Development and evaluation of a self-emulsifying drug delivery system for artemether and lumefantrine

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

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Graduation: May 2019
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ACKNOWLEDGEMENTS

All my thanks and praise goes to my Heavenly Father, who has lead me on this path and gave me the ability and resources to complete my masters. May my masters reflect the gifts He has bestowed upon me and bring honour to Him.

They say that two people together can conquer the world, and with my two study leaders I felt like I could conquer my masters. **Dr Joe** thank you for being a pillar of strength during this whole process, you went above and beyond for me. Always ready to help and always there to listen to all my sorrows and sharing in all my joys. I am at a loss for words in how to display my gratitude for all you have done for me and mean to me. All I can say is thank you for everything, but most of all thank you for being such an awesome study leader for these past two years and allowing me the privilege to be one of your students. I admire you, not only as a study leader, but also as a person. I look up to you in all you have accomplished.

Prof Lissinda thank you for being such an integral part of my masters, thank you for all the time and effort you put into helping me. Always greeting me with a friendly face. Your dedication and passion for research reflects in your work and all the help you offered, which is something to admire. I feel honoured to have had you as one of my study leaders.

Prof. Jan Du Preez, I do not even know where to being. Prof my masters would not have been completed if not for you! Thank you for not giving up on my validations, even when I had given up a couple of times. Thank you for always smiling and always having a positive attitude. Whenever I walk into your offices feeling negative and thinking this is it, you always had a new plan and you were always positive that this next plan will work! Prof's positivity definitely rubbed off on me, giving me the motivation to push through the challenging times. I enjoyed spending time with prof in the lab and learned so much. I am honoured to have been able to tap into a small part of prof's incredible knowledge and to have learned so much from you! Thank you!

I would like to offer my thanks to **Prof Marique Aucamp,** you helped me with my TAM experiments and processing of the data. Thank you for spending the time to help me and to explain the experiments to me. I really learned a lot and have a greater understanding of compatibility studies.

Thank you to **Prof. Wessels** for helping me in the lab and helping me with processing my data. Thank you for your willingness to help, I really appreciated all of prof's help and the time prof took to explain certain concepts to me.

I would not be where I am today if not for the love and support from my family. You guys probably thought I was crazy when I said I wanted to do my masters in pharmaceutics. Since I kicked up such

a fuss about who would ever want to do their masters, let alone in pharmaceutics. Yet, when I came to you guys and said that my plan was to do my master in pharmaceutics you supported me all the way. **Dad** thank you for your endless support and interest in my work, thank you for the endless cups of coffee that you made for me while working. **Mom**, thank you for always listening to the ups and the downs and showing an interest in my masters. Thank you for proof reading my masters for me! **Marki,** thank you for proof reading my masters, even though you still do not know the title, for always being there when I freaked out about my masters, and all the cups of tea to calm me down!

To all my friends that accompanied me on this journey I would like to thank you for everything. **Danielle**, **Jessica**, **Alandi** and **Christi** thank you for all the support you offered and for understanding what I was going through, it was reassuring to know that I could lean on you guys when times were challenging. **Lee**, thank you for all the coffee visits, teasing, pranks and all the words of encouragement, you made these two years fun! Thank you for being there through it all, the tears and the joys!

“I can do everything through Christ, who strengthens me”

Philippians 4:13

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LIST OF ABBREVIATIONS

BSC:	Biopharmaceutical Classification System
CYP:	Cytochrome
h	Hours
HPLC:	High Performance Liquid Chromatography
ICH:	International Conference of Harmonisation
IP:	International Pharmacopoeia
LFCS:	Lipid Formulation Classification System
MSC	Model selection criteria
MDT:	Mean Dissolution Time
Min	Minutes
RSD:	Relative Standard Deviation
SD:	Standard Deviation
SLS	Sodium lauryl sulphate
TAM:	Thermal Activity Monitor
THF:	Tetrahydrofuran
SEDDS:	Self-Emulsifying Drug Delivery System
SMEDDS:	Self-Microemulsifying Drug Delivery System
SNEDDS:	Self-Nanoemulsifying Drug Delivery System
WHO:	World Health Organisation

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ABSTRACT

Malaria is a grave concern globally, however, in sub-Saharan Africa it remains an even more severe problem due to the fact that more than 90% of all malaria cases caused by *Plasmodium falciparum* reside in this region. The World Health Organisation (WHO) set an international goal to eliminate malaria by 2018, however, even though a steady decline in the amount of deaths was noticed, the number of reported cases still increase at an alarming rate. The fight against malaria is disadvantaged by the limit in drug availability, nonetheless, this is not the only concern. Resistance against malaria treatment by the parasite is slowly becoming a more serious issue compared to drug availability. The WHO recommended Coartem®, a fixed-dose combination of artemether and lumefantrine, as first line treatment. However, there have been cases reported of treatment failure which is possibly due to sub-optimal lumefantrine levels available in the systemic circulation, indicating that attention needs to be focussed on attempting to rectify increase the bioavailability of Coartem®.

Artemether and lumefantrine are both classified as poorly aqueous soluble drugs and lumefantrine was found more effective when provided with a highly fatty meal. For this reason, formulating highly lipophilic antimalarial drugs into lipid dosage forms has become a topic of interest as it is postulated that the additional lipophilic delivery system properties may assist in enhancing drug absorption even more. One such formulation being investigated is self-emulsifying drug delivery systems (SEDDSs), which have proven to be physically stable emulsions that are able to be distributed in the gastrointestinal tract. The digestive motility of the stomach and small intestine initiates the self-emulsifying mechanisms, which in turn solubilise the drug(s) incorporated; and this will consequently have a positive effect on the bioavailability of the incorporated drug(s) due to improved absorption.

The main objective of this study is to investigate the effect that the fixed-dose combination of artemether and lumefantrine has on the stability of SEDDS formulations as well as to establish the extent to which artemether and lumefantrine are released from this particular dosage form. The effect of the use of natural oils (avocado-, castor-, coconut-, olive-, and peanut oil) in combination with a surfactant (Sodium lauryl sulphate (SLS) and Tween® 80) and co-surfactant (Span® 60 and Span® 80) was also investigated. Solubility of both artemether and lumefantrine was tested in the selected oils, after which pseudo-ternary phase diagrams were constructed to identify the most optimum ratio of oil to surfactant and co-surfactant in order to produce the most ideal SEDDS formulations. Subsequently, certain SEDDS formulations were chosen due to their emulsion range characteristics and these formulations were tested to determine the physical stability of each of the selected SEDDS formulations together with the incorporated fixed-dose combination of artemether and lumefantrine. Following, dissolution experiments were conducted to conclude the rate and extent of release of the artemether and lumefantrine from the selected SEDDS formulations.

In this study it was identified that the oils improved the solubility of both artemether and lumefantrine exponentially when compared to their individual solubility in water. The combination with the selected oils chosen, the surfactant Tween® 80 in conjunction with the co-surfactant Span® 80 produced the most stable SEDDS formulations. Moreover, the surfactant and co-surfactant combinations that contained SLS either formed no emulsion area, or formed a very small emulsion area that could not be used for further studies. Consequently, the SEDDS formulations that were considered optimal are: avocado oil (4:6) (4 being the surfactant and 6 being the co-surfactant used), castor oil (2:8) S80, castor oil (3:7) S60, coconut oil (6:4), olive oil (3:7), and peanut oil (6:4). Furthermore, these selected SEDDS formulations were subjected to physical stability testing and all of these formulations displayed adequate stability. Droplet size measurements of the selected SEDDS formulations indicated that avocado oil (4:6), castor oil (2:8) S80, castor oil (3:7) S60, coconut oil (6:4), and olive oil (3:7) could be deemed as being in the nano-range, whereas peanut oil (6:4) portrayed an average droplet size that classifies it as being in the micro-range.

Both artemether and lumefantrine were satisfactorily released from the SEDDS formulations; though release was only observed when the pH of the dissolution media was increased. The release of artemether was noted when the pH was increased to 6.8 and lumefantrine was released only when the pH was increased to 7.4. Artemether displayed a superior release from the SEDDS formulations compared to lumefantrine that displayed only moderate release. Both active ingredients displayed Fickian diffusion when released from the SEDDS formulations, as all of their drug release profiles could be fitted to the Peppas Sahlin 2 equation.

Due to the abovementioned results obtained, it could be concluded that the SEDDS formulations: avocado oil (4:6), castor oil (2:8) S80, castor oil (3:7) S60, coconut oil (6:4), olive oil (3:7), and peanut oil (6:4), which comprised the surfactant Tween® 80and the co-surfactant Span 80®, produced physically stable SEDDS formulations that displayed adequate release of both artemether and lumefantrine. Considering the physical stability and the SEDDS formulations that displayed superior release of both artemether and lumefantrine, the avocado oil (4:6) and olive oil (3:7) SEDDS are regarded as being the most optimal SEDDS formulations for the fixed-dose combination of artemether and lumefantrine. However, these dosage forms will need to be investigated further in order to determine the bioavailability of both artemether and lumefantrine from these drug delivery systems.

KEYWORDS: Artemether; Lumefantrine; Malaria; Lipid-based dosage forms; SEDDS

CHAPTER 1

INTRODUCTION, AIM AND OBJECTIVES

1.1 INTRODUCTION

1.1.1 Malaria, a life-threatening mosquito borne blood disease

Malaria continues to be a devastating widespread infectious disease, therefore, placing immense pressure on world health (Benelli *et al.*, 2017; Cohen *et al*., 2012; Feng *et al.*, 2015; Mehlhorn, 2008; Sherrard-Smith *et al*., 2017). Daniel Vasella, the chief executive of Novartis stated in 2006 that: “The fight against malaria is a complex one. Availability of the drug is only one element” (Spar & Delacey, 2006). Currently, drug availability is not the only concern anymore; resistance towards malaria treatment by the parasite is now additionally of global concern (WHO, 2017). The mosquito, depicted in Figure 1.1, has been classified as one of the world’s most deadly creatures, doing more harm to humans than most animals (NDoH, 2017).

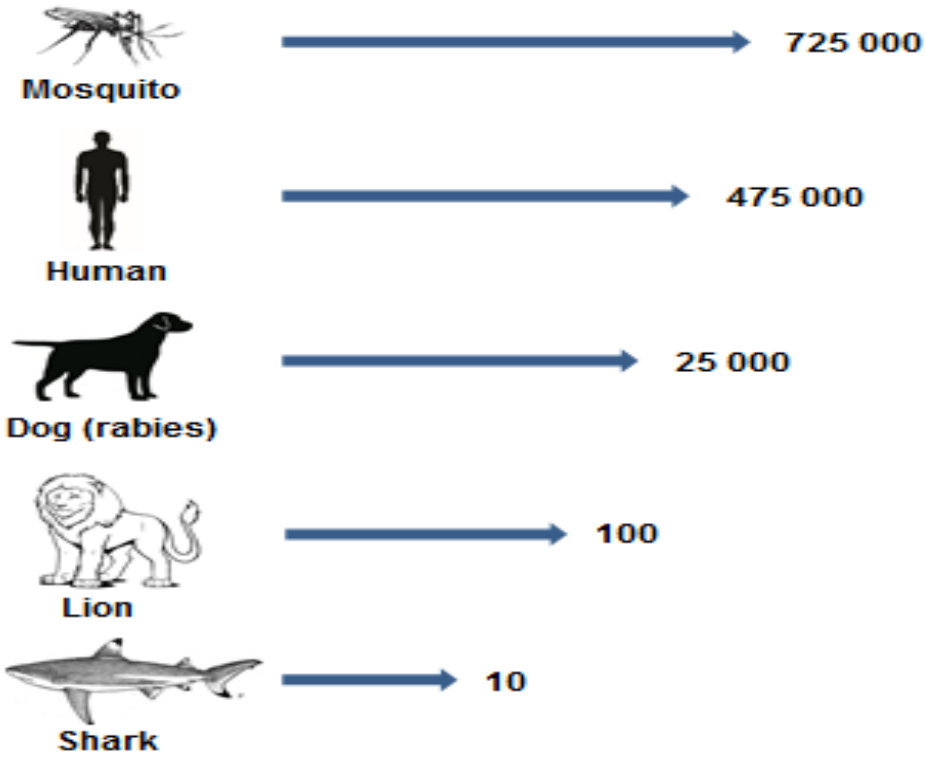

Figure 1.1: *Number of people killed by animals per year (NDoH, 2017)*

Most recent statistics indicate that nearly half of the world's population is at risk of contracting malaria (WHO, 2017). The World Health Organisation (WHO) stated, in their latest malaria report, that the incidence of malaria worldwide has decreased (WHO, 2017). In 2010 there were 237 million cases of malaria reported and in 2016 a clear downward trend was identified, as only 216 million cases were reported (WHO, 2017). However, since 2016 a major setback in malaria control has been experienced by a few countries; South Africa in particular (NDoH, 2017). Table 1.1 portrays the latest statistics of malaria incidences for 2016 and 2017 in South Africa. From this table it can be predicted that the drive to eliminate malaria remains a challenging task, because the amount of newly reported cases has increased in the past year by an astronomical rate. Hence, it is important to remain vigilant and keep up to date with the latest treatment and prevention regimes (NDoH, 2017; NDoH, 2018). According to the WHO, Sub-Saharan Africa has a disproportionately high (90%) incidence of malaria; and approximately 92% of people die once they have contracted this disease (WHO, 2017). This is of an alarming concern for the inhabitants residing in Africa, many who reside in third world/ poverty conditions without access to basic healthcare and lack of access to high fatty meals, thus an answer needs to be found (Chotivanich *et al.,* 2012). South Africa alone has had a sudden increase in seasonal malaria (from September to the end of May); which is possibly due to the recent heavy rains, increase in ambient temperatures, and high humidity (NDoH, 2017; Wits Communications, 2017). Another concern is that people who are born in a malaria area build "semi-immunity" to this disease due to surviving a number of malaria infections which they have contracted, however, when they commute to a non-malaria area they lose their "immunity" within 3-6 months, rendering them just as vulnerable as the rest of the population to contracting malaria (NDoH, 2018). Similarly, when they travel from their hometown to their place of work, mosquitoes can travel with them (NDoH, 2017; NDoH, 2018). In the Western Waterberg district around Lephalale and Thabazimbi, at least 46 cases of malaria have been reported. Although these incidents were not classified as a malaria outbreak, they are particularly distressing as this is an area that has not had many cases of malaria in the past (Tandwa, 2017).

Table 1.1: Incidences of malaria in South Africa for the period of 2016-2017 (NDoH, 2017)

	2016	**2017**	**Percentage increased (%)**
Local cases	11	507	4509
Imported cases	246	945	284
Reported cases	227	438	93

Recently two patients from Doornpoort, a suburb in the northern part of Pretoria, as well as two patients from Swartruggens in the North-West Province contracted malaria, despite these patients not traveling to a malaria area; and more importantly, these parts are not classified as malaria areas. This particular malaria has been labelled 'Odyssean', 'mini-buses' or 'suitcase' malaria; indicating that the mosquito travelled in a vehicle or suitcase from a malaria area to these parts. Cases similar to those mentioned are becoming more and more disturbing, because malaria is not expected in these regions, and medical professionals do not link the symptoms to malaria, which in turn leads to fatal consequences (NDoH, 2017; NDoH, 2018; Wits Communications, 2017). The symptoms that patients experience when contracting malaria can be linked to various different illnesses, which renders the diagnosis of malaria challenging, particularly in areas where malaria is not expected. Patients who contract malaria initially display flu-like symptoms, such as fever, sore muscles, general weakness, and symptoms of acute respiratory tract infections (Bell & Perkins, 2012). This can potentially cause an increase in the mortality and/or morbidity rates of patients who are not correctly diagnosed with malaria in the early stages of the parasite's life cycle (Barnes, 2012; Bell & Perkins, 2012).

Malaria has caused immense concern in world health, causing havoc in endemic countries, many of which are third world countries, such as Sub-Saharan Africa, as mentioned before (WHO, 2017). Malaria remains a continuous distress, especially with patients still being infected with the disease on a daily basis even though preventative measures are implemented in an attempt to avert the spread of this disease (e.g. insecticide, bed nets, insect repellents, etc.). When considering the use of antimalarials and its effects against the transmission of the parasite, the following three components must be taken into consideration, namely:

- the effect of the drug on the early gametocytes and asexual stages,
- the sporontocidal effects which occur in the mosquito, and
- the effect the drug has on the mature infectious gametocytes (Barnes, 2012).

It remains advantageous for the patient to receive treatment in the early stages of malaria, as this will hopefully eliminate the parasite while it is still in its asexual stages. However, investigation into drugs that can eliminate the parasite in its sexual (gametocyte) stages should not be ignored as this plays an integral part in the treatment of malaria (Barnes, 2012). Research has shown that *Plasmodium falciparum* has, for example, grown resistant to artemisinin and its derivatives, when used as monotherapy (Balikagala *et al.*, 2017). This pushed the WHO to consider alternative dosage regimes that could effectively combat this worldwide endemic disease (WHO, 2015). Novel chemical entities/drugs/compounds are being developed, but due to a lack of funding and the time it takes to develop, new treatments

have not yet reached the market. Researchers are now also investigating new methods to improve existing drugs as well as their dosage forms (Feng *et al.*, 2015). Combination therapy, for example, has become a field of interest and is widely used. This type of therapy combines for instance an artemisinin derivative, which is a short acting antimalarial, with a long acting antimalarial (e.g. lumefantrine, which is used in this study). This combination therapy against malaria has a dualistic effect in eliminating the parasite from the blood, thereby minimalising resistance (WHO, 2015). The combination of artemether and lumefantrine has been classified by the WHO as first line treatment against uncomplicated *P. falciparum* malaria (WHO, 2017). A commercially available example of combination therapy against uncomplicated malaria is a product called Coartem®, which consists of a fixed-dose combination of artemether and lumefantrine. The manufacturers developed both a conventional tablet formulation, as well as a dispersible tablet formulation containing this fixed-dose combination of the aforementioned drugs that became the first-line of treatment for uncomplicated malaria (Abdulla & Sagara, 2009).

Furthermore, there has been an immense growth in the development of lipid-based formulations (e.g. by means of hot-melt extrusions, nanostructured lipid carriers, Pheroid™ technology, emulsions, self-emulsifying drug delivery systems, solid emulsions, etc.) to establish whether the dissolution as well as absorption of various antimalarial drugs can be improved (Bhandari *et al.*, 2017; du Plessis *et al*., 2015; Jain *et al.,* 2014; Joshi *et al.*, 2008b; Kate *et al.,* 2016). Another novel dosage form that is being investigated, is a sublingual spray of the antimalarial drug, artemether. This formulation is being considered in terms of the percentage of drug that has been absorbed into the circulatory system *via* the buccal and sublingual routes; and to study whether possible side effects may appear (Salman *et al.,* 2015). Nonetheless, this compound is highly lipophilic and transport through these routes are limited. Another clinical problem that has been observed upon administration of various antimalarial therapies is specifically: a fatty meal is required to improve the solubility and consequently the absorption of lipophilic drugs, particularly when artemether and lumefantrine are utilised as first-line therapy (Mwebaza *et al*., 2017).

1.1.2 Artemether

Artemether (Figure 1.2) is a β-methyl derivative of dihydroartemisinin (Shu-Hua, 2005). Cytochrome P450 3A4 (CYP3A4) and cytochrome P450 3A5 (CYP3A5) rapidly metabolise artemether to dihydroartemisinin, which is responsible for the antimalarial activity observed (Aderibigbe, 2017). Dihydroartemisinin, in turn, is classified as the principal bioactive metabolite and the activity of this drug is thought to be due to the endoperoxide bond (Shu-Hua, 2005). Artemether proves an effective antimalarial compound, however, it is more

lipophilic than other artemisinin compounds (Jelinek, 2013). Its mechanism of action is fast, and an effect is seen almost immediately; even though this compound is rapidly eliminated from the blood (WHO, 2015). Dihydroartemisinin is inactivated by glucuronidation and eliminated in the bile (Aderibigbe, 2017). Due to the rapid onset of action of artemether, the symptoms of malaria can swiftly be alleviated providing the patient with faster symptomatic relief (Prabhu *et al.*, 2016).

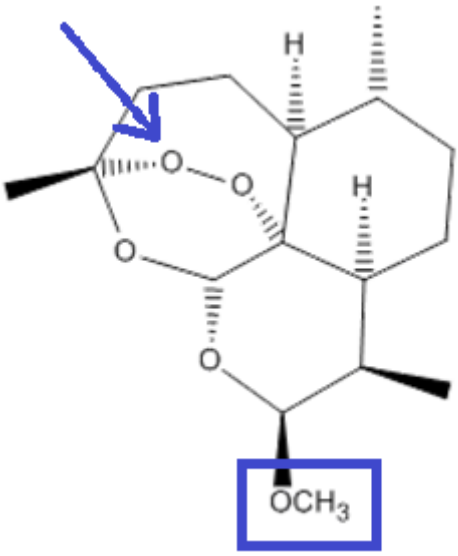

Figure 1.2: *Chemical structure of artemether. The arrow indicates the endoperoxide that is common in all artemisinin derivatives. The box indicates the C10 group that is unique to each artemisinin derivative and determines its water-solubility. In artemether the methyl ether causes the drug to be practically insoluble in aqueous environments (Karunajeewa, 2012)*

Artemether is furthermore classified as a class II drug according to the Biopharmaceutical Classification System (BCS), indicating that the compound has a low aqueous solubility and high permeability (Patil *et al.*, 2013). The high lipophilic character of artemether complicates the formulation of a suitable dosage form which can be effectively administered to eliminate the parasites from the blood. Presently artemether is being administered orally. However, as stated the compound has poor solubility, signifying that the drug is not dissolved appropriately, which in turn limits absorption and subsequently prevents optimal circulatory drug concentrations (Ansari *et al.*, 2014). Another route that is frequently used to administer this compound is the intramuscular route, though the injection is painful, the absorption is slow and also unpredictable (Patil *et al.*, 2013; Prabhu *et al.*, 2016).

1.1.3 Lumefantrine

Lumefantrine (Figure 1.3) is classified as a fluorene derivative belonging to the aryl amino-alcohol group of antimalarials and is not used as monotherapy in the treatment of malaria (WHO, 2015). This drug is not utilised as monotherapy due to its low intrinsic value (the ability of the drug-receptor combination to produce an effect) when compared to other antimalarials (Ezzet *et al.*, 2000). According to the BCS, lumefantrine can be categorised as a class IV drug, specifying that the drug has low aqueous solubility as well as low permeability (Patil *et al.*, 2013). It is a highly lipophilic compound and consequently drug absorption is improved when administered with fatty foods or dairy products (WHO, 2015). It has erratic behaviour in different individuals and the rate of absorption can vary due to its fat dependant absorption (Borrmann *et al.,* 2010; Mwebaza *et al*., 2017; WHO, 2015).

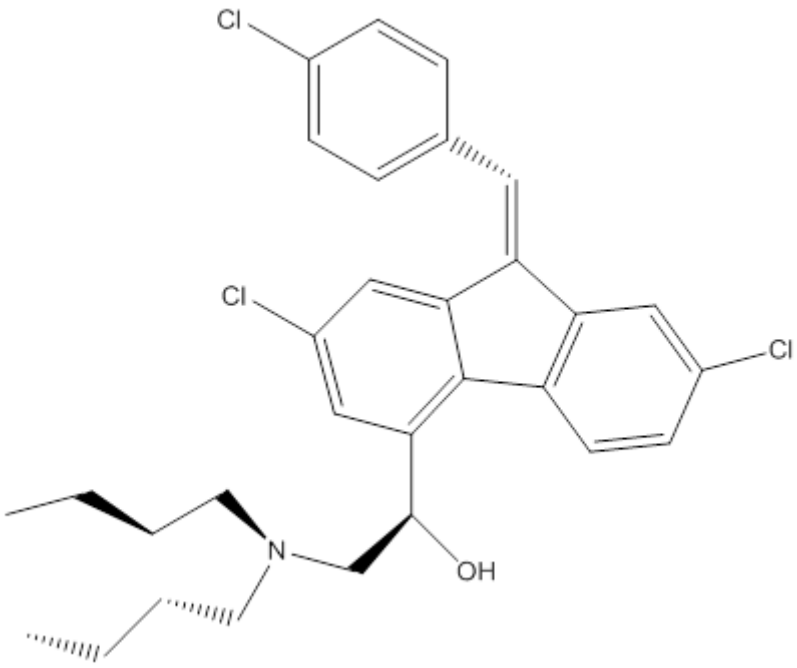

Figure 1.3: *Chemical structure of lumefantrine*

1.1.4 Fixed Dosing with Artemether-Lumefantrine

The WHO declared the artemether-lumefantrine combination as a strongly recommended treatment option for uncomplicated malaria (WHO, 2015). Consequently, the combination of artemether-lumefantrine is marketed as Coartem® in a ratio of 20 mg : 120 mg by Novartis (Spar & Delacey, 2006). The rationale behind this combination therapy is that the artemisinin derivative (in this study artemether) rapidly removes the parasite from the blood, thus, reducing the parasite load in the blood by an exponential factor. Artemether also has an effect on the sexual stages of the parasite; the parasite cannot be transferred to the mosquito

therefore, preventing the spread of malarial parasites (WHO, 2015). As mentioned, the artemisinin derivative has a short half-life and the combination drug (in this study lumefantrine) has a longer half-life. Lumefantrine eliminates any parasites that remain; this in turn protects artemether from becoming ineffective towards the malarial parasites. The longer half-life of lumefantrine also provides a post-treatment prophylaxis (WHO, 2015).

Recent treatment failures started to become more evident in some patients who were using Coartem®. It has been speculated that these treatment failures are due to sub-optimal lumefantrine concentrations (Färnert *et al*., 2012; Mizuno *et al*., 2009). Lumefantrine is highly protein bound (>99%) and, as stated previously, is mainly metabolised by CYP3A4. The absorption of lumefantrine, however, is improved after a small amount of fat has been ingested (du Plessis *et al*., 2015; Mizuno *et al*., 2009). Since lumefantrine is highly protein bound, metabolised by CYP3A4, and the absorption is fat dependent, a high variability of drug plasma concentration in patients who are on this treatment, has been observed. Moreover, the bioavailability of lumefantrine is vital in determining the efficacy of the fixed-dose combination (du Plessis *et al*., 2015; Färnert *et al*., 2012; Mizuno *et al*., 2009). The challenge arose to formulate a preparation which is able to improve the solubilisation of both these drugs. This formulation needs to be designed in order to overcome the metabolism of artemether in the gastrointestinal tract as well as improve the permeability of lumefantrine (Patil *et al.*, 2013). Hence, since fat intake is a vital component in the absorption of this fixed-dose combination, investigations can be conducted into lipid-based formulations that may be beneficial in ensuring sufficient delivery and absorption of this combination therapy. Various lipid formulations produced by means of numerous methods have been tested to establish whether the delivery and absorption of a fixed-dose combination will be improved, namely: nanostructured lipid carriers, Pheroid™ technology, hot-melt extrusion, lipid emulsions for parenteral administration, and solid self-emulsifying drug delivery systems (SEDDS) to name a few (Fule *et al.,* 2015; Jain *et al.,* 2014; Ma *et al*., 2014; Patil *et al.,* 2013).

1.1.5 Lipid-based formulations

As is very well-known and regularly stated, the oral route is still one of the most popular routes for the delivery of numerous medicines; and patients still mostly prefer this route of administration (Agrawal *et al.,* 2015). Many of the drugs that are orally administered are hydrophobic in nature; i.e. the drug exhibits poor water-solubility. Low solubility may lead to sub-therapeutic plasma concentrations, owing to a decrease in the dissolution of the drug (Sprunk *et al.*, 2012). Many approaches have been studied, for example, tablets, injections, dispersible tablets, and capsules, to name a few. However, each method poses its own limitations (Feeney *et al*., 2016; Fule *et al.,* 2015; Jain *et al.,* 2014; Prajapat *et al.,* 2017; Singh

et al., 2011; Sprunk *et al.*, 2012), especially when considering lipophilic drugs. This led to the incentive to formulate lipid-based formulations due to the ability of lipids to solubilise in the small intestine, forming a more lipophilic microenvironment that surrounds the drug particles, allowing them to dissolve more easily into the gastrointestinal secretions. Solubilisation is enhanced by using natural and synthetic lipids to improve the dissolution of poorly water-soluble drugs (Humberstone & Charman, 1997). Extensive research in lipids is due to the effect fatty meals have on enhancing the absorption of poorly water-soluble drugs; it has provided sufficient evidence on the benefits lipids bring to absorption (Borrmann *et al*., 2010; Humberstone & Charman, 1997). The versatility of lipids offers a large variety of formulations such as: solutions, suspensions, emulsions, self-emulsifying systems as well as micro-emulsions (Humberstone & Charman, 1997; Nanjwade *et al.,* 2011).

Studies have shown that the solubility and absorption of artemether and lumefantrine can be increased by lipid-based formulations due to the fact that fatty meals increase the absorption of both these drugs (Mwebaza *et al.,* 2017). The following lipid-based formulations have been investigated: hot-melt extrusion, where both the drugs are stabilised during extrusion inside the polymeric network of the lipid, where after tablets can be formed. A significant improvement was noted in the *in vitro* dissolution and solubility of both drugs when compared to the pure drugs as well as marketed products (Fule *et al.,* 2015). Another approach identified was nanostructured lipid carriers which proved to increase the solubility of both drugs as well as selectively targeting the parasite-infected red cells (Jain *et al*., 2014). Emulsions were examined as a suitable lipid-based formulation for artemether and lumefantrine, however, the emulsions were found to be sensitive and metastable which negatively affected the delivery of both drugs (Patil *et al.*, 2013). Lipid-based formulations are of clinical importance for the administration of artemether and lumefantrine as a fixed-dose combination, because as mentioned earlier, fatty compounds assist in the absorption of this type of fixed-dose combination and many patient groups do not have access to high fatty meals (du Plessis *et al*., 2015; Mizuno *et al*., 2009; Mwebaza *et al.,* 2017). A novel approach that is being investigated in order to improve the solubility of lipophilic drugs is SEDDS. SEDDS have proven significantly effective for poorly soluble drugs (Agrawal *et al.,* 2015; Balata *et al.,* 2016; Chudasama *et al.,* 2015). However, studies have not yet been conducted on artemether and lumefantrine as a fixed-dose drug combination.

1.1.6 Self-emulsifying drug delivery systems

As stated above, poor solubility is exhibited by many commercial medicinal products; this is mainly due to the high lipophilicity of these compounds. High lipophilicity leads to poor aqueous solubility of the drug, which in turn leads to poor bioavailability and variability in the

release of drug particles from the dosage form (Rahman *et al*., 2013). In order to overcome this problem, novel formulations have been investigated, of which lipid-based formulations are showing promising results. One such type of formulation is emulsions; however, emulsions are sensitive and metastable, causing a problem in the effective delivery of a drug. Thus, SEDDSs were developed, generating a physically stable formulation, which is easier to manufacture (Patil *et al*., 2013). SEDDSs spread in the gastrointestinal tract and the self-emulsifying mechanism is activated by the digestive motility of the stomach and small intestine. It has been concluded that these formulations improve the rate and extent of absorption of the drug molecules as well as the bioavailability of the drug tested (Patil *et al*., 2013). Nonetheless, SEDDS have not been investigated or evaluated before as an effective delivery system for artemether and lumefantrine in a fixed-dose combination.

Due to the fact that numerous combinations of various excipients exist for lipid-based formulations, a classification system was established. This classification system is known as the Lipid Formulation Classification System (LFCS) that categorises lipids into four types of lipid-based formulations in accordance with the composition of the formulation and the effect on preventing the drug from precipitating from the formulation (Rahman *et al.*, 2013). Type I formulations include drugs in solutions comprising triglycerides and/or mixed glycerides. Type II formulations are classified as SEDDS. These formulations are isotropic mixtures of lipids and lipophilic surfactants, which self-emulsifies into fine oil-in-water emulsions when the SEDDS come into contact with an aqueous medium (Rahman *et al.*, 2013). SEDDS are designed to dissolve poorly-water-soluble drugs and are advantageous in overcoming delayed dissolution (Porter *et al.*, 2008). In this study natural oils will be utilised in formulating SEDDS, because these oils are more readily accessible, safe for oral consumption and the solubilisation of artemether and lumefantrine may be improved. In *in vivo* studies, SEDDS showed superiority over other dosage forms due to rapid drug release and an increase in drug solubilisation in the gastrointestinal lumen, which improved the bioavailability of the formulation. Furthermore, the rapid release of the drug is accounted for by the finely dissolved drug particles present in the SEDDS (Rahman *et al.*, 2013).

Type III lipid-based formulations are classified as self-microemulsifying drug delivery systems (SMEDDS) and these formulations are formulated with a hydrophilic surfactant and co-surfactant. The term self-nanoemulsifying (i.e. self-nanoemulsifying drug delivery systems or SNEDDS) is also used interchangeably. Type III formulations are further divided into two classes namely; Type IIIA and Type IIIB. This classification is used to distinguish between the hydrophilic and lipophilic character of the formulations. A more hydrophilic system (Type IIIB) comprises more hydrophilic surfactants and co-surfactants and contains a lower lipid

concentration (Porter *et al.*, 2008). Relating Type IIIA and Type IIIB; Type IIIB displays higher dispersion rates compared to Type IIIA, however, the risk of premature drug precipitation on dispersion is higher due to the low lipid content found in Type IIIB (Rahman *et al.*, 2013).

There is still confusion regarding the use of the terminology SEDDS, SMEDDS and SNEDDS. The main difference between SEDDS and SMEDDS transmits to the particle size and the optical clarity of the dispersion. A SEDDS formulation has a particle size larger than 100 nm and the dispersion presents with an opaque appearance. Typically, SMEDDS have a smaller particle size (smaller than 100 nm) and the dispersion has an optically clear appearance (Porter *et al.*, 2008). The composition of SMEDDS might include co-surfactants which SEDDS typically do not consist of. A distinction can also be made on mixing of the various ingredients; i.e., SNEDDS will only form when the surfactant and oil are mixed first, after which the water is added. With SMEDDS, the order in which the ingredients are mixed is not a crucial factor (Chatterjee *et al.,* 2016; Dokania & Joshi, 2015). The input of energy required to form an emulsion is also a distinguishing factor between SNEDDS and SMEDDS. SMEDDS are isotropic and classified as thermodynamically stable, this is due to the co-surfactants that reduce the interfacial tension needed for the SMEDDS to form (Dokania & Joshi, 2015). Typically, SNEDDS, on the other hand, require an input of energy, either by mechanical interference or the chemical potential found within the components. SNEDDS are furthermore classified as thermodynamically unstable, but kinetically stable systems (Chatterjee *et al.,* 2016; Dokania & Joshi, 2015).

1.2 RESEARCH PROBLEM

The commercial product, Coartem®, which contains artemether and lumefantrine in a fixed-dose combination, has been declared as a first-line therapy against uncomplicated malaria. However, this product is a conventional tablet formulation that displays erratic drug release, dissolution, and absorption (Abdulla & Sagara, 2009). A fatty meal is needed to increase the bioavailability of both these drugs (Mizuno *et al*., 2009; Mwebaza *et al.,* 2017; WHO, 2015). Furthermore, patients that have malaria experience amongst other symptoms: nausea, stomach cramps and vomiting, which discourages them from eating, especially fatty foods, which many of the target patients also do not have access to highly fatty meals (Ribera *et al.*, 2016). Thus, the development of a SEDDS containing a fixed-dose of artemether and lumefantrine may prove vital in improving the solubilisation of these lipophilic drugs and consequently the absorption thereof.

1.3 AIMS AND OBJECTIVES

This study is aimed at developing an oral SEDDS containing a fixed-dose combination of artemether and lumefantrine as well as a selected natural oil (peanut oil, coconut oil, olive oil, avocado oil, or castor oil). Surfactants (Tween® 80and SLS) and co-surfactants (Span® 80and Span® 60) were included in the formulations to decrease the interfacial tension, thus, decreasing the input of energy required, rendering the emulsion thermodynamically stable (Dokania & Joshi, 2015). These natural oils were utilised in this study due to them being safe for oral use, relatively accessible, and they may improve the solubilisation of both these drugs. Pseudo-ternary diagrams were used to determine the appropriate concentrations of the various ingredients included in the SEDDS. By determining the appropriate concentrations of oil, surfactants and water needed to formulate SEDDSs, the dissolution or pharmaceutical availability of the fixed-dose combination may be improved (Czajkowska-Kośnik *et al.*, 2015, Wang *et al*., 2015).

The objectives of this study are to:

- Determine the solubility of artemether and lumefantrine individually, and in combination, in the pre-selected oils (i.e. avocado-, olive-, castor-, peanut- and coconut oil) utilised in this study.
- Construct pseudo-ternary diagrams to determine the correct concentrations and ratios of oil, surfactant and co-surfactant needed to formulate SEDDSs.
- Formulate artemether/lumefantrine combination SEDDSs containing one of the selected oils (avocado-, olive-, castor-, peanut- and coconut oil), surfactant (SLS and Tween® 80) and co-surfactant (Span® 60and Span® 80) using the pseudo-ternary diagrams to determine the correct concentrations and ratio.
- Evaluate the artemether/lumefantrine combination SEDDSs by means of appearance, droplet size, zeta-potential, assay of the sample, viscosity, thermodynamic stability and phase separation.
- Evaluate the formulated SEDDS release profile by means of dissolution studies conducted in biorelevant media.

Review Article:

Can Lipid-Dosage Forms Assist in The Fight to Eradicate Neglected Tropical Diseases?

Lauren Cilliers; Lissinda H. du Plessis; and Joe M. Viljoen*

This manuscript has been submitted to the internationally accredited journal: ***International Journal of Pharmaceutics****;* and is written according to the guidelines set by this journal which can be found in Annexure E.

Review article

CAN LIPID-DOSAGE FORMS ASSIST IN THE FIGHT TO ERADICATE NEGLECTED TROPICAL DISEASES?

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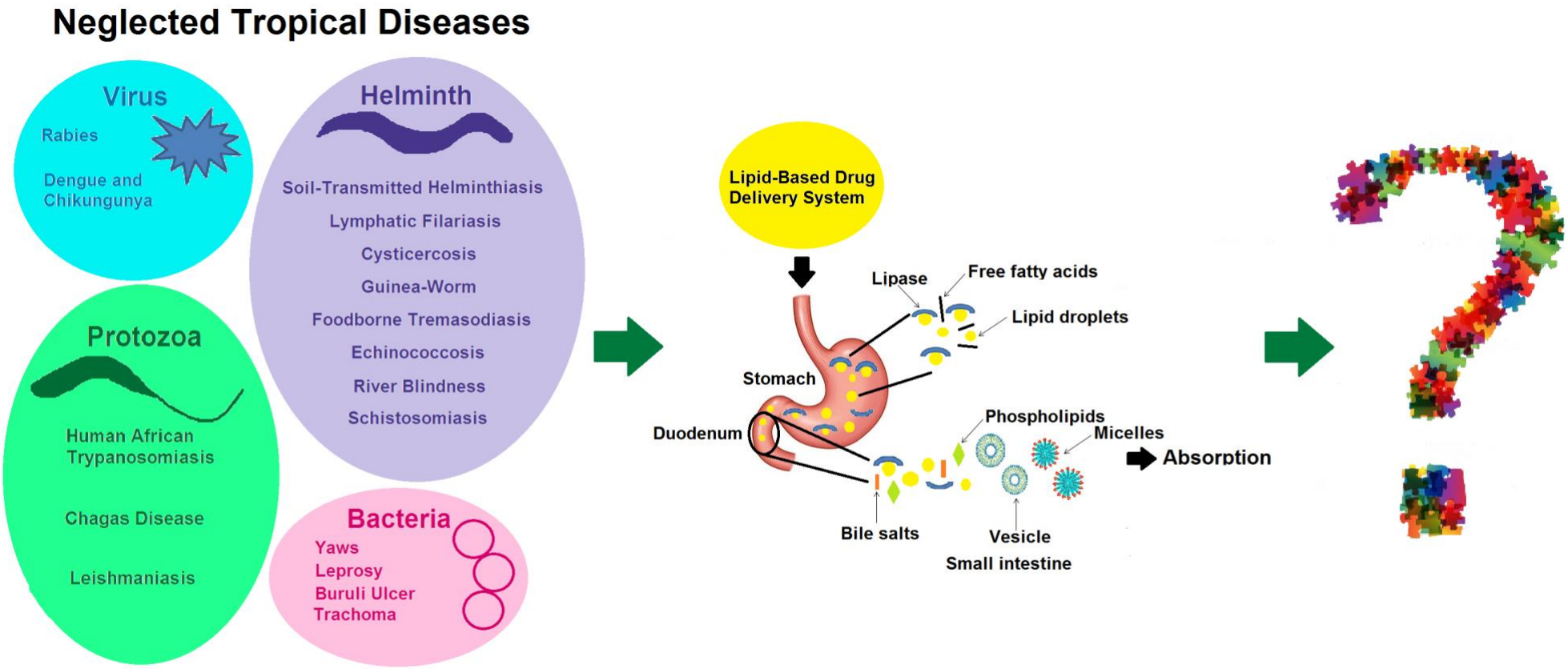
Graphical abstract



Abstract

Neglected tropical diseases (NTDs) have been around for centuries and are predominantly found in poverty-stricken areas. Due to lack of funding, the research on developing new formulations able to restrict NTDs has to some extent stagnated. Rather, a new approach needs to be implemented in order to combat this immense burden that has been placed on the world's healthcare system. Some approaches are to modify the drugs that are already being used to treat NTDs or their dosage forms. Drugs generally used in NTD treatment display poor aqueous solubility, where most of the drugs fall within class II and IV when classified according to the biopharmaceutical classification system (BCS). Lipid-based formulations have recently moved to the forefront of the research field and have proven to display promising developments in the bioavailability of lipophilic drugs. This review aims to highlight the possible mechanisms of lipid dosage forms that may improve the solubility of the lipophilic drugs, which in turn will probably increase the absorption of these said drugs. Lipid based drug formulations have already displayed immense potential when other highly lipophilic drugs have been incorporated into these formulations, rendering this type of dosage form a viable option in aiding in the elimination of NTDs.

Keywords:

Neglected tropical diseases (NTD); Poverty; Lipid-based formulations; SEDDS; SMEDDS; SNEDDS

1.1 Introduction

Neglected tropical diseases (NTDs) are nothing new; in fact, these diseases have been around for thousands of years. Hippocrates and the ancient Egyptians wrote accurate accounts of symptoms observed which later were linked to NTDs (Hotez, 2010). The Public Library of Science for Neglected Tropical Diseases (PLoS NTD) defines NTDs as a group of chronic infectious diseases, that ultimately promote poverty due to their impact on child health and development, pregnancy, and the productivity of workers (di Procolo & Jommi, 2014; PLoS, 2006). NTDs signify a manifold of heterogeneous infectious diseases which the World Health Organization (WHO) has classified under diseases of poverty (Lu *et al*., 2017; WHO, 2015). Diseases of poverty can be divided into two groups namely; the 'big three', which consist of malaria, HIV/AIDS, and tuberculosis. The second group, which is listed in Table 1, comprises of the 17 NTDs that prevail mainly in tropical and subtropical countries (Islan *et al*., 2017). Table 1 represents the 17 NTDs listed by the WHO and the impact they have on populations mainly residing in Sub-Saharan Africa (SSA) as well as their causative agents, their mode of transmission, and their burden on the global populations. Pathogens normally responsible for NTDs have intricate life-cycles, population dynamics, infection processes and epidemiologies, leading to diverse diseases and pathologies which complicate the treatment regimen (WHO, 2013). As aforementioned, NTDs target poverty-stricken areas which lead to further devastation of billions of lives (Crompton & Peters, 2010; Stolk *et al*., 2016; Verrest & Dorlo, 2017). In 2017, there were over 1.4 billion people affected by at least one NTD; and the mortality rate is estimated at 35 000 deaths per day (Aerts *et al.,* 2017; Hotez & Kamath, 2009; Verrest & Dorlo, 2017).

Table 1: *Summary of neglected tropical diseases as well as the endemic areas, causative agents, transmissions, and deaths per year (adapted from Hotez & Kamath, 2009; Verrest & Dorlo, 2017)*

In 2003 it became evident that a change was needed to control, and even eliminate, NTDs as 149 countries were already struggling with these diseases. Approximately 100 of

these countries are endemic to 2 or more NTDs and 30 countries are endemic to 6 or more NTDs (Crompton & Peters, 2010). This caused the WHO to refocus their attention on these specific illnesses and to initiate a paradigm shift. The shift included the WHOs report; 'Global plan to combat neglected tropical diseases 2008–2015', which was a bold approach in eradicating NTDs by providing care and delivering treatment to poverty-stricken populations. Effective use of limited resources as well as the alleviation of illnesses due to poverty were implemented, in order to enable weak health care systems in rural and urban areas (Crompton & Peters, 2010).

Figure 1 represents the burden that NTDs have on society as well as on the surrounding areas. NTDs mainly concentrate in poverty-stricken regions and rarely travel to different districts, therefore presenting a minor threat to high income countries, which resulted in little or no attention being paid to these diseases. The likelihood of NTDs becoming a prominent problem is directly linked to the amount of people living in a rural area. If a rural area experiences a higher influx of people; either by population growth or refugees, the region stands a higher chance of an increased NTD incidence rate (WHO, 2013).

Figure 1: *Common features of neglected tropical diseases (Crompton & Peters, 2010; Hunt et al., 2007)*

The disfigurement and disability caused by NTDs, as well as the fact that these diseases are mainly found in low income countries, has led to stigma and social discrimination; especially for women, whose marriage prospects may lessen, or they are left vulnerable to abuse and abandonment (Crompton & Peters, 2010; Hotez & Kamath, 2009; Hunt *et al.*, 2007; Islan *et al*., 2017). Moreover, a connection has been established between NTDs and human rights. It has been identified that NTDs are either a cause, or consequence of human rights that have been violated (Hunt *et al*., 2007). Individuals and communities are vulnerable to NTDs because their basic human rights are not being met,

i.e., right to clean water, adequate housing, education, health, non-discrimination, privacy, work, and to benefit from scientific progress (Hunt *et al*., 2007; WHO, 2010; WHO, 2015). In order to quantify the burden that NTDs has on communities, the disability-adjusted life year (DALY) is used as a measuring tool. DALY measures the relative impact of permanent or severe deformities as well as disabilities found in local and global populations due to NTDs (Hotez *et al*., 2014; Hunt *et al*., 2007). DALYs can be divided into two groups, namely; years of life lost (YLLs) due to early deaths, or years lived with disability (YLDs) (Hotez *et al*., 2014). Most of the prominent NTDs (intestinal nematode infections, schistosomiasis, food-borne trematodiases, onchocerciasis, cysticercosis, and trachoma) affect people in terms of disability and not death (YLDs). The burden on the communities' health can therefore be quantified, however, the impact these diseases have on child development, school attendance, agriculture, the cost of treatment and preventative measures; and the overall productivity of the workers are not considered when calculating DALYs. Despite these limitations, DALYs provide a relative estimate on the impact that the NTDs have locally and globally (Hotez *et al*., 2014). Hotez *et al*., (2014) added the estimated DALY values of the 17 NTDs classified by the WHO to approximately 48 million. This number is comparable to tuberculosis which has a DALY value of 49 million and is nearly half the value of the world's two major diseases, namely; malaria (83 million) and HIV/AIDS (82 million). These values prove that NTDs are a major concern and affect numerous individuals, thus, controlling these diseases need to become a priority (Hotez *et al*., 2014; Hunt *et al*., 2007).

1.1.1 The drive to eliminate neglected tropical diseases

NTDs contribute to an overall 12% of the global disease burden, nonetheless out of all the drugs approved over the past decade a mere 1% was developed for NTDs (Verrest & Dorlo, 2017). This prompted the WHO and pharmaceutical companies to explore developing lipid-based formulations in order to improve the absorption of the drugs already being used to treat NTDs (Hotez & Aksoy, 2017; WHO, 2010; WHO, 2013).

The drive to eliminate NTDs has been somewhat successful, and the WHO and PLoS NTD have collaborated their efforts into eliminating the 17 NTDs, as listed in Table 1, in order to improve the overall global health care system (Hotez & Aksoy, 2017; PLoS, 2006; WHO, 2010; WHO, 2013). The battle against NTDs has been a challenging and tedious journey, however, a drop in the number of new cases has become evident. For example, from 1989 till 2009 the number of new dracunculiasis cases decreased with more than 99% and the containment of the disease was reduced from 12 to 4 countries (WHO, 2010). The WHO started to endorse preventative chemotherapy in countries that were in dire need and this led to 75 countries and approximately 670 million people benefiting from this initiative (WHO, 2010). However, the situation is still dire, thus emphasizing the importance of exploring new dosage forms in order to treat and ultimately eliminate NTDs (WHO, 2010).

1.2 Lipid formulations and nanopharmaceuticals as new dosage forms

Many new chemical drug entities are highly lipophilic and display extremely poor water-solubility thus prohibiting any further development despite them having favorable pharmacological activity (Ali *et al*., 2008; Dahan & Hoffman, 2007; Sunitha *et al*., 2011). Lipophilic drugs display poor bioavailability because these drugs exhibit poor solubility in gastrointestinal fluid. The poor solubility then further limits the rate at which the drug dissolves in the gastrointestinal tract, which causes only a fraction of the drug to be absorbed, thus very little drug is found systemically (O'Shea *et al,* 2015). Various different approaches have been employed in an attempt to improve the solubility of these drugs so that drug bioavailability will improve (Fatouros *et al*., 2007; Feeney *et al*., 2016; Kalepu *et al*., 2013; Sunitha *et al.,* 2011; Wang & Pal, 2014). The absorption of poorly water-soluble drugs is erratic and unpredictable, which results in slow dissolution of the drug into the gastrointestinal fluid. This has led to the incentive to develop novel formulations which possibly could improve the bioavailability of the drug, which will aid in improving the

absorption of the drug and subsequently the dissolution of the drug (Dahan & Hoffman, 2007; Desai *et al.,* 2009; O'Shea *et al*., 2015).

One such an approach is to modify the physicochemical properties of a drug, e.g., reduce drug particle size and/or salt formation; however, these methods proved to have their own limitations. Salt formation is restricted to synthesizing weak acidic and basic salts that are able to convert back to their original forms, causing aggregation to occur in the gastrointestinal tract which, in turn, results in poor absorption. Particle size reduction is not always desirable since handling difficulties, poor wettability, and agglomeration are experienced with very fine powders (Aulton, 2018; Rahman *et al*., 2013). Novel formulations such as cyclodextrins, nanoparticles, solid dispersions and permeation enhancers have also been investigated and found to be somewhat successful, though mostly significantly expensive. More recently, the focus has shifted to lipid-based drug formulations (Rahman *et al*., 2013).

New drugs are in the process of being developed for some of the NTDs; however, these drugs will still have to undergo clinical trials and could take a long time to reach the commercial market. Additionally, when these drugs reach the market, they might be too expensive for individuals living in rural settings (Vermelho *et al*., 2017). Whilst new drugs are being developed possible changes in current formulations consisting of lipophilic drugs could be made that may provide more acceptable drug release profiles. Lipophilic dosage forms can have many beneficial outcomes on the absorption of lipophilic drugs (Kale & Deore, 2017; Porter *et al.,* 2007). Lipophilic formulations attribute to the drug being more stable in the lipid-based dosage form. These types of formulations can be very versatile, and both lipophilic and hydrophilic drugs can be incorporated into lipid-based formulations, which present more targeted and controlled release of the drug. They are easy and relatively inexpensive to formulate, and the bioavailability of a lipophilic drug has been proven to increase signifying that lipid-based dosage forms are able to increase absorption of a lipophilic drug (Mishra *et al.,* 2018; Morgen *et al.,*2017). Changes made to these formulations may similarly be more affordable for the greater majority of people who suffer

from NTDs. Most of the drugs used for NTDs are poorly soluble, thus improvements in the formulation and/or the method of administration could possibly be beneficial to the bioavailability of the drug. This could further lead to improved efficacy of the drug as well as decreased toxicity (Fatouros *et al*., 2007; Sunitha *et al*., 2011).

The drugs that have been used to date have all been classified according to the biopharmaceutical classification system (BCS), as either class II or class IV indicating that most, if not all, of the drugs used to treat NTDs display poor water-solubility (Cavalcanti *et al.,* 2012; Dawre *et al.,* 2018; Diaz-chiguer *et al.,* 2012; Kumar, 2013; Rodrigues *et al.,* 2011; Siqueira *et al.,* 2017; Spar & Delacey, 2008). Most of the older regime drugs used to treat NTDs have been identified as an effective treatment regime, however, their toxicity profile is often unbearable to patients. The toxicity is generally due to high doses given in order to ensure that an appropriate therapeutic response is achieved (Díaz-Chiguer *et al.,* 2012; Padró *et al.*, 2013; Skiba-Lahiani *et al*., 2015). Therefore, it is worth investigating the benefits lipid formulations may have in reducing the toxicity as well as increasing the solubility, of the drug which would lead to enhance the absorption of the drug. If the solubility of the drug is improved, lower dosages can be given to achieve the same therapeutic effect as before, subsequently possibly reducing toxic effects of these drugs which, in turn, can increase patient compliance (Kalepu *et al*., 2013).

Fatty meals are known to have a significant impact on the absorption of poorly-water-soluble, lipophilic drugs. Two basic principles can be linked to this phenomenon, namely; the effect of the contents present in a meal and the postprandial changes in the gastrointestinal environment. When fatty meals are co-administered with a poorly water-soluble lipophilic drug; a noticeable difference has been observed in the bioavailability of the drug. This is due to how the gastrointestinal tract responds to the ingested lipid (Custodio *et al.,*2008). When a drug is administered together with a lipid into the gastrointestinal tract, there is a considerable increase in the release of tri-, di-, monoglycerides and free fatty acids. The increase in triglycerides is important as this assist with the transport of lipids, leading to the absorption of a lipid drug and therefore, resulting in enhanced absorption of the poorly-

water-soluble drug. After a meal has been ingested, one of the routes lipids follow is to form chylomicrons, which consist of large triglyceride-rich lipoproteins. These chylomicrons transport the ingested lipid to various tissues; distributing the lipids throughout the body (Julve *et al.,* 2016). This has led to researchers noticing that lipid-dosage forms and meals that are high in fat, improve the bioavailability of poorly water-soluble, lipophilic drugs by increasing the distribution of the lipids via various routes in the body (Custodio *et al.,*2008; Julve *et al.,* 2016; Randolph & Miller, 2014). Consequently, investigations are being conducted into the use of lipids as vehicles to identify robust and effective techniques to improve the bioavailability and ultimately the absorption of these lipophilic drugs (Basalious & Ahmed, 2017; Dahan & Hoffman, 2007; Porter *et al.,* 2007). Moreover, it has become apparent that some exogenous components, such as surfactants and co-surfactants may also alter the gastrointestinal fluids, leading to an increase in drug solubilization. Furthermore, lipids have the ability to influence the gastrointestinal solubilization by increasing the solubilization capacity (Porter *et al*., 2007).

Lipids have the ability to self-assemble into bilayers where they form fluid membranes that are fairly impermeable to most water-soluble molecules (Tresset, 2009). Upon oral administration the physical and chemical nature of most of the lipid-based formulations change remarkably. The lipid interacts with biliary and pancreatic secretions in the small intestine, in a similar way to the digestion of food-based lipids (Kauss *et al.*, 2018; Williams *et al.*, 2012). The residence time of the formulation in the stomach plays an important role in the vastness of any effect that will aid digestion (Porter *et al*., 2007). Upon ingestion of the lipid, the lipid emulsifies and enters the duodenum; the duodenum secretes pancreatic lipases and esterases which mediates the digestion of the lipid at the oil-water interface, forming lipid digestion products (Kauss *et al*., 2018; Porter *et al*., 2007; Williams *et al*., 2012). These products formed are solubilized by salt-phospholipid-cholesterol-mixed micelles which are secreted in the bile, forming a colloidal structure in the gastrointestinal fluids. These colloidal structures aid in the solubilization of the administered lipids and the co-administered poorly water-soluble drugs, by preventing the lipophilic drug from prematurely precipitating

from the formulation (Porter *et al.,* 2007; Williams *et al.*, 2012). The drug, which has been incorporated into the lipid, is relocated into the aforementioned colloidal structure which then acts as a shuttle for the drug, as well as digestion products to the area of absorption, encouraging the absorption of the drug by manipulating the lipid digestion and absorption cascade, as illustrated in Figure 2 (Dahan & Hoffman, 2007; Kauss *et al.,* 2018; Williams *et al*., 2012).

Figure 2: *A schematic representation of the lipid digestion and absorption cascade*

The following formulations have been proven effective in the delivery of poorly water-soluble drugs, namely;

- liposomes,
- mixed micelles,
- solid lipid nanoparticles,
- micro- and nanoemulsions,
- self-emulsifying emulsions, and
- hot melt extrusion (Deshmukh, 2014; Fricker *et al*., 2010; Hauss, 2007; Katteboina *et al*., 2009; Miller *et al*., 2007; Pattni *et al*., 2015).

Most lipids are not able to withstand high temperatures due to their low melting points, which could indicate that formulations, prepared by means of for example hot-melt extrusion, might not be ideal for treatment of NTDs. If the lipid matrix tablet, formulated from hot-melt extrusion, starts to melt the integrity of the tablet is compromised and the tablet is of no worth to the patient (Chen, 2008; Miller *et al*., 2007). Most NTDs are present in tropical areas and it is important that the integrity of the formulation is not compromised by these high and humid conditions, therefore, the lipids used must be able to withstand these hot and humid climates (WHO, 2013).

Liposomes, mixed micelles, and self-lipid nanoparticles introduce an alternative lipid carrier structure and have been proven to enhance the solubility of lipophilic drugs. Self-lipid

nanoparticles encapsulate the drug which allows a controlled drug release and the solid lipid-based colloidal carriers support improved stability of the drug (Islan *et al*., 2017). Self-lipid nanoparticles are also small in size and have a narrow size distribution which is beneficial as it provides the potential to create a dosage form that is able to deliver a lipophilic drug to a specific cell (Deshmukh, 2014). Unfortunately, like most dosage forms, self-lipid nanoparticles present with side effects, including; substandard drug loading, expulsion of drug upon storage, fluctuating gelation tendencies, and possible particle growth which could affect the targeted release of the drug (Deshmukh, 2014). Liposomes on the other hand, are also unique in the sense that they are formulated in a way that they promote targeted release of the drug in the disease cells present. What makes liposomes even more appealing is that these dosage forms are able to deliver the drug safely, and it is able to deliver a trigger-like release at the site of action (Pattni *et al*., 2015). Liposomes have already proven effective in the administration of amphotericin B, however they present with certain limitations which include; that the drug content can leak from the liposomes which could lead to potential cytotoxicity, the trigger release offered by liposomes requires a stimulus and if the stimulus is not present the drug will not be released. Moreover, the production of liposomes proves challenging as batch-to-batch reproducibility is often a problem and drug loading into the liposome has found to be erratic and low (Akbarzadeh *et* al., 2013; Islan *et al*., 2017; Pattni *et al*., 2015).

Furthermore, these formulations are perceived as difficult and expensive to formulate: the problem lies in the upscaling of these formulations as this is time consuming and, with upscaling, there could possibly be more room for error. Due to the fact that these formulations take time to manufacture, time and money play important roles in the distribution of these drugs, and therefore donations will need to be provided to aid in distributing the drug to lower income areas that require the specific treatment (Akbarzadeh *et al*., 2013; Pattni *et al*., 2015). Lipid formulations normally used for the purpose of formulating emulsions can be classified into 4 types as depicted in Figure 3.

Figure 3: *Lipid formulation classification system (Rahman et al., 2013)*

Lipid-based formulations can be manipulated into a large variety of formulations for example solutions, suspensions, emulsions, self-emulsifying drug delivery systems and microemulsions. Moreover, blends of several excipients can be formed such as; pure triglyceride oils, blends of various triglycerides, diglycerides and monoglycerides, various surfactants (hydrophilic or lipophilic), and hydrophilic co-solvents can be used in the formulation process (Dahan & Hoffman, 2007; Mishra *et al.,* 2018; Rahman *et al*., 2013).

Emulsions can be divided into micro- and nano-emulsions, and self-emulsifying emulsions. Self-emulsifying drug delivery systems (SEDDS) are classified according to Pouton and Porter (2008) as a type II or III lipid delivery system (Figure 3) and can further be divided into micro-self-emulsifying drugs (SMEDDS) and nano-self emulsifying drugs (SNEDDS) (Kauss *et al*., 2018). The formulation is mainly made up of an oil and surfactant mixture which upon contact with peristalsis (gentle aggregation) and water or gastric media spontaneously forms a fine emulsion (oil-in-water) (Bernkop-Schnürch & Jalil, 2018; Nikolakakis & Partheniadis, 2017; Rohrer *et al.,* 2018). The reason behind formulated SEDDS is that they are said to improve the permeability as well as the solubility of BCS class II and IV drugs. The rationale behind improving the permeability of the drug is that upon administration, the SEDDS is degraded by gastrointestinal enzymes and is absorbed via enterocytes (Kauss *et al*., 2018; Nikolakakis & Partheniadis, 2017; Rohrer *et al*., 2018). Lymphatic absorption is favored, because the lipids are absorbed by the micelles, thus improving permeability and consequently absorption (Kauss *et al*., 2018). Furthermore, SNEDDS have been found to bypass first pass metabolism due to lymphatic absorption (Kauss *et al*., 2018).

SEDDS have proven to be an effective formulation in improving drug solubility. However, a problem that may exist is that at high temperatures the emulsions might break causing aggregation, flocculation, changes in pH, changes in color, changes in particle size and zeta potential, Ostwald ripening, and phase inversion, which could compromise the

integrity of the emulsion leading to a decrease in the efficacy of the drug (Khan *et al*., 2014; Weigel *et al*, 2018). SEDDS are formulated on a trial and error basis, which means the process can often be time consuming (Rohrer *et al*., 2018; Planchette *et al*., 2017). However, the advantages of SEDDSs render this type of formulation desirable. Due to the particles in the SEDDSs being finely dispersed, dissolution of the drug is promoted which leads to improved drug bioavailability. This proves advantageous to the drugs used to treat NTDs, as most of them display poor solubility and thus have poor dissolution (Rahman *et al.*, 2013). SEDDS can also be administered via various routes, allowing for a wide variety of formulations which allows for treatment of various age groups as well as targeted treatment in certain areas of the body, e.g. a topical formulation can be developed to help treat lesions caused by leprosy (Rohrer *et al*., 2018; Planchette *et al*., 2017).

In recent years the focus of emulsions has shifted from SEDDS to SMEDDS and SNEDDS. These lipophilic dosage forms form a protective environment for drugs that are water labile, thus these dosage forms are ideal for poorly water-soluble and water labile drugs. Upon ingestion SMEDDS and SNEDDS undergo a mild agitation in the gastrointestinal tract and an oil-in-water emulsion is formed which initiates the lipid cascade prior to absorption, as stated earlier (Xue *et al.,* 2018). The rationale behind focusing on SMEDDS and SNEDDS is due to these emulsions having a decreased globule size, and these drug delivery systems are isotropic in nature and they have a high thermodynamic stability (Dokania & Joshi, 2014). Micro-emulsions generally comprise water, oil, surfactant, and co-surfactant. The surfactant and co-surfactant create a low interfacial tension allowing the drug to easily move from the SMEDDS to the area of absorption in the gastrointestinal tract (Dokania & Joshi, 2014). The oil droplets present in the SMEDDS also falls within the micro range rendering this particular dosage form ideal, as the smaller oil droplets have a larger surface area and thus the drug particles can easily cross from the dosage form to the area of absorption (Kauss *et al.,* 2018). The advantages and disadvantages of SMEDDS are summarized in Table 2.

Table 2: *Advantages and disadvantages of SMEDDS*

SNEDDS are very similar to SMEDDS and therefore they display similar advantages and disadvantages however, the mean oil droplet size is less than 100 nm, classifying them in the nano range making this dosage form a sought after delivery system. However, the key differences between SMEDDS and SNEDDS are that the SNEDDSs typically have a smaller droplet size than SMEDDSs. SNEDDS are also different due to their high solvent capacity and their exceptional stability. The smaller oil droplet size enhances the bioavailability of the drug, which is due to an increased surface area which enhances the permeation through the intestinal membrane, as well as SNEDDSs display characteristics that eliminate food effects and improves the dissolution rate of the incorporated drug (Mishra *et al.,* 2018; Syukri *et al.,* 2018; Xue *et al.,* 2018).

1.3 Conclusion

NTDs are not homogeneous in nature, however they share similar characteristics. One such characteristic is that, in order to prevent a population from contracting NTDs, we need to ensure that the population has adequate clean water and food, proper sanitation, and good hygiene (Hunt *et al*., 2007). There are five strategies that the WHO has recommended as intervention for NTDs, namely; preventative chemotherapy, intensified case management, vector control, provision of safe water, sanitation and hygiene, and veterinary public health. These strategies are relatively low in cost and therefore feasible in controlling, preventing, and possibly eliminating NTDs (Crompton & Peters, 2010). The fight against NTDs is still a long and difficult battle which, more often than not, requires a large sum of money to combat these diseases. People in rural settings cannot afford expensive treatment, thus it is important to try and improve old regimes in the hope that this battle would dissolve and that NTDs can become a problem of the past. Sadly, NTDs will remain a major burden until basic human rights are met worldwide.

Current chemotherapy has proven to be flawed, and thus improvements need to be made to these formulations. Lipid formulations are a viable option and one that should be considered in improving the solubility of the various drugs used to treat NTDs. By improving the solubility of the drug, inevitably the bioavailability of the drug will also be improved. Many poorly aqueous soluble drugs have been tested in lipid-based formulations and displayed improved bioavailability of the drug (Hauss *et al.,* 1998; Julianto *et al*., 2000; Kang *et al.,* 2004; Kauss *et al.,* 2018; Mueller *et al.,* 1994; Shehatta, 2002; Trull *et al.,* 1994; Yap and Yuen, 2004). These formulations are often inexpensive to manufacture, thus making them a desirable and sought after formulation (Planchette *et al*., 2017).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Table 1: *Summary of neglected tropical diseases as well as the endemic areas, causative agents, transmissions, and deaths per year (adapted from Hotez & Kamath, 2009; Verrest & Dorlo, 2017)*

Disease	Causative agent	Endemic areas	Estimated population infected in SSA	Transmission	Deaths per year (Global estimate)
Protozoal infection					
Chagas disease	*Trypanosoma cruzi*	Latin America	20 000	Triatomine bug	10 300
Human African trypanosomiasis	*Trypanosoma brucei gambiense, T. brucei rhodesiense*	Africa	50 000-70 000 (17 000 annually)	Tsetse fly	9100
Leishmaniasis	*Visceral: Leishmania donovani, L. infantum* *Cutaneous: L. major, L. tropica, L. brazilliensis, L. Mexicana* and other *Leishmania* spp.	Indian subcontinent, Asia, Africa, Mediterranean basin, South America	19 000-24 000 new cases annually in Sudan and Ethiopia	Phlebotomine sandflies	51 600
Malaria	*Plasmodium* spp.	Africa	212 million	Female *Anopheles* mosquito	429 000
Bacterial infection					
Buruli ulcer	*Mycobacterium ulcerans*	Africa, South America, Western pacific regions	>4000	Unknown	Unknown
Leprosy	*Mycobacterium leprae*	Africa, America, South east Asia, Eastern Mediterranean, Western pacific	30 055 (registered prevalence)	Unknown	Unknown
Trachoma	*Chlamydia trachomatis*	Africa, Middle East, Asia, South America, Australia	30 million	Direct or indirect contact with an infected person	Unknown
Endemic treponematoses	*Treponema pallidum, T. carateum*	Global distribution	Unknown	Skin contact	Unknown
Helminthes					

Cysticercosis/ taeniasis	*Taenia solium, Taenia Saginata, diphyllobothrium latum*	Worldwide, mainly Africa, Asia, and Latin America	Unknown	Ingestion of infected pork	1200
Dracunculiasis	*Dracunculus medinesis*	Chad, Ethiopia, Mali, South Sudan	9585	Contaminated water	Unknown
Echinococcosis	*Echinococcus granulosus, Echinococcus multilocularis*	Worldwide	Unknown	Feces of carnivores	1200
Foodborne trematodiases	*Clonorchis* spp., *Opisthorchis* spp.,and *Paragoniums* spp., *Echinostoma* spp., *Fasciolopsis buski, Metagonimus, Metagonimus* spp., *Heterophyidae*	South-east Asia, Central and South America	Unknown	Contaminated food	Unknown
Lymphatic filariasis	*Wuchereria bancrofti, Brugia malayi, B. timori*	Africa, Asia, Central and South America	46-51 million	Mosquitos	Unknown
Onchocerciasis	*Onchocerca volvulus*	Africa, Latin America, Yemen	37 million	Black flies	Unknown
Schistosomiasis	*Schistosona haematobium, S. guineensis, S. intercalatum, S. Japonicum, S. mansoni, S. mekongi*	Africa, South-America, Middle East, East-Asia. Laos, Cambodia	192 million	Contaminated water	11 700
Soil-transmitted helminthiases	*Ascaris lumbricoides, Trichuris trichiura, Necator americanus, Ancylostoma duodenale*	Worldwide	Unknown	Human faeces	2700
		Viral infection			
Dengue	Dengue fever virus (genus: Flavivirus)	Asia and Latin America	>50 million annually	Mosquito	14 700
Rabies	Rabies virus (genus: Lyssavirus)	Worldwide, mostly Africa, Asia, Latin America and western pacific	Unknown	Animals, mostly domestic dogs	26 400

Table 2: *Advantages and disadvantages of SMEDDS*

Advantages	Disadvantages
• Storage is easy and simple, due to SMEDDS being thermodynamically stable • SMEDDS are often very stable, because they are not formulated with water • The SMEDDS formulation can be formulated either as a capsule or a tablet and thus patients are more compliant • Food has no effect on the SMEDDS and the lipid in the SMEDDS aids with absorption • SMEDDS can be filled in capsules, thus making the drug more palatable • SMEDDS are easy to manufacture and to scale-up	• Drug can precipitate upon dilution • The lipids present in the SMEDDS can undergo oxidation and polymorphism

Figure Captions

Figure 1: *Common features of neglected tropical diseases (Crompton & Peters, 2010; Hunt et al., 2007)*

Figure 2: *A schematic representation of the lipid digestion and absorption cascade*

Figure 3: *Lipid formulation classification system (Rahman et al., 2013)*

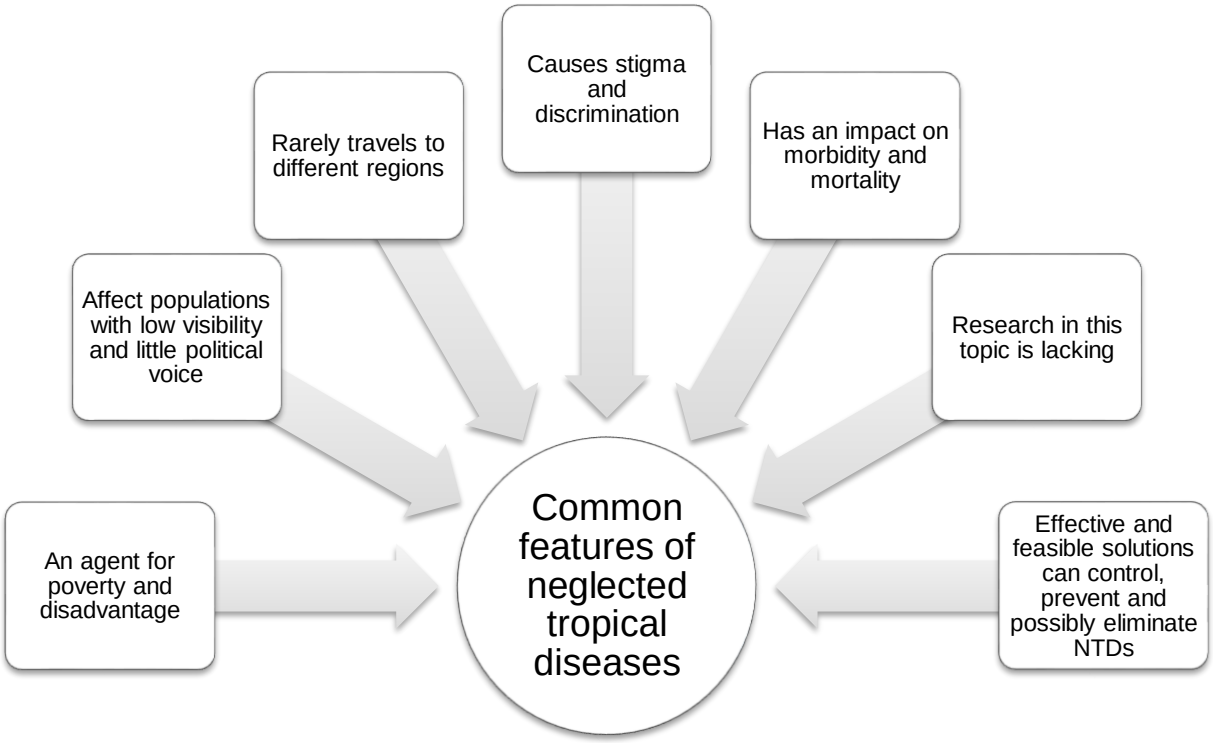


Figure 1: *Common features of neglected tropical diseases (Crompton & Peters, 2010; Hunt et al., 2007)*

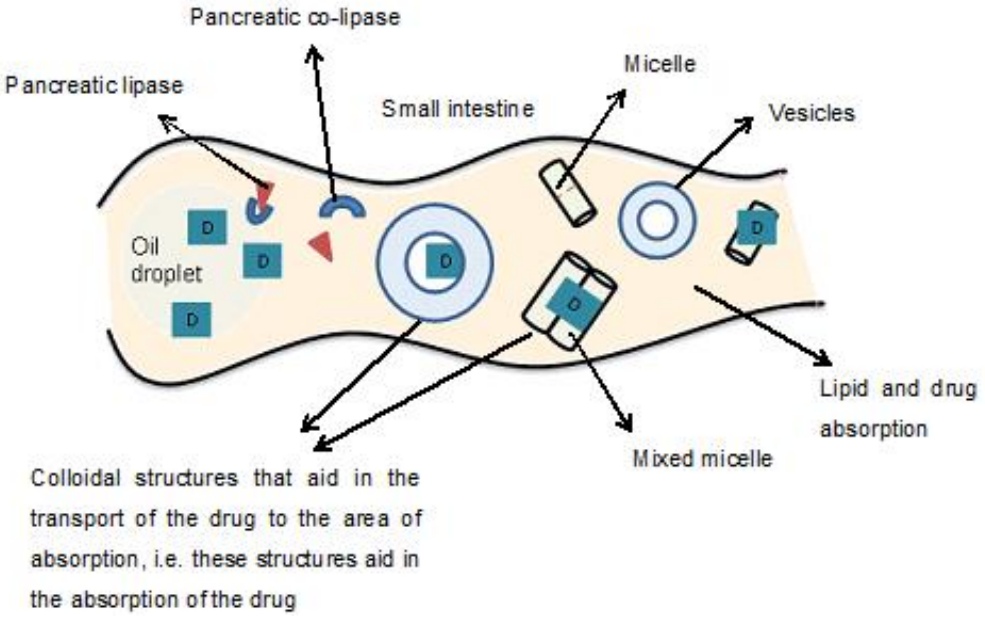


Figure 2: *A schematic representation of the lipid digestion and absorption cascade*

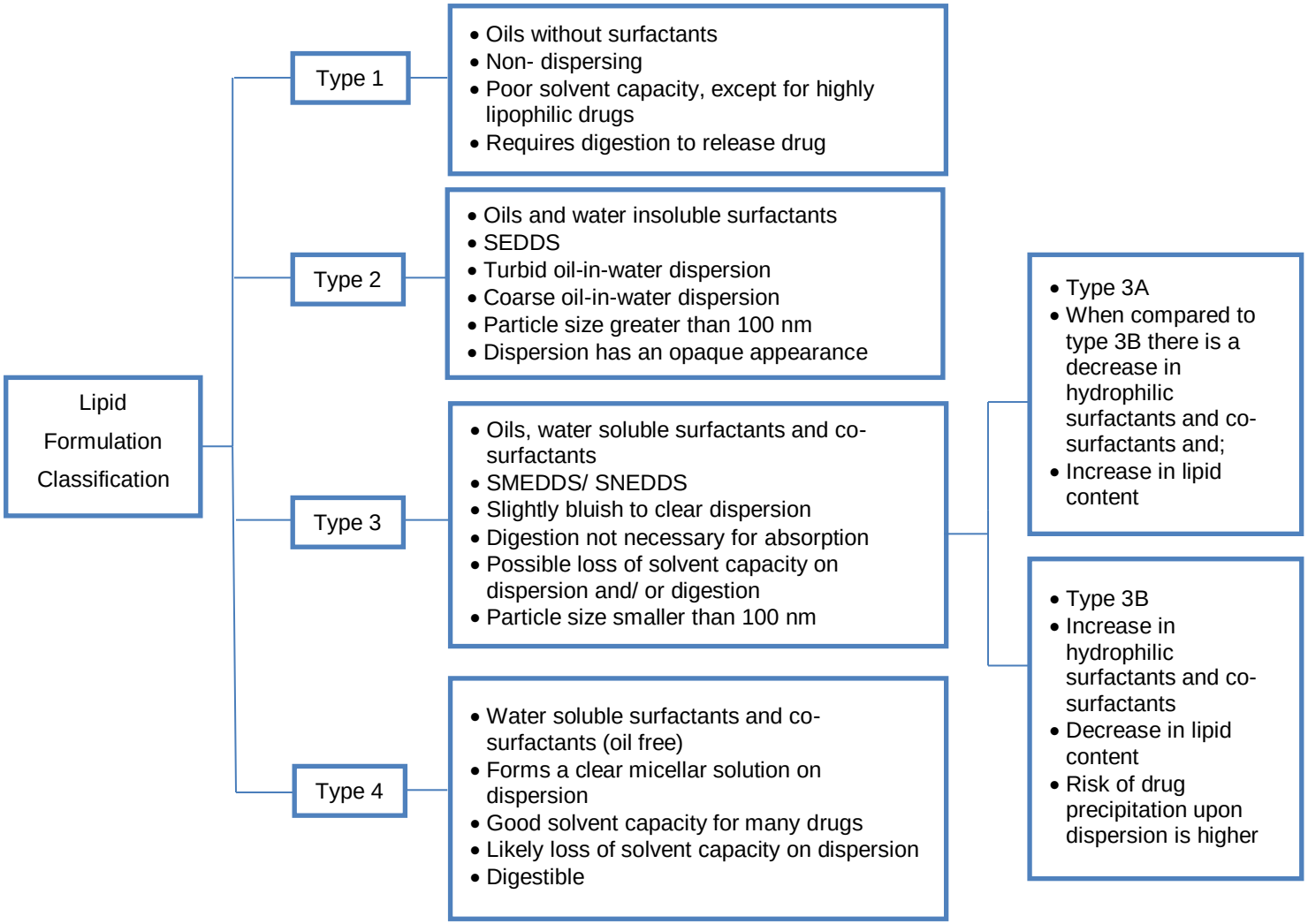


Figure 3: *Lipid formulation classification system (Rahman et al., 2013)*

CHAPTER 3

METHODOLOGY AND MATERIALS

3.1 RESEARCH METHODOLOGY

3.1.1 Introduction

The formulation of a self-emulsifying drug delivery system (SEDDS), for the most part, requires precise mixing of the surfactant phase and oil phase, as well as dissolving the active ingredients in the correct quantities into the delivery system. Thus, a selection of various oils and surfactants were made, and the oils were chosen to compliment the poor solubility of both artemether and lumefantrine. The surfactants chosen, were selected to compliment the hydrophilic-lipophilic balance (HLB) of the oils.

With lumefantrine it is well known that a fatty meal must be taken to assist in enhancing the absorption of this highly lipophilic drug (Borrmann *et al.,* 2010). Hence, natural oils such as olive-, castor-, avocado-, peanut-, and coconut oil were utilised, as these oils will mimic the fatty meal and initiate the same lipophilic pathway as a fatty meal would have, subsequently enhancing the absorption of lumefantrine (Nanjwade *et al.,* 2011). Moreover, the oils chosen are natural oils and are therefore rich in dietary supplements essential for a number of key biological functions, including growth and development. Not only do these oils improve the *in vivo* lipophilic profile of a patient but they could, in addition, possibly reduce cardiovascular morbidity. Most patients that do contract malaria in Africa are often residing in a rural setting where there is immense poverty and thus a lack in receiving proper dietary fats. These oils are therefore a beneficial choice as they will be able to contribute to the dietary health of the patient as well as promote proper absorption of lumefantrine and artemether (Ribeiro-Santos *et al.,* 2018).

This chapter motivates the choice of excipients chosen for the various SEDDS formulations of this study. It also deals with the experimental procedures followed to determine the physical properties, stability profile, drug content, as well as the dissolution profiles of the SEDDS containing both artemether and lumefantrine.

3.1.2 Materials

The materials that were utilised in this study are listed in Table 3.1.

Table 3.1: *Materials and manufactures used in this study*

Material	Manufacturer
Artemether	Kindly donated by Prof Wilna Liebenberg DB FINE CHEMICALS (PTY) LTD
Lumefantrine	Kindly donated by the MRC Flagship Programme – Cipla Mumbai Pty Ltd India
Olive oil	Pick 'n Pay Retailers (Pty) Ltd
Coconut oil	Lifestyle Food, Dis-Chem Pharmacies
Castor oil	Allied Drug Company (Pty) Ltd
Avocado oil	Westfalia Fruit Products, Oil Refinery, Everdon Estates, Howick, KZN
Peanut oil	Allied Drug Company (Pty) Ltd
Tween® 80	Associated Chemical Enterprises (Pty) Ltd
SLS	Merck Chemicals (PTY) LTD
Span® 60	Sigma Aldrich Chemistry GmbH, Steinheim, Switzerland
Span® 80	Industrial Analytical (Pty) Ltd

3.2 Preformulation studies

3.2.1 Infrared spectrum for artemether and lumefantrine

For the purpose of this study, infrared (IR) spectroscopy was used to identify whether the artemether and lumefantrine powders received did not have any impurities present. This was done by comparing the batches of artemether and lumefantrine used in this study to a known, pure reference standard of both lumefantrine and artemether. An aliquot of each powder sample of either artemether or lumefantrine received was measured, and individually placed on the machine where the wavelength of each powder sample was determined. The wavelengths of the individual samples were subsequently compared to the reference standard samples' wavelengths.

An Alpha sample compartment RT-DLaTGS IR (ATR) spectrometer was employed, and the samples were measured at a wavelength between 400–4000 nm. These ATR units are designed with a clamping utility, ensuring good contact of the powders and fine powders. This good clamping utility ensures that respectable reproducibility is achieved. The ATR unit is advantageous as it ensures faster sampling without any preparation of the sample required, the reproducibility is excellent, and there is minimal room for operator error (AzoM, 2014).

3.2.2 High performance liquid chromatography method validation for artemether and lumefantrine

3.2.2.1 Analyses of samples

In order to determine the solubility, concentration, and/or degradation of the different samples, an HPLC method was used, which in turn assisted in identifying and quantifying the analytes. Da Costa César *et al.*, (2007) simultaneously conducted HPLC methods for both artemether and lumefantrine, however, these methods were validated in this study according to the International Conference of Harmonisation (ICH) guidelines (Afosah, 2010; Branch, 2005; da Costa Ce´sar *et al.*, 2008). For the validation of the HPLC method the following parameters were considered: linearity, specificity, range, repeatability, accuracy, limit of detection and limit of quantification (Laxmi *et al.*, 2015).

HPLC analysis was carried out utilising a Hitachi chromatographic system. The system consists of a 5410 UV detector, an auto-sampler (5260) with a sample temperature controller and a solvent delivery module (5160). This system was equipped with a Luna C18-2 column, 150 x 4.6 mm, 5 µm, (Phenomenex, Torrance, CA) and it was used to conduct a concentration assay. The column temperature and flow rate were maintained at 30°C and 1.0 ml/min, respectively. UV detection was conducted at 210 nm and the mobile phase included acetonitrile and water in a 75:25 ratio (Joshi *et al.,* 2008b). For injection into the HPLC system, 20 µl of each sample tested was used and a standard calibration curve was subsequently constructed.

3.2.2.2 Standard and sample preparation

Accurate amounts of 5 mg artemether and 30 mg lumefantrine were weighed and transferred to a 100 ml volumetric flask. To ensure complete solubilisation of both artemether and lumefantrine, 1 ml octane sulphonic acid was added to the dry powder, after which methanol was added up to the 100 ml mark. This solution was ultrasonicated for 3 min. The final solution presented a concentration of 50 µg/ml artemether and 300 µg/ml Lumefantrine (da Costa César *et al*., 2008).

3.2.2.3 Validation

3.2.2.3.1 Specificity

Specificity is a test for interference by determining whether the analytical method employed is capable of delivering responses that have no interference and the results given are true (Rozet *et al*., 2007).

The peaks of artemether and lumefantrine were determined separately as well as the peaks of hydrochloric acid and phosphate buffer, which were employed during the dissolution experiments in order to determine whether any signs of interference occurred between the active ingredients and dissolution media (da Costa César *et al*., 2008). First, a standard solution, which consisted of 30 mg lumefantrine and 5 mg artemether respectively, was weighed and transferred into a 100 ml volumetric flask. Octane sulphonic acid (1 ml) was added to the volumetric flask to aid in the dissolution of lumefantrine. Next, methanol was added to constitute the rest of the volume. The flask was ultrasonicated for 3 min to warrant a uniform dispersion of both drugs in the methanol. This solution was transferred to an HPLC vial and labelled as the standard. Hydrochloric acid and phosphate buffers were each added to their own HPLC vials and labelled accordingly for further analyses. Additionally, two samples were prepared. The first sample consisted of 5 ml placebo (oil and surfactant phase) that was added to a 20 ml flask and filled to volume with tetrahydrofuran (THF). The sample was then ultrasonicated for 3 min and transferred to an HPLC vial and labelled as “the placebo”. The second sample contained 5 ml of the placebo, with 30 mg lumefantrine and 40 mg artemether respectively, in a 20 ml volumetric flask. This was also filled to volume with THF. The sample was ultrasonicated for 3 min, transferred to an HPLC vial, and labelled as “the active”. All of the HPLC vials were consequently analysed on the HPLC.

3.2.2.3.2 Linearity

Linearity is an analytical procedure where a relationship exists, within a specified range between the response (e.g. the area under the curve) and the concentration of the analyte being measured. The range is classified as the interval between the upper and lower limits of the analyte that are determined with accuracy, precision and linearity (Rozet *et al.,* 2007). According to the ICH an R squared (R^2) value of at least 0.99 should be yielded when analysing the linear regression between the response and concentration of the analyte (Branch, 2005).

An aliquot of 25 mg for both artemether and lumefantrine was individually weighed and each transferred to a 100 ml volumetric flask, after which 1 ml orthophosphoric acid (85%) was added to the dry powder in order to assist in the solubilisation of lumefantrine. Methanol was added to the flask to constitute the rest of the 100 ml and the flask was ultrasonicated for 3 min. From this solution 5 ml was reserved and added to a 50 ml volumetric flask. The volume was reconstituted with methanol and, from this second solution 5 ml was withdrawn and added to a different 50 ml volumetric flask. The volume of the flask was made-up with methanol. From these three different volumetric flasks, the concentration of both artemether and lumefantrine ranged from 2.5 µg/ml, 25.0 µg/ml, and 250.0 µg/ml, respectively. Three HPLC

vials were filled, each containing one of the three concentrations and the vials were injected in duplicate at 2.5, 5.0, 7.5, and 10.0 µg/ml, respectively. All data obtained from each compound were used to plot a calibration curve for concentration versus response and the R^2 value calculated (da Costa César *et al*., 2008).

3.2.2.3.3 Accuracy

Accuracy is defined as the proximity between a test result and the true or accepted reference value that should be perceived across its range (Rozet *et al*., 2007). The ICH recommends that the mean percentage recovery should be within 98 and 102%, with a %RSD (relative standard deviation) of less than 15% for the method to be considered accurate (Branch, 2005; da Costa César *et al*., 2008).

A reference standard of 5 mg artemether and 30 mg lumefantrine were accurately weighed and injected as the standard sample. Both active ingredients were weighed at three different concentrations, 33, 66 and 133 µg/ml for artemether and 77, 155 and 310 µg/ml for lumefantrine, respectively. For each concentration the samples were prepared in triplicate and the recovery percentage of each mean value was calculated.

3.2.2.3.4 Precision

Precision is a vital component in a robust analytical method to ensure repeatability and reproducibility of multiple measurements of homogenous samples under specified conditions (Araujo, 2009; Rozet *et al.,* 2007). Therefore, both intra-day and inter-day precision were measured.

Intra-day precision

Intra-day precision requires a minimum of three samples to be injected over the period of one day. Three different concentrations of lumefantrine (240, 300 and 360 µg/ml) and artemether (40, 50 and 60 µg/ml) were individually injected. The end result is normally expressed as the standard deviation (SD) or the %RSD of a statistically significant number of samples (Araujo, 2009; Rozet *et al*., 2007; Stöckl *et al*., 2009). The %RSD value should be less than 15% to be of an accepted standard when determining both intra-day and inter-day precision (da Costa César *et al*., 2008; Rozet *et al.,* 2007).

Inter-day precision

During the determination of inter-day precision, the experiments are normally conducted over a span of 3 days under the same conditions, thus, the analyst and the equipment must remain constant variables (Araujo, 2009; Rozet *et al*., 2007; Stöckl *et al*., 2009).

Lumefantrine (300 ug) and artemether (50 µg), were respectively injected over a period of 3 consecutive days, after which the %RSD for the three measurements was determined (da Costa César *et al*., 2008).

3.2.2.3.5 Limit of quantification and limit of detection

The lowest concentration, or amount of analyte, that can be measured with an acceptable level of precision and accuracy is known as the limit of quantification (LOQ) (Araujo, 2009). There are various approaches to evaluating the LOQ and limit of detection (LOD). However, for the purpose of this study LOQ and LOD were evaluated using the signal/noise ratio approach. This approach is when signals of samples, with known analytical concentrations, are compared to blank samples up to an analytical concentration that produces a signal equivalent to 10x the standard deviation of the blank sample (Araujo, 2009).

Standard solutions of artemether and lumefantrine were prepared by dilution and injected into the HPLC. The standard solutions were prepared in decreasing concentrations, with the final concentration of artemether and lumefantrine being, 10.51 µg/ml and 1.05 µg/ml, respectively. These final concentrations were placed into an HPLC vial and injected seven times at different concentrations (2.5-, 5.0-, 7.5-, and 10.0 µg/ml), in order to determine the LOD and LOQ of the sample. The LOD was considered when a signal/noise ratio of 3, or a %RSD of 30 was obtained. Similarly, the LOQ was considered when a signal/noise ratio of 10, or a %RSD of 10 was obtained (da Costa César *et al*., 2008; ICH Q2(R1), 2005; Katsidzira *et al.,* 2016).

3.2.3 Isothermal microcalorimetry

The foundation on which isothermal microcalorimetry is based on is that all physical and chemical processes coincide with heat exchanges within their surroundings. In the thermal activity monitor (TAM) the sample is maintained at isothermal conditions (Phipps & Mackin, 2000). During this process heat flows between the sample and its surroundings, causing a reaction to occur which creates a temperature gradient between the sample and its surroundings. The heat flow between the sample and its surroundings is measured as a function of time (Phipps & Mackin, 2000). Since heat flow is being measured, it is vital to know the melting points of each component (Table 3.2) as this will determine the temperature at which the TAM will be maintained for the duration of the experiment.

Table 3.2: *Denaturing/melting points of the components used in the study*

Components	Melting/Denaturing points
Artemether	86-90°C
Lumefantrine	125-130°C
Olive oil	193°C
Coconut oil	175°C
Castor oil	229°C
Avocado oil	249°C
Peanut oil	232°C
Tween® 80	>100°C
SLS	170°C
Span® 60	55°C
Span® 80	113°C

Isothermal microcalorimetry was used to determine whether interactions occurred between the various components to be formulated in the SEDDS. A Thermal Activity Monitor (TAMIII) apparatus (TA Instruments, New Castle, Delaware, USA) equipped with an oil bath with a stability of ±100 µK over a period of 24 h was employed in this study. The temperature was maintained at 50°C as this is below the melting points of each of the components. A sample mass of 70 mg was retained for each sample for the duration of the experiment. In order to identify any reactions between the components, a baseline first has to be established, i.e. the heat flow was measured for each component individually. Once a baseline had been recognised, the heat flow of the various combinations of the components constituting the SEDDSs were measured. The calorimetric output observed by the individual component was summarised as a hypothetical response. This hypothetical response is the expected calorimetric output if the two components do not interact with one another. If the components do however interact with one another, the measured calorimetric response will differ from the hypothetical response. An observed change in heat flow higher than 100 µW/g was considered a significant difference; indicating that an interaction between the components was indeed detected.

3.3 Preparation of SEDDS

3.3.1 Solubility studies

The solubility of each of the individual active compounds (artemether and lumefantrine), as well as the fixed-dose combination utilised in the selected solvents (oils, surfactants and co-

surfactants) was determined employing the shake flask method, described by Joshi *et al*. (2008a). A surplus amount (approximately 500 mg) of the specific sample was added to 5.0 ml of a selected oil in a screw capped tube. The opening of the tubes was covered with Parafilm® before the cap was screwed back on. Each of the mixtures were vortexed for approximately 1 min to enable a uniform dispersion. These mixtures were placed in a temperature-controlled bath (37 ± 2°C) and agitated for 48 h to ensure that equilibrium solubility was achieved (Joshi *et al.*, 2008b). The samples were then centrifuged at 3 000 rpm for 15 min. The supernatant was collected and diluted with tetrahydrofuran (THF), and the concentration of the dissolved sample was determined by means of HPLC as discussed in the following section (Czajkowska-Kośnik *et al.*, 2015; Joshi *et al*., 2008a).

3.3.2 Pseudo-ternary phase diagram

SEDDS will form oil-in-water emulsions with only moderate agitation, once these systems are introduced into an aqueous media. The selected surfactant and co-surfactant(s) adsorb at the interface with subsequent reduction of the interfacial energy. Consequently, the thermodynamic stability of the formulation is improved by means of a decrease in the free energy required to form the emulsion. The selection of the oil phase and surfactant therefore plays a vital role in the design of SEDDS (Czajkowska-Kośnik *et al*., 2015). Pseudo-ternary phase diagrams of drugs, oils, surfactant, co-surfactants, and water may be extremely supportive in determining the most appropriate composition of SEDDS. These diagrams identify the self-emulsifying regions and furthermore determine the optimum concentrations and ratios of oil, surfactant and co-surfactant when used in a combination. Once the region of the SEDDS is determined, the feasibility of forming an emulsion can be determined (Czajkowska-Kośnik *et al.*, 2015; Wang *et al*., 2015).

In order to find an appropriate concentration range for all of the components (fixed-dose combination, oil, surfactant, co-surfactant and water) at room temperature (approximately 25°C) in which they form emulsions, pseudo-ternary phase diagrams were constructed utilising the water titration method (Boonme *et al*., 2006; Czajkowska-Kośnik *et al.*, 2015; Wang *et al*., 2015). First, the surfactant and co-surfactant were mixed together. This mixture is referred to as the “surfactant phase”. Kang *et al*. (2004) determined that the ratio of surfactant and co-surfactant should be 1:1 as they found that higher concentration ratios improved the emulsion range, however, a decrease in stability was noted which could lead to precipitation of the incorporated drug. Next, mixtures of the oil and surfactant phase at certain weight ratios (w/w) of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10, in different glass vials, were moderately agitated by means of vortexing for 5 min to form homogenous mixtures. Each mixture was titrated with water in a dropwise fashion until the first sign of turbidity was

noted so as to identify the end-point of the emulsion range. Post equilibrium, if the system became clear, the addition of water was continued. Once equilibrium of the mixture was achieved the mixtures were visually inspected, by means of polarised lenses, for transparency and for optical isotropicity. Inspection of the SEDDS through a polarized lens is a simple way to generally classify the SEDDS. If the observed solution is black, the SEDDS is classified as being in the microemulsion range. However, particle size analysis, utilising a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK), was performed to undoubtedly classify the SEDDS (Boonme *et al*., 2006; Czajkowska-Kośnik *et al*., 2015; Joshi *et al*., 2008b; Wang *et al*., 2015; Wang & Pal, 2015).

3.3.3 Appearance

All of the formulations were visually examined, and their microscopic appearance was noted utilising cross-polarised light microscopy (Nikon® Optiphot PFX Microscope, Thailand, Bangkok).

3.3.4 Formulation of SEDDS

Results obtained from the constructed pseudo-ternary phase diagrams were used to prepare the SEDDS by means of aqueous phase titration. The fixed-dose combination was first dissolved in the chosen oil and subjected to sonication. A selected surfactant phase was mixed separately and added to the oil and fixed-dose combination according to the ratios determined by the pseudo-ternary phase diagram. The ratio of surfactant to co-surfactant was fixed at 1:1 as mentioned previously. Subsequently, deionised water was added in small increments (less than 5% w/w) to the surfactant-oil mixture at room temperature (approximately 25°C) while continuously stirring until a clear emulsion was formed. All samples were stored at room temperature for at least 24 h to achieve equilibrium prior to additional analysis (Aparna *et al.*, 2015; Boonme *et al*., 2006; Fernandez *et al*., 2004; Wang & Pal, 2014).

3.4 Characterisation of SEDDS

3.4.1 Assay

In order to determine the drug content present in the SEDDS a 5 ml sample from each batch was filtered through a 0.45 µm membrane filter. Next, the filtrate was diluted with THF, after which it was further diluted by withdrawing 5 ml and diluting it with methanol to obtain a final volume of 10 ml; this was done in triplicate. The final sample was placed in HPLC vials and analysed by means of HPLC as described in Section 3.3.1. This experiment was conducted in triplicate.

3.4.2 Droplet size and zeta-potential

Droplet size is a crucial factor in self-emulsification performance as it determines the rate and extent of drug release, the absorption rate into the systemic circulation, as well as the stability of the emulsion or SEDDS. Zeta potential measurement, on the other hand, is used to classify the charge of the droplets. The charge on an oil droplet in conventional SEDDS is negative due to the presence of free fatty acids however, incorporation of a cationic lipid, such as oleylamine, at a concentration range of 1–3% will yield cationic SEDDS (Balata *et al*., 2016; Gursoy & Benita, 2004; Mahapatra *et al*., 2014).

Mean droplet size and size distribution were measured utilising a Malvern Mastersizer 2 000, equipped with a wet cell Hydro 2 000 SM dispersion unit (Malvern Instruments, Worcestershire, UK), and the Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) employing photon correlation spectroscopy, was used to determine the zeta potential. Dynamic light scattering analyses of the Brownian motion of the particles, which is due to the fluctuations in light scattering, was furthermore conducted. The light scattering was measured at an angle of 90°, while the temperature was regulated at 25°C (Chudasama *et al*., 2015; Mahapatra *et al*., 2014).

3.4.3 Determination of self-emulsification

The mechanism of action of self-emulsification is not yet fully understood. However, it has been suggested that self-emulsification occurs when the entropy change favouring dispersion is significantly higher than the energy required to increase the surface area of the dispersion. Emulsification occurs spontaneously with SEDDS due to the free energy required, this is either classified as low and positive, or low and negative. It is however compulsory for the interfacial structure to display no resistance against surface shearing in order for emulsification to occur. The ease of emulsification is suggested to be related to the simplicity of water penetration into the various liquid crystal structures or gel phases formed on the surface of the droplet (Gursoy & Benita, 2004).

The self-emulsification times of the different SEDDSs were assessed through testing in a type II Distek 2500 dissolution system apparatus (2501049, North Brunswick, New Jersey, USA, in-house: NWU). A sample from each formulation (1 ml) was added to 100 ml distilled water, maintained at 37°C (± 0.5°C), with the paddle speed set to rotate at 50 rpm for gentle agitation. The time taken for each formulation to form a clear homogenous system after dilution was noted and recorded utilising the following grading system (Basalious *et al*., 2010, Czajkowska-Kośnik *et al*., 2015, Dangre *et al*., 2016):

- Grade 1: Rapidly forming (within 1 min) emulsion, with a clear or bluish appearance;

- Grade 2: Rapidly forming (within 1 min), slightly less clear emulsion, with a bluish white appearance;
- Grade 3: Fine milky emulsion that formed within 2 min;
- Grade 4: Dull, greyish white emulsion having a slightly oily appearance that is slow to emulsify (longer than 2 min);
- Grade 5: Formulation exhibiting either poor or minimal emulsification with large oil droplets on the surface.

3.4.4 Cloud point determination

The cloud point of a SEDDS is defined as the temperature above which an aqueous solution of a water-soluble surfactant, particularly non-ionic, becomes turbid or unclear. This point is an indication of the effective formation of a stable emulsion. Once the temperature to which a SEDDS is exposed to is higher than the cloud point, an irreversible phase separation will occur, and the cloudiness of the preparation will have a detrimental effect on drug absorption due to dehydration of the hydrophilic moiety (i.e. surface active component). Therefore, the cloud point for SEDDS should be higher than 37°C in order to avoid phase separation occurring in the gastrointestinal tract (Agrawal *et al*., 2015; Chudasama *et al*., 2011).

Each fixed-dose combination SEDDS was diluted (1:100) with purified water and the sample was placed in a water bath where the temperature was gradually increased at 5°C increments (or 2°C intervals upon approaching the cloud point). Once the sample became cloudy in appearance, the cloud point was classified (i.e. photos were taken for record purposes) (Agrawal *et al*., 2015; Chudasama *et al*., 2011).

3.4.5 Thermodynamic stability studies

Thermodynamic stability tests are conducted to assess the physical stability of SEDDS. All of the fixed-dose combination SEDDSs were subjected to the following thermodynamic stability tests (Aparna *et al*., 2015, Czajkowska-Kośnik *et al*., 2015):

- Freeze-thaw cycles: Three cycles between refrigerator temperature (i.e. 4°C) and 45°C, with the storage at each temperature for 24 h were conducted. The SEDDS formulations were visually inspected for any physical instability at all of these temperatures.
- Centrifugation test: The SEDDS formulations were centrifuged at 3 500 rpm for 30 min, where after they were examined for phase separation.
- Dilution test: The SEDDS formulations were diluted 100-fold with distilled water and stored at 25°C for 24 h prior to visual inspection for phase separation and precipitation of the active compounds.

3.4.6 Viscosity

Viscosity of the SEDDS formulations was measured without dilution of the samples (10 ml) using a Brookfield® Viscometer model DV-II+ (Stoughton, United States of America). The temperature of the water circulating in the water bath was maintained at 25°C by a Brookfield® temperature controller. A SC4-34 LV spindle and helipath was chosen to ensure optimum torque depending on the consistency of the formulations. The Helipath stand D20733 slowly raises and lowers the viscometer at a rate of 7/8-inches per minute, during which a T-bar spindle rotates in the formulation. The LV spindle was immersed in the formulation without causing a disturbance. Viscosity readings were taken every 10 sec for 5 min at different shear rates (5; 10; 20; 30; 50; 60; and 100 rpm) and approximately 32 measurements were provided at each shear rate from which an average was determined. The rheological behaviour of the dispersed system was analysed by construction of rheograms of shear stress *vs.* shear rate (Jain & Soni, 2012).

3.4.7 Dissolution studies

A Distek® dissolution system, model 2500, (Distek® Inc, North Brunswick, New Jersey), which was connected to the Distek® Evolution 4300 auto sampler, was implemented to determine the pharmaceutical availability of both artemether and lumefantrine from the different SEDDS formulations at a regulated temperature of 37°C (± 0.5°C), and a paddle speed of 100 rpm. This experiment was extended over a period of 12 h and each formulation was tested in 6 fold (Bashaiwoldu *et al*., 2011). The dissolution media initially consisted of 600 ml hydrochloric acid (0.1 M) for the first 120 min in order to maintain a pH value of 1.2. This 120 min period mimics the typical gastric emptying time of the stomach (Obitte *et al*., 2010). A 1.5 ml sample was removed using a syringe attached to a 0.45 µm membrane filter and placed into test tubes at specified time intervals of 5, 13, 21, 30, 60, 90 and 120 min. This volume was immediately replaced with fresh dissolution media of the same temperature (Vertzoni *et al*., 2004). Subsequently, after the 120 min had elapsed, a 0.2 M phosphate buffer (300 ml) was added to increase the pH of the dissolution media to 6.8. The dissolution media volume added up to 900 ml; where 5 min was allocated for adjusting the pH. All pH adjustments were conducted using either a 2 M hydrochloric acid solution or a 2 M sodium hydroxide solution. Further samples were withdrawn at time intervals of 150, 180, 240, 300 min. Lastly, a third dissolution media, containing phospholipids and bile salts, was prepared by dissolving the phospholipids and bile salts in fresh dissolution media. The third dissolution media was added after five and a half hours to achieve a pH of 7.4, this mimicked the ileocecal pH. The increased pH and the fact that the phospholipid acts as a natural surfactant should create desirable conditions to release the remainder of the lumefantrine and artemether in the SEDDS (Obitte *et al.,* 2010).

The samples were then withdrawn at the time intervals of 360, 420, 480 and 500 min, replacing the dissolution media after every withdrawal. Once the last sample was taken, the paddle speed was adjusted to 150 rpm (infinity sample) and stirred for an additional 30 min to ensure maximum drug release. The artemether and lumefantrine concentrations in the samples were measured utilising a validated HPLC method (Section 3.3.1).

3.5 Statistical analysis

A one-way analysis of variance (ANOVA) was used to statistically analyse the collected data in order to indicate whether any significant differences and variations were found ($p < 0.05$). From the data obtained in the dissolution studies, the parameters described in the following sections were calculated.

3.5.1 Mean dissolution time

The average time it takes for the entire drug dose to be released into the solution from the dosage form is classified as the mean dissolution time (MDT). The mean dissolution time for both artemether and lumefantrine was calculated, using Equation 3.1.

$$MDT = \frac{\sum_{j=1}^{n} t_{mid} \Delta x_d}{\sum_{j=1}^{n} \Delta x_d} \qquad \textbf{(3.1)}$$

From the equation 3.1 the following can be deduced: j is the sample number, n, is the total number of samples, t_{mid}, is the midpoint between j and j-1, and Dx_d, is the additional mass of drug dissolved between j and j-1 (Costa & Lobo, 2001).

3.5.2 Fit factors

Fit factors were used as a way to compare the dissolution profiles of the sample formulations and a control formulation. The fit factor, f_1, is classified as the difference factor, which determines the percentage error between the two curves. Curves that are indistinguishable from one another were represented with a value of 0, and as the value increases so does the variation between the two curves. Ideally, the f_1-value should be ≤ 15, as this indicates that the amount of time taken to dissolve the drug correlates for both the sample and control formulations. Fit factor, f_2, is an indication of the similarity factor between the two curves and if the value is ≥ 50 it indicates that both the sample and control formulations are fairly similar. If the value obtained for f_2 is equal to 100, this indicates that the two curves are identical. The

following equations (Equation 3.2 and 3.3) were used to calculate the fit factors, f_1 and f_2 (Moore & Flanner, 1996).

$$f_1 = \frac{\sum_{j=1}^{n} |R_J - T_J|}{\sum_{j=1}^{n} (R_J - T_J)/2} \times 100 \quad \textbf{(3.2)}$$

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{j=1}^{n} |R_J - T_J|^2\right]^{-0.5} \times 100\right\} \quad \textbf{(3.3)}$$

Where: R_j is the reference assay at time point. T_J, is the test assay at time point., and n is the number of pull points.

3.5.3 Mathematic modelling

The purpose of mathematic modelling is to simplify an already complex drug release profile, and to understand the release mechanisms of both artemether and lumefantrine from the SEDDS formulations used in this study. A computer program, namely DDSolver, was used to fit the release kinetics of both artemether and lumefantrine to a specific model (Zhang *et al.,* 2010).

CHAPTER 4

RESULTS

4.1 Introduction

Successful application of self-emulsifying drug delivery systems (SEDDSs) depends on the appropriate selection of oils and surfactants in which lumefantrine and artemether are soluble and chemically compatible, i.e. stable in. This chapter deals with the results obtained from the various experiments that were executed in order to determine the drug content, stability and dissolution properties of the different SEDDSs. The solubility of lumefantrine and artemether, in the various selected oils chosen, were first off established. These experiments were followed by determining the optimal ratio of oil to surfactant, in order to formulate the most optimal SEDDSs for the fixed-dose combination of artemether and lumefantrine. After the most optimal SEDDS formulations were chosen, they were characterised utilising the following tests: zeta potential and droplet size; viscosity; cloud point; self-emulsification time; assay; and pharmaceutical availability of the two drugs. The physical stability of the SEDDS formulations was also determined through these experiments. The surfactant and co-surfactant combinations used are: Tween® 80 and Span® 80; Tween® 80 and Span® 60; SLS (SLS) and Span® 80; and SLS and Span® 60. These surfactant/co-surfactant mixtures were combined in a set ratio of 1:1 and termed the “surfactant phase”. The different surfactant phases were subsequently tested in various ratios with the selected oils (i.e. coconut-, olive-, peanut-, avocado-, and castor oil).

4.2 Preformulation studies

4.2.1 Infrared spectrum for artemether and lumefantrine

IR spectroscopy is used to identify various functional groups as different functional groups have different IR spectra. These differences include changes in frequency, intensity, band contours and the number of bands present on the IR spectrum (Hachula, 2018). Thus, IR spectroscopy is a reliable method of identifying different samples by comparing their subsequent wavelengths and intensities.

Figure 4.1 depicts the IR spectra of a standard reference of artemether as well as the sample artemether utilised in this study. From this figure it is clear that the peaks as well as the

intensity of the peaks between the two samples are similar, thus confirming that no impurities were present in the study sample during analysis. The IR spectra of a standard reference of lumefantrine and a sample of the lumefantrine used in this study were also compared as depicted in Figure 4.2. Again, it was found that the lumefantrine contained no impurities as the peaks elucidated at approximately the same wavelength with approximately the same intensity.

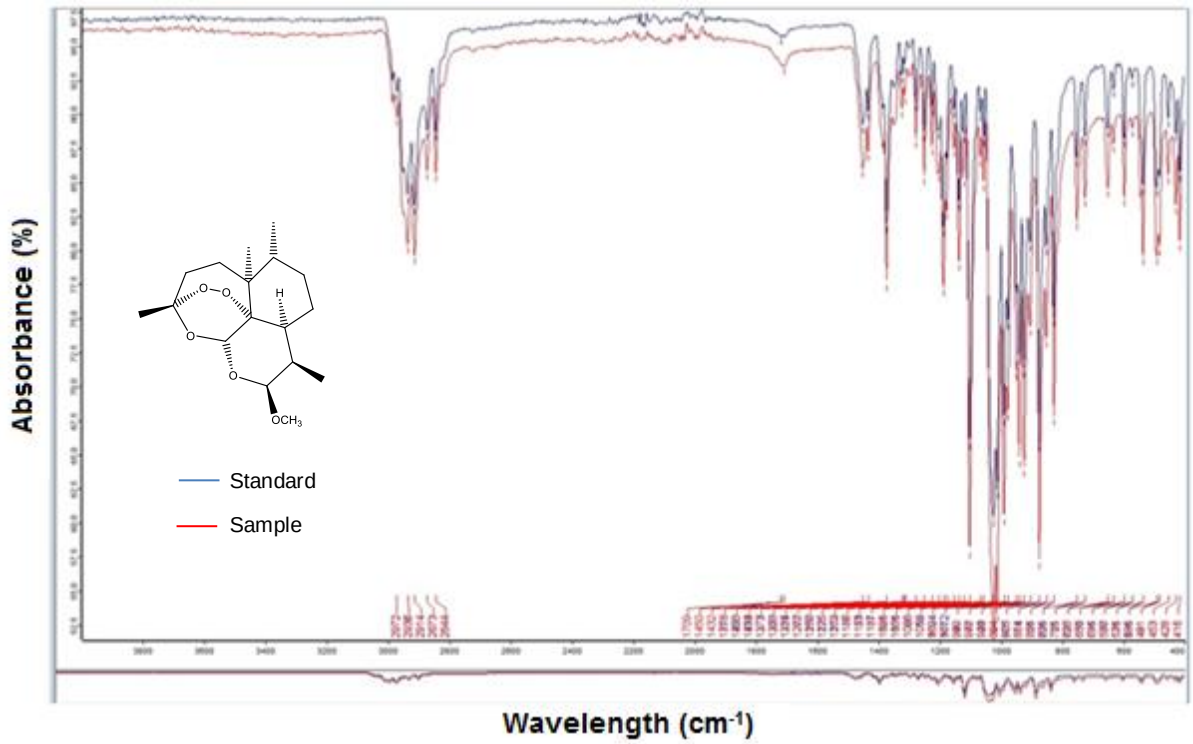


Figure 4.1: *IR spectra of artemether and the reference standard utilised*

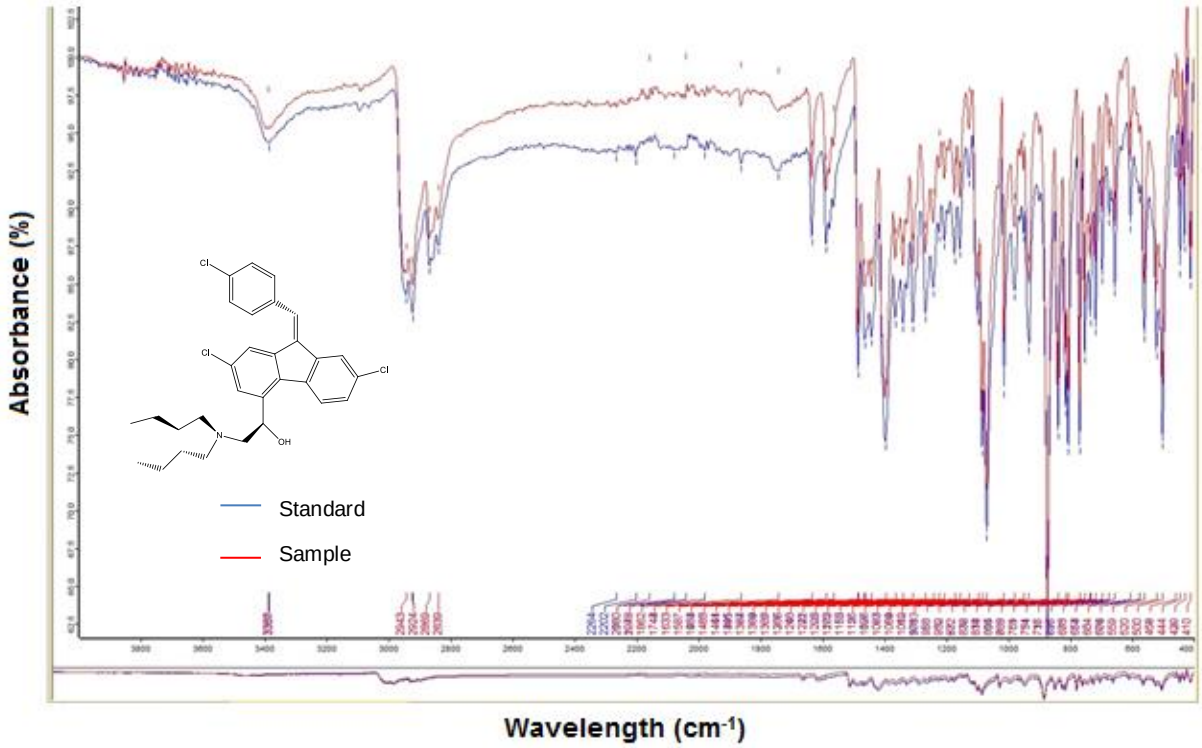


Figure 4.2: *IR spectra of lumefantrine and the reference standard utilised*

4.2.2 High performance liquid chromatography method validation of artemether and lumefantrine

4.2.2.1 Introduction

The word validation is derived from the Latin word *validus*; denoting: strong; and implies that through various tests something has to be proved to be of adequate standard, true and useful (Araujo, 2009; Rozet *et al*., 2007; Stöckl *et al*., 2009). The ICH requires that the validation is confirmed through examination and objective evidence that the set requirements were fulfilled (Stöckl *et al*., 2009). Therefore, the chromatographic method described in chapter 3.2.1 was validated, in order to verify whether this analytical method is reliable and sensitive.

4.2.2.2 Specificity

Specificity for lumefantrine and artemether was determined by comparing the chromatograms of the active ingredients with chromatograms obtained for hydrochloric acid and phosphate buffer, respectively. This was done to determine whether any interference occurred between these products (Figure 4.3).

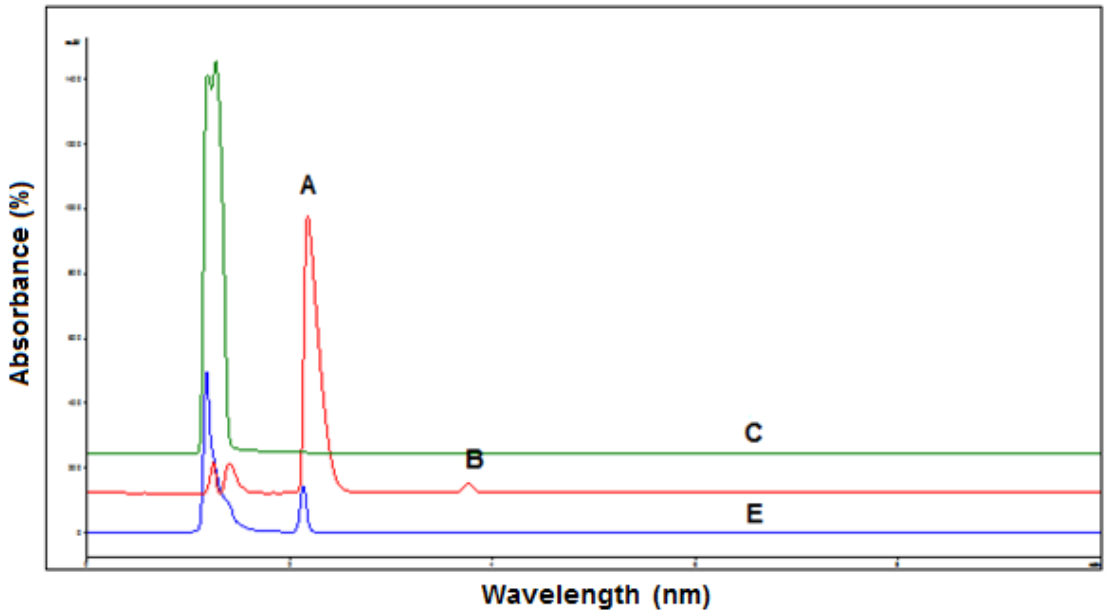


Figure 4.3: *High performance liquid chromatography (HPLC) chromatogram of artemether (B) and lumefantrine (A) in the presence of hydrochloric acid (E) and phosphate buffer (C)*

From the chromatogram it is evident that the active ingredient, artemether, does not seem to display any interference with hydrochloric acid or the phosphate buffer. No interference was noted between lumefantrine and the phosphate buffer. However, a slight interference is seen between the active ingredient, lumefantrine, and the hydrochloric acid. Nonetheless, the experiment is still deemed acceptable due to the fact that it is a negligible small difference in interference, and thus should not have any significant effect on the dissolution experiments conducted.

4.2.2.3 Linearity

The peak areas for both lumefantrine and artemether were determined by preparing a standard solution containing both these active ingredients and injecting them in three different concentrations and at three different injection volumes, as described in the method section (Chapter 3, Section 3.2.4.3.2). Each solution was injected in duplicate. The peak areas for lumefantrine and artemether are presented in Tables 4.1 and 4.2, respectively.

Table 4.1: *Peak areas obtained for a series of lumefantrine standard solutions*

Standard Solution (µg/ml)	Injection volume (µl)	Final Concentration (µg/ml)	Peak Area (mAu)		Average Peak Area (mAu)
2.51	2.5	0.628	0.308	0.293	0.300
	5.0	1.255	0.606	0.619	0.613
	7.5	1.883	0.945	0.000	0.473
	10.0	2.510	1.272	1.260	1.266
25.1	2.5	6.275	3.098	3.075	3.086
	5.0	12.550	6.326	6.297	6.312
	7.5	18.825	9.463	9.453	9.458
	10.0	25.100	12.701	12.675	12.688
251	2.5	62.750	31.205	31.291	31.248
	5.0	125.500	63.483	63.545	63.514
	7.5	188.250	95.303	95.080	95.192
	10.0	251.000	126.972	126.767	126.869

The chromatographic results attained were utilised to establish if a linear relationship exists between peak area and concentration when employing the suggested HPLC method. A clear trend could be observed in both Tables 4.1 and 4.2 indicating that, as the peak areas increased, the artemether and lumefantrine concentrations subsequently increased. The peak area values acquired for the duplicate injections at each injection volume did not display any significantly large variations; therefore, indicating that a high level of repeatability existed. Additionally, a linear regression graph of both artemether (Figure 4.4) and lumefantrine (Figure 4.3) could be constructed by plotting the peak area versus concentration values.

Table 4.2: *Peak areas obtained for a series of artemether standard solutions*

Standard Solution (µg/ml)	Injection volume (µl)	Final Concentration (µg/ml)	Peak area (mAu)		Average Peak Area (mAu)
2.5	2.5	0.625	0.000	0.000	0.000
	5.0	1.250	0.010	0.010	0.010
	7.5	1.875	0.000	0.000	0.000
	10.0	2.500	0.000	0.000	0.000
25	2.5	6.250	0.060	0.063	0.062
	5.0	12.500	0.123	0.123	0.123
	7.5	18.750	0.176	0.179	0.178
	10.0	25.000	0.231	0.235	0.233
250	2.5	62.500	0.560	0.555	0.557
	5.0	125.000	1.113	1.119	1.116
	7.5	187.500	1.672	1.659	1.665
	10.0	250.000	2.215	2.210	2.213

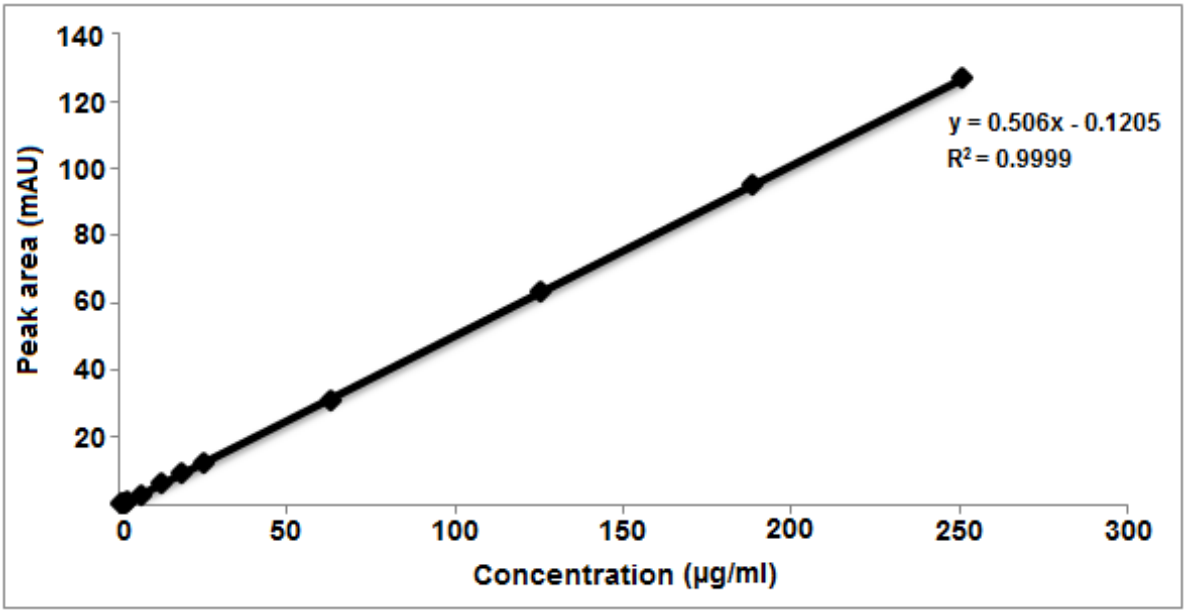


Figure 4.4: *Linear regression curve for lumefantrine standard solutions*

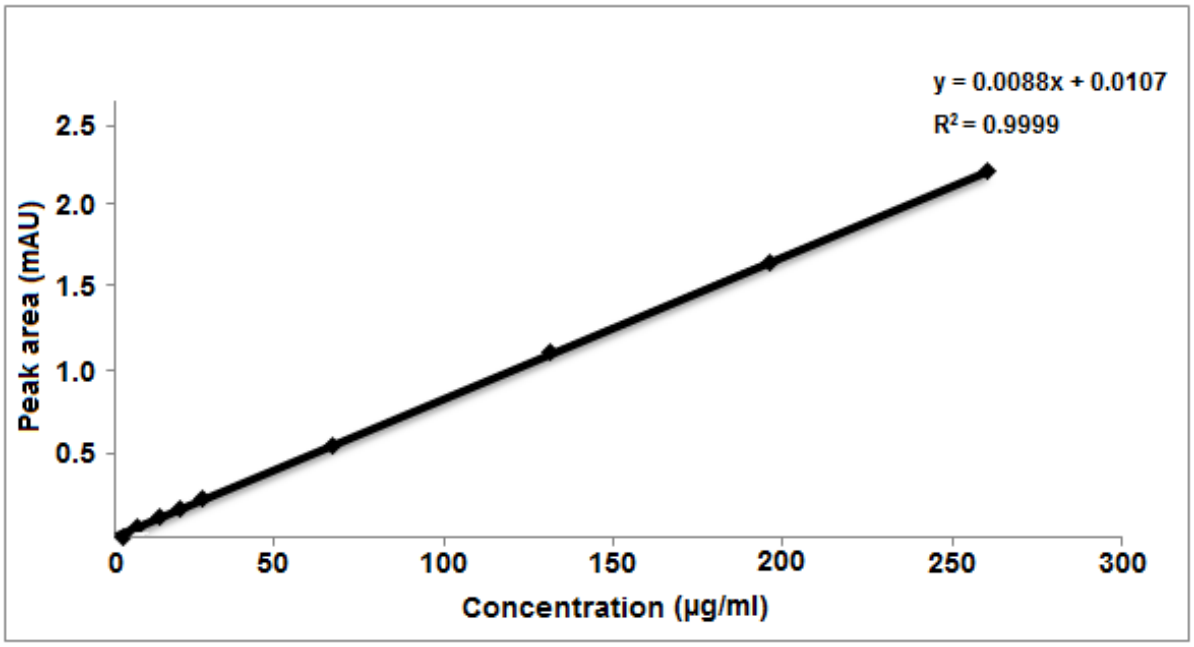


Figure 4.5: *Linear regression curve for artemether standard solutions*

In order for any data to be considered linear, the linear regression analysis should yield an R^2 value as close to 1 as possible, however, a 0.995 R^2 value is also deemed acceptable (Araujo, 2009). From Figures 4.4 and 4.5 it is evident that both lumefantrine and artemether depicted R^2 values of 0.9999, thus meeting the required criteria.

4.2.2.4 Accuracy

Standard solutions of lumefantrine and artemether were prepared and consequently diluted (133, 66, and 33 µg/ml for artemether; and 309, 155, and 77 µg/ml for lumefantrine) in order to compare the results to a sample solution containing the active ingredients and excipients to be used in the study. Accuracy is determined to establish how reliable the test results are to that of the true value. The acceptable criteria for accuracy, according to the Food and drug administration (FDA), is that the percentage recovery should be between 98–102%. The %RSD should be lower than 15 (FDA, 2001; ICH, 2005). Results obtained for lumefantrine and artemether are tabled in Table 4.3 and Table 4.4, correspondingly. From these tables it could be concluded that the %RSD for artemether and lumefantrine are 5.8% and 1.0%, respectively.

Table 4.3: *Accuracy results for artemether*

Concentration Spiked			Recovery		
Standard Solution (µg/ml)	**Peak area (mAu)**		**Mean peak area (mAu)**	**µg/ml**	**%**
133.900	136.145	139.234	137.745	136.434	101.927
132,700	137.000	133.183	135.177	133.837	100.976
132.500	140.884	130.735	135.863	134.574	101.565
66.900	64.841	65.371	65.164	64.568	96.316
66.300	71.774	80.411	76.116	75.434	113.636
66.250	67.113	63.721	65.435	64.875	97.886
33.425	29.447	33.524	31.535	31.278	93.376
33.175	38.253	33.154	35.718	35.353	106.577
33.125	36.722	36.455	36.679	36.245	109.443
				Mean	101.547
				SD[1]	5.943
				%RSD[2]	5.811

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

Table 4.4: *Accuracy results for lumefantrine*

Concentration Spiked			Recovery		
Standard Solution (µg/ml)	**Peak area (mAu)**		**Mean peak area (mAu)**	**µg/ml**	**%**
309.100	17997.333	18128.913	18063.112	309.747	100.253
310.400	18131.854	18065.324	18098.646	310.357	100.057
310.900	18089.647	18124.143	18106.937	310.439	99.957
154.550	9064.085	9027.147	9045.657	155.146	100.395
155.200	8990.746	8986.327	8988.536	154.196	99.336
155.450	9040.943	9057.643	9049.327	155.362	99.846
77.275	4402.000	4382.336	4392.238	75.354	97.400
77.600	4418.046	4426.016	4422.035	75.825	97.754
77.725	4438.948	4502.234	4470.612	76.675	98.600
				Mean	99.256
				SD	1.000
				%RSD	1.000

1. Standard deviation (SD)
2. Relative standard deviation (%RSD)

4.2.2.5 Precision

Intra-day variation

Precision determines the repeatability or reproducibility of the HPLC method used. The requirements remain the same as for accuracy, where the %RSD should be lower than 15 (FDA, 2001; ICH, 2005). Intra-day precision was determined for both artemether and lumefantrine by injecting three samples over a period of a day and calculating the %RSD for each active ingredient. Tables 4.5 and 4.6 display the intra-day precision results for artemether and lumefantrine, respectively, where it is clear that both the active ingredients' results adhered to the stated criteria.

Table 4.5: *Intra-day precision results for artemether*

Concentration Spiked				**Recovery**	
Standard Solution (µg/ml)	**Peak area (mAu)**		**Mean peak area (mAu)**	**µg/ml**	**%**
60.000	393.500	437.240	415.371	62.023	103.370
60.942	379.717	384.650	382.185	58.167	95.450
59.770	469.087	471.519	470.303	68.407	114.450
50.000	418.224	402.271	410.248	61.428	122.860
50.599	435.200	428.000	431.601	63.909	126.310
50.819	311.273	311.400	311.337	49.934	98.260
39.947	300.801	274.731	287.765	47.194	118.140
39.9147	172.181	128.501	150.341	31.225	78.230
42.248	201.462	200.519	200.990	37.110	87.840
				Mean	104.990
				SD	15.580
				%RSD	14.840

Artemether contains chromophores that display poor absorption of UV light and lack functional groups, rendering it difficult to detect reliable peaks on the chromatogram (White *et al.,* 1999). Thus, the FDA states that in such a case, the %RSD can be lower than 15 (FDA, 2001; ICH, 2005).

Table 4.6: *Intra-day precision results for lumefantrine*

Concentration Spiked				Recovery	
Standard solution (µg/ml)	**Peak area (mAu)**		**Mean peak area (mAu)**	**µg/ml**	**%**
359.000	10187.223	10099.367	10143.339	350.454	97.600
358.000	9359.532	10027.759	9693.645	334.837	93.523
358.500	9398.865	9376.001	9387.412	324.253	90.423
298.000	8621.541	8025.109	8323.346	287.387	96.457
299.000	8050.377	8100.376	8075.337	278.748	93.276
298.000	7708.381	7710.587	7709.457	266.108	89.300
239.000	7468.9	7454.590	7461.734	257.500	107.768
238.500	6529.311	6490.123	6509.744	224.576	94.100
238.000	6661.929	6753.899	6707.911	231.436	97.234
				Mean	95.512
				SD	5.122
				%RSD	5.343

Inter-day variation

Inter-day variation needs to be conducted over a three day time period, where the repeatability of the data is determined. In order for this test to prove repeatability of the method used, it is vital that the same conditions are maintained during the entire testing period and thus, the same equipment is also used. A fresh batch of samples was prepared each day, as previously described in intra-day variation, and the samples were injected into the HPLC in duplicate. Table 4.7 portrays the results acquired for the two active ingredients, individually. Both artemether and lumefantrine adhered to the set requirements (%RSD≤15).

Table 4.7: *Inter-day precision results for artemether and lumefantrine*

		Concentration spiked				Recovery				
		Standard solution (µg/ml)	Peak area (mAu)		Mean peak area (mAu)	µg/ml	%	Mean peak area (mAu)	SD	%RSD
Artemether	Day 2	50.000	233.800	241.880	237.842	45.542	89.912	99.050	8.080	8.160
		46.150	227.498	249.202	238.354	45.135	97.635			
		50.000	292.417	287.135	289.781	54.832	109.600			
	Day 3	50.000	558.621	581.164	569.893	51.675	103.387	98.230	3.920	3.990
		50.000	523.234	510.981	517.182	46.825	93.799			
		46.000	505.344	486.174	495.784	44.957	97.700			
Lumefantrine	Day 2	305.000	5647.102	5676.933	5662.032	320.125	104.976	101.507	2.500	2.400
		320.750	5691.424	5623.822	5657.643	319.824	99.700			
		325.000	5712.522	5755.034	5733.711	324.176	99.747			
	Day 3	319.500	5787.074	5810.094	5798.551	334.254	104.647	103.300	1.500	1.500
		320.700	5791.153	5797.086	5794.111	333.979	104.132			
		325.100	5679.388	5736.625	5708.000	328.953	101.254			

4.2.2.6 Limit of quantification (LOQ) and limit of detection (LOD)

The LOD and LOQ are deemed acceptable for an active ingredient if the %RSD is below 30 for the LOD and if the %RSD is below 10 for LOQ (da Costa César *et al*., 2008; ICH Q2(R1), 2005; Katsidzira *et al.,* 2016). Results obtained for LOQ and LOD experiments conducted for both artemether and lumefantrine are listed in Tables 4.8 and 4.9, respectively. Artemether produced a LOQ of 2.628 µg/ml (%RSD≤10) and a LOD of 1.051 µg/ml (%RSD≤30); whereas lumefantrine displayed a LOQ of 0.105 µg/ml (%RSD ≤10) and a LOD of 0.525 µg/ml (%RSD≤30). Therefore, in conclusion, both active ingredients adhere to the set criteria for LOD and LOQ.

Table 4.8: *LOD/LOQ results obtained for artemether*

Injection Volume (µl)	1.000	2.500	5.000	7.500
Concentration (µg/ml)	1.051	2.628	5.255	7.883
Peak area (mAu)	0.490	1.272	2.337	4.000
	0.488	1.186	2.432	3.600
	0.437	1.194	2.163	4.000
	0.490	1.289	2.416	3.900
	0.441	1.268	2.560	4.600
	0.406	1.195	2.365	4.195
Mean	0.460	1.230	2.380	4.050
SD	0.030	0.040	0.120	0.300
%RSD	7.130	3.460	5.010	7.500
LOQ = 2.628 µg/ml (%RSD≤10)				
LOD = 1.051 µg/ml (%RSD≤30)				

Table 4.9: *LOD/LOQ results obtained for lumefantrine*

Injection Volume (µl)	1.000	2.500	5.000	7.500
Concentration (µg/ml)	1.000	2.500	5.000	7.500
Peak area (mAu)	0.105	0.263	0.525	0.788
	3.200	8.500	17.100	25.600
	3.200	8.500	16.900	25.600
	3.600	8.400	17.200	25.600
	3.300	8.600	17.100	25.900
	3.400	8.800	17.300	25.700
Mean	3.400	8.520	17.120	25.650
SD	0.190	0.160	0.120	0.130
%RSD	5.630	1.850	0.710	0.490
LOD = 0.105µg/ml (%RSD≤10)				
LOQ = 0.525µg/ml (%RSD≤30)				

4.2.3 Isothermal microcalorimetry

Compatibility studies between artemether and lumefantrine, and the different excipients used in the SEDDS formulations were performed by means of isothermal microcalorimetry. Calorimetry refers to measuring techniques that are used for direct determination of the rate of heat production, heat, and heat capacity as a function of temperature and time (Wadsö, 2010). Microcalorimetry is a robust method for determining incompatibilities and instabilities between active pharmaceutical ingredients and/or excipients. This method is a credible way of detecting incompatibilities, because almost all physical and chemical processes are accompanied by heat exchange. Therefore, it is sensitive to all physical and chemical processes associated with heat flow. The high sensitivity of this method renders it possible to conduct measurements at temperatures close to real conditions and to detect noticeably slow reactions. Heat flow data will contain contributions from either one process, or several processes. To be able to distinguish specific contributions, careful experimental planning is

required, as well as sufficient background knowledge pertaining to the sample being analysed (Wadsö, 2010).

When calculating the heat flow of the various components in order to determine whether any interactions were detected, it is of utmost importance to first establish a baseline. The baseline is calculated by individually measuring the heat flow of each component. Subsequently, artemether, lumefantrine and the various oils and surfactants used were weighed and mixed in a 1:1:1:1:1 ratio. The various combinations were then placed in the Thermal Activity Monitor (TAMIII) apparatus (TA Instruments, New Castle, Delaware, USA) for 24 h and the temperature was maintained at 50°C. Subsequently, each of the combinations were compared to the baseline values. The calorimetric output of the individual components is summarised as the hypothetical response. This hypothetical response is the anticipated calorimetric output if the two components measured do not interact with each other. If an interaction is observed, the observed calorimetric output will differ remarkably from the hypothetical response. Moreover, an interaction between the two components will be detected if the change in heat flow from the observed heat flow, compared to the hypothetical response is higher than 100 µW/g.

Figures 4.6–4.9 depict the observed heat flow versus the hypothetical response for olive oil in combination with artemether, lumefantrine and the selected surfactants. The interaction heat flow for the various graphs are: 5.37 ± 6.55 µW/g; 1.15 ± 1.49 µW/g; 24.75 ± 21.52 µW/g; and 7.12 ± 6.58 µW/g, respectively. All of the acquired interaction heat flow results are below 100 µW/g. It can therefore be concluded that no interaction was observed between olive oil and the various components.

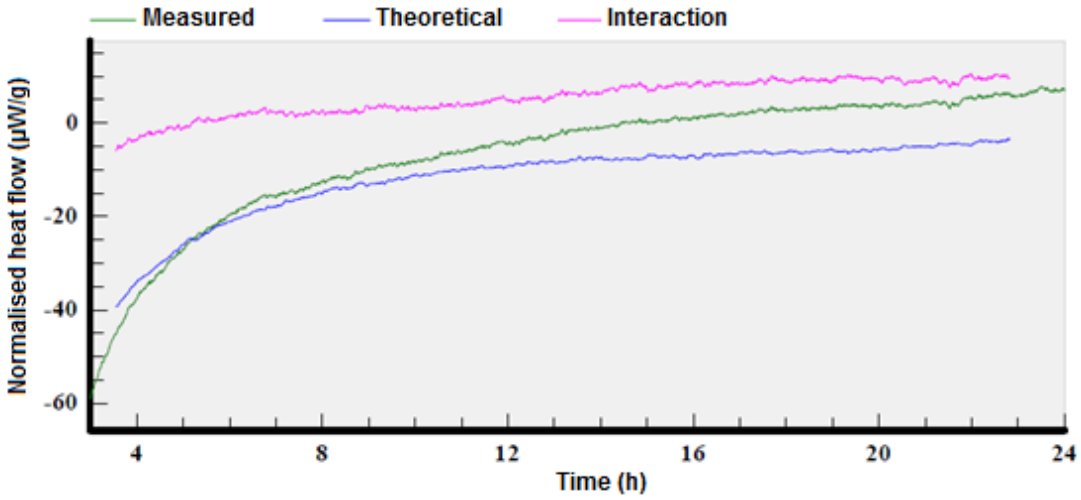


Figure 4.6: *Heat flow data obtained with the combination of artemether, lumefantrine, olive oil, Tween® 80 and Span® 60 in a 1:1:1:1:1 ratio*

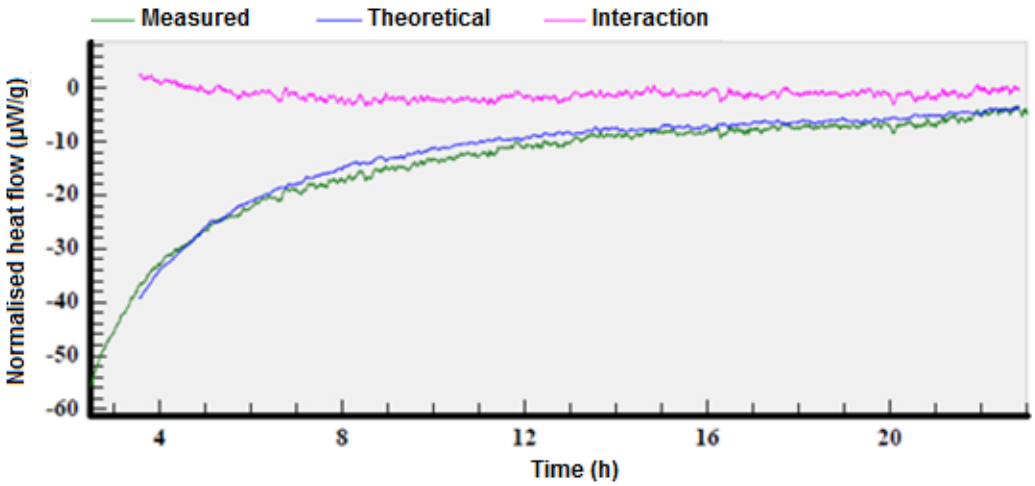


Figure 4.7: *Heat flow versus time graph obtained for an artemether, lumefantrine, olive oil, Tween® 80 and Span® 80 in a 1:1:1:1:1 ratio*

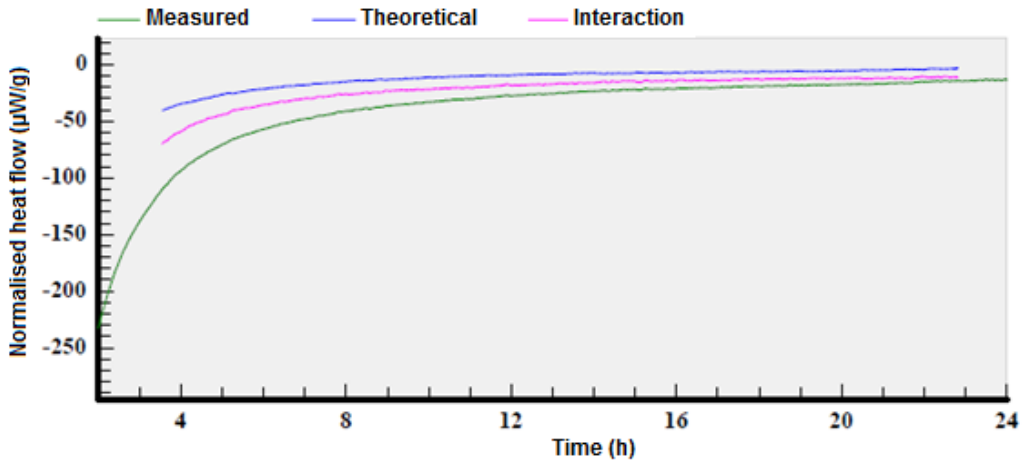


Figure 4.8: *Heat flow versus time graph obtained for a combination of artemether, lumefantrine, olive oil, SLS and Span® 60 in a 1:1:1:1:1 ratio*

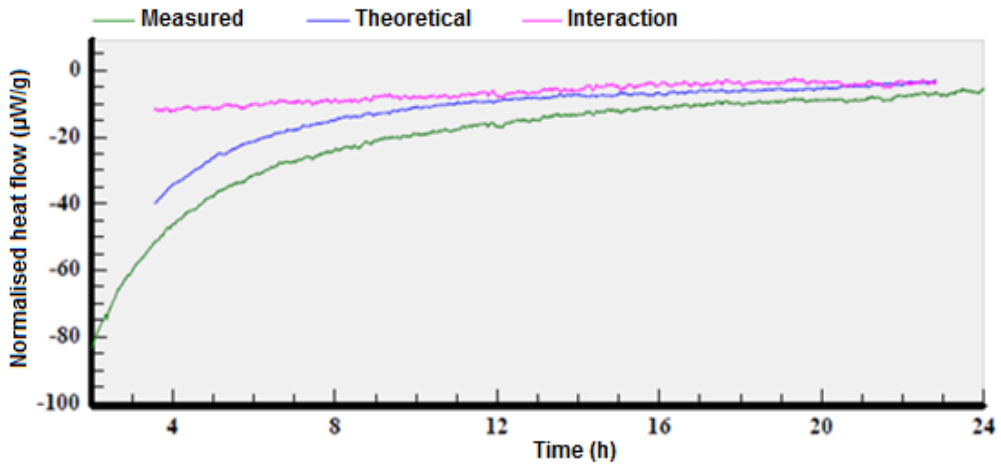


Figure 4.9: *Heat flow versus time graph obtained for a combination of artemether, lumefantrine, olive oil, SLS and Span® 80 in a 1:1:1:1:1 ratio*

In a similar fashion, avocado oil's heat flow measurement was compared to artemether, lumefantrine, and the selected surfactants to ascertain whether any interactions coexist between these various components. Figures 4.10–4.13 exhibit the observed normalised heat flow versus the hypothetical response. The interaction heat flow values attained for the different combinations are: 4.78 ± 4.15 µW/g; 9.58 ± 3.85 µW/g; 14.12 ± 1.42 µW/g; and 19.91 ± 19.52 µW/g, individually. These interaction heat flow values are, according to compatibility studies, noted as slightly high values. However, because all of the interaction heat flow values remain below 100 µW/g; and due to the fact that no trough is seen on the interaction curve, no incompatibilities could be identified, and the combinations are considered compatible.

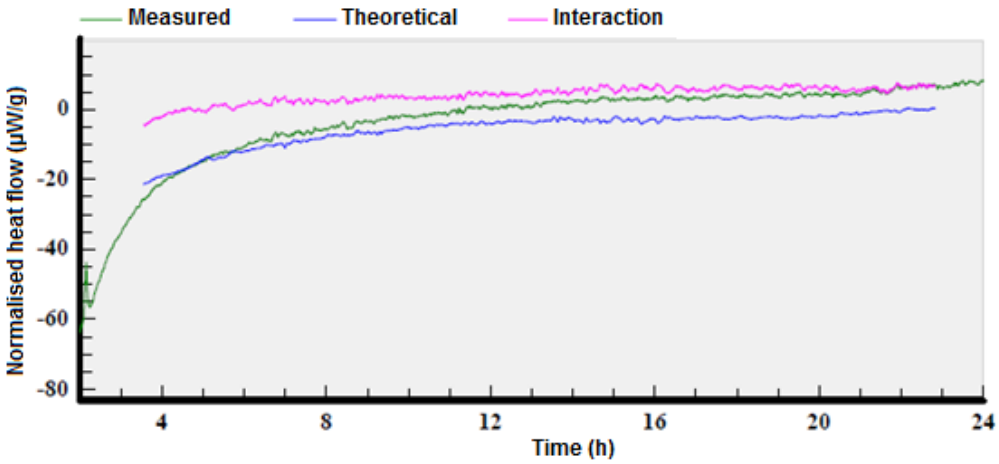


Figure 4.10: *Heat flow versus time graph obtained for artemether, lumefantrine, avocado oil, Tween® 80 and Span® 80 combination in a 1:1:1:1:1 ratio*

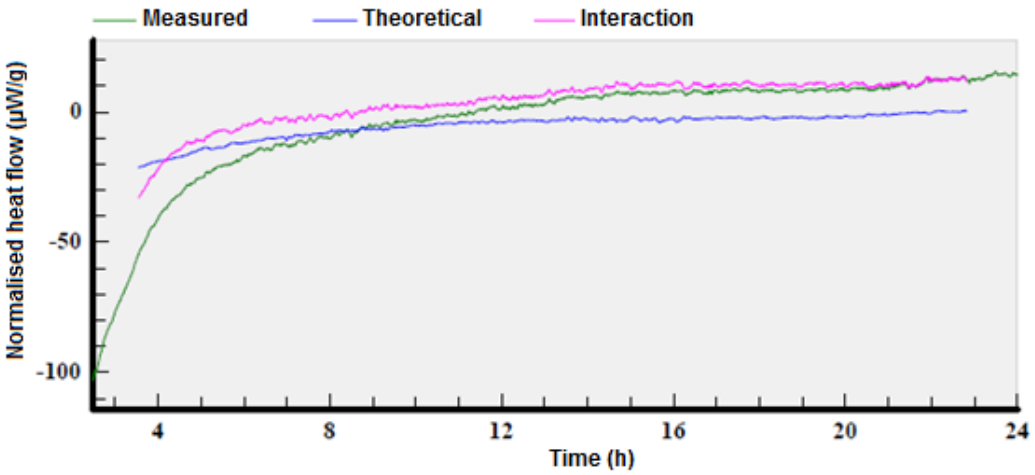


Figure 4.11: *Heat flow versus time graph obtained for artemether, lumefantrine, avocado oil, Tween® 80 and Span® 60 combination in a 1:1:1:1:1 ratio*

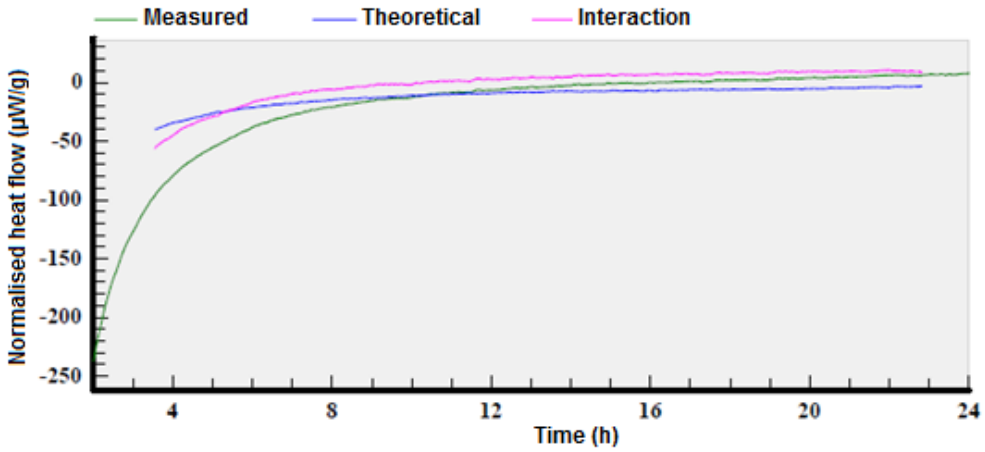


Figure 4.12: *Heat flow data obtained for artemether, lumefantrine, avocado oil, SLS and Span® 60 combination in a 1:1:1:1:1 ratio*

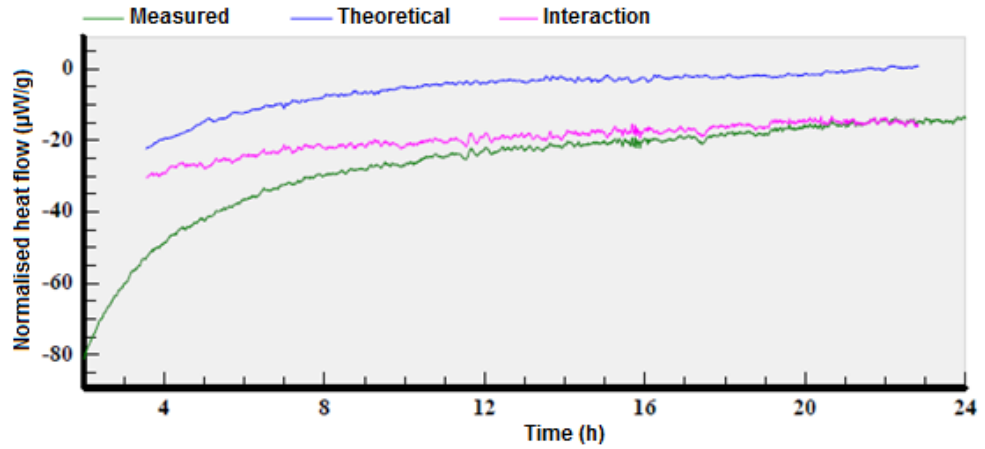


Figure 4.13: *Heat flow data obtained for artemether, lumefantrine, avocado oil, SLS and Span® 80 combination in a 1:1:1:1:1 ratio*

The interaction heat flow results for combinations of castor oil, artemether, lumefantrine, and the selected surfactants are illustrated in Figures 4.14-4.18. Interaction heat flow values achieved for Figures 4.16-4.18 are 9.70 ± 11.4 µW/g, 20.58 ± 27.06 µW/g, and 1.29 ± 4.02 µW/g, respectively, which indicated that no incompatibilities were detected. Data obtained for the castor oil, artemether, lumefantrine, Tween® 80 and Span® 60 combination indicated that some degree of incompatibility exists between the various components (Figure 4.14) as the average interaction heat flow measured is 3.99 ± 5.36 mW/g. In microcalorimetry this is considered a relatively high value, however, this experiment was deemed inconclusive. Thus, in order to confirm whether any incompatibilities truly do exist between any of the aforementioned components, various combinations of the original mixtures' components were further investigated. This combination was also repeated to ensure the accuracy of the initial results. Figure 4.15 depicts the repeated heat flow

experiment where the interaction heat flow was calculated as 4.92 µW/g with an interaction error of 9.93 µW/g, thus signifying that no incompatibility exists between the various components.

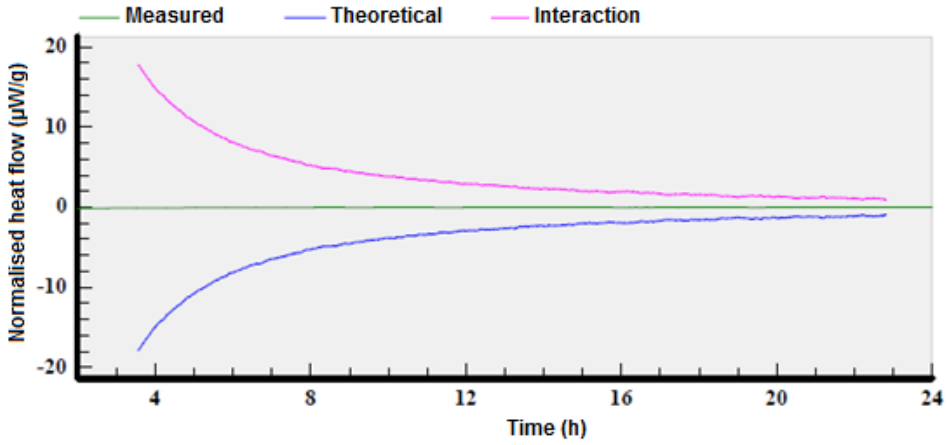


Figure 4.14: *Heat flow data obtained for artemether, lumefantrine, castor oil, Tween® 80 and Span® 60 combination in a 1:1:1:1:1 ratio*

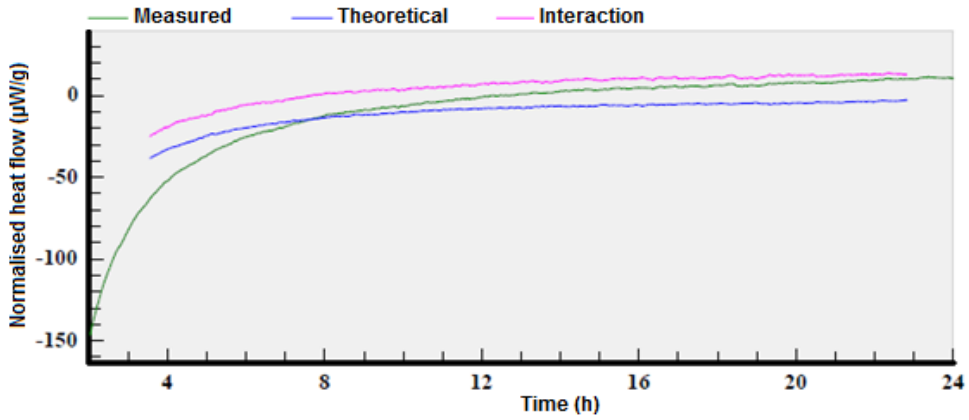


Figure 4.15: *Heat flow data obtained for artemether, lumefantrine, castor oil, Tween® 80 and Span® 60 combination in a 1:1:1:1:1 ratio*

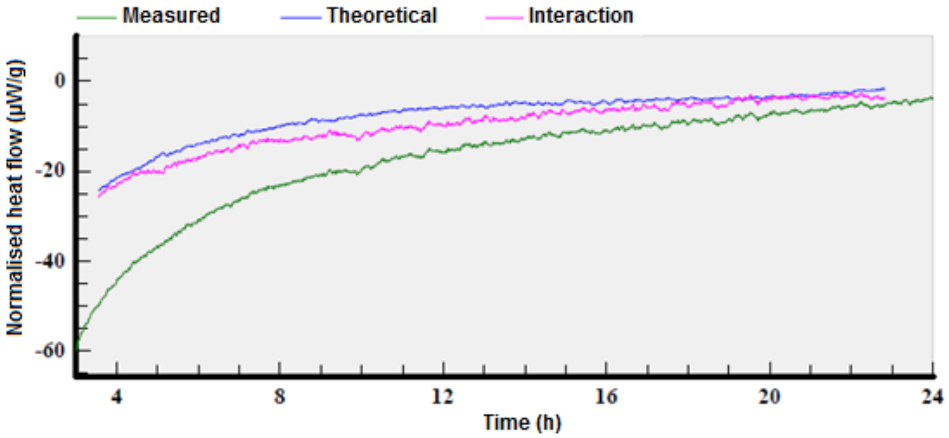


Figure 4.16: *Heat flow data obtained for artemether, lumefantrine, castor oil, Tween® 80 and Span® 80 combination in a 1:1:1:1:1 ratio*

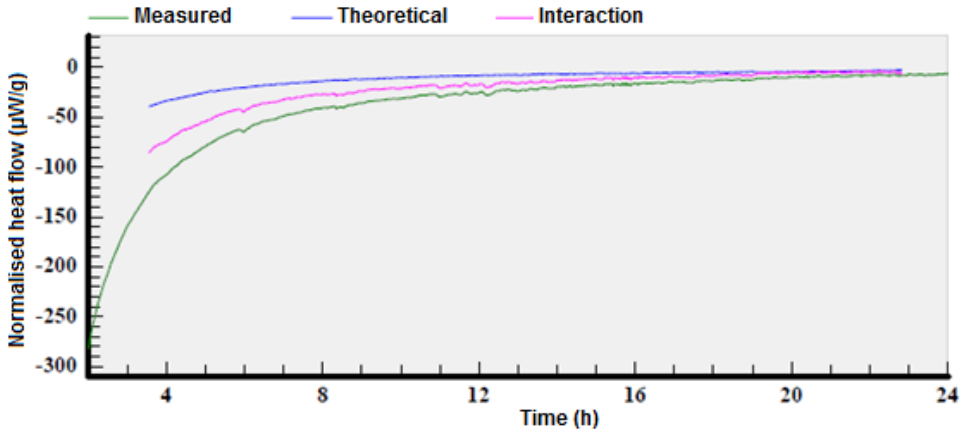


Figure 4.17: *Heat flow data obtained for artemether, lumefantrine, castor oil, SLS and Span® 60 combination in a 1:1:1:1:1 ratio*

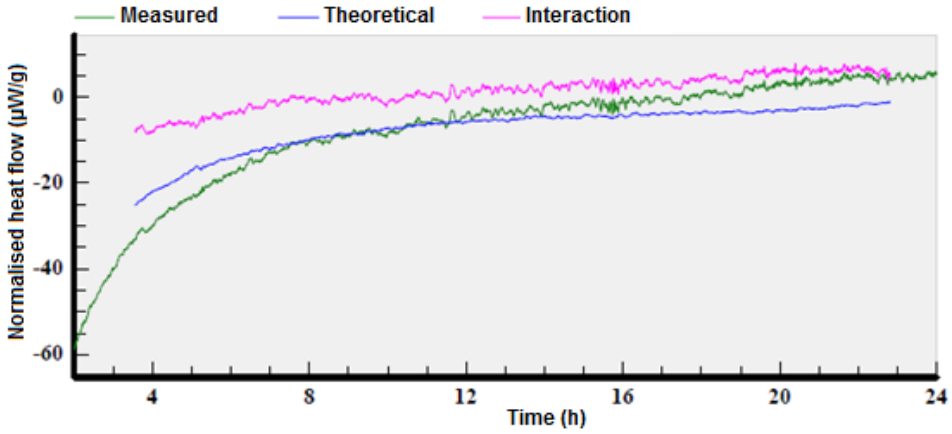


Figure 4.18: *Heat flow data obtained for artemether, lumefantrine, castor oil, SLS and Span® 80 combination in a 1:1:1:1:1 ratio*

The various combinations of peanut oil, artemether, lumefantrine, and selected surfactants are exhibited in Figures 4.19–4.22. No incompatibilities were detected as the interaction heat flow values calculated for each combination are: 698.1 nW/g ± 9.24 nW/g, 16.91 ± 17.31 µW/g, 21.57 ± 24.54 µW/g, and 8.77 ± 9.34 µW/g, respectively. These values are once again below 100 µW/g, thus confirming that these components are compatible with one another.

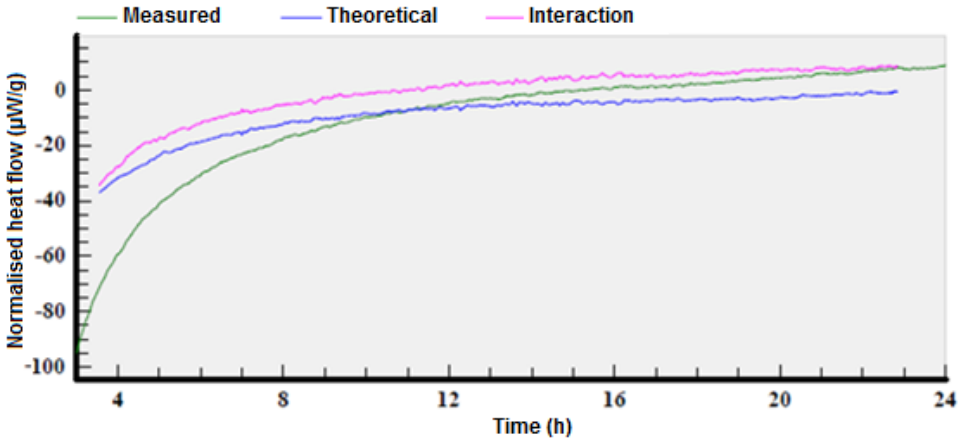


Figure 4.19: *Heat flow data obtained for artemether, lumefantrine, peanut oil, Tween® 80 and Span® 60 combination in a 1:1:1:1:1 ratio*

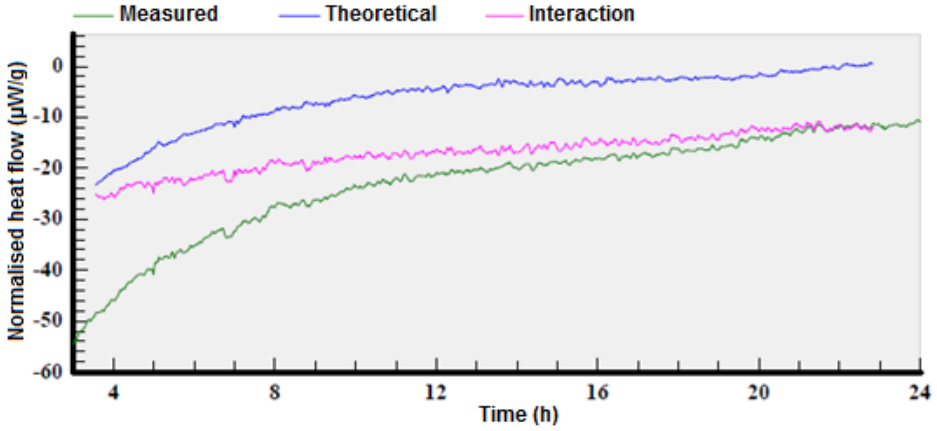


Figure 4.20: *Heat flow data obtained for artemether, lumefantrine, peanut oil, Tween® 80 and Span® 80 combination in a 1:1:1:1:1 ratio*

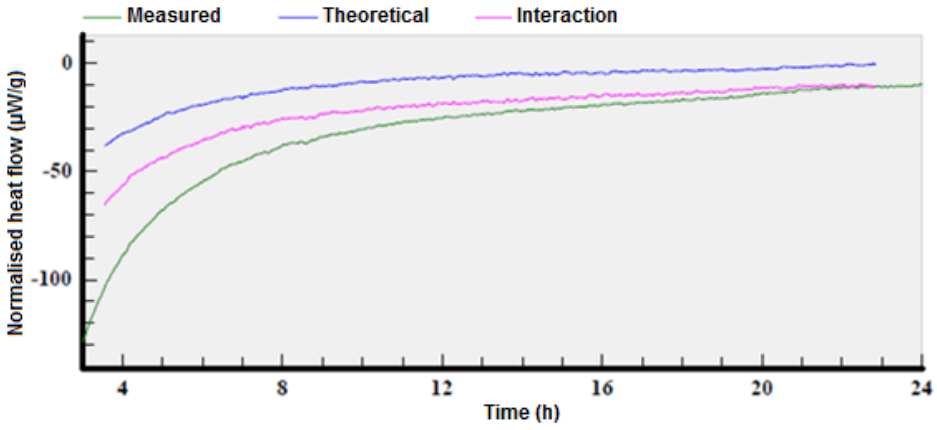


Figure 4.21: *Heat flow data obtained for artemether, lumefantrine, peanut oil, SLS and Span® 60 combination in a 1:1:1:1:1 ratio*

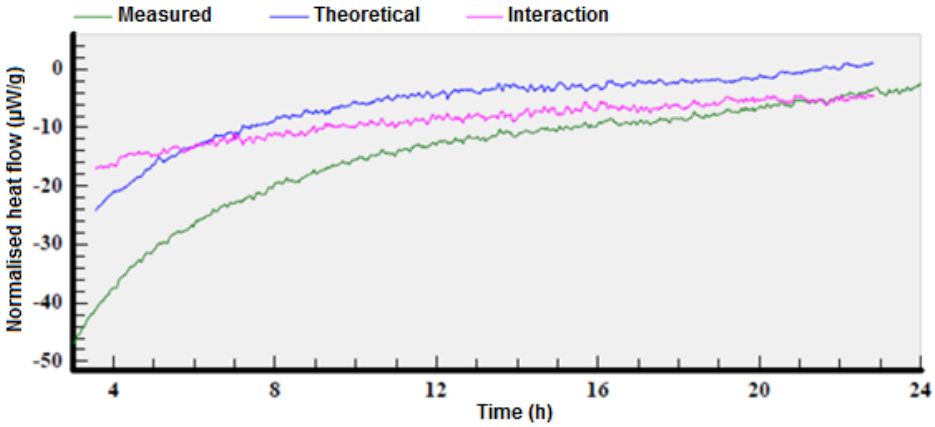


Figure 4.22: *Heat flow data obtained for artemether, lumefantrine, peanut oil, SLS and Span® 80 combination in a 1:1:1:1:1 ratio*

The interaction heat flow data of coconut oil, artemether, lumefantrine, and selected surfactant combinations were measured and are portrayed in Figures 4.23-4.26. The interaction heat flow values for these combinations are: 12.67 ± 12.78 µW/g, 11.99 ± 12.40 µW/g, 15.32 ± 16.60 µW/g, and 5.44 ± 6.42 µW/g, respectively. The calculated interaction heat flow values of the said combinations are below 100 µW/g, hence, proving that the different components are compatible with one another. There is a small endothermic event visible in both Figure 4.25 and Figure 4.26, respectively; nonetheless, these events are not an indication that an incompatibility was detected, as these changes in heat flow may be considered relatively small (15.32 ± 16.60 µW/g and 5.44 ± 6.42 µW/g) and therefore seen as negligible.

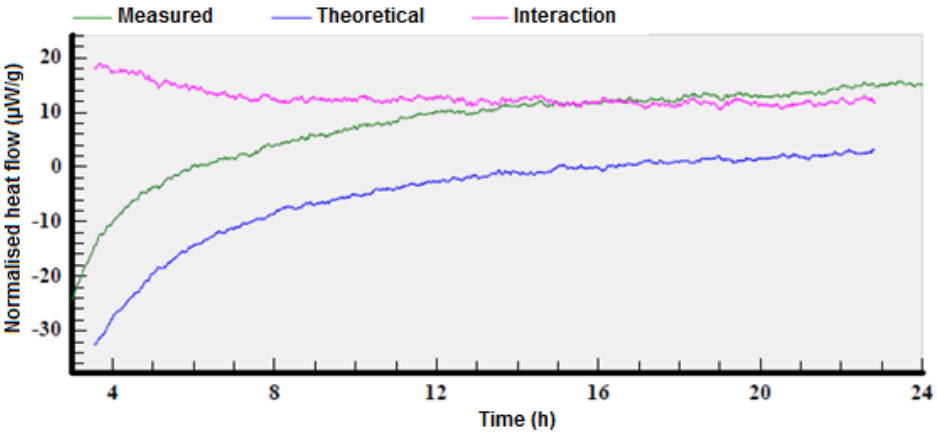


Figure 4.23: *Heat flow data obtained for artemether, lumefantrine, coconut oil, Tween® 80 and Span® 60 combination in a 1:1:1:1:1 ratio*

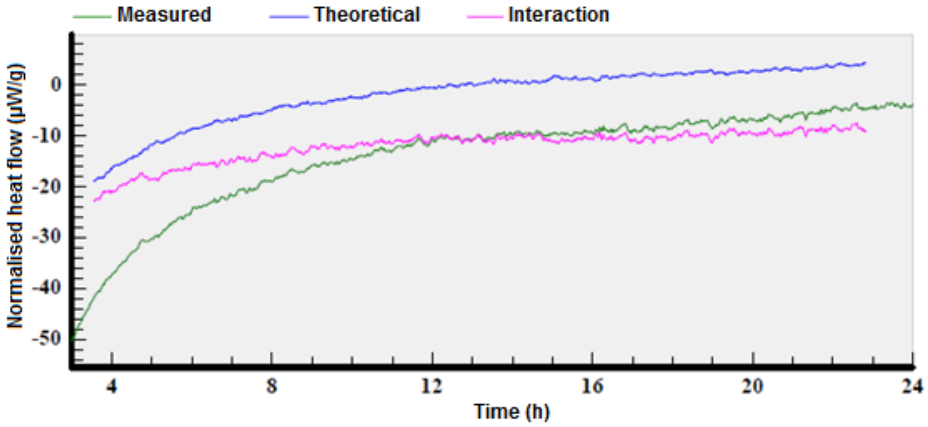


Figure 4.24: *Heat flow obtained for artemether, lumefantrine, coconut oil, Tween® 80 and Span® 80 combination in a 1:1:1:1:1 ratio*

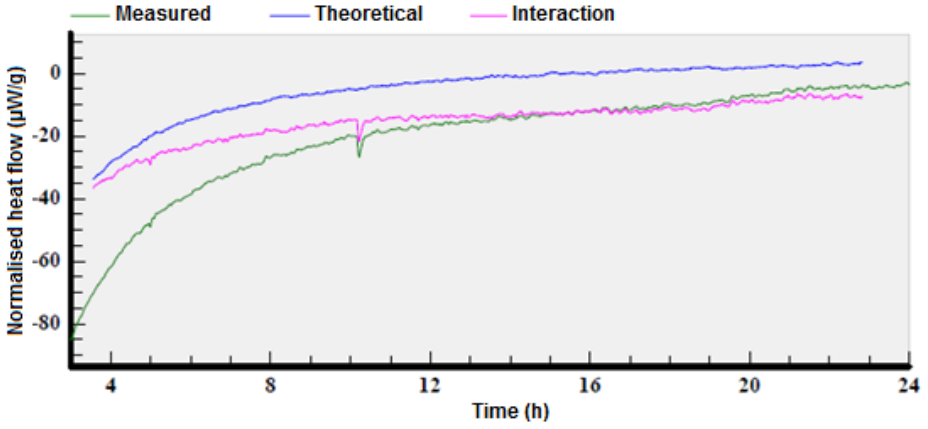


Figure 4.25: *Heat flow data obtained for artemether, lumefantrine, coconut oil, SLS and Span® 60 combination in a 1:1:1:1:1 ratio*

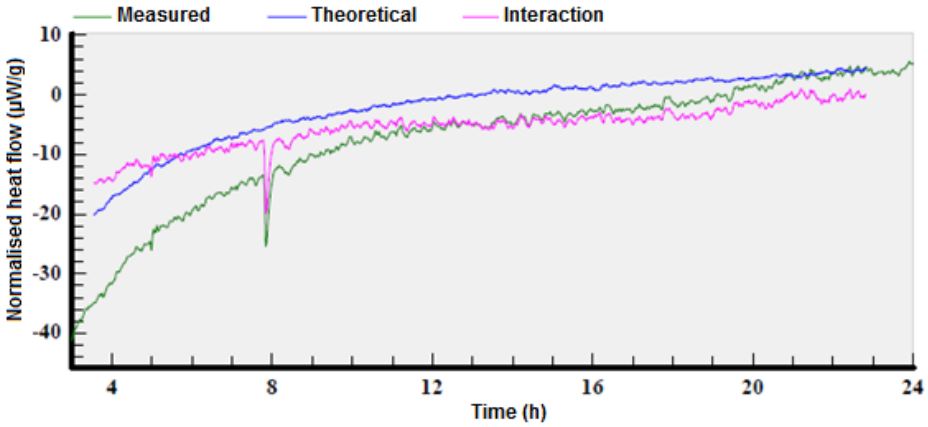


Figure 4.26: *Heat flow data obtained for artemether, lumefantrine, coconut oil, SLS and Span® 80 combination in a 1:1:1:1:1 ratio*

From the results obtained it is evident that no incompatibilities were detected for any oil, drug, and surfactant combination, which may be considered ideal for the purpose of formulating SEDDS formulations containing a fixed-dose of artemether and lumefantrine. When formulating emulsions, it is vital that the components are compatible with one another due to incompatibilities having the potential to influence the stability of a given formulation, which could further compromise the integrity of emulsions. Invariably the particle size of drug particles plays a dynamic role in the stability of an emulsion. Unstable particles can aggregate, thus creating a smaller surface area, which in turn will inhibit the particles from moving freely through the emulsion, causing phase separation or creaming to occur. If the components are incompatible with one another, the overall zeta potential of the emulsion will most probably also be affected, causing oil droplets to aggregate which can cause flocculation or coalescence (Fustier *et al*., 2010). Polymorphism of the crystalline structure of drug particles may also occur when the physical chemical properties of the components in an emulsion are influenced. The change in crystalline structure of drug particles can negatively influence the solubility of these drug particles, therefore decreasing the absorption and thus the potency of the drug. Furthermore, a change in the physicochemical properties of the components can affect the pH of an emulsion, which may lead to a change in the colour of the emulsion as well as the emulsion becoming rancid (Rousseau, 2000). All of these aforementioned factors can cause instability within an emulsion, causing: flocculation, creaming, phase separation, Ostwald ripening (this phenomenon is when smaller particles dissolve in solution and aggregate to the larger particles, creating an inhomogeneous solution which is unstable in nature) , and coalescence; hence, emphasising the importance that all the components should be compatible in order to maintain the integrity of the given emulsion (Fustier *et al*., 2010; Khan *et al.,*2014).

4.3 Preparation of SEDDS

4.3.1 Solubility studies

Solubility, dissolution rate and the permeability of pharmaceutical compounds are vital parameters in achieving oral therapeutic efficiency (Ujhelyi *et al.,* 2018). Solubility may be defined as the maximum mass or volume of a solute that dissolves in a given mass or volume of solvent at a specific temperature and at equilibrium. The United Sates Pharmacopoeia (USP) and European Pharmacopoeia (EP) have provided terms for describing solubility as listed in Table 4.10 (Aulton, 2018). Artemether and lumefantrine are classified according to the BCS classification system as having low aqueous solubility (Patil *et al.*, 2013). The solubility results obtained for both artemether and lumefantrine are displayed in Table 4.11. In this study, it was determined that the aqueous solubilities of artemether and lumefantrine are

2 mg/ml and 0.13 mg/ml, respectively, at 25ºC ± 0.5ºC. According to Table 4.10 it can be derived that artemether is slightly soluble in water; whereas lumefantrine is practically insoluble in water. The results attained are therefore corresponding with what the literature has concluded concerning these two drugs (Patil *et al.*, 2013; Rivelli *et al.,* 2018).

Table 4.10: *Expressive solubility: USP and EP terms for reporting solubility of substances at a temperature between 15–25°C (adapted from Aulton, 2018)*

Describing term	Solubility range in water (mg/ml)
Freely soluble	100–1000
Soluble	33–100
Sparingly soluble	10–33
Slightly soluble	1–10
Very slightly soluble	0.1–1
Practically insoluble	<0.1

For the purpose of this study, it is crucial to establish whether the inclusion of artemether and lumefantrine into an oil will increase their solubility. This is essential to be able to ascertain if it is possible to formulate a dosage form capable of releasing the drugs in their solubilised form so that it can be considered a viable option to pursue further. According to Patil *et al.* (2013) and Rivelli *et al.* (2018) both these drugs are highly lipophilic and thus should display significantly higher solubility in lipophilic vehicles. Overall, artemether displayed a conspicuously higher solubility in the oils compared to lumefantrine. (Table 4.11). The solubility of both artemether and lumefantrine in coconut oil is considered highest compared to the other oils tested. Nonetheless, the solubility of the drugs was notably higher in all of the lipophilic vehicles tested, and the following rank order in terms of lipophilic vehicle solubility may be considered: coconut oil >> avocado oil >> olive oil ≥ peanut oil >>> castor oil. Castor oil comprises a dominant fatty acid, namely $C_{18:1}$ acid, which contains numerous hydroxy functional groups. Literature states that castor oil demonstrates an increase in solute solubility due to the presence of these specific hydroxy functional groups. However, for the solubility of the solute to be increased in castor oil, the solute must possess hydrogen donor and accepting properties. Artemether and lumefantrine present with poor hydrogen donor and accepting properties; this may be the reason why the solubility of both drugs in castor oil is deemed poorer compared to the other oils. However, the solubility of both artemether and lumefantrine increased exponentially when introduced to castor oil (Göke & Bunjes, 2017; Larsen *et al.,* 2002). Lumefantrine solubility changed according to the descriptive solubility terms (Table 4.10) from being practically insoluble in water to slightly soluble in the various oils;

whereas the solubility of artemether improved from being slightly soluble in water to freely soluble in the various oils tested. These results are considered highly positive for this study, and thus, SEDDS formulations comprising one of the selected oils tested in combination with artemether and lumefantrine should be investigated further.

Table 4.11: *Solubility of artemether and lumefantrine in the oils used in this study*

	Artemether	**Lumefantrine**
Vehicle	**Solubility (mg/ml)***	**Solubility (mg/ml)***
Water	2 ± 0.260	0.13 ± 0.015
Olive oil	118 ± 0.577	1 ± 0.057
Peanut oil	113 ± 0.577	1 ± 0.057
Coconut oil	135 ± 0.577	2 ± 0.000
Castor oil	74 ± 1.000	0.7 ± 0.064
Avocado oil	127 ± 1.000	1 ± 0.000

*Data is presented as mean ± standard deviation

4.3.2 Pseudo-ternary diagrams

Following the solubility determination, pseudo-ternary diagrams were constructed for all the various selected oil and surfactant combinations. The ratios of oil to surfactant phase utilised in this study were; 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:0, respectively. All of the different ratios oil/surfactant phases were mixed according to these aforementioned ratios after which, water was added in a drop wise fashion until the first signs of turbidity in the emulsion was visually observed. The emulsion range was consequently determined as the area in the pseudo-ternary phase diagram were no turbidity was observed.

Pseudo-ternary diagrams are a different approach to a factorial design; this is due to the fact that, in the pseudo-ternary diagram a range is highlighted where the particular oil and surfactant in conjunction with water formulates a stable SEDDS. Figure 4.27 is an example of one of the pseudo-ternary diagrams constructed for one of the oils, water and surfactant ratios that formed a stable SEDDS. The pseudo-ternary diagram consists of three well-defined regions: the uncoloured region representing the coarse dispersion region; the light green area indicating the liquid crystal region; and the dark green region signifying the nano-emulsion area. The pseudo-ternary diagrams for the six chosen SEDDS formulations are presented in Figures 4.29, and the resultant pseudo-ternary diagrams for all of the different SEDDS formulations that were considered unstable are presented in Annexure A.

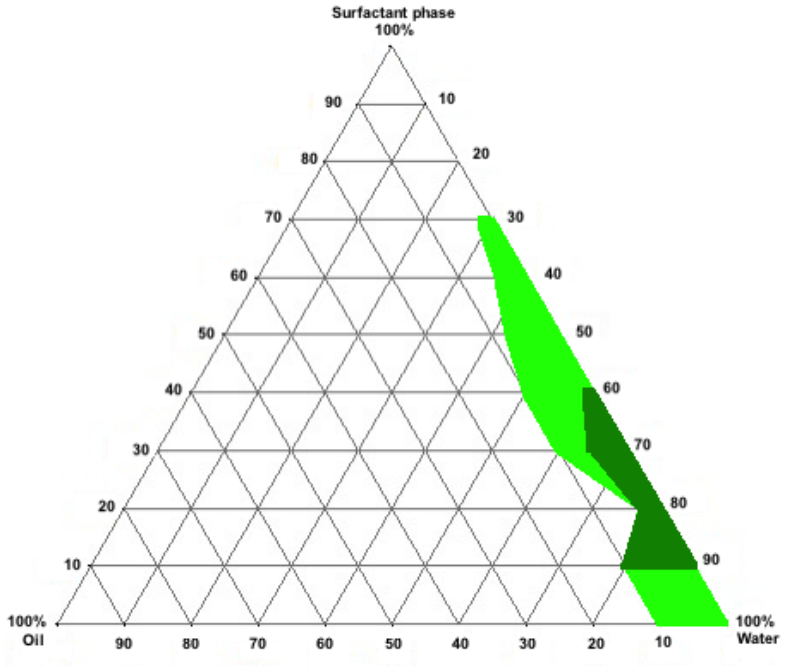


Figure 4.27: *Pseudo-ternary phase diagram for the olive oil/Tween®80/Span®80 system. The green area represents the single phase emulsion region. The uncoloured region represents the coarse dispersion region; the light green area indicates the liquid crystal region; and the dark green region signifies the nano-emulsion area. All compositions are shown as weight ratios*

Post construction of the pseudo-ternary phase diagrams, systems that constituted SLS as surfactant were disregarded from the study as these systems, containing either Span®80 or Span®60 as co-surfactant, produced either a relatively stable range that was too narrow to experiment with; or no stable or clear SEDDS formulations developed upon preparation. The reason for SLS not forming a proper emulsion range can most likely be attributed to the hydrophile lipophile balance (HLB) value of the surfactant. The choice of surfactants plays an imperative role in the emulsification of the SEDDSs, moreover, the HLB value of the oil has also been acknowledged as a vital component in the emulsification process (AboulFotouh *et al.,* 2017; Costa *et al.,* 2014; Wang *et al.,* 2009). The HLB system acts as a scientific approach in predicting the best surfactant and co-surfactant required to produce an optimal emulsifying system. An optimal emulsifying system is formed when the HLB values of the oil phase and surfactant phase match (AboulFotouh *et al.,* 2017; Costa *et al.,* 2014; Fernandes *et al.,* 2012; Wang *et al.,* 2009; Wang & Pal, 2014). Consequently, if the HLB values of the surfactant phase coincide with the HLB values of the oil phase, a stable emulsion will form. SLS has an HLB value of 40 (significantly hydrophilic), which is notably higher than the values of the oils, having HLB values in the range of 6–12 (Fernandes *et al.,* 2012). SLS was chosen because of its high HLB value; according to Wang & Pal (2014), who stated that the higher the HLB value the more hydrophilic the surfactant is. Subsequently, the more hydrophilic surfactant will lead

to an easier formation of an oil-in-water emulsion (Wang & Pal, 2014). However, apart from the advantage of SLS displaying hydrophilic characteristics, the HLB value did not match the HLB values of the different oils used in this study, thus concluding that SLS was, for the purpose of this study, not the optimum choice of surfactant.

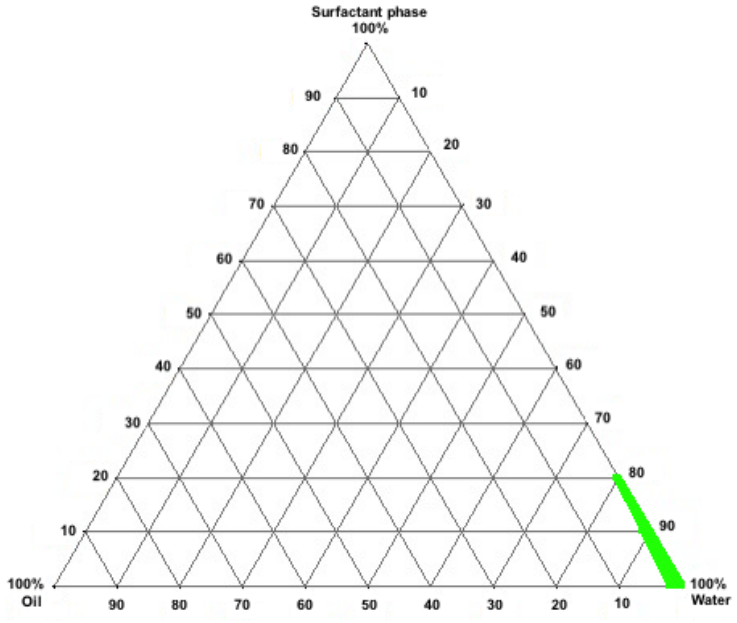


Figure 4.28: *Pseudo-ternary phase diagram for the peanut oil/SLS/Span®80 system. The green area represents the single phase emulsion region. The uncoloured region represents the coarse dispersion region; the light green area indicates the liquid crystal region; and the dark green region signifies the nano-emulsion area. All compositions are shown as weight ratios*

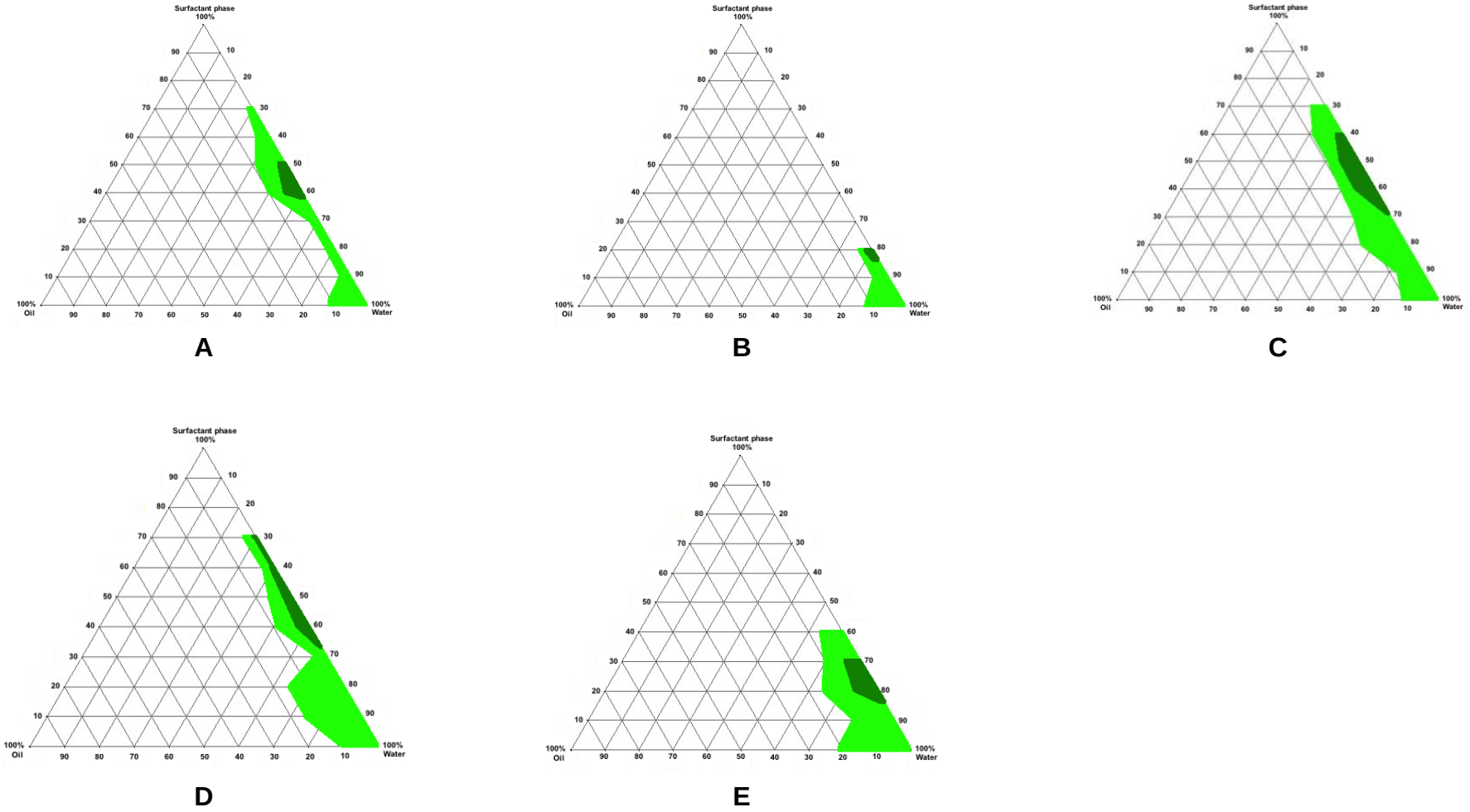
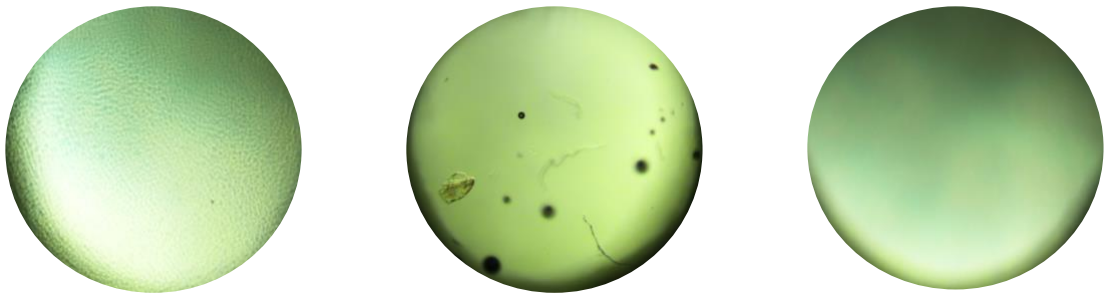


Figure 4.29: *Pseudo-ternary phase diagrams for (A) the peanut oil/Tween®80/Span®80 system; (B) the castor oil/Tween®80/Span®80 system; (C) the coconut oil/Tween®80/Span®80 system; (D) the avocado oil/Tween®80/Span®80 system; and (E) the castor oil/Tween®80/Span®60 system. The green area represents the single phase emulsion region. The uncoloured region represents the coarse dispersion region; the light green area indicates the liquid crystal region; and the dark green region signifies the nano-emulsion area. All compositions are shown as weight ratios*

After each addition of water, the SEDDS formulations were visually examined under a microscope, with a polarised lens, to visually ascertain if the emulsion is in the micro- or nano-range; as well as to inspect if any impurities were included in the emulsion. Emulsions appearing clear and which fell within the emulsion range, were chosen as optimal and stability experiments were conducted on these SEDDS formulations. Figure 4.30A depicts an example of a SEDDS formulations chosen due to it being visually clear; whereas Figure 4.30B portrays an example that was deemed visually unacceptable under the microscope.



A **B** **C**

Figure 4.30: *Examples of SEDDS formulations that were visually inspected where (A) is considered an acceptable SEDDS formulations possibly in the micro-emulsion range; (B) an unacceptable SEDDS formulation containing crystallisation of the surfactant phase; and (C) a possible nano-emulsion due to the addition of more water*

In Figure 4.30B it seems as though the surfactant phase precipitated when the emulsion reached the edge of the emulsion range. Thus, even though to the naked eye the SEDDS formulation may seem clear, when examined under the microscope, it is obvious that this SEDDS formulation ratio is not within the nano-range and cannot be considered stable. Moreover, for this specific SEDDS formulation, the emulsion ranges seem to be notably narrow. The droplet size of the SEDDS formulation displayed in Figure 4.30A is visible under the microscope. This is an indication that this emulsion will probably fall within the micro-range. However, upon further addition of the water phase, in a dropwise fashion, the emulsion became more clear and the droplets were no longer microscopically visible. The addition of water to the SEDDS formulation seems to decrease the droplet size, and move the emulsion from the micro-range into the nano-emulsion range (Figure 4.30C). This conclusion needs to be confirmed upon droplet size measurement discussed in the characterisation section of this chapter.

Based upon the pseudo-ternary phase diagrams and visual inspection, the SEDDS formulations that adhered to the set criteria, and in which the amount of oil phase completely solubilised the fixed-dose artemether and lumefantrine, were selected for further stability investigation. These SEDDS formulations were prepared and left to stand for 24 h in order to establish whether phase separation occurred within these formulations. All of the SEDDS formulations, except for the castor oil SEDDS, that contained a surfactant phase consisting of the surfactant Tween® 80 and co-surfactant Span® 60, displayed phase separation. Therefore, the SEDDS formulations that consisted of a selected oil and the surfactant phase comprising Tween® 80 and Span® 80; as well as the castor oil/Tween® 80/Span® 60 system was further characterised. These formulations are listed in Table 4.12 where the different oil/surfactant phase ratios that formed acceptable SEDDSs are defined.

Table 4.12: *SEDDS formulations that were further characterised*

Selected oil phase	Oil/Surfactant phase ratio	Surfactant phase (1:1)	Abbreviated SEDDS referral
Avocado oil	4:6	Tween® 80/Span® 80	Avocado oil (4:6)
Castor oil	2:8		Castor oil (2:8) S80
Coconut oil	6:4		Coconut oil (6:4)
Olive oil	3:7		Olive oil (3:7)
Peanut oil	6:4		Peanut oil (6:4)
Castor oil	3:7	Tween® 80/Span® 60	Castor oil (3:7) S60

4.4 Characterisation of SEDDS

4.4.1 Assay

The HPLC method described in section 3.3.1 was employed to determine the respective artemether and lumefantrine drug content in the chosen SEDDS formulations. The International Pharmacopoeia (IP) states that oral dosage forms containing artemether and lumefantrine should have a drug content between 90–110%, respectively (IP, 2008). None of the other Pharmacopoeia provided any guidelines as to what the artemether and lumefantrine content should be within a dosage form. Artemether typically displayed a higher percentage drug content compared to the lumefantrine content acquired in the SEDDS formulations (Table 4.13). However, generally the percentage drug content of both lumefantrine and artemether still fell within the parameters set by the IP; and the drug content remained fairly constant within the SEDDSs comprising the different oils. Only the peanut oil (6:4) SEDDS

depicted an artemether content of 112.375% which is slightly higher than the prescribed IP criteria. From the various SEDDS formulations, it is evident that the SEDDSs containing castor oil (castor oil (3:7) S60 and castor oil (2:8) S80) exhibited an overall lower drug content of both lumefantrine and artemether compared to the other SEDDSs. This could possibly be attributed to the poorer solubility of both artemether and lumefantrine in the castor oil.

Table 4.13: *Assay of the percentage lumefantrine and artemether solubilised in the selected SEDDS formulations*

Artemether (%)	SEDDS	Lumefantrine (%)
105.513 (1.963)*	Avocado oil (4:6)	96.553 (0.070)*
95.493 (2.449)*	Castor oil (2:8) S80	95.200 (0.591)*
96.723 (1.710)*	Castor oil (3:7) S60	95.735 (0.040)*
105.600 (0.431)*	Coconut oil (6:4)	96.310 (0.400)*
107.588 (2.580)*	Olive oil (3:7)	96.000 (0.010)*
112.375 (1.263)*	Peanut oil (6:4)	96.211 (0.010)*

*Castor S80= Castor oil, Tween® 80, and Span® 80; Castor S60= Castor oil, Tween® 80, and Span® 60

*The %RSD is displayed in parenthesis

The drug content obtained for artemether in the SEDDS formulations is in the range of 95.493–112.375%; similarly, the drug content for lumefantrine present in the SEDDS formulations ranged from 95.200–96.553%. The %RSD for artemether ranged from 0.431–2.580%; whereas lumefantrine ranged from 0.010–0.591%, signifying that these SEDDS formulations probably display a uniform dispersion of droplets due to the narrow deviation range.

4.4.2 Droplet size and zeta potential

Droplet size distribution ensuing self-emulsification is an important element when assessing a self-emulsifying system. The smaller the droplet size, the larger the interfacial surface area that is available, which in turn leads to faster drug release into an aqueous medium for drug absorption (Chudasama *et al.*, 2011; Czajkowska-Ko´snik *et al.*, 2015; Gershanik & Benita, 2000; Kang *et al.*, 2004; Pouton, 2000; Rao *et al.*, 2013). Gershanik *et al.* (1998) investigated the effect of emulsion droplet size on the penetration ability of the dosage form through intestinal mucosa. They concluded that the optimal droplet size range is between 100–

500 nm. From the six SEDDS formulations (all containing both active ingredients artemether and lumefantrine) tested in this study, the SEDDS formulations containing olive-, avocado-, coconut-, and castor oil all displayed droplet sizes that fell within the nano-range (Figure 4.31); and their %RSD regarding droplet sizes are relatively narrow as it resides below 5% for all of the SEDDS formulations (Annexure B). Only the SEDDS formulation comprising peanut oil is defined as being a SMEDDS formulation, having an average droplet size of 1452.667 nm (16.305%RSD). Therefore, according to the results obtained by Gershanik and co-workers (1998), the SMEDDS containing peanut oil will not be able to permeate the intestinal mucosa to the same extent as the SEDDS constituting the other oils phases. The coconut oil (6:4) SEDDS, although it depicted a droplet size that fell within the nano-range (Figure 4.31), will also not be able to permeate the intestinal mucosa to the same extent as the SEDDS constituting the other oil phases. The olive oil (3:7), castor oil (3:7) S60 and avocado oil (4:6) SEDDS all display a droplet size smaller than 250 nm, indicating that these formulations will probably easily transverse the intestinal mucosa.

Increased droplet sizes attained for the peanut oil (6:4), coconut oil (6:4) and castor oil (2:8) S80 SEDDS could presumably be attributed to the ratio oil phase/surfactant phase. Generally, as the oil phase increased, and the surfactant phase decreased, the droplet increased. Therefore, the less oil phase included in the SEDDS, the higher the probability of the SEDDS diffusing the gastrointestinal tract. Moreover, the small %RSD values indicate that the droplet size in each of these emulsions were fairly uniform. A uniform droplet size is more acceptable as it can play a major role in the stability of an emulsion (AboulFotouh *et al.,* 2017). Literature states that the smaller and the more relatively uniform the droplet size is, the more robust the SEDDS formulation is to any kind of instabilities, for example: flocculation or coalescence, thus indicating that the SEDDS formulation is kinetically stable (AboulFotouh *et al.,* 2017). On the other hand, and as stated, the peanut oil (6:4) SEDDS displayed an average droplet size that fell within the micro-range, however, the %RSD was 16.3%, signifying that the droplets formed in this SEDDS are not of a uniform size. This may thus affect the stability of the SEDDS. To clarify whether the droplet sizes influenced the stability of these formulations, additional thermodynamic stability tests were conducted, which are discussed in the following sections.

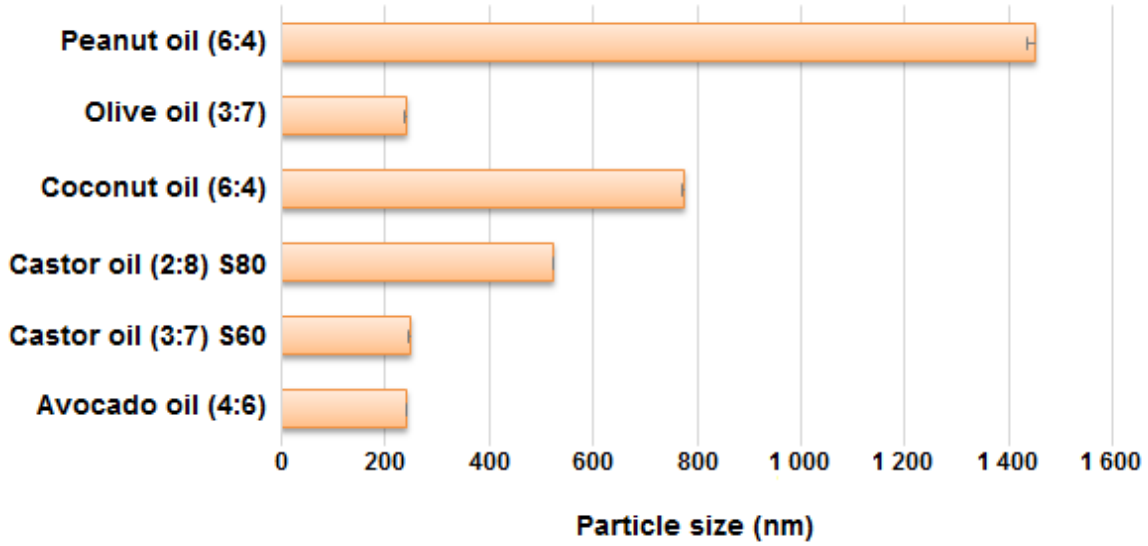


Figure 4.31: Average *droplet size (nm) obtained for the selected SEDDS formulations*

Zeta potential identifies the charge on a droplet of an emulsion. The value obtained for zeta potential defines the degree of electrostatic repulsion between the droplets in a dispersion. Literature states that a higher zeta potential value is indicative of a more stable dispersion where aggregation is prevented. A value above 30 mV or below -30 mV typically indicates that the specific emulsion is highly stable. In addition, traditional SEDDS formulations normally depict an oil droplet charge that is negative due to the presence of free fatty acids in the oil phase (Eid *et al.,* 2014; Silva *et al.,* 2012). As demonstrated in Figure 4.32 all of the SEDDS formulations, except the castor oil (3:7) S60 SEDDS, portrayed a zeta potential value below -30 mV (i.e. higher than a value of 30), which is indicative of highly stable SEDDS formulations. From these results it is postulated that the type of co-surfactant included may play a role in the zeta potential obtained. Although the castor oil (3:7) S60 presented with a lower zeta potential (-20.8 mV), the software of the Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) still indicated that this SEDDS may be considered stable. A stable emulsion has often been linked in literature to a high zeta potential, typically a value of higher than 30 mV, regardless of the sign; and inherently a lower zeta potential value, usually ranging between -11 to -20 mV, would approach the verge of agglomeration (Losso *et al.,* 2005; Wang *et al.,* 2009). However, Roland *et al.* (2003) identified that no direct relationship existed between the overall physical stability and zeta potential. According to the experiments they conducted, emulsions with lower zeta potential values could be classified as more stable during stability testing. Thus, it could be concluded that a zeta potential value not differentiating by more than 10 mV during stability testing is required to be able to predict the absolute stability of an emulsion. Hence, the zeta potential value observed for castor oil (3:7) S60 (-

20.8 mV) is seen as an acceptable value and this SEDDS formulation typically may be classified as being relatively stable.

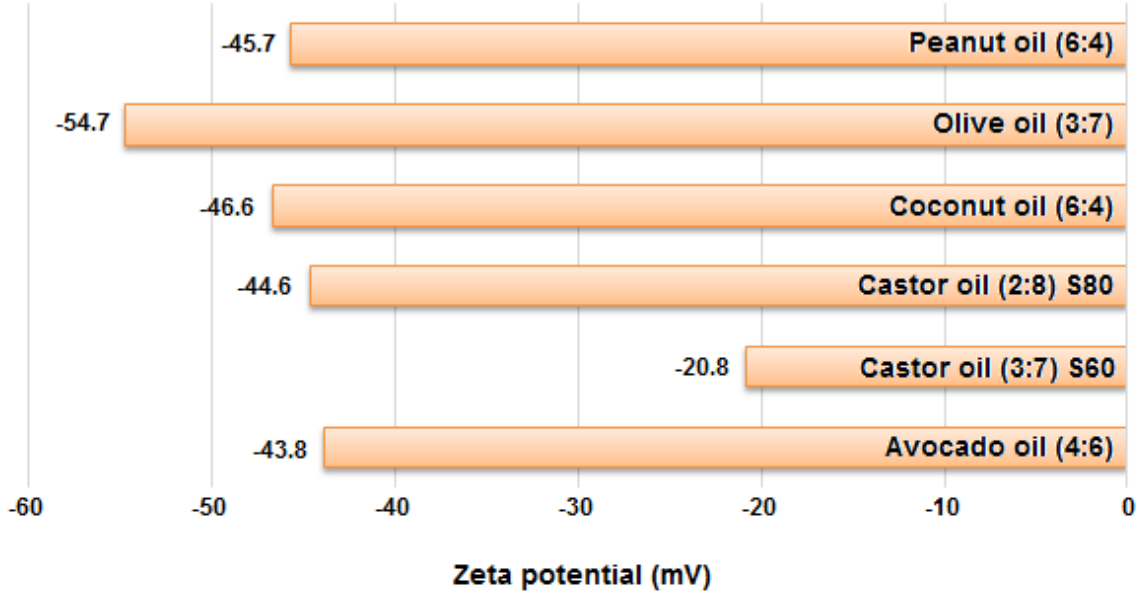


Figure 4.32: Average *zeta potential (mV) obtained for the selected SEDDS formulations*

4.4.3 Determination of self-emulsification

The mechanism of self-emulsification is not completely understood, nonetheless, this mechanism is thought to occur when the dispersion is favoured due to entropy changes, which requires more than the dispersions surface area. Self-emulsification time of SEDDS mimics *in vivo* agitation that the dosage form is exposed to in the gastrointestinal tract. The self-emulsification time of the SEDDS can be divided into 5 groups, namely; Group A, B, C, D, and E, as described in section 3.4.3 (Czajkowska-Kośnik *et al*., 2015, Dangre *et al*., 2016).

It was found that the less time the SEDDS takes to self-emulsifying the more positive the result is. This is because if the SEDDS emulsifies quickly a small amount of energy was required for emulsification, meaning that emulsification of the emulsion occurred spontaneously which is very desirable for this specific dosage form (Basalious *et al*., 2010, Czajkowska-Kośnik *et al*., 2015, Dangre *et al*., 2016). Table 4.14, summarises the emulsification time of the different SEDDS.

Table 4.14: *Determining and grading the emulsification time*

Oil	Emulsification time (s)	Grade
Avocado oil (4:6)	35	A
Castor oil (2:8) S80	59	B
Coconut oil (6:4)	55	A
Olive oil (3:7)	40	A
Peanut oil (6:4)	50	A
Castor oil (3:7) S60	120+	C

Group A and B were deemed as acceptable; group C, D, and E were deemed as a failed result. avocado oil (4:6), castor oil (2:8) S80, coconut oil (6:4), olive oil (3:7), and peanut oil (6:4) were classified in either group A or B, indicating that these SEDDS formulations displayed acceptable self-emulsification mechanisms. The SEDDS formulations comprising castor oil (3:7) S60 interestingly displayed a slightly milky emulsion after 2 min. This possibly has to do with the HLB value of the chosen oil, surfactant, and co-surfactant. The HLB value is a vital component when choosing the right surfactant and co-surfactant to match with the oil phase utilised in the SEDDS formulation. If the HLB values are close to one another it can safely be assumed that a stable emulsion will form, however this is based on theory and upon making the emulsion, the emulsion can still present as stable, even if the HLB values of the surfactant phase differ dramatically from the oil phase. In this study, the ratio of surfactant to co-surfactant used was 1:1, therefore the HLB value of Tween® 80 is 15 and Span® 60 is 4.7. Because the ratio of 1:1, 50% of Tween® 80 and Span® 60, is used and the respective HLB values are 7.5 and 2.35, thus making the total HLB of the surfactant phase 9.85. Castor oil has an HLB value of 14, thus the surfactant phase's HLB values is lower than the HLB value of the oil phase, allowing a safe assumption to be made that the surfactant phase was not efficient enough to lower the interfacial tension, thus the energy required to spontaneously emulsify the emulsion favoured the dispersion surface area and not the entropy changes of the emulsion (Fernandes *et al.,* 2012).

4.3.1 Cloud point determination

Cloud point is defined as the temperature at which the emulsion's clarity becomes cloudy. This point is of specific interest in especially SEDDS utilising non-ionic surfactants. At temperatures higher than the identified cloud point, irreversible phase separation occurs due to the dehydration of the included ingredients; and this may severely affect drug absorption. It is therefore of utmost importance that the cloud point of the SEDDS must be higher than body temperature (i.e. ±37°C); if lower, phase separation of the SEDDS may transpire in the gastrointestinal tract, leading to poor absorption of the incorporated drug (AboulFotouh *et al.,*

2017; Agrawal *et al.,* 2015). The cloud point of each of the selected SEDDS formulations are summarised in the Table 4.15.

Table 4.15: *Cloud point values of the various SEDDS*

Oil	Cloud point (°C)
Avocado oil (4:6)	62
Castor oil (2:8) S80	55
Coconut oil (6:4)	45
Olive oil (3:7)	60
Peanut oil (6:4)	60
Castor oil (3:7) S60	55

All of the selected SEDDS formulations portrayed a cloud point above 37°C, thus implying that the SEDDS should remain stable in the gastrointestinal tract and no phase separation will ensue.

4.3.2 Thermodynamic stability studies

SEDDS formulations experience *in situ* solubilisation with the purpose of forming microemulsion systems. These systems should have stability in order to avoid precipitation of the drugs including; creaming; phase separation; flocculation; Ostwald ripening; and/or cracking of the said formulations. Most often, sustained storage might cause the solubilised drugs to precipitate from these systems; seed crystals may start to appear; and it might grow to larger crystalline materials that will precipitate on the bottom of the container that the specific SEDDS formulation was stored in (Agrawal *et al.*, 2015). For these reasons, the selected SEDDS formulations were exposed to various thermodynamic stress experiments.

The first thermodynamic stress test was the heating and cooling cycle experiment. The avocado oil (4:6), castor oil (2:8) S80, olive oil (3:7), and castor oil (3:7) S60 SEDDS formulations did not depict any physical instabilities during this test. Upon placing the coconut oil (6:4) SEDDS in the fridge, the coconut oil solidified due to its relatively low melting point (24°C). Nonetheless, solidification of this SEDDS formulation was not considered a thermodynamic instability due to the fact that when this formulation was placed in the oven and, when the temperature increased, the coconut oil returned to its original liquid state and no physical instabilities such as phase separation, cracking, or creaming occurred. The peanut oil (6:4) SEDDS also solidified during storage in the fridge and also liquefied during the heating cycle but, on day 6 (the last test day), apparent phase separation transpired. However, to confirm the results acquired, this SEDDS was stirred post testing and was left for 24 h, after

which it was observed that no phase separation occurred. The peanut oil (6:4) SEDDS seemed physically stable when not exposed to varying temperature.

Following, the selected SEDDS formulations were subjected to centrifugation to ascertain if phase separation might develop during exposure to high rotational speed. These formulations were subsequently placed in centrifuging tubes and centrifuged at 3 500 rpms for 30 min. After centrifugation the SEDDS formulations were visually examined. No phase separation was noted with any of the selected SEDDS formulations.

The last thermodynamic stress test conducted was dilution of the SEDDS formulations as this mimics' similar conditions that may occur in the gastrointestinal tract. Therefore, this dilution experiment tests the robustness of the formulation, providing an estimate at how stable the SEDDS will be after oral administration (Agrawal *et al.,* 2015). Again, post testing, the selected SEDDS formulations were visually inspected for any physical instabilities. No physical instabilities could be observed for any of the SEDDS formulations. In conclusion, none of the formulations tested showed any form of physical instability and thus passed the thermodynamic stability stress test.

4.3.3 Viscosity

The internal friction present in a fluid or its tendency to resist flow is known as viscosity (Jain & Soni, 2012; Viswanath *et al.,* 2007). If an emulsion has strong attraction forces between its molecules, the viscosity of the emulsion increases. Inversely, the viscosity of an emulsion will be low if the attraction forces of the molecules in the emulsion are weak (Jain & Soni, 2012). Furthermore, temperature has a notable effect on the viscosity of an emulsion; at high temperatures the attraction forces between the molecules in the emulsion decrease, which causes the emulsion to be less viscous, and vice versa (Jain & Soni, 2012). Viscosity is an important parameter during dispersion of a SEDDS formulation in the aqueous phase. The higher the viscosity the slower the emulsification rate, which in turn may affect the *in vivo* drug release and subsequent bioavailability profiles. It moreover affects handling of SEDDS. The lower the viscosity of the SEDDS formulations, the easier filling of this dosage form into hard or soft gelatin capsules will be (Agrawal *et al.,* 2015; Nasr *et al.*, 2016).

The rheological properties of fluids can be divided into two categories;

1. Newtonian: These fluids have the same viscosity at different shear rates (rpms) and are classified as Newtonian over the shear rate range they are measured at.
2. Non-Newtonian: These fluids have different viscosities at different shear rates. Non-Newtonian fluids can further be divided into two groups;

- Time dependent, and;
- Time independent

The rheological properties of a fluid that is dependent on time, changes when the spindle speed changes and different viscosities are observed. Time dependent rheological properties are classified as thixotropic; meaning the fluid's viscosity decreases when maintained under a constant shear rate. Time independent rheological properties are classified as pseudoplastic; displaying a decrease in viscosity when the shear rate is increased. This term is also known as 'shear thinning'. Most fluids are found to be Non-Newtonian. Representations of Newtonian and non-Newtonian rheological properties are seen in Figure 4.33.

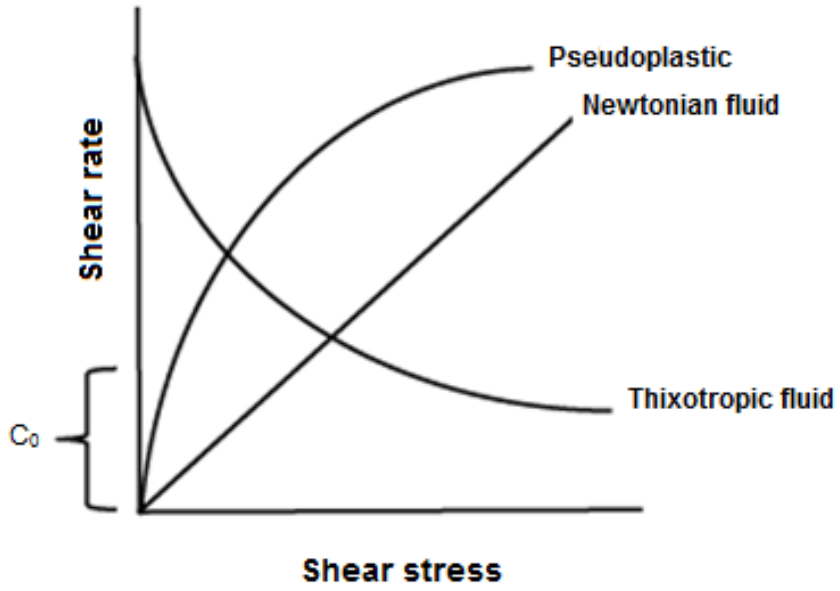


Figure 4.33: *Various types of rheological properties of different fluids*

The viscosity results obtained for the selected SEDDS formulations differed noticeably between the different formulations (Tables 4.17–4.22 and Figures C1–C6, Annexure C). Table 4.16 exhibits results found for the castor oil (2:8) S80 SEDDS. Initially, the castor oil (2:8) S80 SEDDS displayed an increase in viscosity as the shear rate increased; however, when the shear rate was increased to 30 rpm, the viscosity of the emulsion started to decrease upon increase in speed. This indicates that the castor oil (2:8) S80 SEDDS exhibits a time independent rheological behaviour, known as pseudoplastic behaviour, as variation in the shear rate cause a differentiation in the viscosity.

Table 4.16: *Viscosity and shear rate of the castor oil (2:8) S80 SEDDS*

Speed (rpm)	Shear rate (1/s)	Viscosity (cP)	Temperature (ºC)
5	1.4	360.0	23.3
10	2.8	372.0	23.4
20	5.6	372.0	23.2
30	8.4	388.0	23.4
50	14.0	385.2	23.3
60	16.8	384.0	23.3
100	28.0	381.0	23.4

It is evident from Table 4.17 that when the viscosity of the castor oil (3:7) S60 SEDDS is tested, this SEDDS formulation appears fairly viscous at 5 rpms. As the rotational speed is increased, the viscosity of the castor oil (3:7) S60 SEDDS decreases, typically indicating that this SEDDS demonstrates pseudoplastic rheological behaviour.

Table 4.17: *Viscosity and shear rate of the castor oil (3:7) S60 SEDDS*

Speed (rpm)	Shear rate (1/s)	Viscosity (cP)	Temperature (ºC)
5	6.6	544.8	23.2
10	2.8	312.0	23.3
20	5.6	312.0	23.2
30	8.4	311.0	23.2
50	14.0	281.4	23.4
60	16.8	312.0	23.3
100	28.0	300.0	23.2

In Table 4.18 it is apparent that the viscosity of coconut oil (6:4) SEDDS increases as the rotational speed is increased, and only once the stirring rate was increased to 100 rpm did the rheological properties of the SEDDS present with pseudoplastic behaviour. The phenomenon where the viscosity of the SEDDS increases as the stirring rate increases is termed dilatancy. Dilatancy is defined as the tendency of a liquid, in this case the SEDDS, to almost “solidify” as the stirring rate is increased, in other words as the stirring rate increases the emulsion becomes more viscous. Dilatancy ensues due to rapid shearing of the emulsion which forces particles out of their close packing and a void space is formed between the particles. This whole formation can be defined as the system dilates. These newly formed void spaces remain as the system has insufficient liquid to fill the spaces, resulting in a noticeable resistance with

further shearing. Dilatancy is not a positive flow property for an emulsion; and it can also occur when flocculation arises during storage. The sediment that forms is then classified as a dilatant, resisting any attempts at stirring or shaking to form a uniform emulsion, which could lead to caking (Jain & Soni, 2012; Rognon *et al.,* 2011).

Coconut oil normally solidifies at room temperature ($\pm$25°C). Upon conduction of this experiment, room temperature was below 25°C and the water bath of the viscometer could not be controlled ($\pm$23°C). For these reasons the coconut oil (6:4) SEDDS might have portrayed tendencies of dilatancy. However, once the rotational stirring speed was increased to 100 rpm, the increased speed must have created an increase in temperature, causing a decrease in viscosity, and thus possibly pseudoplastic behaviour was observed. In Figure C3, Annexure C, the dilatancy behaviour can clearly be detected from 5–60 rpm; and at 100 rpm the viscosity starts to decrease, displaying possible pseudoplastic behaviour.

Table 4.18: *Viscosity and shear rate of the coconut oil (6:4) SEDDS*

Speed (rpm)	Shear rate (1/s)	Viscosity (cP)	Temperature (ºC)
5	1.4	192.0	23.2
10	2.8	180.0	23.3
20	5.6	200.0	23.2
30	8.4	202.0	23.2
50	14.0	206.1	23.4
60	16.8	216.0	23.3
100	28.0	213.2	23.2

Observing results obtained for the olive oil (3:7) SEDDS (Table 4.19), it is evident that the rheological behaviour is pseudoplastic in nature due to the fact that the viscosity of the olive oil (3:7) SEDDS increases up to 30 rpm and then it starts to decrease from there on. This observation is confirmed with Figure C5 (Annexure C) where a typical pseudoplastic curve with an initial increase and decrease may be seen.

Table 4.19: *Viscosity and shear rate of the olive oil (3:7) SEDDS*

Speed (rpm)	Shear rate (1/s)	Viscosity (cP)	Temperature (ºC)
5	1.4	492.0	23.2
10	2.8	503.9	23.3
20	5.6	510.0	23.2
30	8.4	512.0	23.2
50	14.0	507.6	23.4
60	16.8	505.0	23.3
100	28.0	499.3	23.2

The peanut oil (6:4) SEDDS likewise depicted pseudoplastic behaviour, as tabled in Table 4.20. This SEDDS formulation displayed pseudoplastic behaviour early on during an increase in the rotational stirring speed (i.e. 10 rpm). On the other hand, the avocado oil (4:6) SEDDS presented a typical non-Newtonian, time independent rheological behaviour, and at 30 rpms the emulsion displayed shear thinning as the viscosity of the oil started to decrease at 30 rpms. In Table 4.21 the rheological behaviour of the emulsion can clearly be identified.

Table 4.20: *Viscosity and shear rate of the peanut oil (6:4) SEDDS*

Speed (rpm)	Shear rate (1/s)	Viscosity (cP)	Temperature (ºC)
5	1.4	279.7	23.2
10	2.8	282.0	23.3
20	5.6	278.8	23.2
30	8.4	278.0	23.2
50	14.0	276.0	23.4
60	16.8	275.0	23.3
100	28.0	273.5	23.2

Table 4.21: *Viscosity and shear rate of the avocado oil (4:6) SEDDS*

Speed (rpm)	Shear rate (1/s)	Viscosity (cP)	Temperature (ºC)
5	1.4	372.0	23.2
10	2.8	398.5	23.3
20	5.6	402.0	23.2
30	8.4	400.0	23.2
50	14.0	399.6	23.4
60	16.8	397.9	23.3
100	28.0	393.6	23.2

In conclusion, it was generally observed that the viscosity increased with an increase in the surfactant phase ratio in the selected SEDDS formulations. These results can be attributed to the surfactant phase displaying a higher viscosity than the selected oils. All the above-mentioned SEDDS formulations furthermore displayed plastic rheological properties. Most pharmaceutical preparations are pseudoplastic in nature; this is because this particular flow holds numerous advantageous aspects for pharmaceutical dosage forms. The rationale is that the high viscosity of pseudoplastic flow at a lower shear enables the emulsion to stabilise the insoluble particles present in the SEDDS formulations, which subsequently will prevent these particles from rapidly sedimenting from the SEDDS formulations. Additionally, pseudoplastic flow has a shear thinning characteristic which allows for easy flow when pouring the emulsion (Jain & Soni, 2012). Therefore, this particular rheological behaviour is beneficial for the purpose of this study, as not only will this type of behaviour allow for a more stable SEDDS formulation during storage, but it will also assist with easy handling and pouring of the dosage form.

4.3.4 Dissolution studies

SEDDS are said to promote dissolution of a drug due to a phenomenon known as the negative interfacial tension theory. Negative interfacial tension theory can be defined as the entropy change that occurs during self-emulsification, which is higher for the dispersion than the energy needed to increase the surface area of the dispersion. Another positive of SEDDSs is that the homogeneity of the emulsion droplet size, as well as the polarity of the droplet size, aid in the rapid release of the drug from the SEDDS (Xue *et al.,* 2018). Artemether and lumefantrine are well-known for their poor solubility profiles, thus when the selected SEDDS formulations proved to increase the solubility of both these drugs, it was important to determine whether these SEDDSs are furthermore able to noticeably improve the drug release properties

of both drugs from a given dosage form, as it pertains to the absorption and bioavailability of a drug (Conti *et al.*, 2007). Consequently, the dissolution characteristics of the six chosen SEDDS formulations were analysed to establish both the artemether and lumefantrine release profiles. Due to the already known fact that both artemether and lumefantrine are poorly soluble, phospholipids and bile salts were introduced into the dissolution media after 5.5 h with the intent to mimic the gastrointestinal tract more closely. The addition of bile salts and phospholipids increased the pH to 7.4, imitating the ileum pH (Lipert *et al.,* 2015).

Mean dissolution time (MDT) is a term that may be defined as the average dissolution time of the amount of drug released at all the tested time intervals during dissolution studies. MDT values naturally tend to increase with time and it is more evident when a formulation displays prolonged release. In other words, it is a measure of the rate of the dissolution process. Thus, a higher MDT value is indicative of a slower drug release profile due to a drug-retarding ability of the formulation (Esterhuizen-Rudolph, 2015; Qiu, 2009). The MDT values for both drugs released from the various SEDDS formulations are presented in the following sections. Furthermore, the f_1- and f_2-values for both artemether and lumefantrine released from each of the selected SEDDS formulations were calculated (Table 4.24 and Table 4.26) to conclude whether the artemether and lumefantrine release profiles exhibited a significant difference comparative to each of the SEDDS formulations. No statistical difference is observed when the f_1-value is less than 15% and f_2-value is higher than 50% (Costa & Lobo, 2001; Moore & Flanner, 1996).

4.3.4.1 Dissolution properties of artemether

Dissolution profiles were constructed from the average percentage drug in solution as a function of time. Figure 4.34 illustrates the different dissolution profiles of artemether from the selected SEDDS formulations. Log P is defined as the partition coefficient of a drug, and is the ratio of concentrations of the said drug in a mixture of two immiscible phases (predominantly water and octanol) at equilibrium (Amin *et al.,* 2103; van Zyl *et al.*, 2016). The log P value of artemether is 3.48, rendering it as having a higher affinity for the lipid phase, which is confirmed by the solubility experiments conducted in this study. Artemether will consequently prefer to reside within the lipid phase of the SEDDS formulations. This, however, creates a concern as the gastrointestinal media is hydrophilic. Therefore, surfactants are added to the highly lipophilic formulations, as this will lower the interfacial tension between the emulsion and the gastrointestinal media, allowing artemether to diffuse more easily from a formulated SEDDS into the surrounding media (Amin *et al.,* 2103).

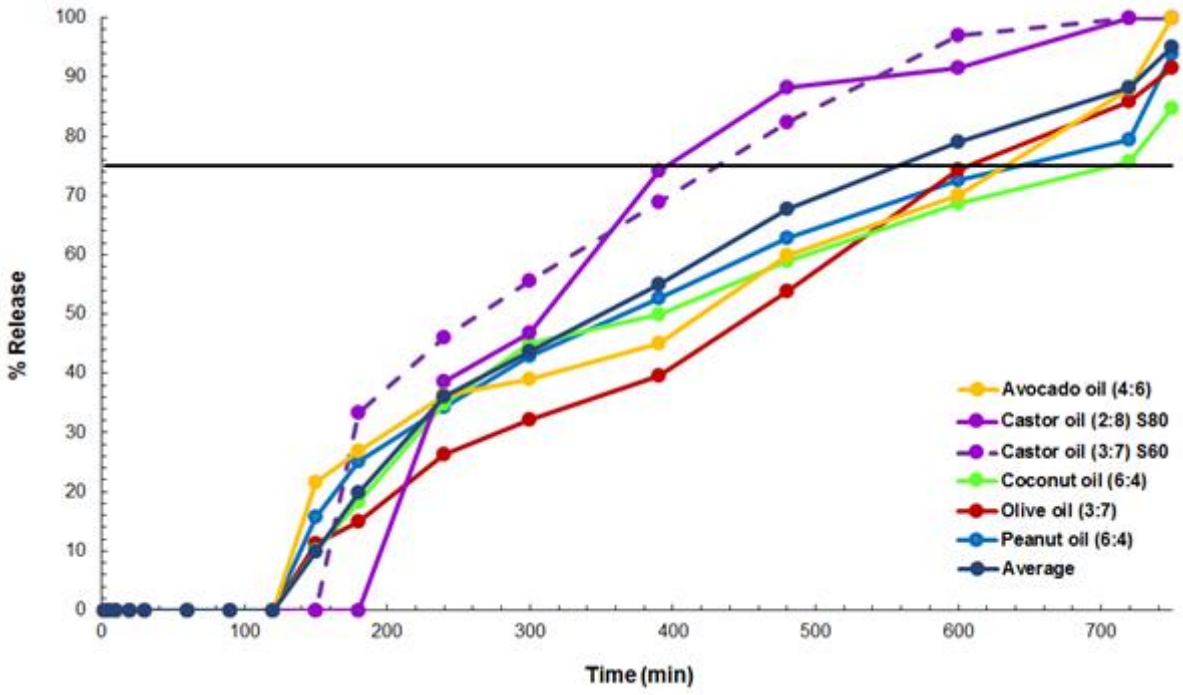


Figure 4.34: *Dissolution profiles of artemether as a function of time from the optimised SEDDS formulations. The horizontal black line indicates the 75% drug release set by the IP (2008)*

From Figure 4.34 it is apparent that all of the selected SEDDS formulations displayed an initial delayed release profile up until approximately 120 min. This was confirmed by the fact that all of the selected SEDDS formulations illustrated high MDT values (Table 4.22), proving that prolonged release was observed during dissolution. Artemether was only released when the pH was increased from 1.2 to 6.8. This is due to the higher pH creating a more favourable environment for artemether to be more highly ionised and therefore becoming more soluble in the dissolution media. Artemether possesses a pKa value of -3.9, indicating that the drug has strong basic properties, which explains why a correlation can be seen in the release of artemether as the pH increases (Amin *et al.,* 2013). In addition, the two SEDDS formulations comprising castor oil as the oil phase portrayed a slower onset of release, where the castor oil (2:8) S80 SEDDS started releasing the artemether at only 180 min. This observation might be due to the castor oil having a higher viscosity compared to the other oil phases of the selected SEDDS formulations, and first had to be diluted by the dissolution media and/or the artemether first had to slowly diffuse through these SEDDS formulations.

Table 4.22: *MDT values of artemether from the various SEDDS formulations*

	Avocado oil (4:6)	Castor oil (2:8) S80	Castor oil (3:7) S60	Coconut oil (6:4)	Olive oil (3:7)	Peanut oil (6:4)
MDT (min)	411.828	332.745	310.876	366.417	412.135	385.094

Once the artemether started to release from the selected SEDDS formulations a relatively constant release rate is observed for all of these formulations ($r^2 \geq 0.982$). The castor oil (3:7) S60 SEDDS and castor oil (2:8) S80 SEDDS displayed a relatively faster artemether release rate compared to the other SEDDS formulations; and artemether was completely released (i.e. 100%) from these formulations. The MDT values for these castor oil SEDDS also indicate that these SEDDS formulations were able to release the artemether faster, as these values are relatively lower comparatively. Moreover, the only other SEDDS formulation that was able to fully release artemether (100%), is the avocado oil (4:6) SEDDS. The coconut oil (6:4), olive oil (3:7) and peanut oil (6:4) SEDDSs, however, did depict artemether release profiles higher than 80%. The avocado oil (4:6) SEDDS and olive oil (3:7) SEDDS presented the highest MDT values indicating a higher artemether retarding ability. A rank order could be established for the artemether release rate from the selected SEDDS formulations (slowest to fastest release rate): olive oil (3:7) ≥ avocado oil (4:6) >> peanut oil (6:4) > coconut oil (6:4) >> castor oil (2:8) S80 > castor oil (3:7) S60.

Considering the fit-factors (Table 4.23) in order to ascertain whether significant differences concerning the release profiles of the selected SEDDS could be observed, it could be concluded that only the castor oil (2:8) S80 and castor oil (3:7) S60 SEDDS formulations differed statistically significantly from the other SEDDS formulations. This observation confirmed that these two formulations depicted longer delayed release profiles of artemether from the SEDDS, as well as higher and faster artemether release once drug release was initiated. The resultant SEDDS formulations thus released artemether in a similar manner.

Table 4.23: *Fit factors displaying the statistical similarities and differences of the SEDDS formulations with artemether*

Comparison	No statistical difference	Statistical difference
Avocado oil (4:6) : Castor oil (2:8) S80		f_1: 38.579 f_2: 42.101
Avocado oil (4:6) : Castor oil (3:7) S60		f_1: 36.041 f_2: 44.454
Avocado oil (4:6) : Coconut oil (6:4)	f_1: 12.197 f_2: 64.662	
Avocado oil (4:6) : Olive oil (3:7)	f_1: 14.840 f_2: 63.111	
Avocado oil (4:6) : Peanut oil (6:4)	f_1: 9.104 f_2: 72.216	
Castor oil (2:8) S80 : Castor oil (3:7) S60	f_1: 15.067 f_2: 52.472	
Castor oil (2:8) S80 : Coconut oil (6:4)		f_1: 30.583 f_2: 43.773
Castor oil (2:8) S80: Olive oil (3:7)		f_1: 45.315 f_2: 41.869
Castor oil (2:8) S80 : Peanut oil (6:4)		f_1: 35.166 f_2: 44,503
Castor oil (3:7) S60 : Coconut oil (6:4)		f_1: 39.220 f_2: 44.282
Castor oil (3:7) S60 : Olive oil (3:7)		f_1: 49.511 f_2: 41.255
Castor oil (3:7) S60 : Peanut oil (6:4)		f_1: 33.574 f_2: 46,702
Coconut oil (6:4) : Olive oil (3:7)	f_1: 16.883 f_2: 62.575	
Coconut oil (6:4) : Peanut oil (6:4)	f_1: 7.576 f_2: 76.321	
Olive oil (3:7) : Peanut oil (6:4)	f_1: 18.791 f_2: 61.081	

One of the reasons for the differences in the artemether release profiles is possibly that, as stated previously, the solubility of artemether in the various oil phases differed notably. Artemether is less soluble in the castor oil, whereas similar solubility profiles were achieved in the avocado-, coconut-, olive-, and peanut oils. Poor solubility can lead to faster release of the drug from a formulation, where increased solubility in the dispersed phase may lead to

reluctance of the drug to diffuse from the formulation (Xue *et al.,* 2018). Likewise, the viscosity values of the different oil phases were nearly identical for the avocado-, coconut-, olive-, and peanut oils, which again differed markedly from the values obtained for castor oil; explaining why the aforementioned SEDDS formulations demonstrated similar drug release profiles compared to the SEDDS comprising castor oil and displaying longer lag times (Costa *et al.,* 2014).

4.3.4.2 Dissolution properties of lumefantrine

Considering the dissolution profiles constructed for lumefantrine from the selected SEDDS formulations, as depicted in Figure 4.35, it is again clear that lumefantrine was only released after the biorelevant media, containing phospholipids and bile salts, was added and the pH increased to 7.4.

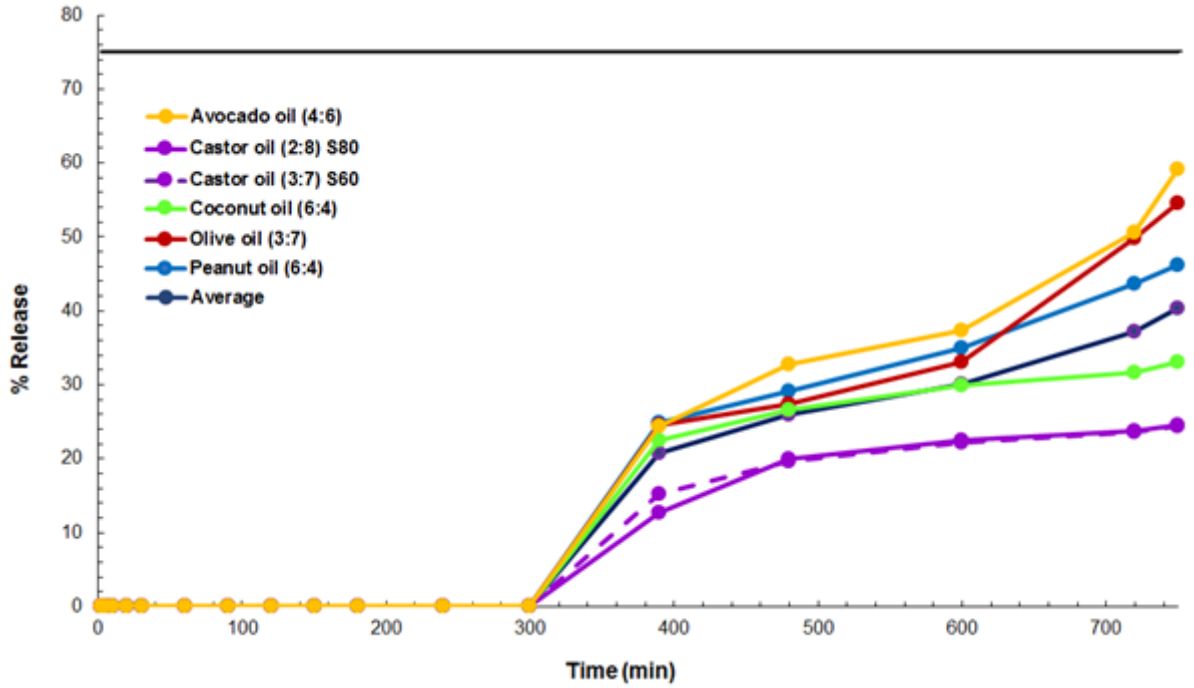


Figure 4.35: *Dissolution profiles of lumefantrine as a function of time from optimised SEDDS formulations. The horizontal black line indicates the 75% drug release set by the IP (2008)*

Lumefantrine has a log P value of 9.19, indicating that it has an expressively higher affinity towards the oil phase (Amin *et al.,* 2013). Phospholipids and bile salts meaningfully influence the solubility and bioavailability of poorly soluble drugs. This is due to the phospholipids and bile salts being physiologically relevant surfactants. Hence, the combination with the synthetic surfactants added to the SEDDS formulations, seemed to markedly decrease the interfacial tension between the SEDDS and the surrounding media, almost forcing the lumefantrine into

the surrounding media (Lipert *et al.,* 2015). The interfacial tension could be reduced due to the entropy changes favouring the dispersion.

However, the biorelevant media used in this study was only a basic composition to simulate conditions in the small intestine and not to improve the dissolution of the two drugs utilised. Most biorelevant media normally contain oleic acid which aids in the solubilisation of lipids, however when adding oleic acid, the biorelevant media becomes more complex. There are generally two types of biorelevant media, namely, type I and type II. Type I mimics' the fasting state of the gastrointestinal tract and type II simulates the fed state. For the purpose of this study type I was used, due to many patients who contract malaria being located in rural areas, and a proper nutritious (fatty) meal while taking their medication is not always possible (Andreas *et al.,* 2016). However, this can be detrimental to the efficacy of the release of lumefantrine during dissolution studies. Type II dissolution media, on the other hand, includes lecithin which facilitates the digestion of lipids. In the gastrointestinal tract there are also various enzymes found which will additionally facilitate in the digestion of lipids, probably improving the release of lumefantrine from the SEDDS formulations (Andreas *et al.,* 2016). There are also lipid transport systems in the gastrointestinal tract where micelles and chylomicrons assist in the transport of lipids to the area of absorption (Kauss *et al*., 2018).

Thus, there are many additional mechanisms present in the gastrointestinal tract that are able to contribute to improving the bioavailability of lumefantrine. Therefore, although the release profiles of lumefantrine obtained in this study indicate relatively poor lumefantrine release, the study was still deemed successful as lumefantrine was indeed released from all of the SEDDS formulations. Moreover, lumefantrine was released more slowly (delayed release of approximately 300 min for all of the selected SEDDS formulations and the MDT values are comparatively higher) from the SEDDS formulations compared to the artemether as expected; and it seems from Figure 4.35 that, given additional analysis time, more lumefantrine may still be released from these formulations. This statement is justified by the higher MDT values obtained for lumefantrine (Table 4.24).

Table 4.24: *MDT values of lumefantrine from the various SEDDS formulations*

	Avocado oil (4:6)	**Castor oil (2:8) S80**	**Castor oil (3:7) S60**	**Coconut oil (6:4)**	**Olive oil (3:7)**	**Peanut oil (6:4)**
MDT	499.684	419.952	412.001	499.515	500.348	458.927

Lumefantrine displays delayed absorption and elimination (Garg *et al.,* 2017). However, this is beneficial because lumefantrine targets the blood schizontocide stages of the disease, and exhibits no antimalarial activity against the pre-erythrocytic liver stages. Thus, when used in

conjunction with artemether, lumefantrine will remove any of the remaining parasites after artemether has reduced the initial parasite load (Garg *et al.,* 2017; Prabhu *et al.,* 2016). Therefore, the delayed release of lumefantrine observed in this study coincides with the delayed working mechanism of this anti-malarial. What is more, unless *in vivo* studies are conducted on these dosage forms, the true bioavailability of the lumefantrine cannot be accurately predicted.

The avocado oil (4:6); coconut oil (6:4); olive oil (3:7); and peanut oil (6:4) SEDDS formulations displayed similar release profiles (Table 4.25) up to approximately 600 min. Interestingly, the castor oil (2:8) S80 and castor oil (3:7) S60 SEDDS formulations exhibited similar delayed lumefantrine release (f_1 = 3.995; f_2 = 96.554), however, the lumefantrine release from these formulations was significantly lower compared to the other selected SEDDS formulations (Table 4.23) which also depicted delayed lumefantrine release. Once again, a rank order could be recognised for the lumefantrine release rate from the selected SEDDS formulations (slowest to fastest release rate): olive oil (3:7) ≥ avocado oil (4:6) = coconut oil (6:4) >>> peanut oil (6:4) >>> castor oil (2:8) S80 > castor oil (3:7) S60. The rationale behind castor oil (3:7) S60 and castor oil (2:8) S80 SEDDS formulations showing lower MDT values, is due to the similar poor solubility of lumefantrine in castor oil as explained in section 4.3.1.1. The lumefantrine will tend to reside within the lipid phase of these SEDDS formulations restricting drug release (Xue *et al.*, 2018). In addition, the SEDDS formulations comprising castor oil exhibited higher viscosity that could have limited fast lumefantrine release (Costa *et al.*, 2014). A rank order for cumulative percentage lumefantrine released up to 750 min could be constructed: avocado oil (4:6) (59.1%) > olive oil (3:7) (54.5%) > peanut oil (6:4) (46.2%) > coconut oil (6:4) (33.1%) > castor oil (2:8) S80 (24.5%) = castor oil (3:7) S60 (24.4%).

Table 4.25: *Fit factors determining the similarities and differences of the SEDDS formulations with lumefantrine*

Comparison	No statistical difference	Statistical difference
Avocado oil (4:6) : Castor oil (2:8) S80		f_1: 45.553 f_2: 53.277
Avocado oil (4:6) : Castor oil (3:7) S60		f_1: 44.299 f_2: 53.535
Avocado oil (4:6) : Coconut oil (6:4)	f_1: 23.703 f_2: 63.949	
Avocado oil (4:6) : Olive oil (3:7)	f_1: 7.253 f_2: 85.537	
Avocado oil (4:6) : Peanut oil (6:4)	f_1: 9.264 f_2: 82.649	
Castor oil (2:8) S80 : Castor oil (3:7) S60	f_1: 3.995 f_2: 96.554	
Castor oil (2:8) S80 : Coconut oil (6:4)		f_1: 40.131 f_2: 69.844
Castor oil (2:8) S80: Olive oil (3:7)		f_1: 41.424 f_2: 55.720
Castor oil (2:8) S80 : Peanut oil (6:4)		f_1: 39.043 f_2: 58.709
Castor oil (3:7) S60 : Coconut oil (6:4)		f_1: 26.995 f_2: 71.210
Castor oil (3:7) S60 : Olive oil (3:7)		f_1: 40.075 f_2: 56.114
Castor oil (3:7) S60 : Peanut oil (6:4)		f_1: 40.416 f_2: 58.196
Coconut oil (6:4) : Olive oil (3:7)	f_1: 17.917 f_2: 66.854	
Coconut oil (6:4) : Peanut oil (6:4)	f_1: 16.504 f_2: 73.354	
Olive oil (3:7) : Peanut oil (6:4)	f_1: 7.420 f_2: 86.100	

To summarise, all of the selected SEDDS formulations were able to release both artemether and lumefantrine. Artemether portrayed an average lag time (ALT) of 135 min and an average MDT value of 369.849 min from the selected SEDDS formulations, which indicated a faster release rate compared to lumefantrine release (ALT = 300 min; average MDT = 465.071 min).

Artemether release further varied between the different SEDDS formulations, as it was released more slowly from the castor oil (2:8) S80 and castor oil (3:7) S60 SEDDS formulations. Although the release rates of the two drugs differed, a similar trend was followed where the castor oil (3:7) S60 SEDDS formulation released both drugs the fastest; and the olive oil (3:7) followed by the avocado oil (4:6) SEDDS formulation depicted a slower release rate for both artemether and lumefantrine. The selected SEDDS formulations were able to release artemether (95.0%) to a meaningfully higher extent compared to lumefantrine (40.3%). However, no clear comparative trend could be established between the %drugs released from the selected SEDDS formulations. Interestingly though, it was noticed that as the surfactant phase of the SEDDS increased, the percentage artemether released subsequently also increased. Therefore, due to the fact that the avocado oil (4:6) and olive oil (3:7) SEDDS formulations portrayed the highest %drug release for lumefantrine; showed exceptional artemether release (100% and 91.6%, respectively); and displayed acceptable modified drug release, these formulations may be considered the most optimum when formulating a SEDDS that comprises a fixed-dose of artemether and lumefantrine.

4.3.4.3 Pharmacokinetics of the release profiles of the SEDDS

In vitro dissolution analysis is not only utilised to supervise the reliability and stability of drug delivery systems, but it can furthermore be used as a reasonably fast and inexpensive procedure to predict *in vivo* absorption of a said drug. For these reasons, quantitative assessment of drug dissolution properties is of immense interest; and although a wide variety of mathematical models exist in order to fit drug release results, all of these are obtained by means of nonlinear equations. DDSolver is a menu-driven add-in program for Microsoft Excel written in Visual Basic for Applications and may be employed to ease drug release model fitting. The purpose of fitting the dissolution profile to mathematic modelling is to simplify the complex release profile of the drug and gain an insight into the release mechanism of a specific dosage form (Zhang *et al.*, 2010).

The release kinetics of both artemether and lumefantrine were fitted with DDSolver to all of the models implemented in the programme and lag time release properties are considered. The DDSolver programme offers various statistical principles for analysing the goodness of fit of a model. These include the correlation coefficient, the coefficient of determination, the adjusted coefficient of determination, the mean square error, the standard deviation of the residuals, sum of squares, weighted sum of squares, the Akaike Information Criterion, and the Model Selection Criterion (MSC). For the release kinetics of the two drugs assessed in this study and to identify the best fitted model, the correlation coefficient (r^2) and the MSC were employed. The best fit of the drug release profile is the model where the calculated r^2

approaches a value of 1, in other words, the highest r^2 value; and also, where the largest MSC value is obtained. In general, a MSC value of more than two to three designates a good fit (Mayer *et al.*, 1999; Zhang *et al.*, 2010).

Both artemether and lumefantrine displayed release properties from all of the selected SEDDS formulations that best fitted the Peppas-Sahlin 2 model (Table 4.25; Equation 4.1). The Peppas-Sahlin model explains the release profile of a drug, fitting the release profile of the drug to either Fickian diffusional release or case- II relaxational release. Fickian diffusion can be described as the solute transport process where the polymer relaxation time is greater than the solvent diffusion time. Fickian release ensues by molecular diffusion of the drug from the dosage form to the gastrointestinal media due to a chemical potential gradient. If the Fickian diffusion constant (k_1) value is higher than 1, it can safely be assumed that Fickian diffusion transpired. Case- II relaxational release, conversely, can be transcribed to the release of drug due to stresses and state-transition in hydrophilic glassy polymers which swell upon contact with biological fluids or water, in a similar fashion the lipid swells upon contact with biological fluids (Fu & Kao, 2010).

$$F = k_1\left(t - Tlag\right)^{0.5} + k_2\left(t - Tlag\right) \quad \textbf{(4.1)}$$

Where: F, is the fraction of drug released; k_1, is the Fickian diffusion constant; k_2, is the Case-II relaxational constant; T_{lag}, is the lag time; and, t, is time.

In Table 4.26 the r^2 and MSC values of all of the selected SEDDS formulations are tabled. Note that all of the values are higher than 0.990 and 3, respectively; thus, implying that the Peppas-Sahlin 2 model is the best fit for all of the formulations considered. It may also be observed that the k_1 values are above 1, signifying that Fickian diffusion occurred from all of the SEDDS formulations analysed. The graphs depicting the best fitted model for the selected SEDDS formulations can be seen in Annexure D.

Table 4.26: *The pharmacokinetic release profiles of the selected SEDDS formulations*

	SEDDS	Model	R^2	MSC	K_1
Artemether	Avocado oil (4:6)	Peppas-Sahlin 2	0.997	5.028	1.030
	Castor oil (2:8) S80		0.996	4.710	6.578
	Castor oil (3:7) S60		0.997	5.037	5.414
	Coconut oil (6:4)		0.997	4.867	3.338
	Olive oil (3:7)		0.994	3.374	2.378
	Peanut oil (6:4)		0.995	4.576	2.794
Lumefantrine	Avocado oil (4:6)		0.991	4.021	2.035
	Castor oil (2:8) S80		0.999	6.991	2.386
	Castor oil (3:7) S60		0.999	8.005	2.003
	Coconut oil (6:4)		0.999	6.359	2.874
	Olive oil (3:7)		0.995	3.586	1.708
	Peanut oil (6:4)		0.996	4.882	2.506

CHAPTER 5

SUMMARY AND FUTURE PROSPECTS

5.1 Summary

As stated, malaria remains a distressing widespread infectious disease that is extremely complex and it places enormous pressure on world health (Benelli *et al.*, 2017; Cohen *et al*., 2012; Feng *et al.*, 2015; Mehlhorn, 2008; Sherrard-Smith *et al*., 2017; Spar & Delacey, 2006). Moreover, resistance towards malaria treatment is now also a global threat (WHO, 2017). Although new chemical entities are being developed, progress is slow due to a lack of funding, and the time it takes to develop novel dosage forms is torturous. For these reasons, scientists are now researching methods to improve existing drugs as well as their dosage forms (Feng *et al.*, 2015).

Combination therapy has become a field of interest and is currently widely used. For example, artemether and lumefantrine are not newly synthesised compounds; but they have been formulated into a fixed-dose combination named Coartem®. Artemether is a short acting compound, whereas lumefantrine is a longer acting antimalarial drug. This combination has been classified by the WHO as first line treatment against uncomplicated *P. falciparum* malaria (WHO, 2017) and the efficacy of this combination therapy has been proven effective (Besufikad, 2017; Makanga *et al*., 2011; Makanga & Krudsood, 2009; Nega *et al*., 2016; Vaughan *et al*., 2004).

However as explained, these drugs are highly lipophilic and formulation of them into an effective dosage form remains challenging. Therefore, it has been prescribed that when taking Coartem®, it must be accompanied by a fatty meal (Mwebaza *et al*., 2017), which is very difficult for patients residing in a third world country, that are not only constantly nauseous, but who live in poverty-stricken areas and do not have the means to obtain proper nutrition (Oldewage-Theron *et al.,* 2006). Pharmaceutical formulation scientists are regularly faced with the problem of insufficient drug solubility as it directly affects the therapeutic ability of a drug. Currently, most novel drugs synthesised are similar to artemether and lumefantrine, i.e. highly lipophilic in nature, highlighting the predicament we are in **(**Mahapatra *et al*., 2014). Thus, poor bioavailability, poor-water-solubility and often the short half-life of drugs have driven

researchers to advance delivery systems that are able to improve the therapeutic efficacy of these types of drugs (Aderibigbe, 2017).

Lipid-based formulations, such as SEDDSs, is a promising strategy to formulate lipophilic composites. This may be an alternative approach to improve orally absorbed drugs, as they have shown to significantly improve oral bioavailability. Literature has indicated that these systems are able to avoid the dissolution step upon oral administration and may even bypass the first pass effect. Researchers have suggested that several mechanisms exist through these systems, which may improve the bioavailability of hydrophobic drugs, including:

- increased membrane fluidity facilitating transcellular absorption;
- the opening of tight junctions, allowing paracellular transport;
- inhibition of P-glycoprotein-mediated drug efflux and/metabolism by gut membrane-bound CYP450 enzymes;
- improved lymphatic transport in combination with stimulation of lipoprotein/chylomicron production;
- facilitation of *in vivo* dispersion through supplementary surfactants; and
- lipolysis of constituent lipids, to name a few.

Due to the fact that SEDDSs are the most dispersed lipid-based formulations, these systems appear to be most favourable. However, the effectiveness of a SEDDS formulation is ordinarily incident-explicit; consequently, the composition of a SEDDS formulation should be very carefully determined (Gursoy & Benita, 2004; Porter & Charman, 2001; Zanchetta *et al.*, 2015). This study therefore aimed to formulate SEDDSs comprising a fixed-dose combination of artemether and lumefantrine, to not only improve the solubilisation of these lipophilic drugs but to also attempt to enhance the erratic drug release, dissolution, and subsequent absorption (Abdulla & Sagara, 2009) of the commercial product, Coartem®, which necessitates the inclusion of a fatty meals upon administration (Mizuno *et al*., 2009; Mwebaza *et al.,* 2017; WHO, 2015).

From the isothermal microcalorimetry experiments conducted it was evident that the selected natural oils (avocado-, castor-, coconut-, olive-, and peanut oil), surfactants (Tween® 80 and SLS) and co-surfactants (Span® 80 and Span® 60) chosen for this study were indeed compatible with one another. Furthermore, solubility studies indicated that the solubility of both artemether and lumefantrine were exponentially enhanced. Following, the selected natural oils and surfactant phase, which comprised one of the surfactants and co-surfactants (1:1 ratio), were employed to prepare SEDDSs. Not only is appropriate selection of components vital, but the amount of each component included is also deemed important in the development of a robust self-dispersible lipid formulation to be delivered orally (Ahmad *et al*., 2013).

Pseudo-ternary phase diagrams, which are significant assessment tools, were utilised to assess the effect of the selected formulation components on *in-vitro* performance as it provides a scientific basis for the selection of the different constituents in the various concentrations (Wang & Pal, 2013).

The pseudo-ternary diagrams where successfully constructed for the following systems: avocado oil (4:6), castor oil (2:8) S80, coconut oil (6:4), olive oil (3:7), peanut oil (6:4), and castor oil (3:7) S60. The nano-emulsion area of each of the SEDDS formulations was carefully identified as the clear area upon visual assessment of the various ratios of oil phase to surfactant phase. Additionally, the impact that the selected oil phases and surfactant phases ratios had on the nano-emulsion area was identified, and it was noted that the stability of the SEDDS formulations and the nano-emulsion area was dependent on the type of surfactant phase used (Wang & Pal, 2013). In this study it was established that SEDDSs are physically stable formulations when the correct surfactant and co-surfactant are chosen to match the HLB values of the oils incorporated (AboulFotouh *et al.,* 2017; Costa *et al.,* 2014; Fernandes *et al.,* 2012). It was evident that, with the natural oils used, that the combination of Tween® 80 and Span® 80 used as surfactant phase, proved to display the most physical stable formulations as well as more clearly defined nano-emulsion areas. However, the SEDDS formulation comprising castor oil, Tween® 80, and Span® 60 displayed only adequate physical stability and a well-defined nano-emulsion area. The loading of the fixed-dose combination artemether and lumefantrine in the oil phase had no significant effect on the nano-emulsion area.

Gastrointestinal absorption is significantly influenced by the charge and size of the oil droplet formed in an emulsion. The size of the oil droplet required to ensure optimal absorption through the intestinal mucosa is generally between 100–500 nm. This is due to smaller oil droplets displaying a decrease in the tendency of the droplets to agglomerate to the intestinal membrane's surface. Thus, concluding that the smaller the oil droplets, the higher the absorption of the oil droplet (Gershanik *et al.*, 1998; Gursoy and Benita, 2004; Tummons *et al.,* 2016). Both the droplet size and zeta potential of emulsions plays an integral part in the stability of these dosage forms. The higher the uniformity of the oil droplets, the more stable the emulsion. Furthermore, if an emulsion displays a zeta potential not differentiating more than 10 mV during stability testing, the emulsion can be assumed as stable (AboulFotouh *et al.,* 2017; Roland *et al.,* 2003). This study demonstrated that the selected SEDDS formulations displayed adequate stability, with avocado oil (4:6), castor oil (2:8) S80, coconut oil (6:4), olive oil (3:7), and castor oil (3:7) S60 presenting with oil droplets sizes that fell within the nano-

range, which could possible conclude that these emulsions would probably display a superior absorption from the gastrointestinal tract.

Cloud point determination and self-emulsification time are experiments to determine the physical stability of SEDDS in the gastrointestinal environment. Cloud point determination indicates the temperature at which the aqueous solution of the water-soluble surfactant displays signs of turbidity. If the temperature is higher than the cloud point of a SEDDS formulation, irreversible phase separation of the formulation will occur, which in turn will lead to poor absorption of the drug. Ideally, the cloud point temperature of a SEDDS should be higher than 37ºC (body temperature). In this study all of the SEDDS formulations depicted a cloud point higher than 37ºC, thus safely assuming that the selected SEDDS will remain stable in the gastrointestinal environment (Chudasama *et al.,* 2015). In a similar fashion, self-emulsification time determines the efficacy of the emulsification of a SEDDS when the SEDDS is introduced into the gastrointestinal media (Balata *et al.,* 2016). The self-emulsification time of avocado oil (4:6), castor oil (2:8) S80, coconut oil (6:4), olive oil (3:7), and peanut oil (6:4) proved that these systems displayed effective emulsification, indicating that the entropy changes of these systems were favoured.

Thermodynamic stability tests evaluate SEDDSs ability to become instable after being stored for a period of time. After an extended storage period, SEDDSs may start to display physical instabilities such as seed crystals that begin to form and agglomerate to one another, causing precipitation, as well as cracking and creaming (Agrawal *et al.,* 2015). The selected SEDDS formulations in this study did not display any physical instabilities, rendering these formulations stable during prolonged storage periods.

All of the selected SEDDS formulations exhibited pseudoplastic rheological behaviour. Ideally a pharmaceutical preparation must display pseudoplastic flow, due to this flow being able to stabilise insoluble particles in SEDDS formulations, which prevents sedimentation of particles (Ujhelyi *et al.,* 2018). Determining the viscosity of SEDDSs identifies if the system is an oil-in-water or water-in-oil SEDDS. If the system displays a lower viscosity, it is regarded as an oil-in-water system; similarly, if the system displays high viscosity, it can be classified as a water-in-oil system. All of the SEDDS formulations demonstrated high viscosity, rendering them water-in-oil systems. Moreover, water-in-oil systems are considered ideal, because these SEDDSs are isotropic in nature and contain a mixture of lipids and surfactants, which when in contact with gastric medium, self-emulsify into an oil-in-water system, allowing enhanced dissolution, transport, and subsequent absorption of the fixed-dose combination of artemether and lumefantrine (Rahman *et al.*, 2013).

Succeeding the physical stability tests, the selected SEDDS formulations were subjected to dissolution studies. All selected SEDDS formulations exhibited release of both artemether and lumefantrine. However, artemether displayed a superior %drug release from the selected SEDDS formulations when compared to lumefantrine. Release of lumefantrine was only detected once the biorelevant media was added to the dissolution media which subsequently increased the pH of the media to 7.4. Interestingly, the release profiles of both artemether and lumefantrine could be fitted to the Peppas-Sahlin 2 model, and thus the release of both drugs can be explained by Fickian diffusion. Fickian diffusion describes the release of artemether and lumefantrine as drug particles moving from an area of a high concentration in the SEDDS formulation to an area of low concentration in the surrounding gastric medium.

Considering the overall physical stability of the selected SEDDS formulations as well as the dissolution profiles obtained for both drugs, it may be concluded that avocado oil (4:6) and olive oil (3:7) SEDDS formulations may be considered the optimal formulations for the fixed-dose combination of artemether and lumefantrine. This study confirms that the use of artemether and lumefantrine in conjunction with natural oils to formulate SEDDSs, are potentially a viable drug delivery system that should be further investigated in order to treat uncomplicated *Plasmodium falciparum* malaria more effectively.

5.2 Future prospects

SEDDS formulations are advantageous as they protect the incorporated drug in the gastrointestinal environment. This type of dosage form is able to provide targeted drug release, enhance oral bioavailability, provide a more constant absorption profile, offer controlled drug delivery profiles, and protect sensitive drug molecules. These advantages render this a versatile dosage form, making these SEDDSs easy to manipulate into different dosage forms, such as capsules, tablets, suppositories, etc. This particular drug delivery system is therefore a desired system, which can be custom made to fit particular needs in order to treat a particular disease or group of people. Tablets can be produced from SEDDS through the addition of a highly absorbent carrier, namely, silicates and cellulose derivatives. These absorbent carriers are able to provide increased content uniformity and allow for an even more stable dosage form compared to liquid dosage forms. Although solid SEDDSs are similar to an amorphous solid dispersion, these systems remain entirely unique due to the fact that the drug remains in the adsorbed non-volatile solvent or lubricant state (O'Reilly Beringhs *et al.,* 2018). Solid SEDDSs furthermore afford a wider range of dosage forms that can be explored, thus making the addition of an adsorbent something to investigate for future use.

Oils often utilised in formulating SEDDSs are normally medium and long chain triglycerides that vary in their degree of saturation. Natural oils are frequently considered for their less toxic profiles and are generally more cost effective; however, these oils are restricted due to their low self-emulsification time and the drugs incorporated are often not as soluble in natural oils compared to more modified oils (Sapra *et al.,* 2012). Hence, modified vegetable oils may be investigated further to determine whether the fixed-dose combination of artemether and lumefantrine, or any poorly soluble artemisinin drug will depict higher solubility and an enhanced self-emulsification time.

A stable, more optimal SEDDS can be manufactured if the HLB values of the oil- and surfactant phases are matched (AboulFotouh *et al.,* 2017; Costa *et al.,* 2014). For this reason, it is suggested that a larger variety of different surfactants and co-surfactants that portray HLB values close to that of the selected oils should be investigated. Also, the incorporation of different hydrophilic surfactants, in order to ensure that an oil-in-water SEDDS is formulated, should be considered (AboulFotouh *et al.,* 2017; Costa *et al.,* 2014; Fernandes *et al.,* 2012).

From the dissolution studies conducted it was evident that there was a noticeable drug release of lumefantrine from the selected SEDDS formulations. However, less than 70% of lumefantrine was released, hence, dissolution studies over a longer time period, for example 18–24 h, should be considered in order to establish the complete drug release profile of lumefantrine from the drug delivery system. Furthermore, *in vivo* studies are encouraged in order to establish whether this dosage form will aid in the transport of this fixed-dose combination across cell membranes. This will also provide insight into how the enzymes in the membranes will react to the SEDDSs.

Given the fact that most artemisinin-based combination therapies are poorly soluble in water (WHO, 2015), the various different artemisinin-based drugs can be tested in SEDDSs in order to determine the physical stability as well as the percentage drug release from this dosage form. Subsequently, the effect that SEDDSs have on the physical stability and release profiles of other poorly-water-soluble drugs should be investigated.

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Annexure A

Pseudo-ternary phase diagrams of the different oils and surfactants and co-surfactants tested

Figure A.1: Pseudo-ternary phase diagrams for the avocado oil/Tween® 80/Span® 60 system

Figure A.2: Pseudo-ternary phase diagrams for the coconut oil/Tween® 80/Span® 60 system

Figure A.3: Pseudo-ternary phase diagrams for the olive oil/Tween® 80/Span® 60 system

Figure A.4: Pseudo-ternary phase diagrams for the peanut oil/Tween® 80/Span® 60 system

Figure A.5: Pseudo-ternary phase diagrams for (A) the avocado oil/SLS/Span® 80 system, (B) the castor oil/SLS/Span® 80 system, (C) the coconut oil/SLS/Span®80 system, (D) the olive oil/SLS/Span® 80 system, and (E) the peanut oil/SLS/Span® 80 system

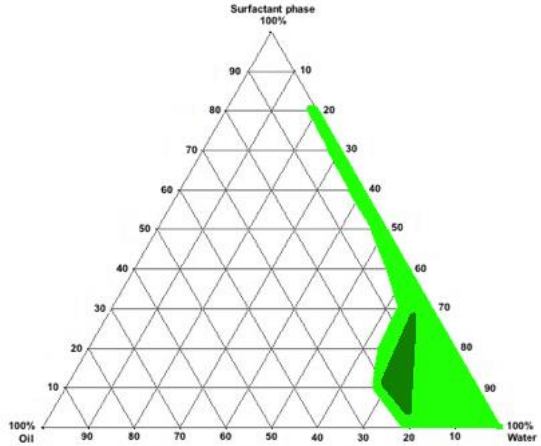

Figure A.1: *Pseudo-ternary phase diagrams for the avocado oil/Tween® 80/Span® 60 system*

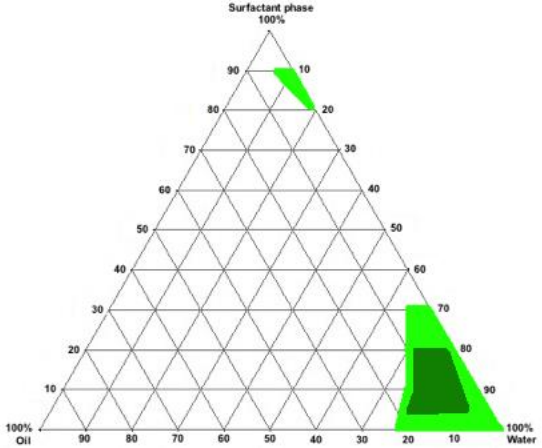

Figure A.2: *Pseudo-ternary phase diagrams for the coconut oil/Tween® 80/Span® 60 system*

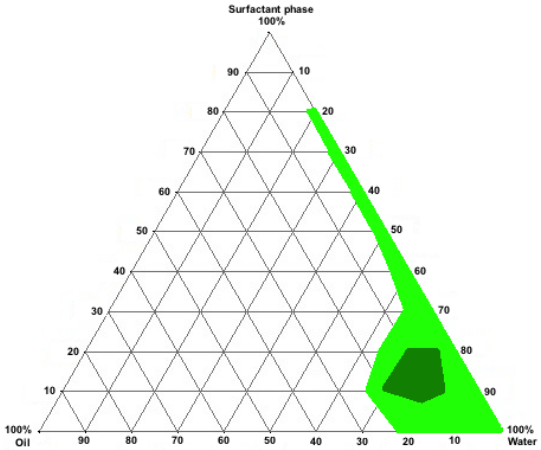

Figure A.3: *Pseudo-ternary phase diagrams for the olive oil/Tween®80/Span® 60 system*

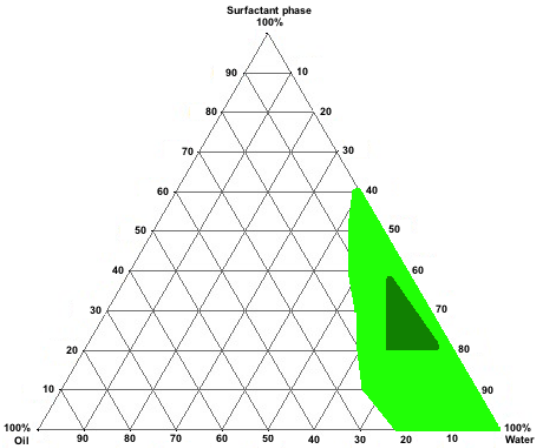

Figure A.4: *Pseudo-ternary phase diagrams for the peanut oil/Tween® 80/Span® 60 system*

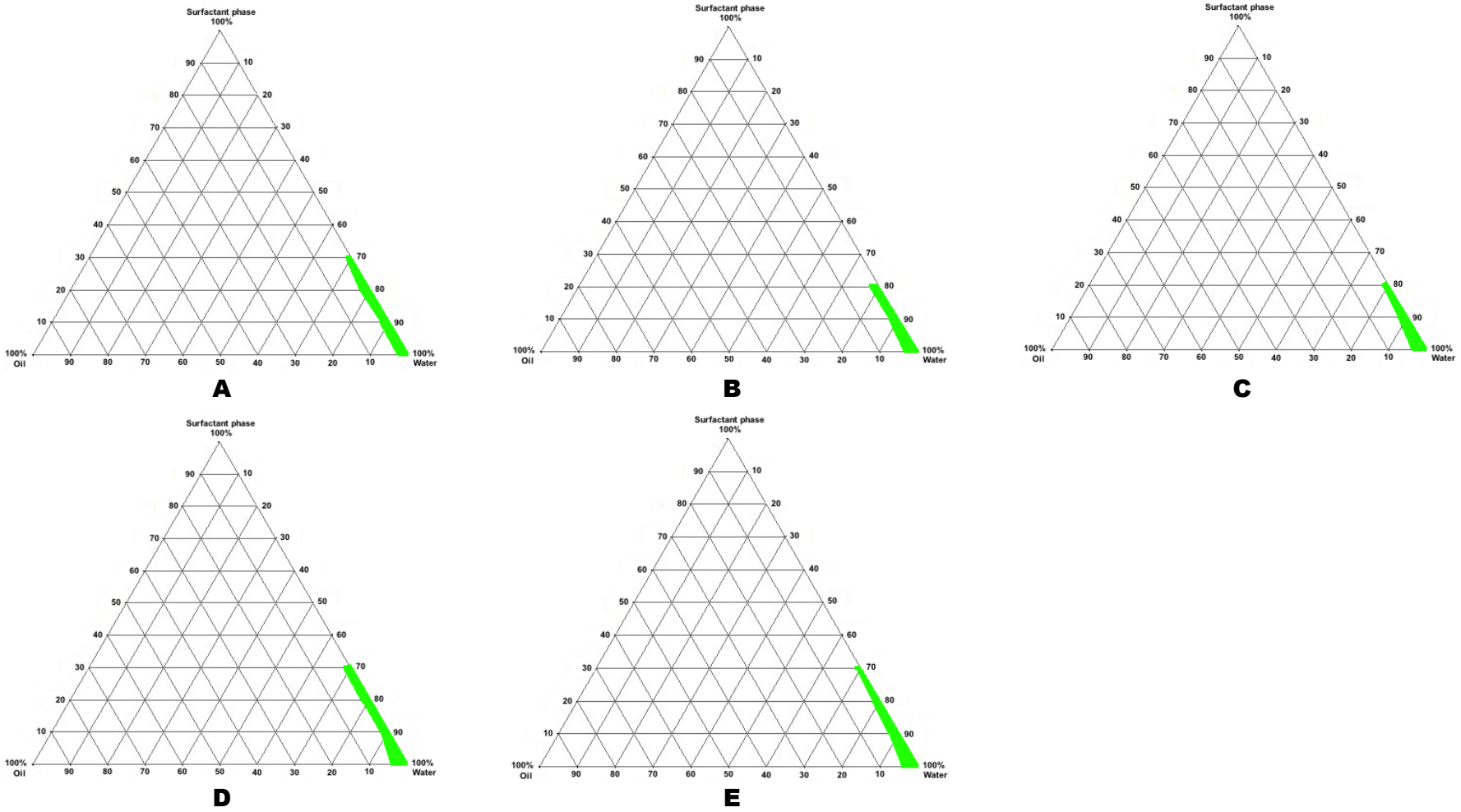

Figure A.5: *Pseudo-ternary phase diagrams for (A) the avocado oil/SLS/Span® 80 system, (B) the castor oil/SLS/Span® 80 system, (C) the coconut oil/SLS/Span® 80 system, (D) the olive oil/SLS/Span® 80 system, and (E) the peanut oil/SLS/Span® 80 system*

Annexure B

Droplet size and zeta potential

Table B.1: Average droplet size and %RSD obtained for the avocado oil (4:6) SEDDS formulation

Table B.2: Average droplet size and %RSD obtained for the castor oil (2:8) S80 SEDDS formulation

Table B.3: Average droplet size and %RSD obtained for the castor oil (3:7) S60 SEDDS formulation

Table B.4: Average droplet size and %RSD obtained for the coconut oil (6:4) SEDDS formulation

Table B.5: Average droplet size and %RSD obtained for the olive oil (3:7) SEDDS formulation

Table B.6: Average droplet size and %RSD obtained for the peanut oil (6:4) SEDDS formulation

Figure B.1: Zeta potential obtained for the avocado oil (4:6) SEDDS formulation

Figure B.2: Zeta potential obtained for the castor oil (2:8) S80 SEDDS formulation

Figure B.3: Zeta potential obtained for the castor oil (3:7) S60 SEDDS formulation

Figure B.4: Zeta potential obtained for the coconut oil (6:4) SEDDS formulation

Figure B.5: Zeta potential obtained for the olive oil (3:7) SEDDS formulation

Figure B.6: Zeta potential obtained for the peanut oil (6:4) SEDDS formulation

TableB.1: *Average droplet size and %RSD obtained for the avocado oil (4:6) SEDDS formulation*

Particle size (nm)	Average particle size (nm)	%RSD
239.5	241.3	3.0
244.9		
238.8		
237.9		
244.2		
242.5		

Table B.2: *Average droplet size and %RSD obtained for the castor oil (2:8) S80 SEDDS formulation*

Particle size (nm)	Average particle size (nm)	%RSD
522.9	524.1	2.6
524.8		
529		
522.5		
522.1		
523.4		

Table B.3: *Average droplet size and %RSD obtained for the castor oil (3:7) S60 SEDDS formulation*

Particle size (nm)	Average particle size (nm)	%RSD
251.3	249.4	3.7
247.7		
251.7		
253.3		
243		
249.2		

Table B.4: *Average droplet size and %RSD obtained for the coconut oil (6:4) SEDDS formulation*

Particle size (nm)	Average particle size (nm)	%RSD
779.6	776.2	5.3
772.5		
778.6		
766.9		
780.4		
778.9		

Table B.5: *Average droplet size and %RSD obtained for the olive oil (3:7) SEDDS formulation*

Particle size (nm)	Average particle size (nm)	%RSD
239.5	241.2	3.0
244.9		
238.4		
237.9		
244.2		
242.2		

Table B.6: *Average droplet size and %RSD obtained for the peanut oil (6:4) SEDDS formulation*

Particle size (nm)	Average particle size (nm)	%RSD
1473.0	1452.7	16.3
1429.0		
1443.0		
1448.0		
1468.0		
1455.0		

Malvern

Sample Details

Sample Name:	Olive oil T80 and S80 1		
SOP Name:	mansettings.nano		
General Notes:	DRUPPEL:1 drop in 200ml water		
File Name:	Avo oil T80 S80.dts	Dispersant Name:	Water
Record Number:	1	Dispersant RI:	1.330
Date and Time:	16 April 2018 02:49:26 PM	Viscosity (cP):	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	Zeta Runs:	12
Count Rate (kcps):	154.6	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	7

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-43.8	Peak 1:	-43.8	100.0	5.12
Zeta Deviation (mV):	5.12	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.0102	Peak 3:	0.00	0.0	0.00

Result quality Good

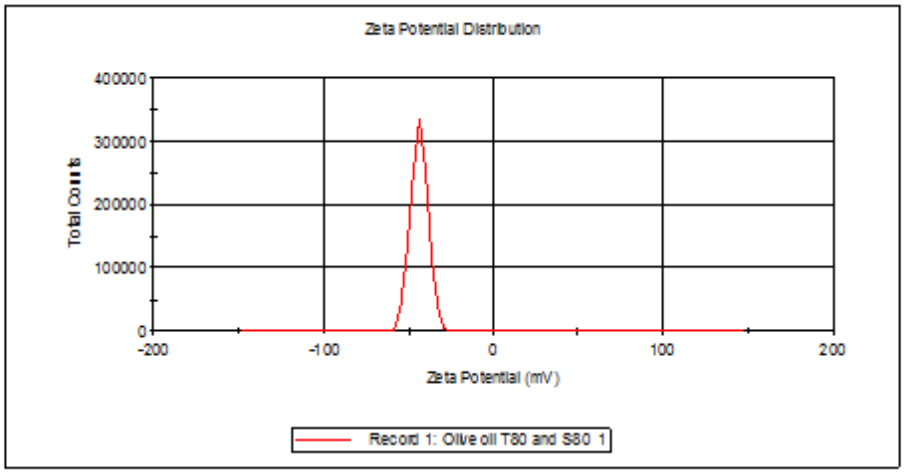

Figure B.1: *Zeta potential obtained for the avocado oil (4:6) SEDDS formulation*

Malvern

Sample Details

Sample Name:	Olive oil T80 and S80 6		
SOP Name:	mansettings.nano		
General Notes:	DRUPPEL:1 drop in 200ml water		
File Name:	Castor T80 S80.dts	Dispersant Name:	Water
Record Number:	12	Dispersant RI:	1.330
Date and Time:	17 April 2018 04:53:35 PM	Viscosity (cP):	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	Zeta Runs:	12
Count Rate (kcps):	356.1	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	7

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-44.6	Peak 1:	-44.6	100.0	5.10
Zeta Deviation (mV):	5.10	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.00137	Peak 3:	0.00	0.0	0.00

Result quality Good

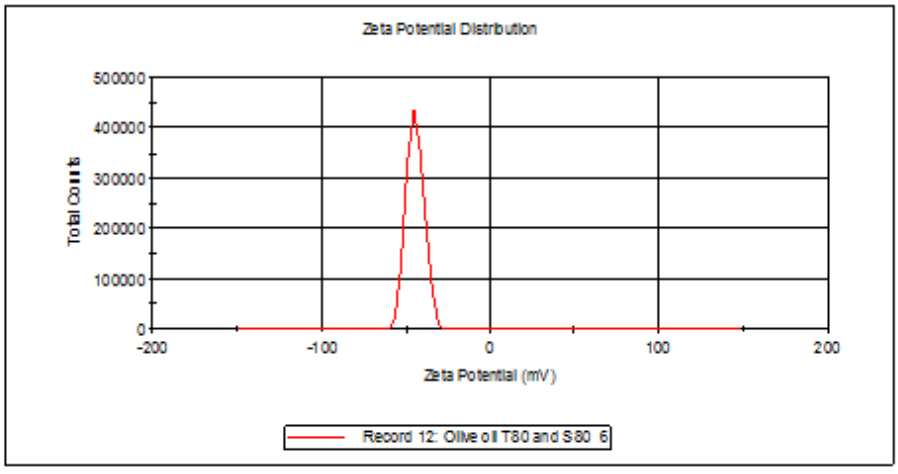

Figure B.2: *Zeta potential obtained for the castor oil (2:8) S80 SEDDS formulation*

Malvern

Sample Details

Sample Name:	Olive oil T80 and S80 3
SOP Name:	mansettings.nano
General Notes:	DRUPPEL:1 drop in 200ml water

File Name:	Castor oil T80 S60.dts	Dispersant Name:	Water
Record Number:	9	Dispersant RI:	1.330
Date and Time:	17 April 2018 06:17:13 PM	Viscosity (cP):	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	Zeta Runs:	12
Count Rate (kcps):	206.6	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	7

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-20.8	Peak 1:	-20.8	100.0	5.03
Zeta Deviation (mV):	5.03	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.00169	Peak 3:	0.00	0.0	0.00

Result quality Good

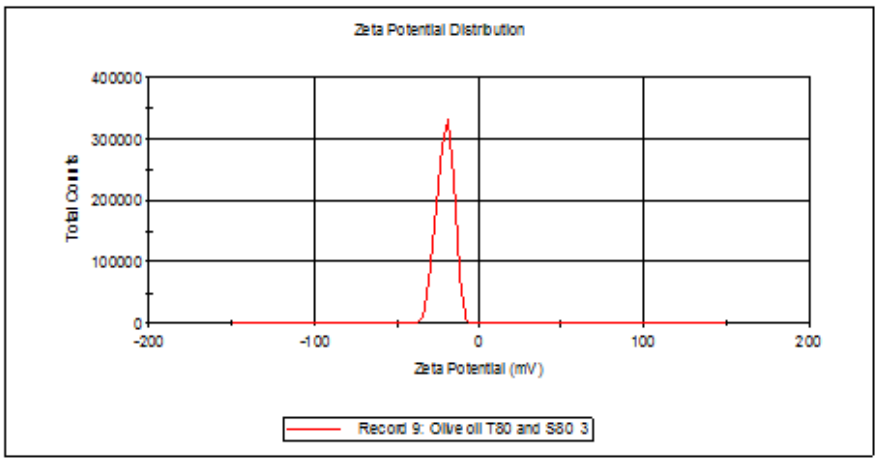

Figure B.3: *Zeta potential obtained for the castor oil (3:7) S60 SEDDS formulation*

Malvern

Sample Details

Sample Name:	Olive oil T80 and S80 1		
SOP Name:	mansettings.nano		
General Notes:	DRUPPEL:1 drop in 200ml water		
File Name:	Coconut T80 S80.dts	**Dispersant Name:**	Water
Record Number:	7	**Dispersant RI:**	1.330
Date and Time:	17 April 2018 05:18:26 PM	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	13
Count Rate (kcps):	58.0	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	7

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-46.6	**Peak 1:**	-46.6	100.0	4.82
Zeta Deviation (mV):	4.82	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0103	**Peak 3:**	0.00	0.0	0.00

Result quality Good

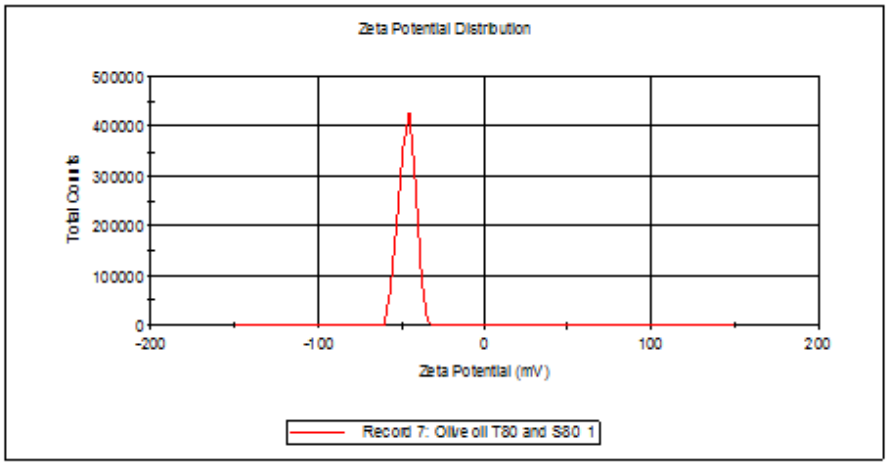

Figure B.4: *Zeta potential obtained for the coconut oil (6:4) SEDDS formulation*

Malvern

Sample Details

Sample Name:	Olive oil T80 and S80 1		
SOP Name:	mansettings.nano		
General Notes:	DRUPPEL:1 drop in 200ml water		
File Name:	Olive oil T80 S80.dts	Dispersant Name:	Water
Record Number:	1	Dispersant RI:	1.330
Date and Time:	16 April 2018 02:10:38 PM	Viscosity (cP):	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	Zeta Runs:	12
Count Rate (kcps):	172.8	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	7

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-54.7	Peak 1:	-54.7	100.0	5.62
Zeta Deviation (mV):	5.62	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.0148	Peak 3:	0.00	0.0	0.00

Result quality Good

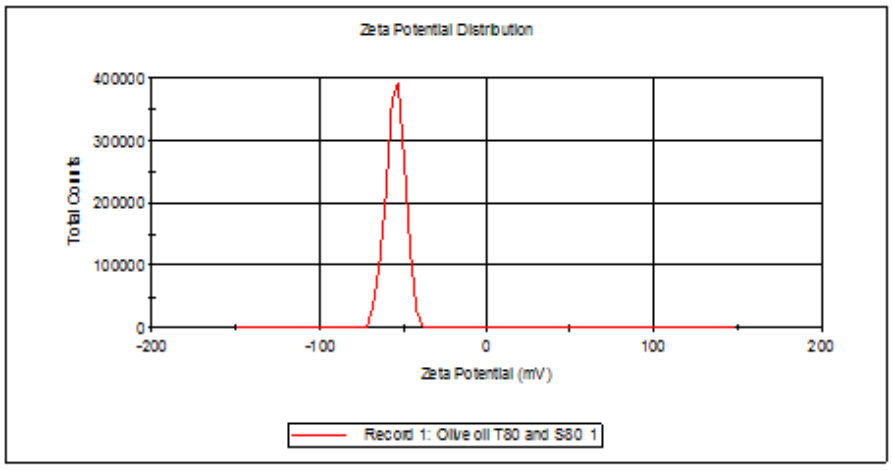

Figure B.5: *Zeta potential obtained for the olive oil (3:7) SEDDS formulation*

Malvern

Sample Details

Sample Name:	Olive oil T80 and S80 2		
SOP Name:	mansettings.nano		
General Notes:	DRUPPEL:1 drop in 200ml water		
File Name:	Peanut oil T 80 S80.dts	Dispersant Name:	Water
Record Number:	8	Dispersant RI:	1.330
Date and Time:	17 April 2018 05:50:50 PM	Viscosity (cP):	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	Zeta Runs:	12
Count Rate (kcps):	152.5	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	6

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-45.7	Peak 1:	-45.7	100.0	4.46
Zeta Deviation (mV):	4.46	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.00679	Peak 3:	0.00	0.0	0.00

Result quality Good

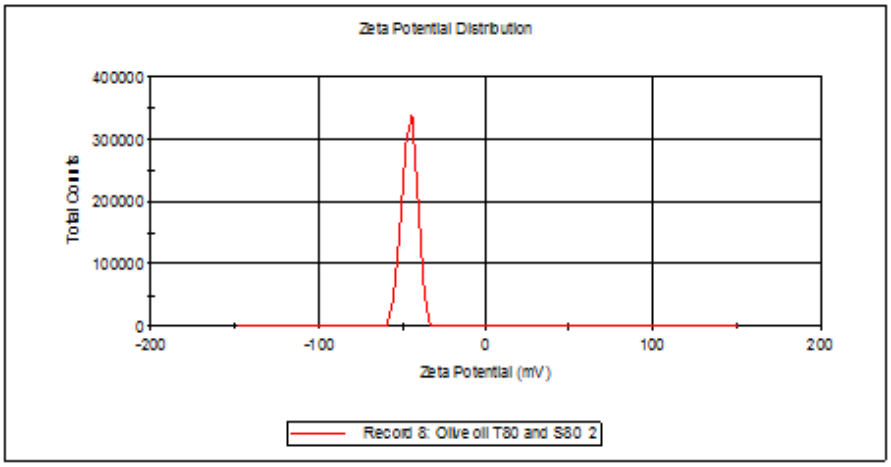

Figure B.6: *Zeta potential obtained for the peanut oil (6:4) SEDDS formulation*

Annexure C

Viscosity results for the selected SEDDS formulation

Figure C.1: Diagram of the viscosity of avocado oil (4:6) at different rotational speeds

Figure C.2: Diagram of the viscosity of castor oil (2:8) S80 at different rotational speeds

Figure C.3: Diagram of the viscosity of castor oil (3:7) S60 at different rotational speeds

Figure C.4: Diagram of the viscosity of coconut oil (6:4) at different rotational speeds

Figure C.5: Diagram of the viscosity of olive oil (3:7) at different rotational speeds

Figure C.6: Diagram of the viscosity of peanut oil (6:4) at different rotational speeds

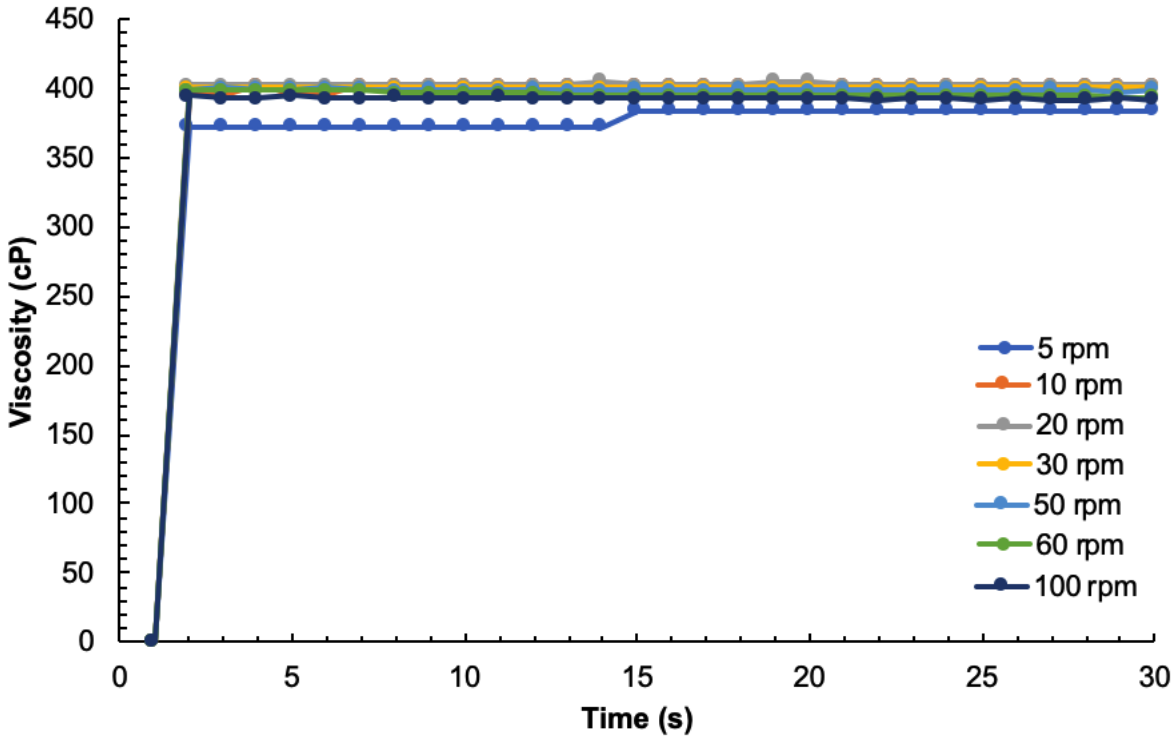

Figure C.1: *Diagram of the viscosity of avocado oil (4:6) at different rotational speeds*

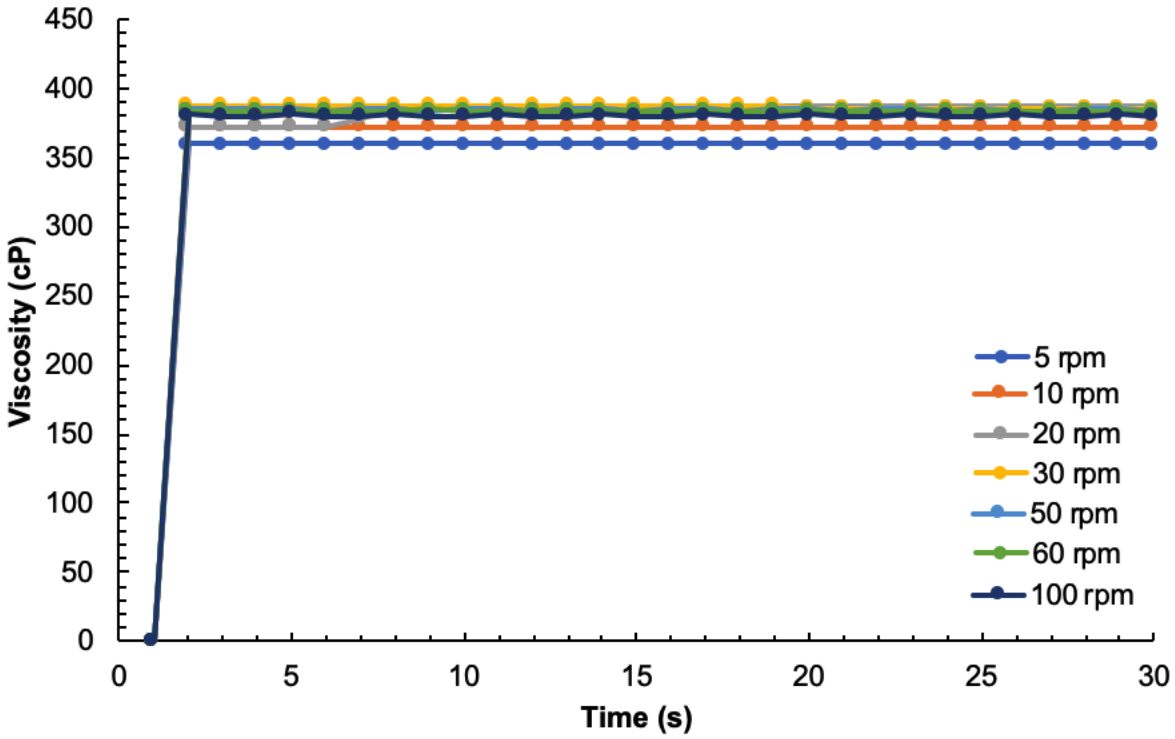

Figure C.2: *Diagram of the viscosity of castor oil (2:8) S80 at different rotational speeds*

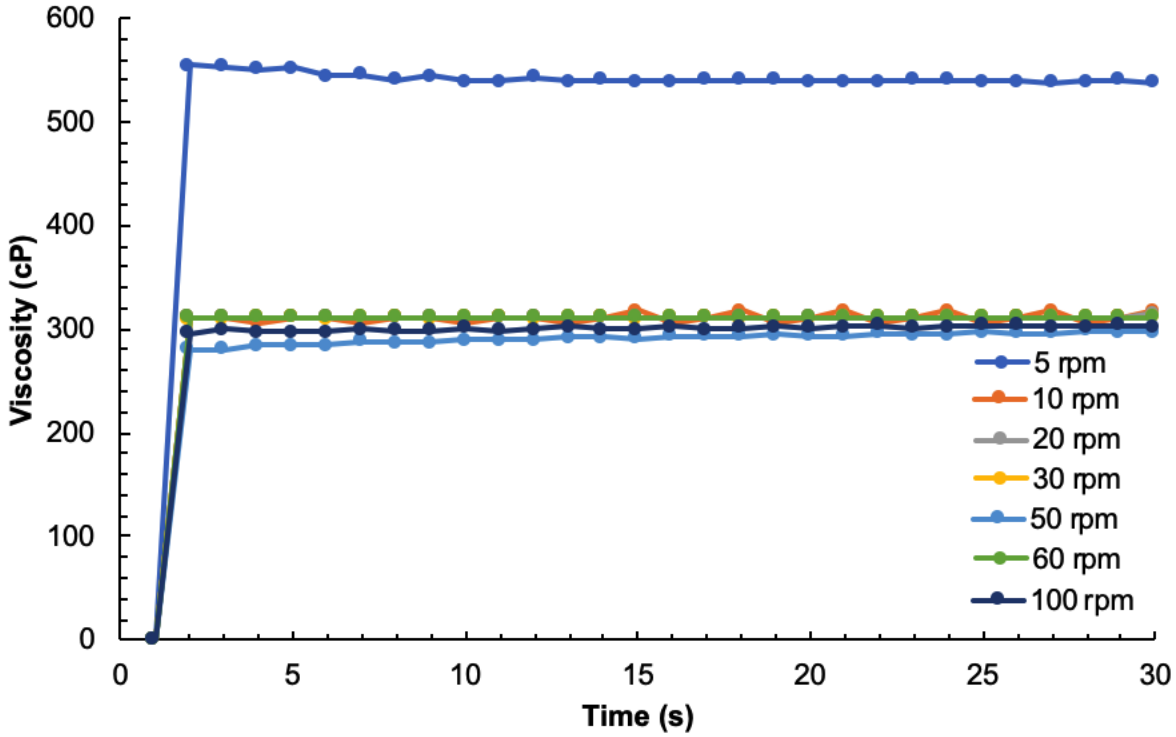

Figure C.3: *Diagram of the viscosity of castor oil (3:7) S60 at different rotational speeds*

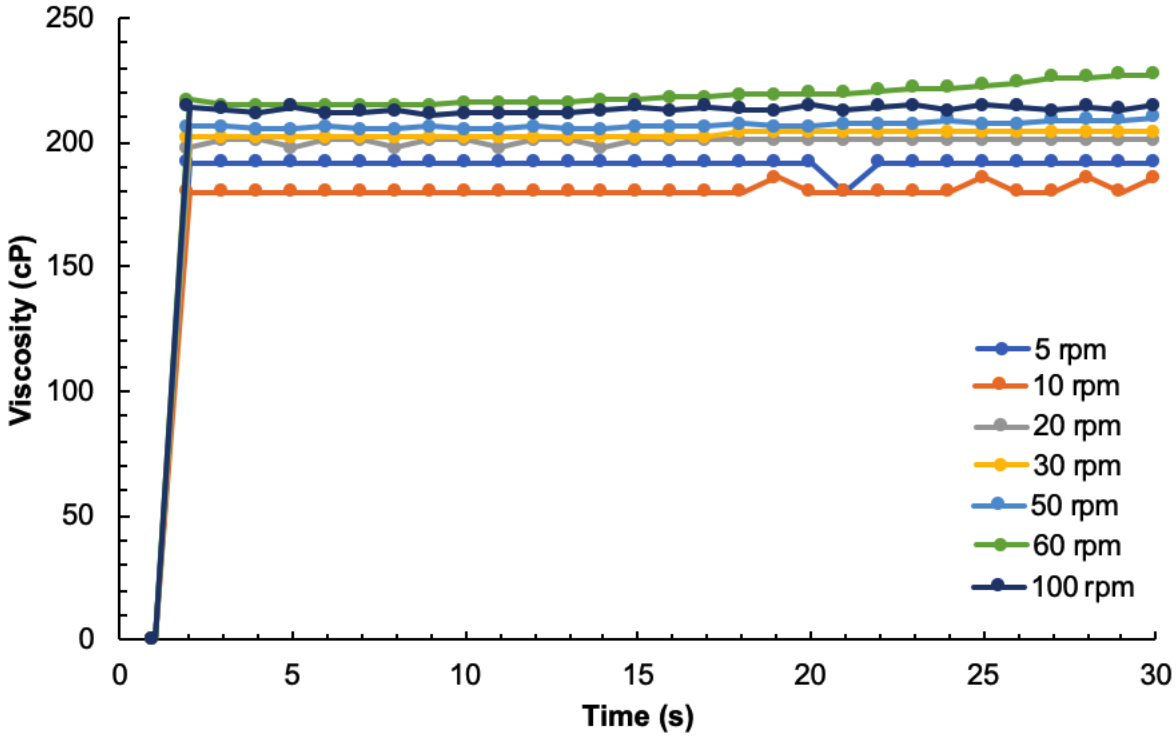

Figure C.4: *Diagram of the viscosity of coconut oil (6:4) at different rotational speeds*

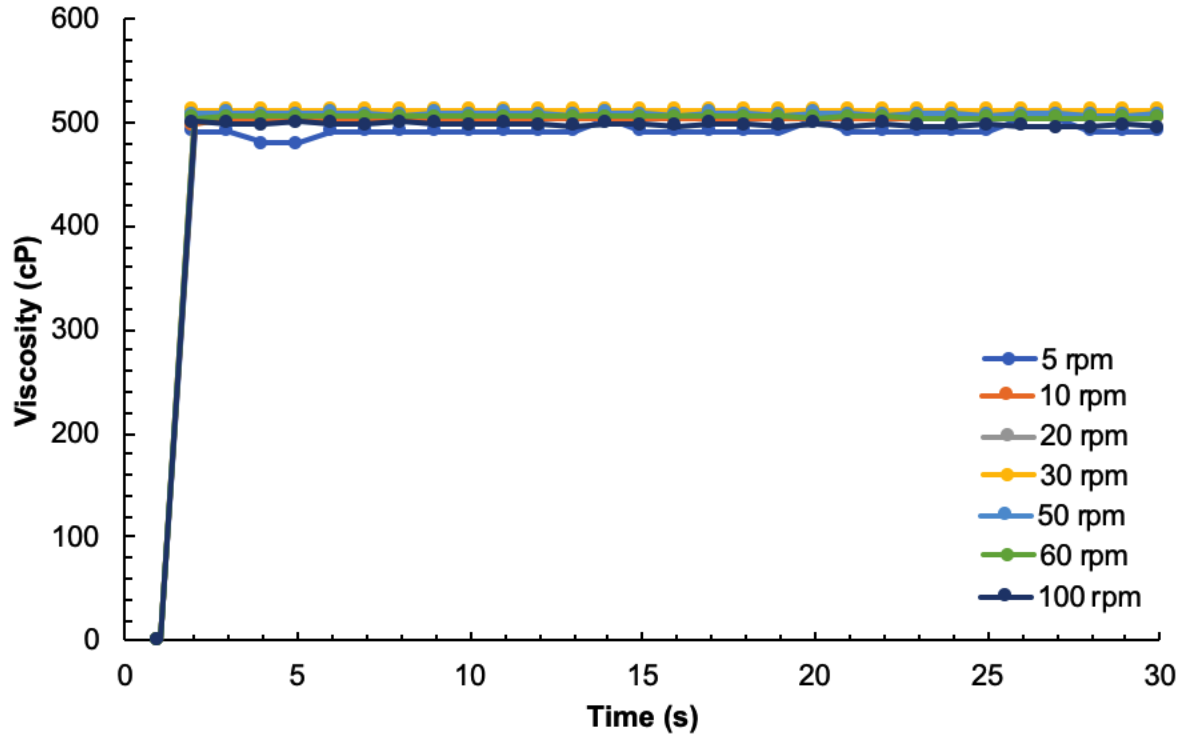

Figure C.5: *Diagram of the viscosity of olive oil (3:7) at different rotational speeds*

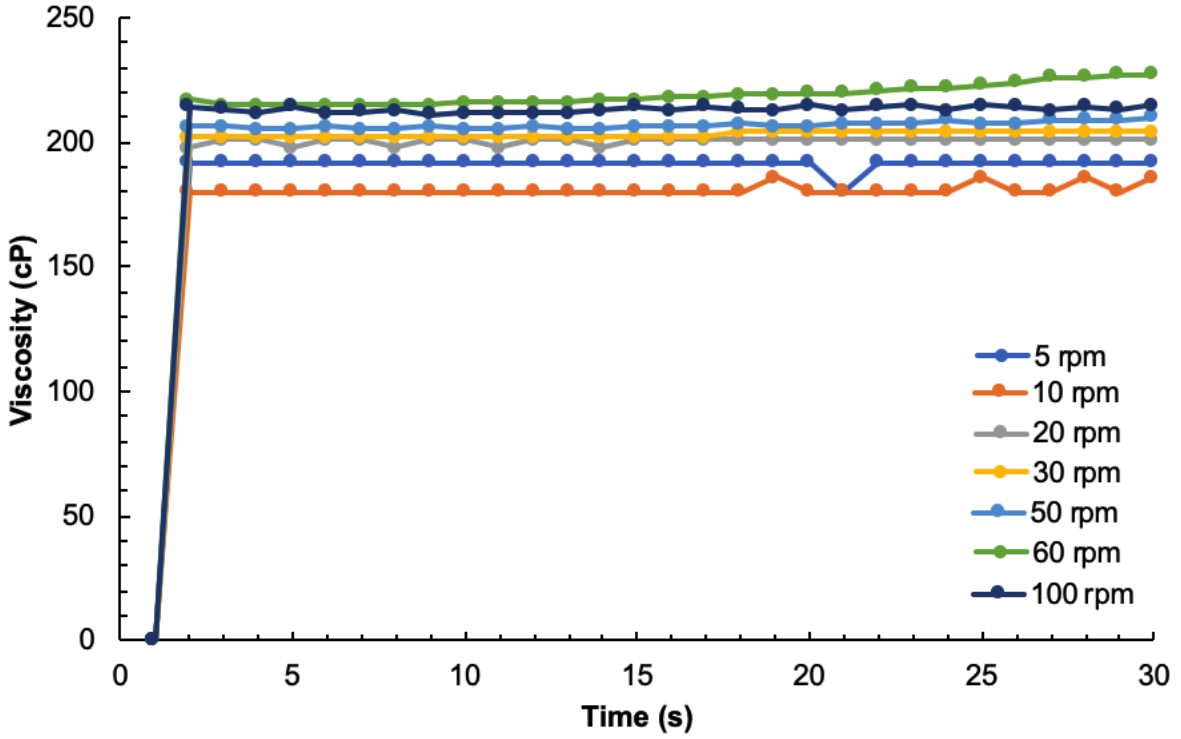

Figure C.6: *Diagram of the viscosity of peanut oil (6:4) at different rotational speeds*

Annexure D

Pharmacokinetics results for the selected SEDDS formulation

Figure D.1: Diagram of the best fit line for the Peppas-Sahlin 2 model of avocado oil (4:6) for artemether

Figure D.2: Diagram of the best fit line for the Peppas-Sahlin 2 model of castor oil (2:8) S80 for artemether

Figure D.3: Diagram of the best fit line for the Peppas-Sahlin 2 model of castor oil (3:7) S60 for artemether

Figure D.4: Diagram of the best fit line for the Peppas-Sahlin 2 model of coconut oil (6:4) for artemether

Figure D.5: Diagram of the best fit line for the Peppas-Sahlin 2 model of olive oil (3:7) for artemether

Figure D.6: Diagram of the best fit line for the Peppas-Sahlin 2 model of peanut oil (6:4) for artemether

Figure D.7: Diagram of the best fit line for the Peppas-Sahlin 2 model of avocado oil (4:6) for lumefantrine

Figure D.8: Diagram of the best fit line for the Peppas-Sahlin 2 model of castor oil (2:8) S80 for lumefantrine

Figure D.9: Diagram of the best fit line for the Peppas-Sahlin 2 model of castor oil (3:7) S60 for lumefantrine

Figure D.10: Diagram of the best fit line for the Peppas-Sahlin 2 model of coconut oil (6:4) for lumefantrine

Figure D.11: Diagram of the best fit line for the Peppas-Sahlin 2 model of olive oil (3:7) for lumefantrine

Figure D.12: Diagram of the best fit line for the Peppas-Sahlin 2 model of peanut oil (6:4) for lumefantrine

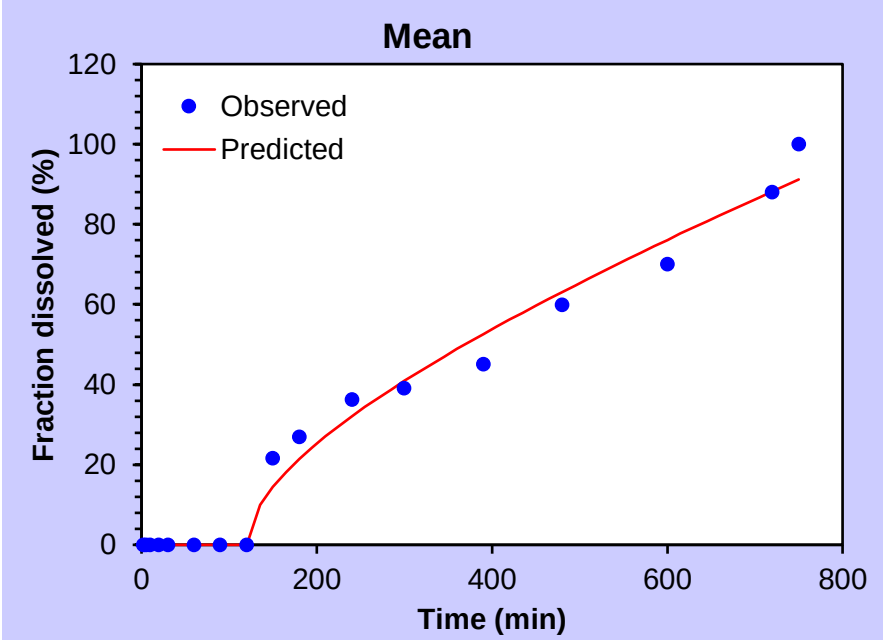

Figure D.1: *Diagram of the best fit line for the Peppas-Sahlin 2 model of avocado oil (4:6) for artemether*

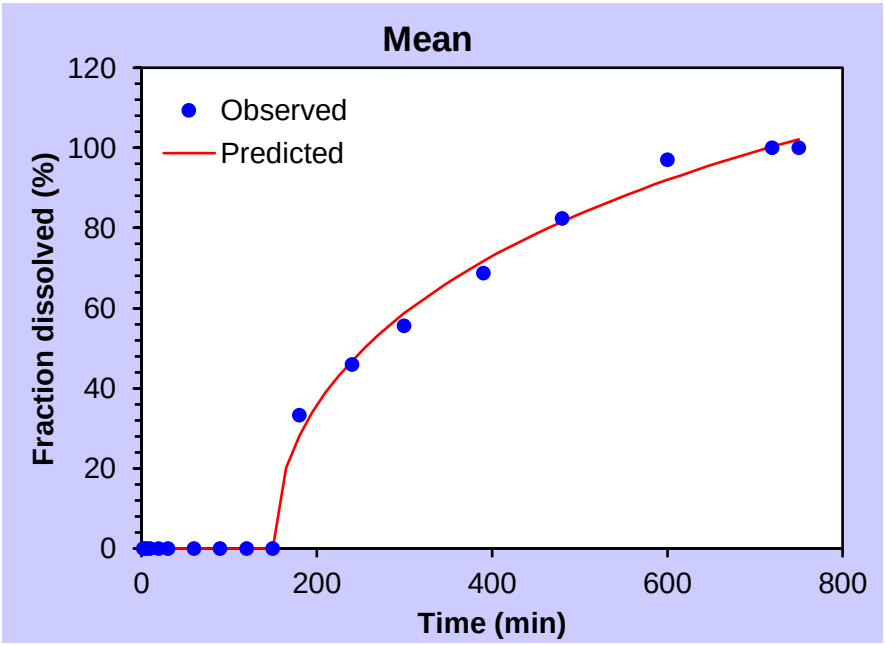

Figure D.2: *Diagram of the best fit line for the Peppas-Sahlin 2 model of castor oil (2:8) S80 for artemether*

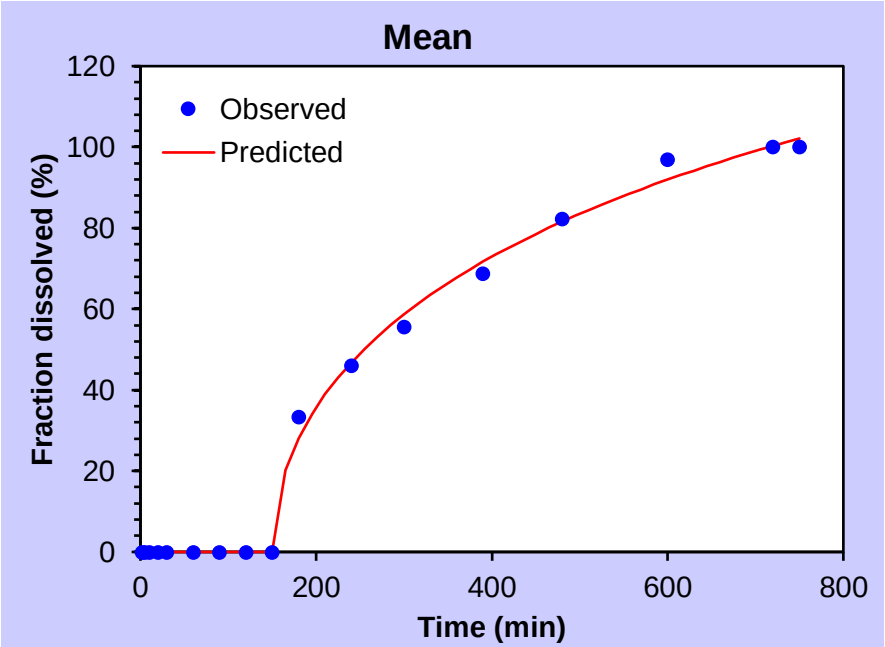

Figure D.3: *Diagram of the best fit line for the Peppas-Sahlin 2 model of castor oil (3:7) S60 for artemether*

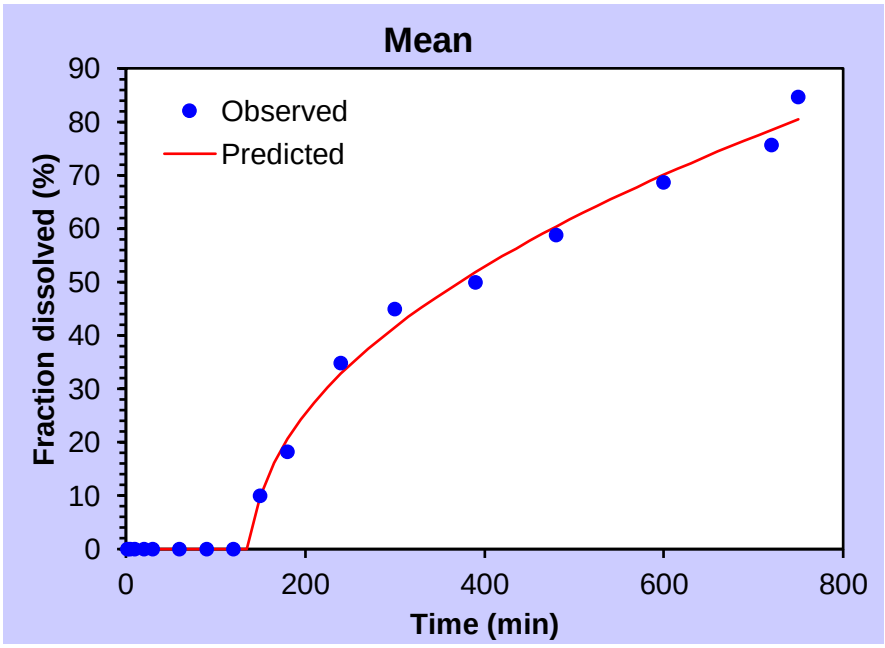

Figure D.4: *Diagram of the best fit line for the Peppas-Sahlin 2 model of coconut oil (6:4) for artemether*

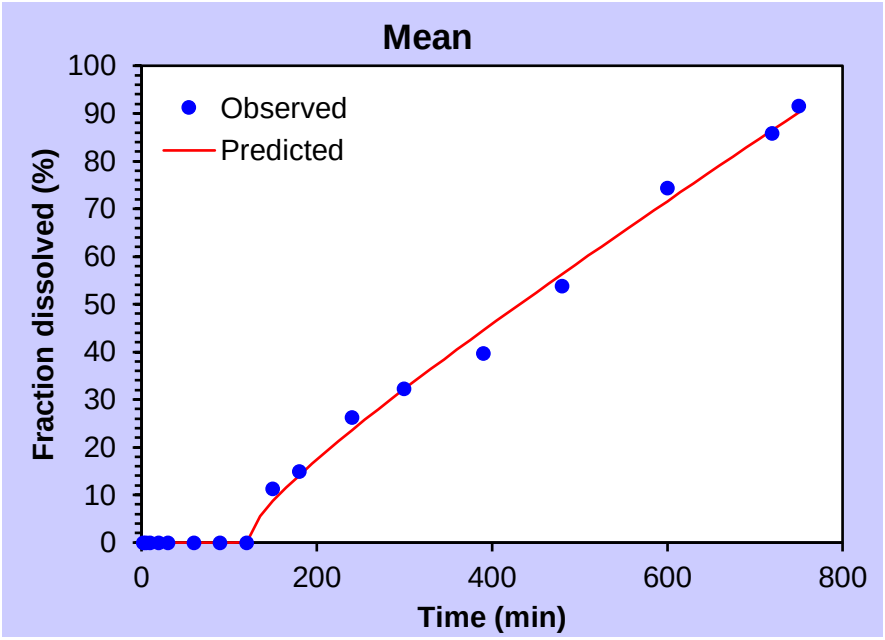

Figure D.5: *Diagram of the best fit line for the Peppas-Sahlin 2 model of olive oil (3:7) for artemether*

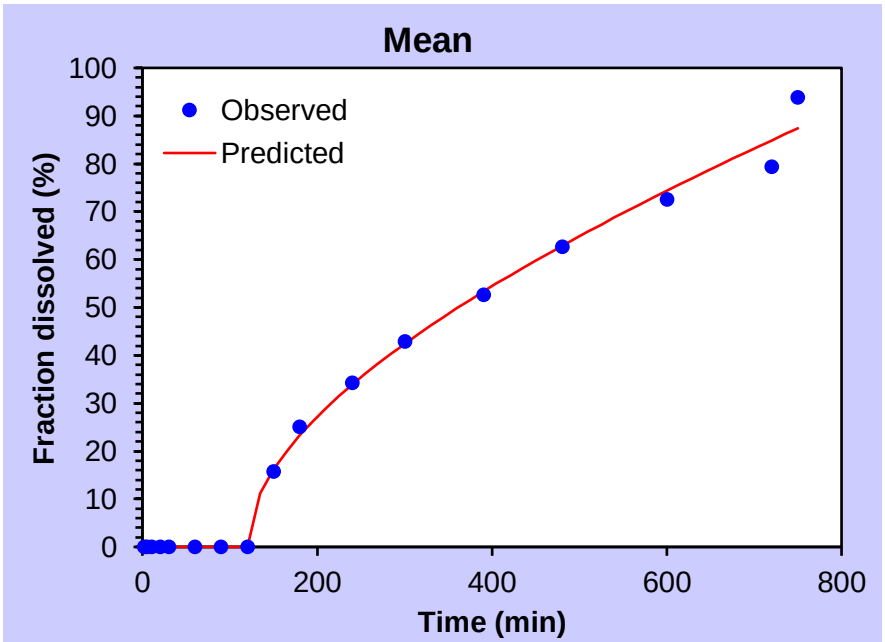

Figure D.6: *Diagram of the best fit line for the Peppas-Sahlin 2 model of peanut oil (6:4) for artemether*

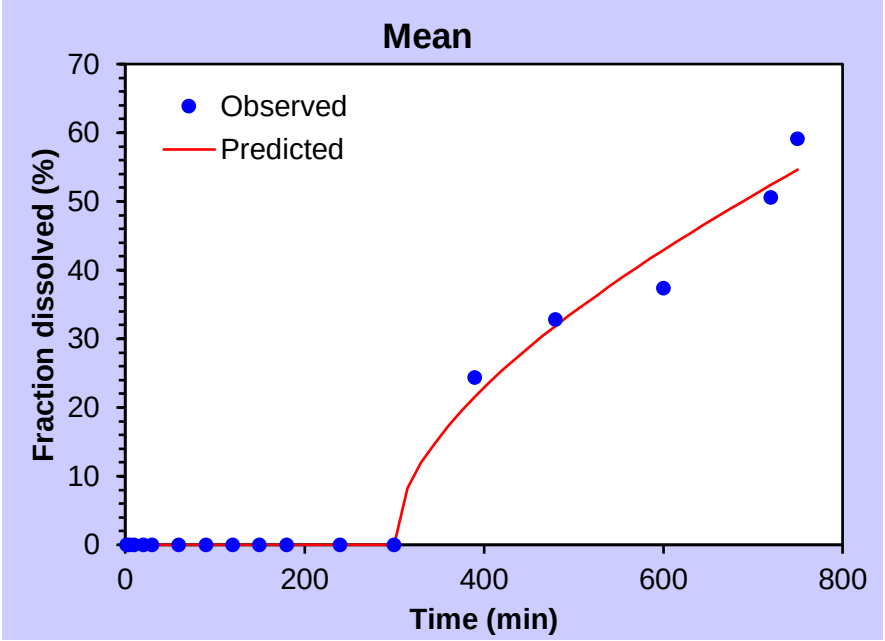

Figure D.7: *Diagram of the best fit line for the Peppas-Sahlin 2 model of avocado oil (4:6) for lumefantrine*

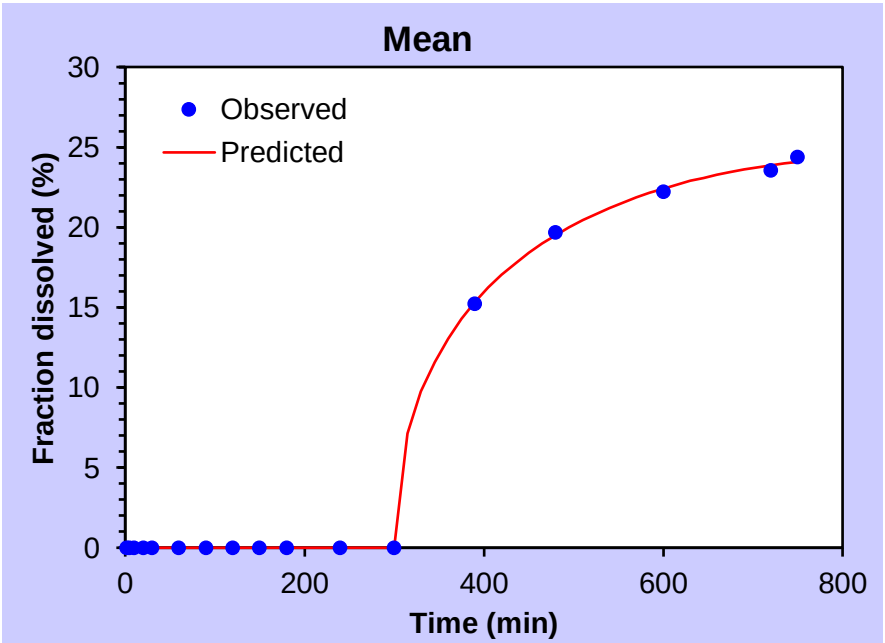

Figure D.8: *Diagram of the best fit line for the Peppas-Sahlin 2 model of castor oil (2:8) S80 for lumefantrine*

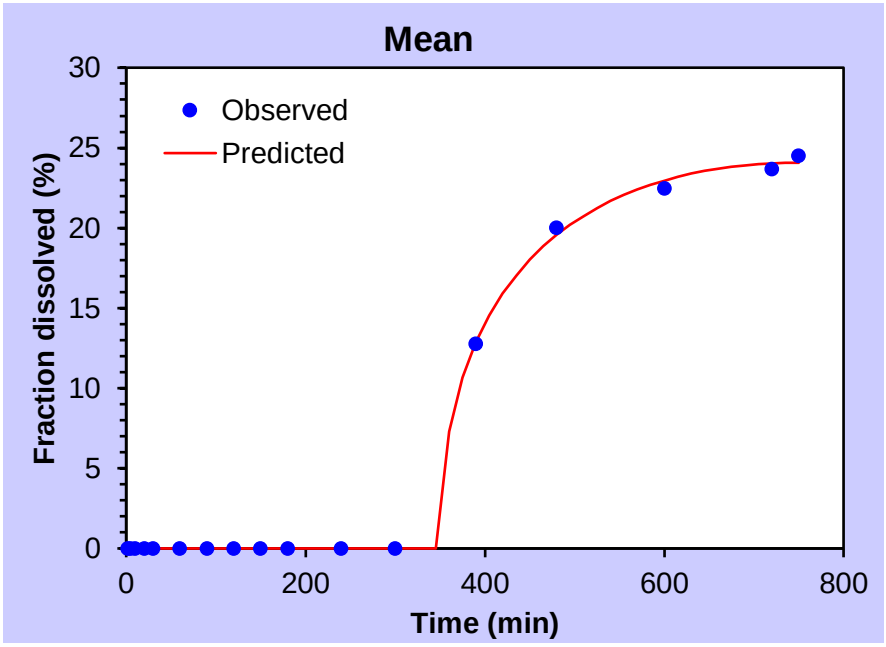

Figure D.9*: Diagram of the best fit line for the Peppas-Sahlin 2 model of castor oil (3:7) S60 for lumefantrine*

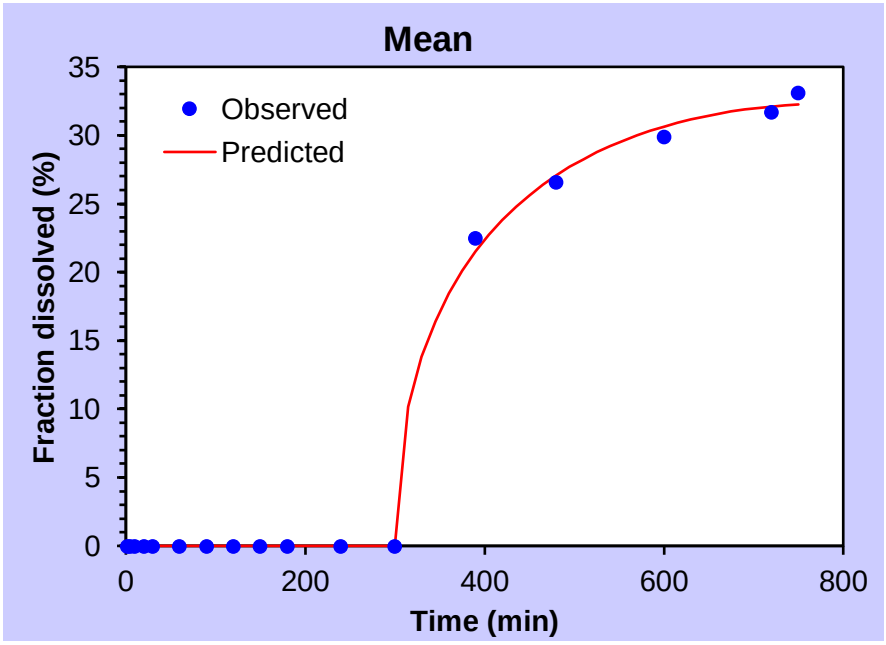

Figure D.10: *Diagram of the best fit line for the Peppas-Sahlin 2 model of coconut oil (6:4) for lumefantrine*

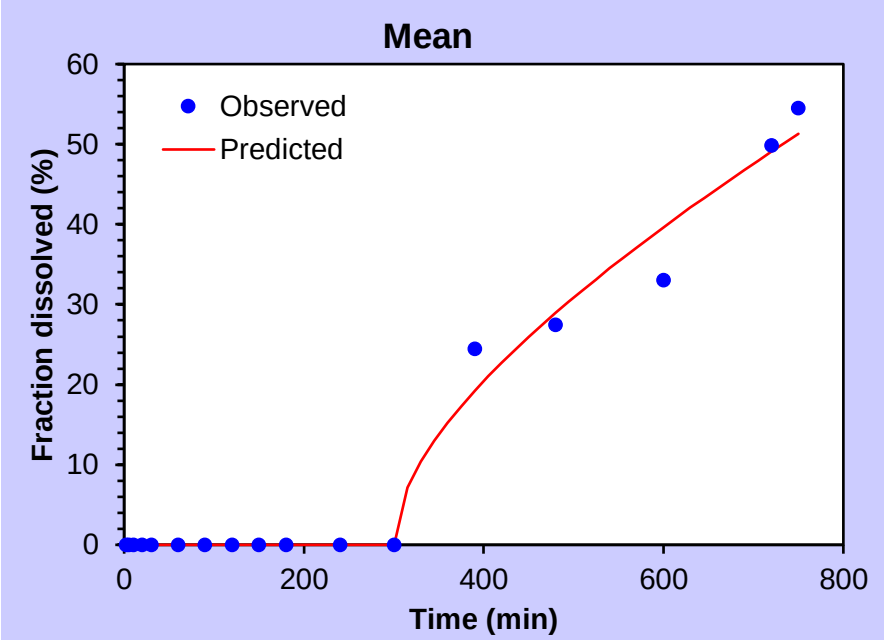

Figure D.11: *Diagram of the best fit line for the Peppas-Sahlin 2 model of olive oil (3:7) for lumefantrine*

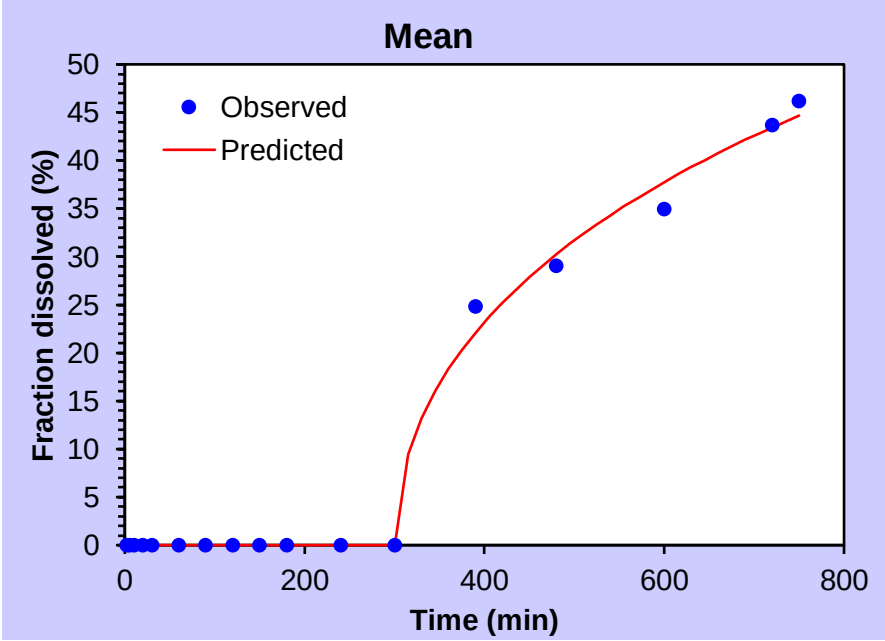

Figure D.12: *Diagram of the best fit line for the Peppas-Sahlin 2 model of peanut oil (6:4) for lumefantrine*

Annexure E

Author's guidelines

INTERNATIONAL JOURNAL OF PHARMACEUTICS

AUTHOR INFORMATION PACK

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Areas of particular interest include: pharmaceutical nanotechnology; physical pharmacy; polymer chemistry and physical chemistry as applied to pharmaceutics; excipient function and characterisation; biopharmaceutics; absorption mechanisms; membrane function and transport; novel routes and modes of delivery; responsive delivery systems, feedback and control mechanisms including biosensors; applications of cell and molecular biology to drug delivery; prodrug design; bioadhesion (carrier-ligand interactions); and biotechnology (protein and peptide formulation and delivery).

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