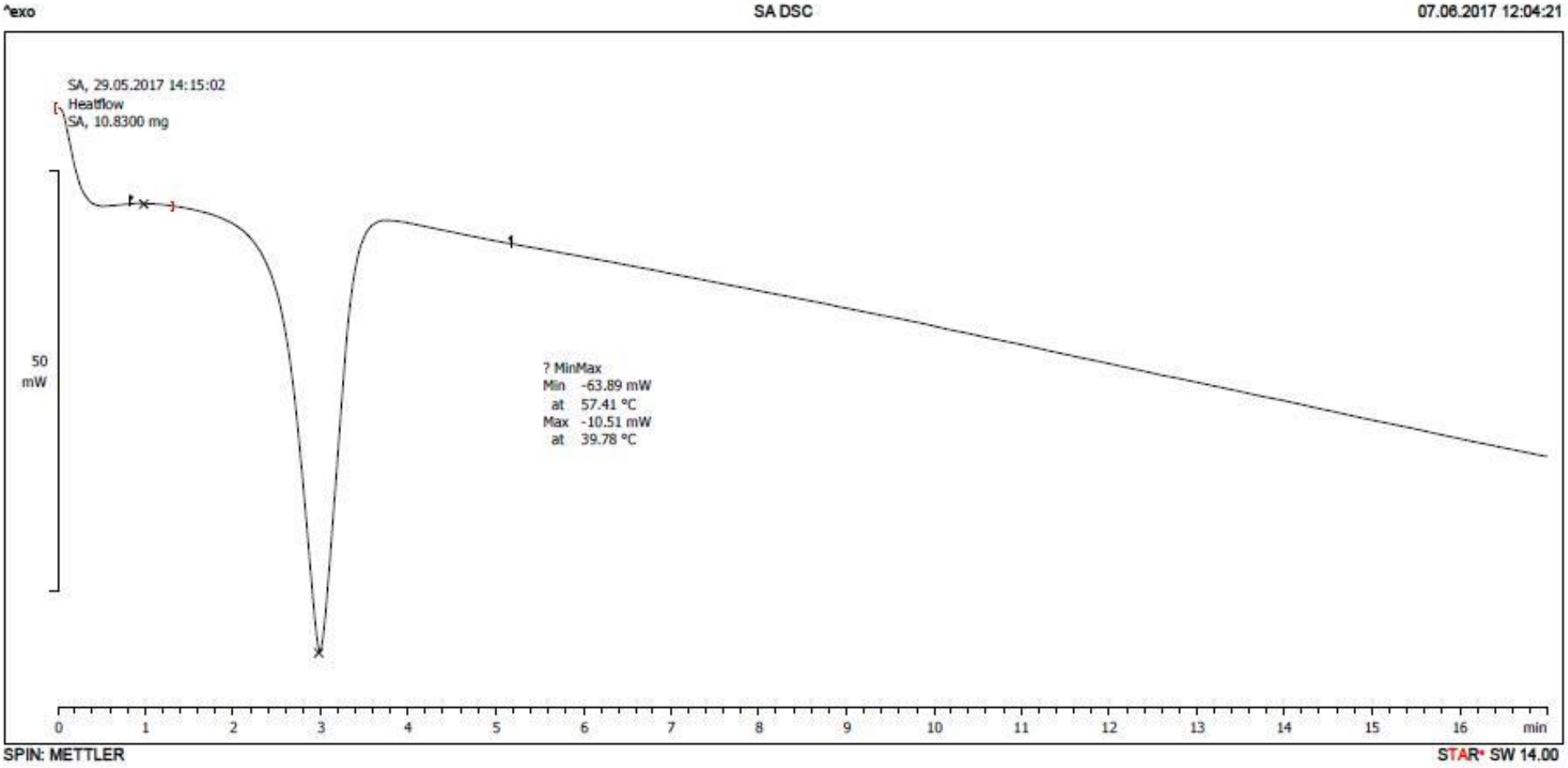
B.1: DSC curve of Glycerol Monostearate



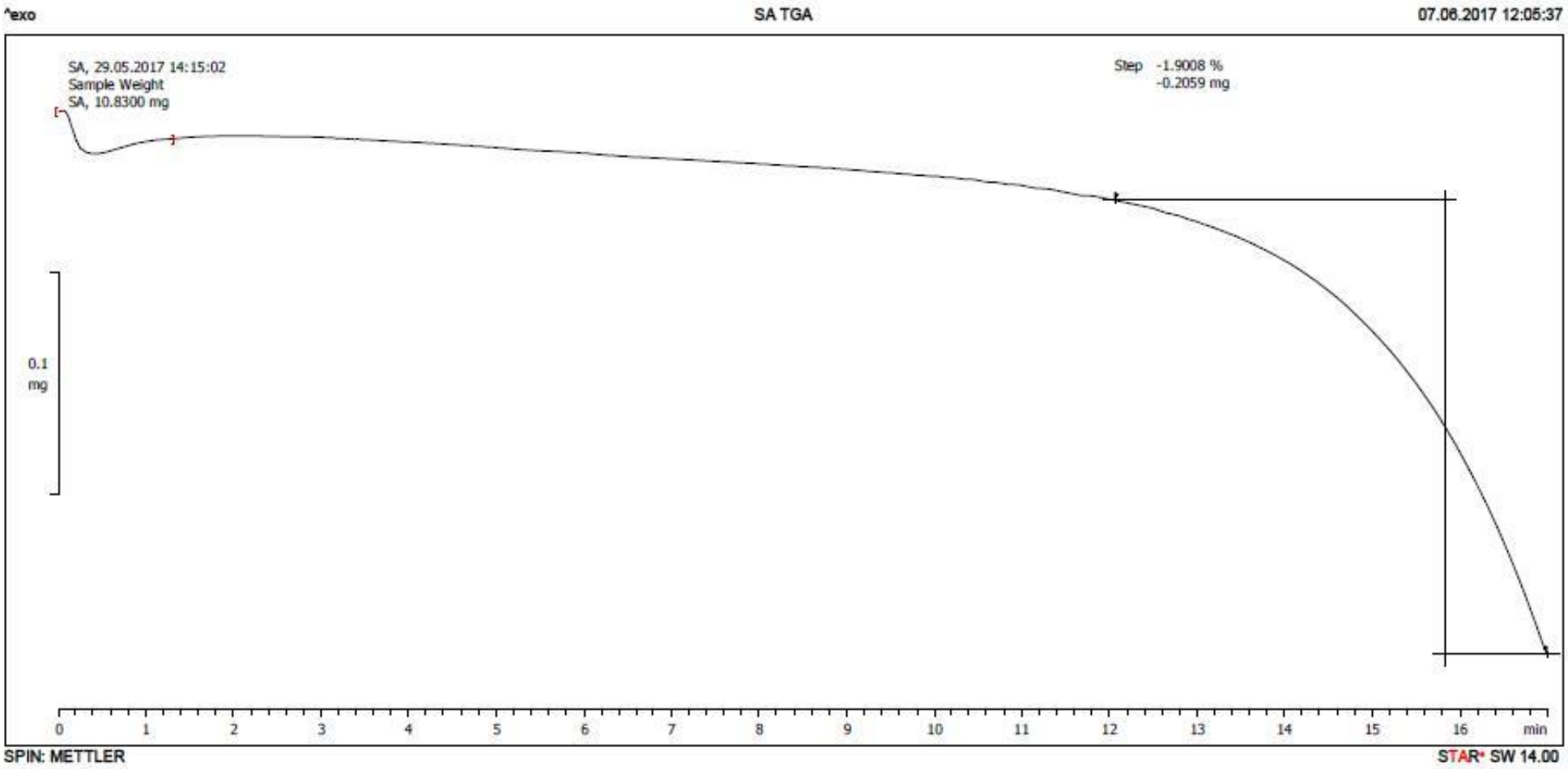
B.2: TGA curve of Glycerol Monostearate



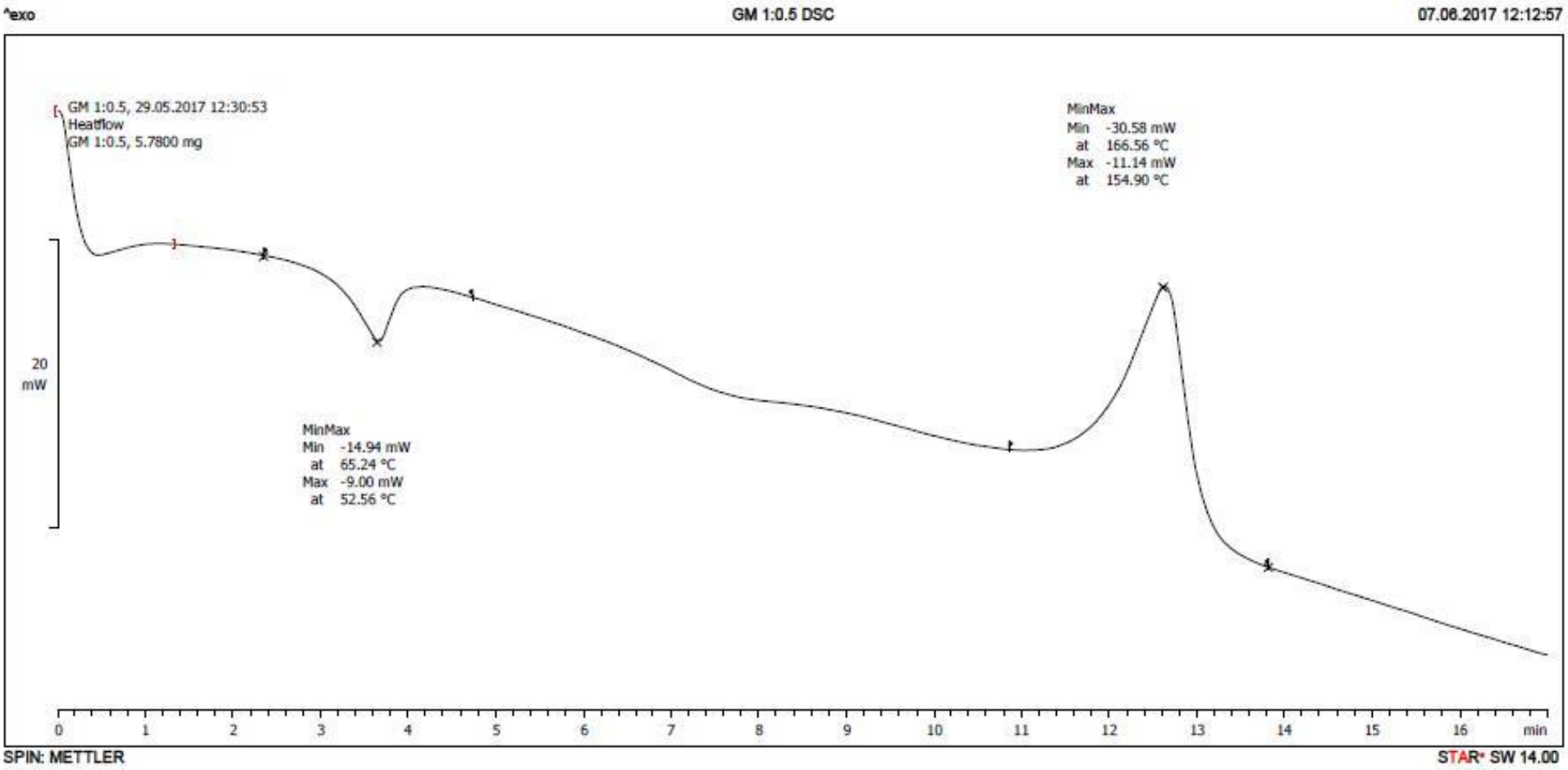
B.3: DSC curve of Stearic Acid



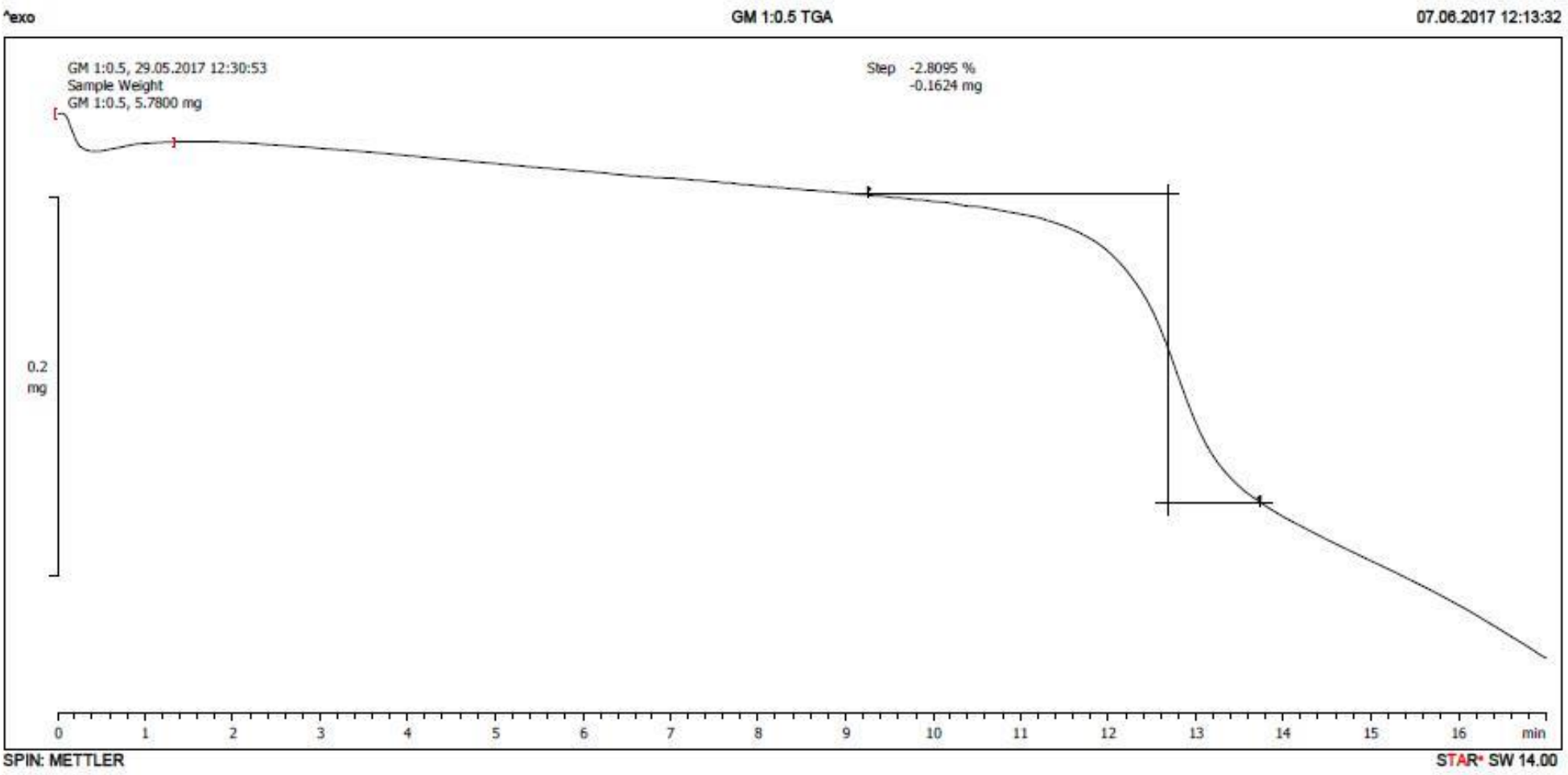
B.4: TGA curve of Stearic Acid



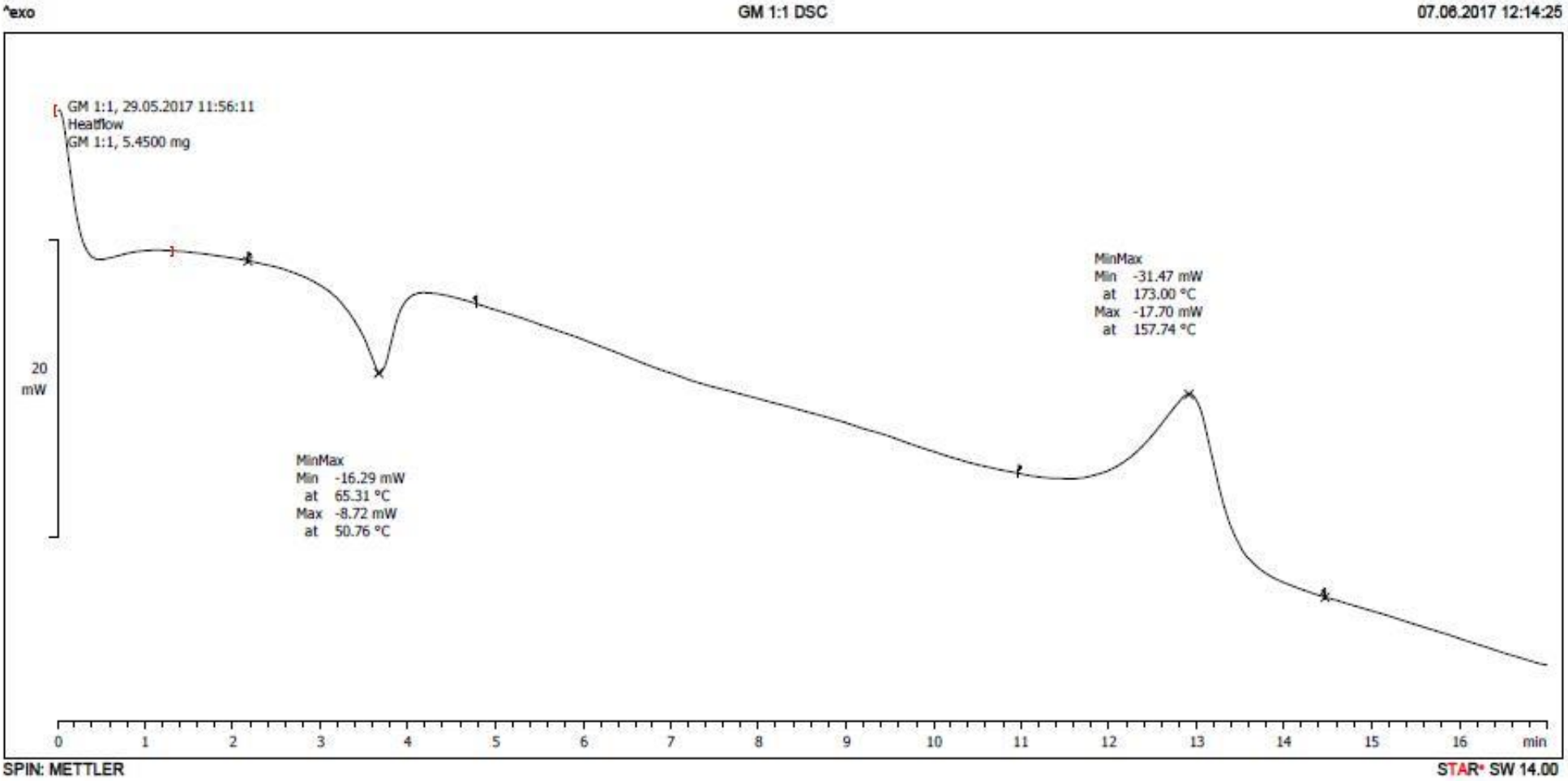
B.5: DSC curve of Glycerol Monostearate 1:0.5 Lipid Dispersion



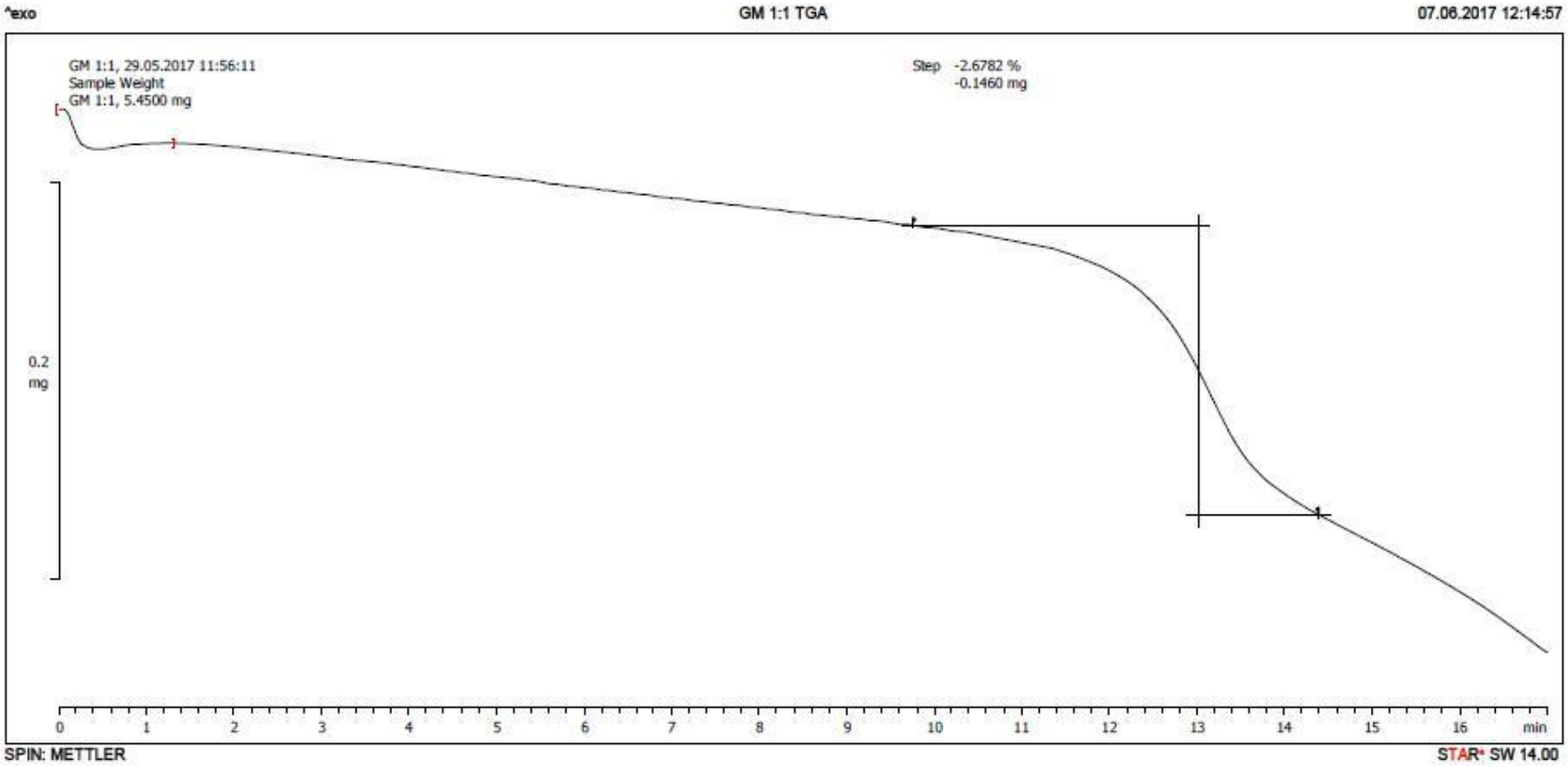
B.6: TGA curve of Glycerol Monostearate 1:0.5 Lipid Dispersion



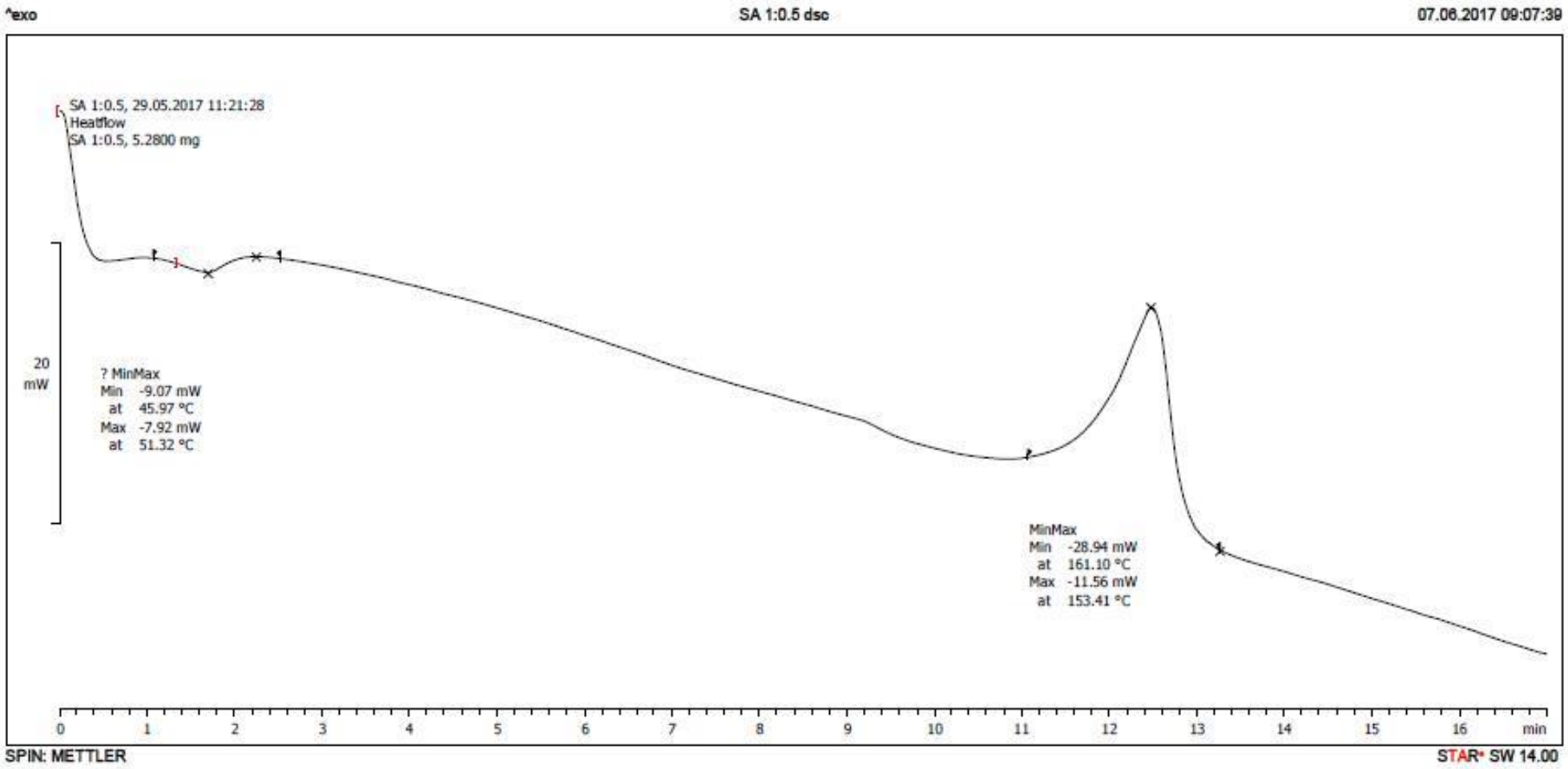
B.7: DSC curve of Glycerol Monostearate 1:1 Lipid Dispersion



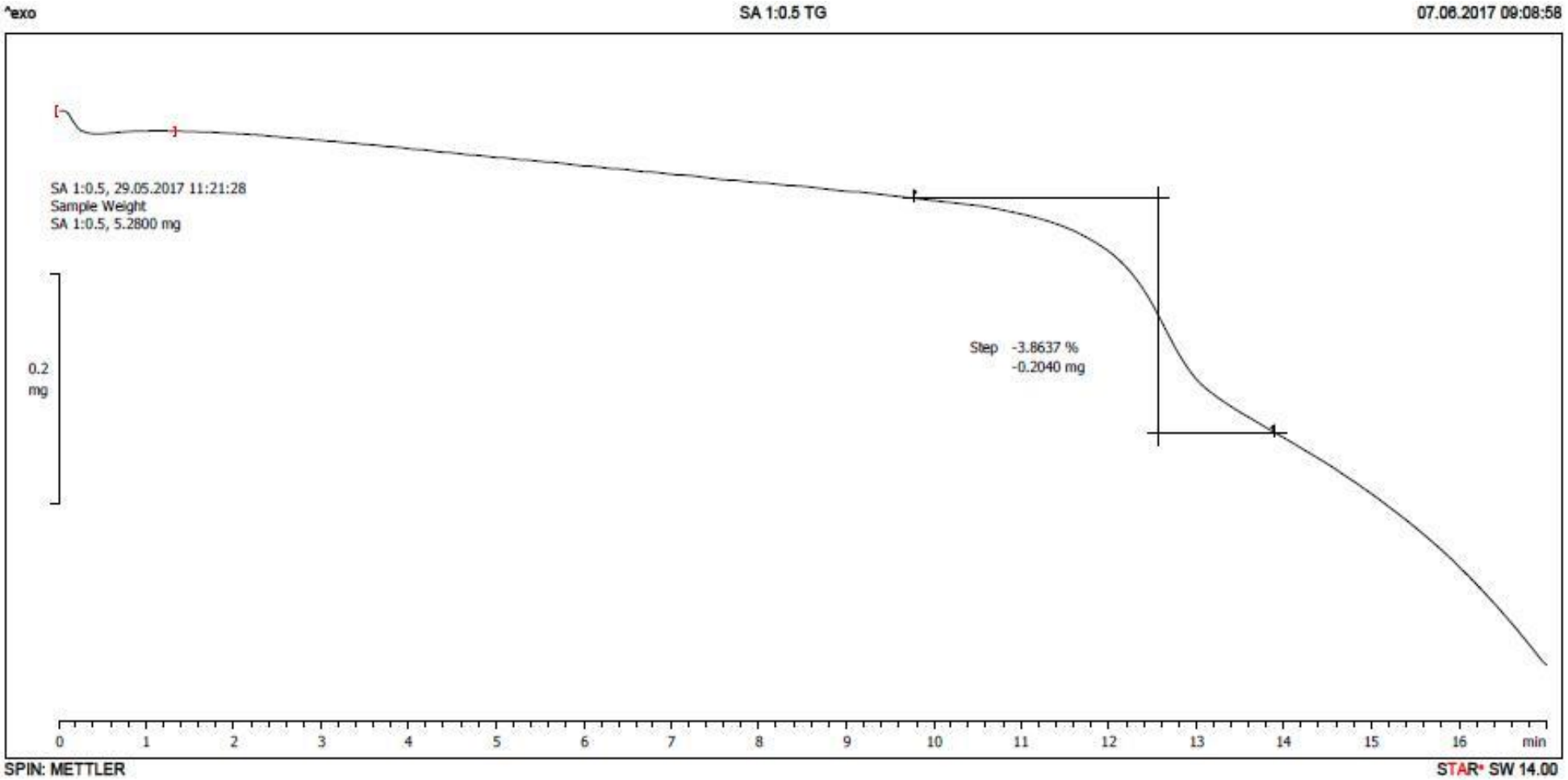
B.8: TGA curve of Glycerol Monostearate 1:1 Lipid Dispersion



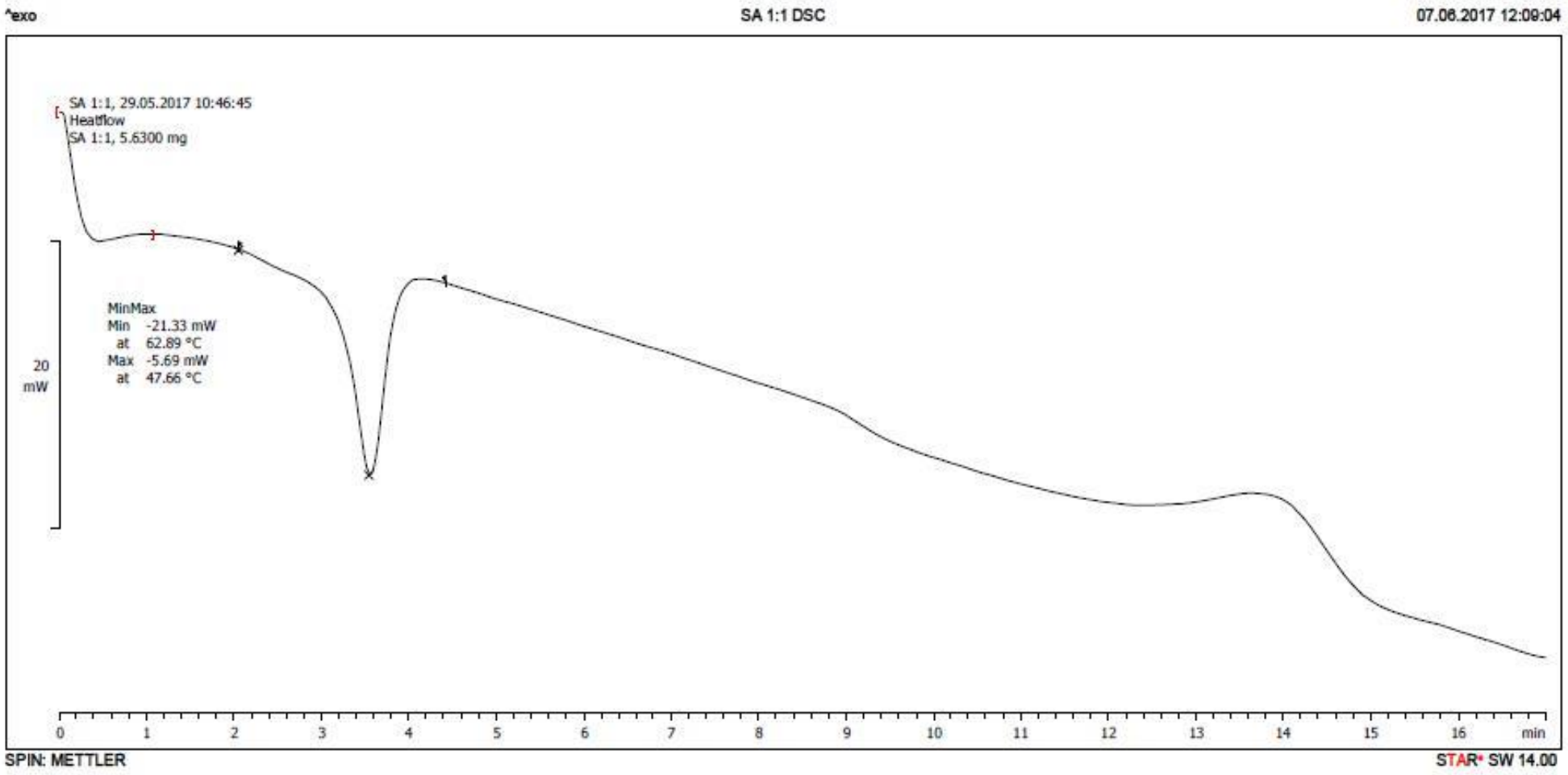
B.9: DSC curve of Stearic Acid 1:0.5 Lipid Dispersion



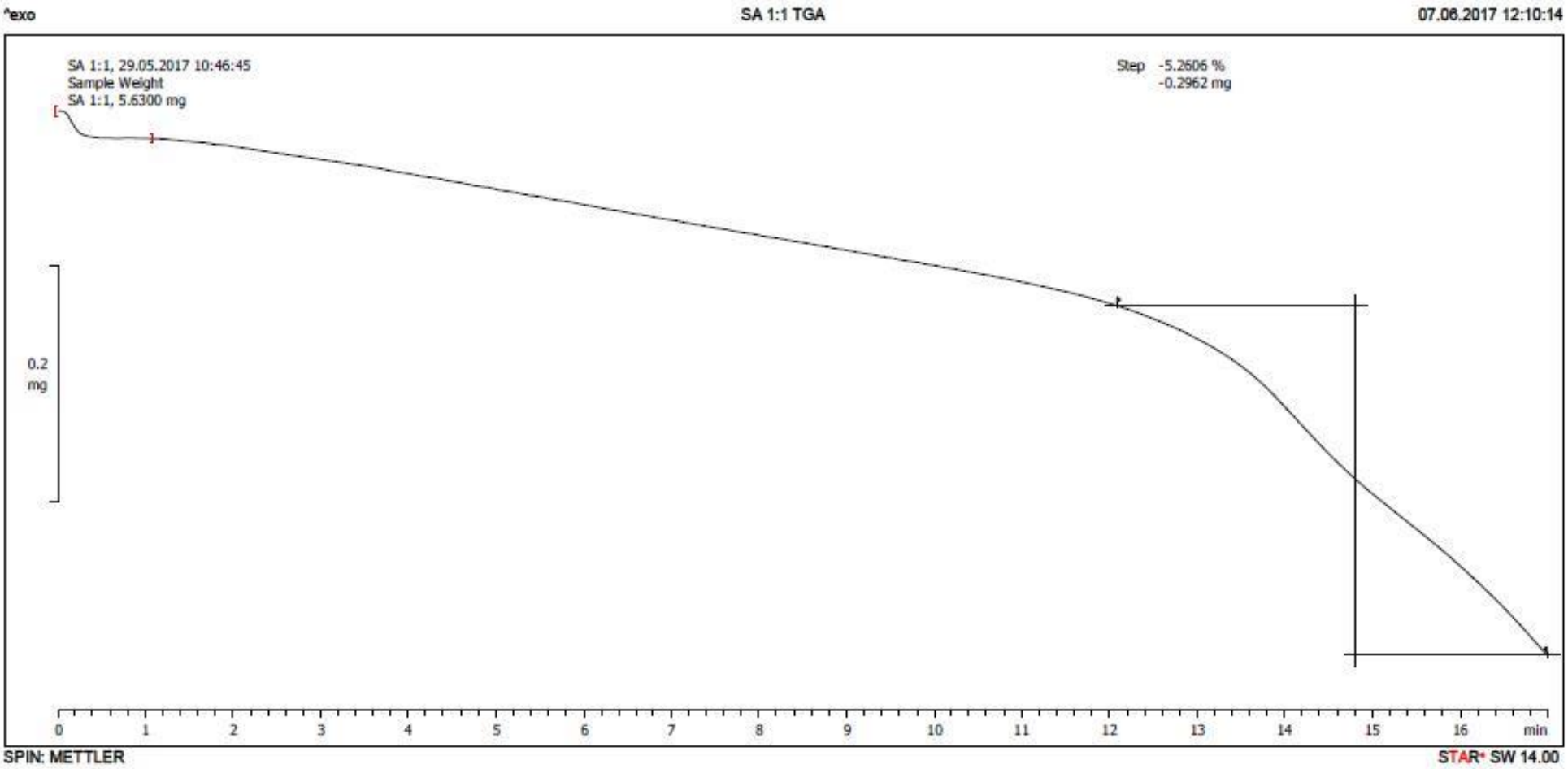
B.10: TGA curve of Stearic Acid 1:0.5 Lipid Dispersion



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



B.12: TGA curve of Stearic Acid 1:1 Lipid Dispersion



ANNEXURE C

Antwort: Consent to use SEM images

From:	<Margit.Bonnetsmueller@meggle.de>
To:	"Suzanne Hatting" <23392983@nwu.ac.za>
CC:	<christoph.adler@meggle.de>
Date:	Wednesday - September 13, 2017 12:13 PM
Subject:	Antwort: Consent to use SEM images
Attachments:	TEXT.htm; Part.002; Part.003; Mime.822

Hello Suzanne,

It's great to hear that our productswere used for your dissertation. You can certainly use the attached imagesfor it.

Good luck,

Margit Bonnetsmüller

Project Manager Marketing
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Von: "Suzanne Hatting"<23392983@nwu.ac.za>
An: <service.pharma@meggle.de>,
Datum: 13.09.2017 10:47
Betreff: Consent to useSEM images

Good day,

I am a Pharmaceutics Masters student at North-West Universityof Potchefstroom in South-Africa. I am writing a dissertation onthe *Development of lipid matrix tablets containing a double fixed-doseof artemisone and lumefantrine.* The fillers I used in the productionof my tablets were RetaLac, CombiLac and MicroceLac that was donated byMeggle.

I would like to request permission to used the attachedimages in my dissertation. Copyright on photos is currently a bigissue and I do not want to cause trouble.

Kind Regards

Suzanne Hattingh

ANNEXURE D

Table D.1:

Swelling and erosion for glycerol monostearate 1:0.5 formulations (*%RSD indicated in parenthesis).*

		G0.5R1		G0.5C1		G0.5M1	
		Tablet (mg)	**% Swelling**	**Tablet (mg)**	**% Swelling**	**Tablet (mg)**	**% Swelling**
Time (min)	**0**	**540.00** (0.017)	0.000	**493.333** (0.062)	0.000	**503.333** (0.050)	0.000
	1	**716.667** (0.043)	32.823	**573.333** (0.036)	16.342	**563.333** (0.020)	12.044
	2	**770.00** (0.039)	42.588	**580.000** (0.046)	17.740	**565.321** (0.021)	12.316
	5	**780.001** (0.017)	44.475	**585.112** (0.035)	18.604	*	*
	10	**790.000** (0.0.17)	46.361	**586.667** (0.035)	19.074	*	*
	15	**803.333** (0.007)	48.877	*	*	*	*
	20	**813.333** (0.019)	50.763	*	*	*	*
	30	*	*	*	*	*	*
	60	*	*	*	*	*	*
	90	*	*	*	*	*	*
	120	*	*	*	*	*	*
	150	*	*	*	*	*	*
Erosion (%)		**6.222** (0.396)		**5.289** (0.535)		**1.354** (0.732)	

**Dark background is an indication where no further swelling occurred*

Table D.2:

Swelling and erosion for glycerol monostearate 1:1 formulations *(%RSD indicated in parenthesis).*

		G1R1		G1C1	
		Tablet (mg)	**% Swelling**	**Tablet (mg)**	**% Swelling**
Time (min)	**0**	**543.100** (0.038)	0.000	**506.667** (0.030)	0.000
	1	**700.210** (0.049)	28.801	**570.000** (0.035)	12.516
	2	**717.001** (0.056)	31.845	**564.667** (0.034)	11.645**
	5	**747.000** (0.081)	37.294	**559.735** (0.037)	10.474**
	10	**756.667** (0.101)	39.079	*	*
	15	*	*	*	*
	20	*	*	*	*
	30	*	*	*	*
	60	*	*	*	*
	90	*	*	*	*
	120	*	*	*	*
	150	*	*	*	*
Erosion (%)		**6.693** (0.396)		**57.077** (1.673)	

**Dark background is an indication where no further swelling occurred **Indication of erosion*

Table D.3:

Swelling and erosion for stearic acid 1:0.5 formulations *(%RSD indicated in parenthesis).*

		S0.5R1		S0.5C1		S0.5M1	
		Tablet (mg)	**% Swelling**	**Tablet (mg)**	**% Swelling**	**Tablet (mg)**	**% Swelling**
Time (min)	**0**	**523.333** (0.011)	0.000	**536.678** (0.01)	0.000	**550.000** (0.182)	0.000
	1	**640.000** (0.027)	22.315	**613.334** (0.032)	14.283	**603.333** (0.069)	9.810
	2	**676.667** (0.023)	29.294	**614.521** (0.040)	14.505	*	*
	5	**733.333** (0.044)	40.167	*	*	*	*
	10	**760.00** (0.0.13)	45.234	*	*	*	*
	15	*	*	*	*	*	*
	20	*	*	*	*	*	*
	30	*	*	*	*	*	*
	60	*	*	*	*	*	*
	90	*	*	*	*	*	*
	120	*	*	*	*	*	*
	150	*	*	*	*	*	*

Erosion (%)	**13.377** (0.144)	**6.790** (1.502)	**9.810** (0.973)

**Dark background is an indication where no further swelling occurred*

***Indication of erosion*

Table D.4:

Swelling and erosion for stearic acid 1:1 formulations *(%RSD indicated in parenthesis).*

		S1R1		**S1C1**		**S1M1**	
		Tablet (mg)	**% Swelling**	**Tablet (mg)**	**% Swelling**	**Tablet (mg)**	**% Swelling**
Time (min)	**0**	**506.667** (0.011)	0.000	**490.00** (0.353)	0.000	**483.333** (0.032)	0.000
	1	**613.333** (0.047)	21.085	**620.000** (0.032)	26.723	**510.000** (0.020)	5.615
	2	**630.000** (0.073)	24.366	**623.344** (0.512)	27.433	**600.020** (0.246)	23.615
	5	**660.001** (0.030)	30.248	**615.364** (0.512)	25.585**	*	*
	10	**673.333** (0.0.17)	32.915	**513.479** (0.985)	4.792**	*	*
	15	*****	*	**473.215** (2.542)	-3.426**	*	*
	20	*	*	*	*	*	*
	30	*	*	*	*	*	*
	60	*	*	*	*	*	*
	90	*	*	*	*	*	*
	120	*	*	*	*	*	*

	150	*	*	*	*	*	*
Erosion (%)		**36.627** (0.396)		-		**16.247** (0.798)	

**Dark background is an indication where no further swelling occurred **Indication of erosion*

ANNEXURE E

Figure E.1:

Diffr

actogram of G5M1 formulation

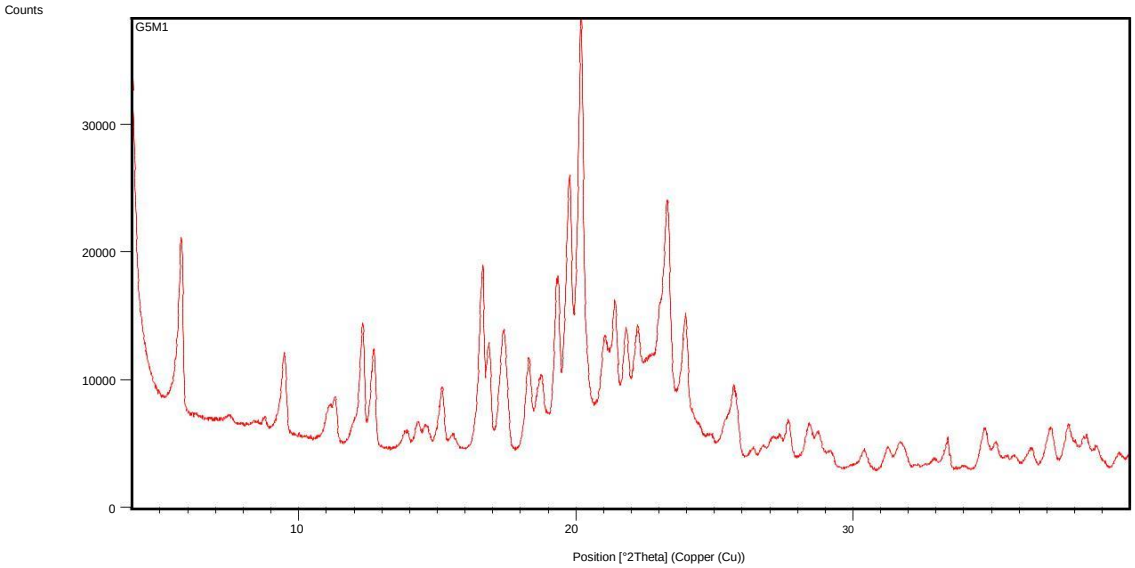

Figure E.2:

Diffr

actogram of G5C1 formulation

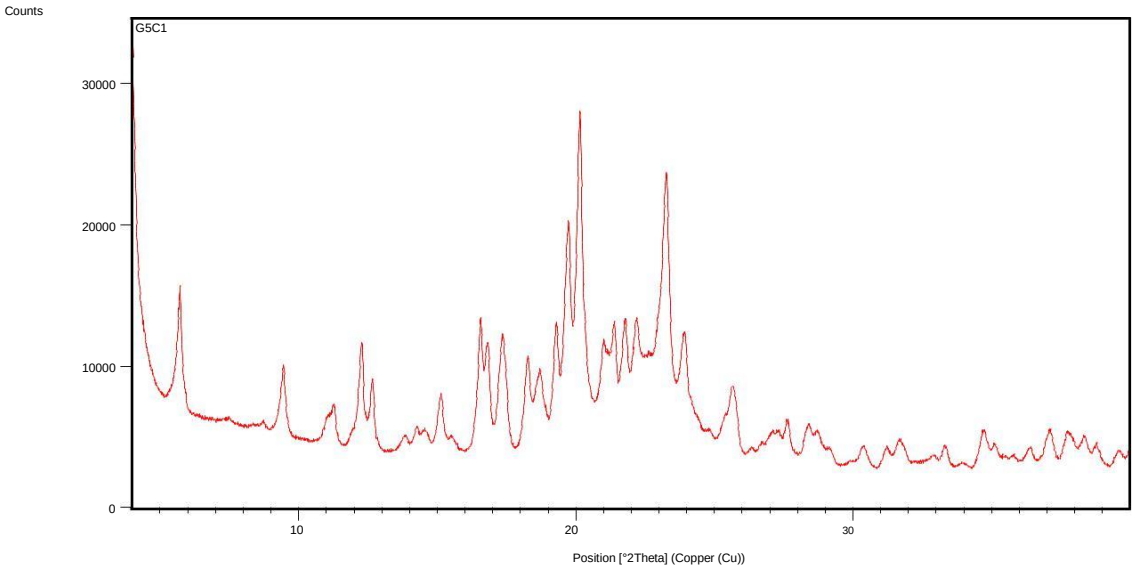

Figure E.3:

Diffr

actogram of G5R1 formulation

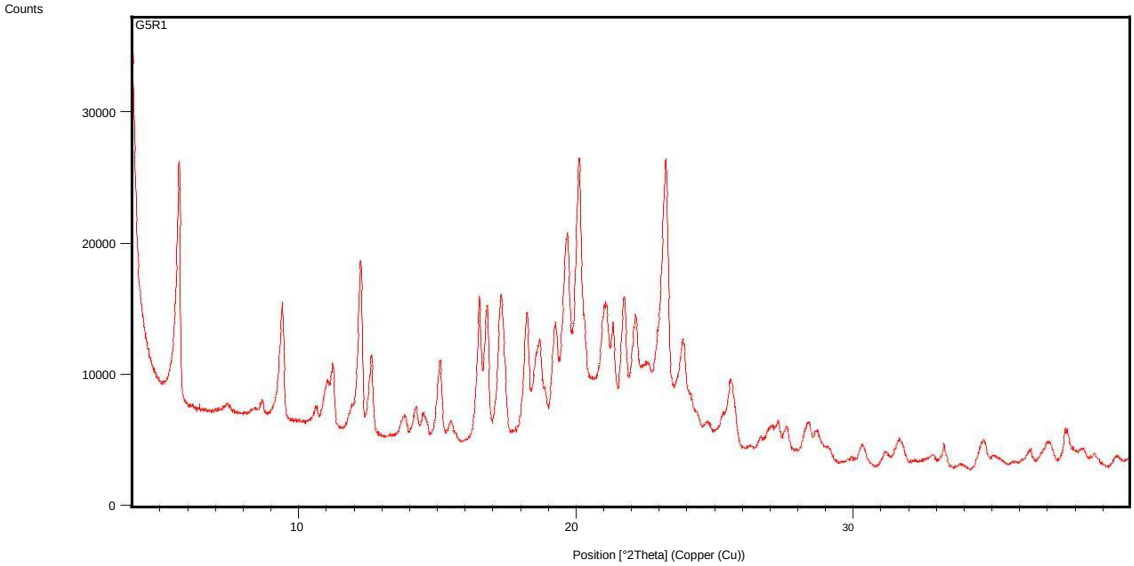

Figure E.4:

Diffr

actogram of G1M1 formulation

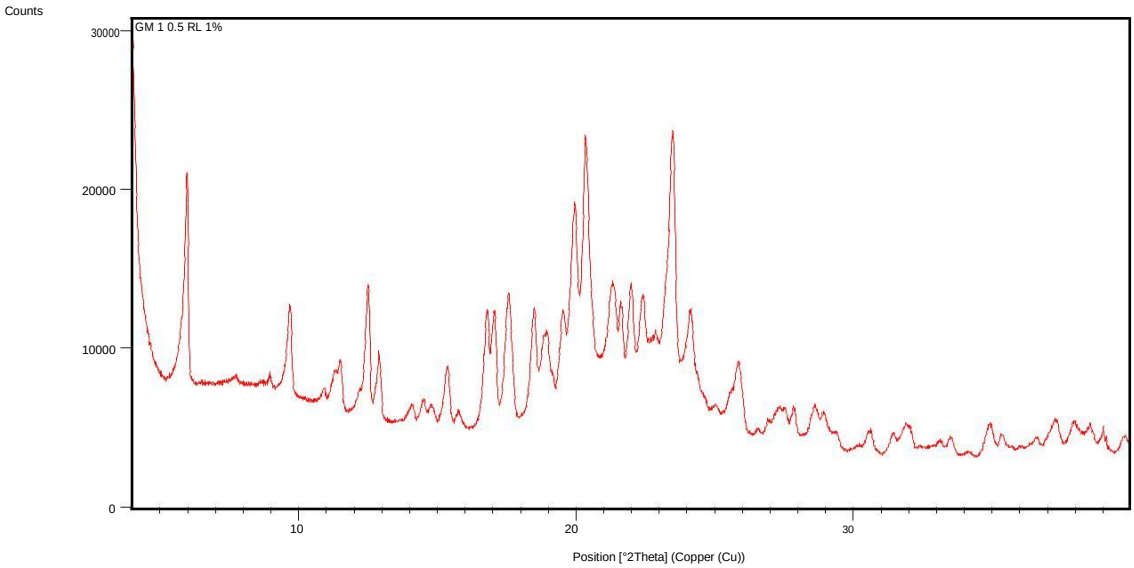

Figure E.5:

Diffr

actogram of G1C1 formulation

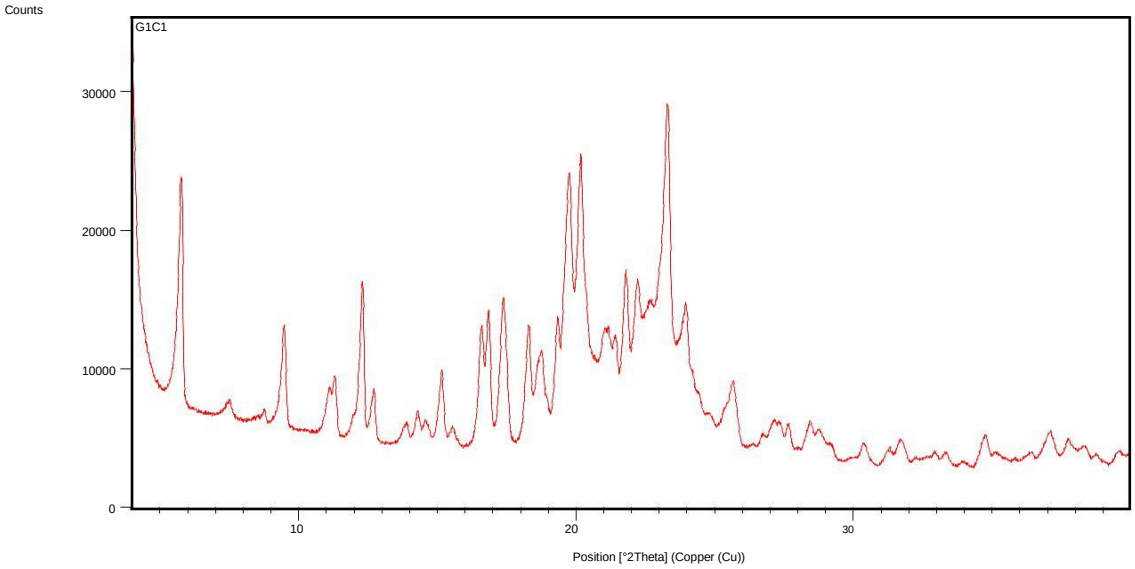

Figure E.6:

Diffr

actogram of G1R1 formulation

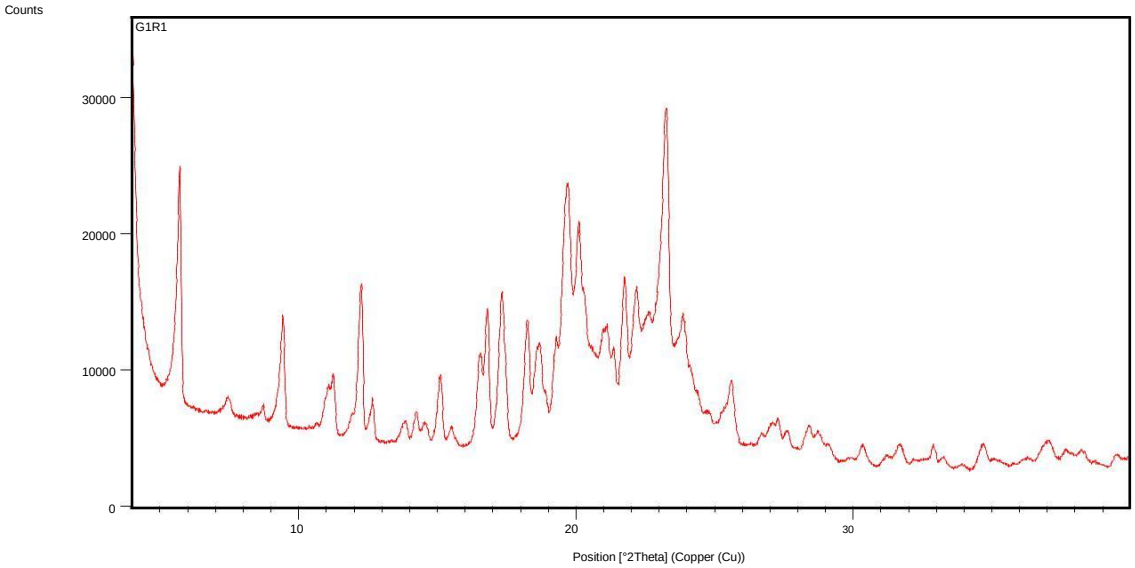

Figure E.7:

Diffr

actogram of S5M1 formulation

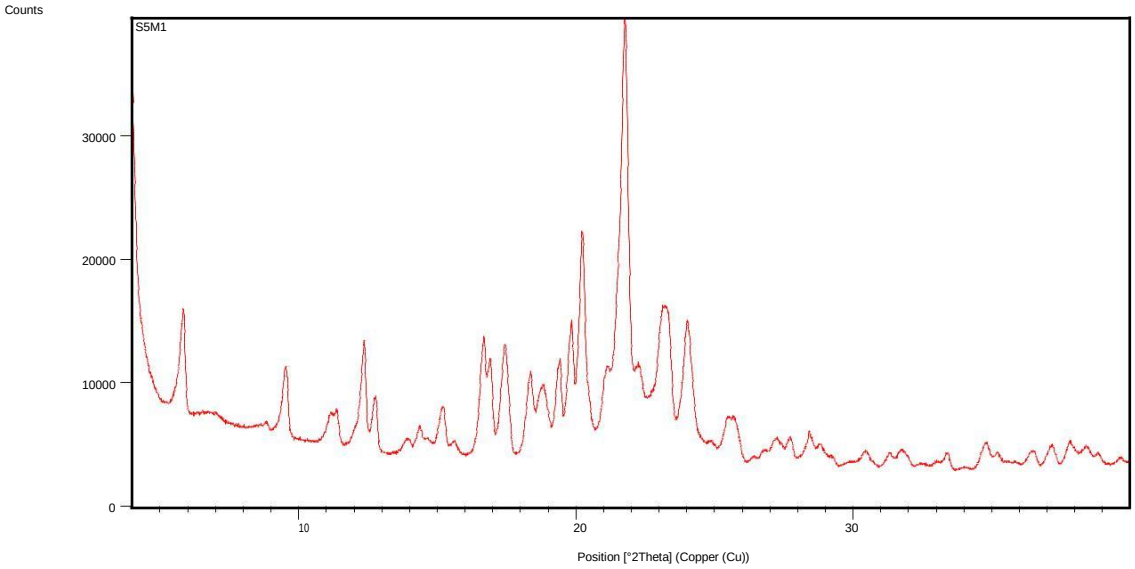

Figure E.8:

Diffr

actogram of S5C1 formulation

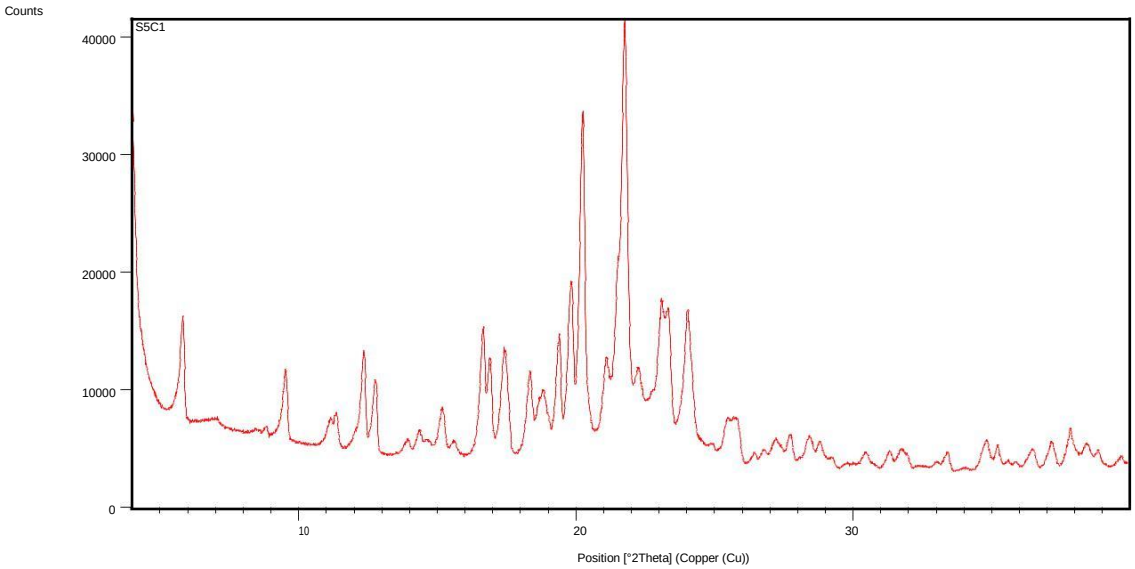

Figure E.9:

Diffr

actogram of S5R1 formulation

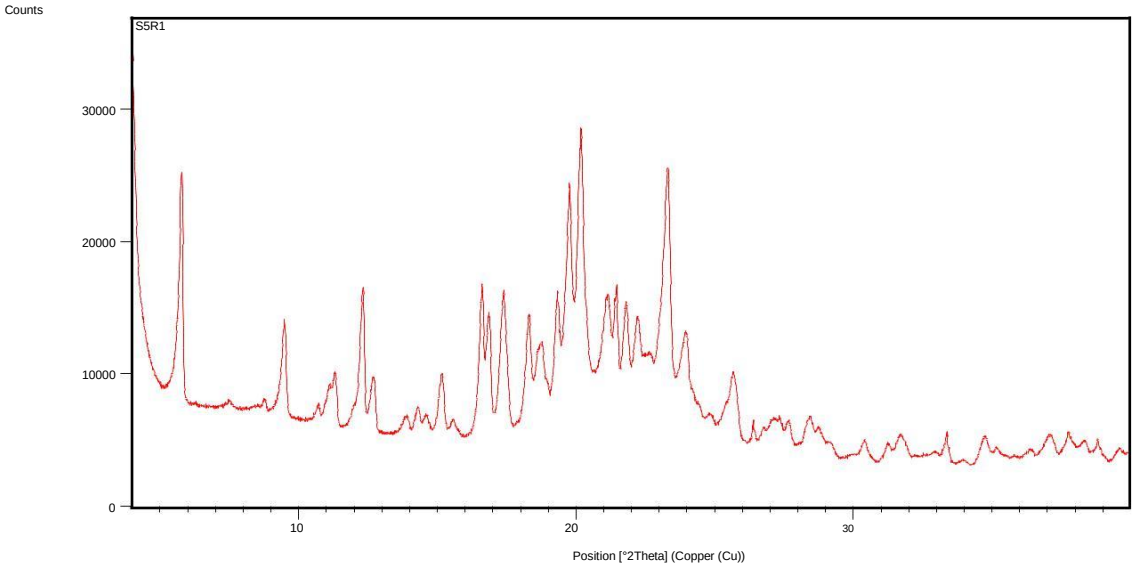

Figure E.10:

Diffr

actogram of S1M1 formulation

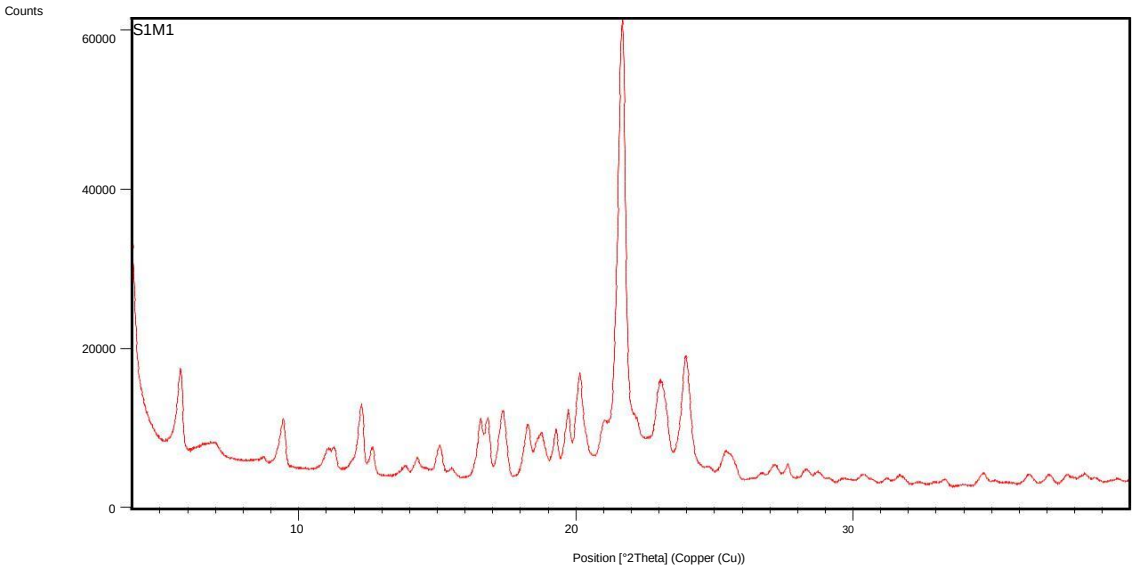

Figure E.11:

Diffr

actogram of S1C1 formulation

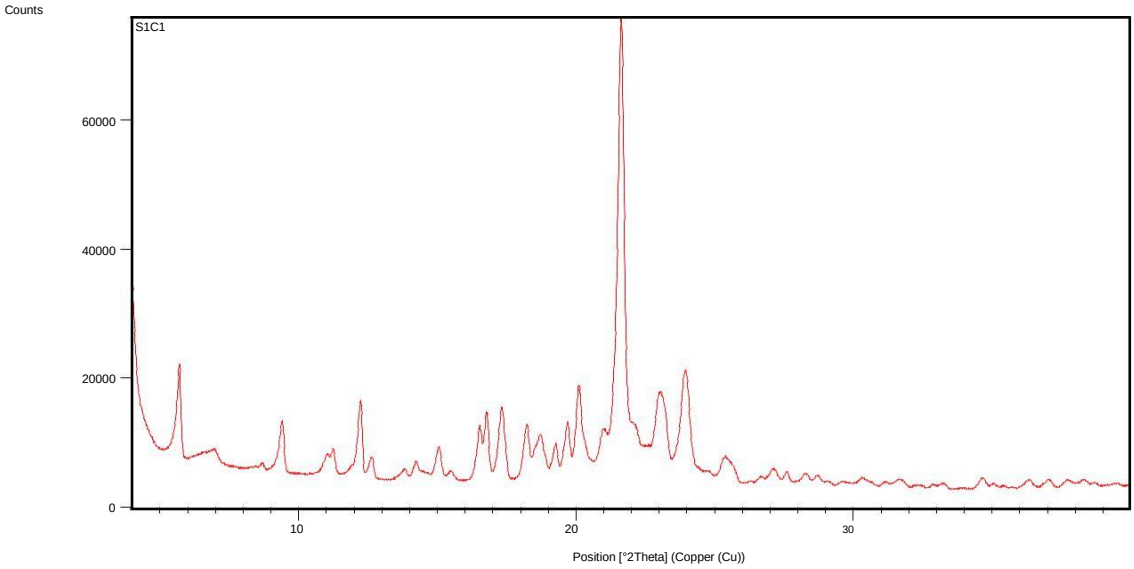

Figure E.12:

Diffr

actogram of S1R1 formulation