Development and evaluation of a solid oral dosage form for an artesunate and mefloquine drug combination

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Dedicated to Stephan.

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AIM AND OBJECTIVES OF THE INVESTIGATION

AIM

The aim of the study was to design an oral fixed-dose combination of mefloquine hydrochloride and artesunate.

BACKGROUND

More than 250 million people worldwide experience a malarial illness annually. Furthermore, between one and three million deaths are caused by malaria in sub-Saharan Africa, and it remains an important cause of death worldwide (Balint, 2001:261; WHO 2010:1). The development of resistance to antimalarial drugs by *Plasmodium falciparum* poses a major threat to the tropical areas of the world. Mefloquine was introduced in 1984, but the decline in efficacy since 1990 has been so rapid that monotherapy is no longer indicated. Recent evidence indicates that mefloquine is more effective when used in combination with an artemisinin compound. Artemisinin and its derivatives represent a new class of antimalarials that is effective against drug-resistant *Plasmodium falciparum* strains (Baker & Burgin, 1996:372) and, therefore, they are of utmost importance in the current antimalarial campaign (WHO, 2010:17). Artesunate is an artemisinin with a high clinical efficacy but has a problematic short elimination half-life (Price *et al.,* 1995:526). A fixed-dose combination of mefloquine and artesunate may improve medication compliance by reducing the treatment burden of patients. Although fixed-dose combination formulations are technically difficult to design, they are strongly preferred and recommended over blistered co-packaged or loose tablet combinations to promote adherence to treatment and to reduce the potential selective use of the medicines as monotherapy (WHO, 2010:17).

The rationale behind the effectiveness of a mefloquine and artesunate combination lies in the fact that mefloquine possess a long elimination half-life and artesunate has a rapid onset of action against *Plasmodium falciparum*. However, the problematic short half-life of artesunate has to be overcome by modifying the release of artesunate to render artesunate to be absorbed further down in the gastrointestinal

tract. To facilitate this desired absorption of artesunate, the objective would be to design an oral solid dosage form with a dual release action of artesunate.

The challenge lies in designing a compressible free-flowing and stable powder mixture of an artemisinin-based combination therapy (ACT) dosage form in conjunction with mefloquine. For a solid oral dosage form of artesunate and mefloquine to be taken as few times as possible during the day and over a short period of time would be a technically difficult task to achieve (WHO, 2010:17).

The World Health Organization (WHO) recommends an oral dose of artesunate (4 mg/kg/day once daily for three days) with mefloquine either split over 2 days as 15 mg/kg on day one and 10 mg/kg on day two or over 3 days as 8.3 mg/kg/day once a day for 3 days (WHO, 2010:20). The total mass of mefloquine and artesunate to be taken orally would amount to 615 mg/day for a patient weighing 50 kg. The active pharmaceutical ingredients (APIs) in itself presents a tremendous challenge for the formulation of a solid oral dosage form considering the physical and micromeritic properties of mefloquine (Yadav *et al*., 2010:1037) and artesunate (Kauss *et al*., 2010:198).

Artesunate and mefloquine are characterised by poor flowability and compressibility. Good flowability and compressibility are just two of the properties, powders for direct compression have to possess. The poor flowability can be the result of artesunate having asymmetrical, jagged shaped particles and mefloquine having symmetrical match-type structures. Powders can be granulated prior to compression to overcome poor flowability and to improve compressibility. Granulation is a process of particle size enlargement, and the most frequently used granulation technique is wet granulation. Wet granulation is a size enlargement technique that facilitates operations of solid processing through wetting. Appropriately applied, wet granulation should conserve the pharmaceutical properties of the granulated drug. Additionally, a granulated powder is sought-after in many solid processing and handling applications. It contains little or no dust, flows freely for easy metering, and has good storage and handling characteristics (Iveson *et al*., 2001:4).

The enteric coating technique is used industrially as a size enlargement method although its kinetics and mechanisms are poorly understood (Evonik, 2010:1). Even

though the process of enteric coating is inadequately understood, it bears some advantages. The coating procedure is widely adopted to optimise specific site release of the coated drug (Sherif *et al*., 1969:28) and as a size enlargement technique.

To enhance the micromeritic properties of the APIs for tableting purposes it was suggested to subject artesunate and mefloquine to the coating and wet granulation processes respectively. The enteric granulation process of artesunate particles was aimed to render delayed-release of a fraction of the artesunate dose for the double fixed-dose combination.

The mefloquine particles have to dissolve rapidly in the acidic environment of the stomach, requiring a disintegrant to be included in the granules. Artesunate has to be subjected to wet granulation for tableting purposes as well as enteric coating to facilitate a release of artesunate at a later stage in the gastrointestinal tract, thus causing a secondary release of the potent rapid acting artesunate.

OBJECTIVES

The aim of the study necessitated the following objectives:

- Investigation on the viability of an ACT double fixed-dose combination of artesunate and mefloquine for the treatment of uncomplicated *falciparum* malaria.

- A study of the processes which would provide the most suitable manufacturing conditions, and materials which would produce a superior and stable product in close accordance with the FDA guidelines for pharmaceutical manufacturing.

- Investigation of the compressibility of artesunate and mefloquine with the minimum amount of additional pharmaceutical excipients and manufacturing stages to ultimately deliver a viable and effective finished pharmaceutical product (FPP).

- Investigation of the various processes and materials contributing to the manufacturing of a double fixed-dose combination of artesunate and mefloquine intended to be taken once a day over three consecutive days.

- Identification of the optimal granulation parameters which would lead to a tablet comprising an initial rapid release of artesunate and mefloquine followed by a delayed release of artesunate.

- Evaluation of the micromeritic properties, compressibility and possible incompatibilities of the APIs and mixtures prior to and after granulation and coating.

- To establish whether preformulation studies done in accordance with the quality by design concept were viable in designing a FPP.

- Development of an analytical procedure to measure and evaluate artesunate and mefloquine.

- Investigation of the dissolution behaviour of artesunate and mefloquine.

- Investigate the kinetics of drug release of artesunate and mefloquine from the double fixed-dose combination.

- Comment on the applicability, effectiveness and economic viability of an artesunate and mefloquine double fixed-dose combination.

ABSTRACT

Malaria affects about forty percent of the world's population. Annually more than 1.5 million fatalities due to malaria occur and parasite resistance to existing antimalarial drugs such as mefloquine has already reached disturbingly high levels in South-East Asia and on the African continent. Consequently, there is a dire need for new drugs or formulations in the prophylaxis and treatment of malaria. Artesunate, an artemisinin derivative, represents a new category of antimalarials that is effective against drug-resistant *Plasmodium falciparum* strains and is of significance in the current antimalarial campaign. As formulating an ACT double fixed-dose combination is technically difficult, it is essential that fixed-dose combinations are shown to have satisfactory ingredient compatibility, stability, and dissolution rates similar to the separate oral dosage forms.

Since the general deployment of a combination of artesunate and mefloquine in 1994, the cure rate increased again to almost 100% from 1998 onwards, and there has been a sustained decline in the incidence of *Plasmodium falciparum* malaria in the experimental studies (Nosten *et al*., 2000:297; WHO, 2010:17). However, the successful formulation of a solid oral dosage form and fixed dosage combination of artesunate and mefloquine remains both a market opportunity and a challenge.

Artesunate and mefloquine both exhibited poor flow properties. Furthermore, different elimination half-lives, treatment dosages as well as solubility properties of artesunate and mefloquine required different formulation approaches. To substantiate the FDA's pharmaceutical quality by design concept, the double fixed-dose combination of artesunate and mefloquine required strict preliminary formulation considerations regarding compatibility between excipients and between the APIs. Materials and process methods were only considered if theoretically and experimentally proved safe. Infrared absorption spectroscopy (IR) and X-ray powder diffraction (XRPD) data proved compatibility between ingredients and stability during the complete manufacturing process by a peak by peak correlation.

Scanning Electron Micrographs (SEM) provided explanations for the inferior flow properties exhibited by the investigated APIs. Particle size analysis and SEM micrographs confirmed that the larger, rounder and more consistently sized particles of the granulated APIs contributed to improved flow under the specified testing conditions.

A compressible mixture containing 615 mg of the APIs in accordance with the WHO recommendation of 25 mg/kg of mefloquine taken in two or three divided dosages, and 4 mg/kg/day for 3 days of artesunate for uncomplicated *falciparum* malaria was developed. Mini-tablets of artesunate and mefloquine were compressed separately and successfully with the required therapeutic dosages and complied with pharmacopoeial standards. Preformulation studies eventually led to a formula for a double fixed-dose combination and with the specific aim of delaying the release of artesunate due to its short half-life.

A factorial design revealed the predominant factors contributing to the successful wet granulation of artesunate and mefloquine. A fractional factorial design identified the optimum factors and factor levels. The application of the granulation fluid (20% w/w) proved to be sufficient by a spraying method for both artesunate and mefloquine. A compatible acrylic polymer and coating agent for artesunate, Eudragit® L100 was employed to delay the release of approximately half of the artesunate dose from the double fixed-dose combination tablet until a pH of 6.8.

A compressible mixture was identified and formulated to contain 200 mg of artesunate and 415 mg of mefloquine per tablet. The physical properties of the tablets complied with BP standards.

An HPLC method from available literature was adapted and validated for analytical procedures. Dissolution studies according to a USP method were conducted to verify and quantify the release of the APIs in the double fixed-dose combination. The initial dissolution rate (DRi) of artesunate and mefloquine in the acidic dissolution medium was rapid as required. The enteric coated fraction of the artesunate exhibited no release in an acidic environment after 2 hours, but rapid release in a medium with a pH of 6.8.

The structure of the granulated particles of mefloquine may have contributed to its first order release profile in the dissolution mediums. A linear correlation was present between the rate of mefloquine release and the percentage of mefloquine dissolved (R^2 = 0.9484). Additionally, a linear relationship was found between the logarithm of the percentage mefloquine remaining against time (R^2 = 0.9908). First order drug release is the dominant release profile found in the pharmaceutical industry today and is coherent with the kinetics of release obtained for mefloquine.

A concept pre-clinical phase, double fixed-dose combination solid oral dosage form for artesunate and mefloquine was developed. The double fixed-dose combination was designed in accordance with the WHO's recommendation for an oral dosage regimen of artesunate and mefloquine for the treatment of uncomplicated *falciparum* malaria. The specifications of the double fixed-dose combination were developed in close accordance with the FDA's quality by design concept and WHO recommendations. An HPLC analytical procedure was developed to verify the presence of artesunate and mefloquine. The dissolution profiles of artesunate and mefloquine were investigated during the dissolution studies.

Keywords: artemisinin-based combination therapy, artesunate, enteric coating, first order drug release, granulation, malaria, mefloquine, pharmaceutical quality by design, tableting, two-drug fixed-dose combination.

UITTREKSEL

Malaria affekteer omtrent veertig persent van die wêreld se populasie. Jaarliks sterf meer as 1.5 miljoen mense, en weerstand teen tans gebruikte geneesmiddels soos meflokien het al reeds kommerwekkende vlakke bereik in Suidoos-Asië en Afrika. Daar is dus gevolglik ‘n dringende vraag na nuwe geneesmiddels of formulerings vir die profilakse en behandeling van malaria. Artesunaat is ‘n artemisinienderivaat en verteenwoordig ‘n nuwe kategorie geneesmiddels wat effektief is teen weerstandbiedende *Plasmodium falciparum*-kulture, en is dus van uiterste belang in die huidige veldtog teen malaria. Omdat die formulering van vastedosiskombinasieprodukte van artemisinien-gebaseerde-kombinasieterapie tegnies baie moeilik is, is dit noodsaaklik dat bestanddele verenigbaarheid en stabiliteit toon. Farmaseutiese beskikbaarheid, soortgelyk aan die vlakke van vrystelling van afsonderlike tablette, is noodsaaklike vereistes vir ‘n vastedosiskombinasieproduk.

Sedert die algemene orale toediening van ‘n kombinasie van artesunaat en meflokien in 1994, het die graad van genesing gestyg tot bykans 100% vanaf 1998 en was daar ook ‘n volgehoue afname in die voorkoms van *Plasmodium falciparum* malaria tydens eksperimentele studies (Nosten *et al*., 2000:297). Desnieteenstaande is daar ‘n uitdagende leemte vir ‘n suksesvolle soliede orale doseervorm en vastedosiskombinasieproduk van artesunaat en meflokien.

Beide artesunaat en meflokien vertoon swak vloeieienskappe, maar omrede die twee aktiewe bestanddele oor verskillende eliminasiehalfleeftye, oplosbaarhede en doserings beskik, is verskillende benaderings ten opsigte van formulering nodig. Om die “*Food and Drug Administration”* (FDA) se farmaseutiese kwaliteitsontwerpkonsep te handhaaf, het noukeurige voorafgaande studies aangaande verenigbaarheid tussen hulpstowwe en die betrokke aktiewe farmaseutiese bestanddele vereis. Grondstowwe en prosesse is slegs in gebruik geneem indien dit teoreties veilig en effektief kon wees, en eksperimenteel veilig en effektief bewys is. Verenigbaarheid tussen die bestanddele en stabiliteit tydens natgranulering en enteriese bedekking is bevestig deur x-straalpoeierdiffraksie en infrarooi-spektroskopie.

Skandeerelektron-mikroskopiefoto's het bewys gelewer vir moontlike verklarings vir die swak vloei- en tabletteringseienskappe van die betrokke aktiewe bestanddele. Die evaluering van deeltjiegrootteverspreiding het bewys dat groter en meer eenvormige grootte deeltjies van die betrokke aktiewe bestanddele, verkry deur middel van natgranulering, 'n verbetering van vloei- en tabletteringseienskappe teweeggebring het.

'n Saampersbare formule, bevattende 615 mg aktiewe bestanddele gelykstaande aan 'n dosis van 25 mg/kg meflokien, geneem oor drie dae in verdeelde dosisse, en 4 mg/kg/dag artesunaat vir 3 dae, soos aanbeveel deur die Wêreldgesondheidsorganisasie (WGO) vir ongekompliseerde *falciparum* malaria, is suksesvol geformuleer. Minitablette van beide geneesmiddels is suksesvol getabletteer wat voldoen het aan die standaarde van die farmakopieë, met die gedefinieerde kombinasie bestanddele en vereiste terapeutiese dosisse. Intensiewe preformuleringstudies rakende formulering het uiteindelik gelei tot 'n prototipe formule vir 'n vastedosiskombinasieproduk met die spesifieke doel om die vrystelling van artesunaat te verleng en sodoende artesunaat se problematiese kort eliminasie-halfleeftyd te oorkom.

Eudragit® L100, 'n akriliese polimeer, was geskik om as verenigbare beddekingmateriaal vir artesunaat op te tree en sodoende die vrystelling van 'n gedeelte van die artesunaatdosis te vertraag tot by 'n pH van 6.8.

Preformuleringstudies het die belangrikste faktore uitgesonder wat bygedra het tot die suksesvolle natgranulering van artesunaat en meflokien. Die optimum-faktore en vlakke is met behulp van 'n faktoriaalontwerp geïdentifiseer. Die sproeidroog aanwending van Eudragit® L100 was voldoende om die vrystelling van 'n fraksie van die gegranuleerde artesunaat te modifiseer.

Die saampersbare formule het 200 mg artesunaat en 415 mg meflokien per tablet bevat en die fisiese eienskappe van die tablette het voldoen aan die spesifikasies van die Britse Farmakopie (BP).
'n Hoë druk vloeistofchromatografie-metode (HDVC) is uit beskikbare literatuur geïdentifiseer. Die HDVC-metode is ontwikkel en aangepas om die teenwoordigheid

en konsentrasie van die aktiewe farmaseutiese bestanddele tydens dissolusiestudies te ondersoek.

Die gegranuleerde deeltjies van meflokien kon die rede wees vir die eerste orde vrystellingsmodel wat getoon is tydens dissolusie. ‘n Liniêre verwantskap is gevind tussen die dissolusietempo van meflokien en die persentasie meflokien reeds opgelos (R^2 = 0.9484). ‘n Bykomende liniêre verwantskap is gevind tussen die logaritme van die persentasie meflokien onopgelos en tyd (R^2 = 0.9908). Eerste orde vrystelling is die dominante vrystellingsmodel in die farmaseutiese industrie en is waargeneem tydens die dissolusie van meflokien.

‘n Konsep pre-kliniese fase, soliede orale doseervorm vir ‘n artesunaat en meflokien vastedosiskombinasieproduk is ontwikkel. Die doelmatigheid en spesifikasies rakende die vastedosiskombinasieproduk is ontwerp volgens konsepriglyne van die FDA en in terme van dosis en behandelingsperiode soos voorgestel deur die Wêreldgesondheidsorganisasie.

Sleutelwoorde: artemisinien-gebaseerde kombinasieterapie, artesunaat, enteriese bedekking, eerste orde geneesmiddelvrystelling, farmaseutiese kwaliteitsontwerp, granulering, malaria, meflokien, tablettering, vastedosiskombinasieproduk.

1. CHAPTER ONE

MALARIA, ANTIMALARIALS AND ASPECTS OF PHARMACEUTICAL DOSAGE FORM DESIGN

1.1 INTRODUCTION

Approximately fourty percent of the earth's population is at risk of malaria infection. Every year, more than 250 million individuals experience a malarial infection, and more than 1.5 million fatalities (frequently African youngsters) occur. In patients with severe and complicated disease, the death rate is among twenty to fifty percent (Balint, 2001:261). Of the four human malaria parasites, *Plasmodium falciparum* is the overwhelming cause of serious disease and death (WHO, 2000:1; Gkrania-Klotsas & Lever, 2007:73).

Parasite resistance to prevailing antimalarial drugs has already reached disturbingly high levels in South-East Asia and Africa (WHO, 2000:1; WHO, 2010:7). Fixed-dose combinations of ACTs can treat malaria effectively, however, formulating fixed-dose combinations of ACTs is technically difficult (WHO, 2010:17), generating a dire need for novel drugs and formulations in the prophylaxis and treatment of malaria.

1.2 MALARIA

Malaria is a protozoan disease that is spread to humans via the bite of the female *Anopheles* mosquito (Breman *et al*., 2006:65). There are four types of human malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. Recently, several human cases of malaria have also occurred with *Plasmodium knowlesi*, a primate malaria. Among the above mentioned, *Plasmodium falciparum* is the deadliest (Gkrania-Klotsas & Lever, 2007:73).

1.3 HISTORY OF MALARIA

Humans have been affected by malaria for thousands of years. In ancient Egypt malaria probably occurred in lowland areas as evident in the enlarged spleens of several Egyptian mummies. Tutankhamen, king of ancient Egypt from 1333 to 1323 BC, may have been suffering from malaria. In 2010, researchers recovered traces of

malaria parasites from the mummified remains of his blood (Hawass *et al*., 2010:638).

In ancient Greece, malaria appeared annually in summer and autumn as a fever, as described by Hippocrates (Cunha & Cunha, 2008:194). From the descriptions made by Hippocrates, some researchers have assumed that malaria occurring in Greece in ancient times was possibly caused by *Plasmodium vivax* and *Plasmodium malariae* (Cunha & Cunha, 2008:195).

Malarial fevers were also linked with swamps and marshlands as early as classical Greece, however, the role of mosquitoes in spreading the infection was entirely unknown. Since ancient Greek times, attempts were made to control malaria by draining swamps and stagnant marshlands (Konradsen *et al*., 2004:99). Several of the early Greeks believed malaria was contracted by consuming swamp water. Later, since the Romans linked the disease to the inhalation of "*miasmas*," or vapours, arising from bodies of stagnant water, the illness came to be named "*mal aria"*, or "*bad air*." (Cunha & Cunha, 2008:197).

During the later stages of the Roman Empire, nevertheless, malaria was a much more fierce disease than it had earlier been in the countries alongside the northern coastline of the Mediterranean Sea, and the connotation of malaria with the Pontine Marshes of the Roman Campagna was well-known (Sérandour *et al*., 2007:115). Scientists have recognised this upsurge in the fierceness of malaria to environmental changes related with the removal of forestland that had accompanied increased agricultural activities. These agricultural modifications permitted different species of mosquitoes from the northern parts of Africa to be introduced and effectively based in the southern parts of Europe. The newly established mosquito species were superior transmitters of *Plasmodium falciparum* than several of the indigenous European mosquitos (Encyclopædia Britannica Online, 2013:1).

A unique cure for malaria failed to become accessible in Europe up until the 1630s, when the bark of the cinchona tree was brought to Spain from Peru in South America. This revolutionary drug became generally obtainable by the mid 1800s, after the active ingredient of the bark of the cinchona tree, quinine, was effectively isolated and the Dutch started to grow cinchona trees in plantations on the Indonesian island of Java (Ferreira Júnior *et al*., 2012:107).

Although the treatment helped bring down the infection rate, the direct link was never established until the discovery of the *Anopheles* mosquito (*Figure 1.1*) as the vector of malaria by Giovanni Grassi and his colleagues in 1898 (Bynum, 2010:1534).

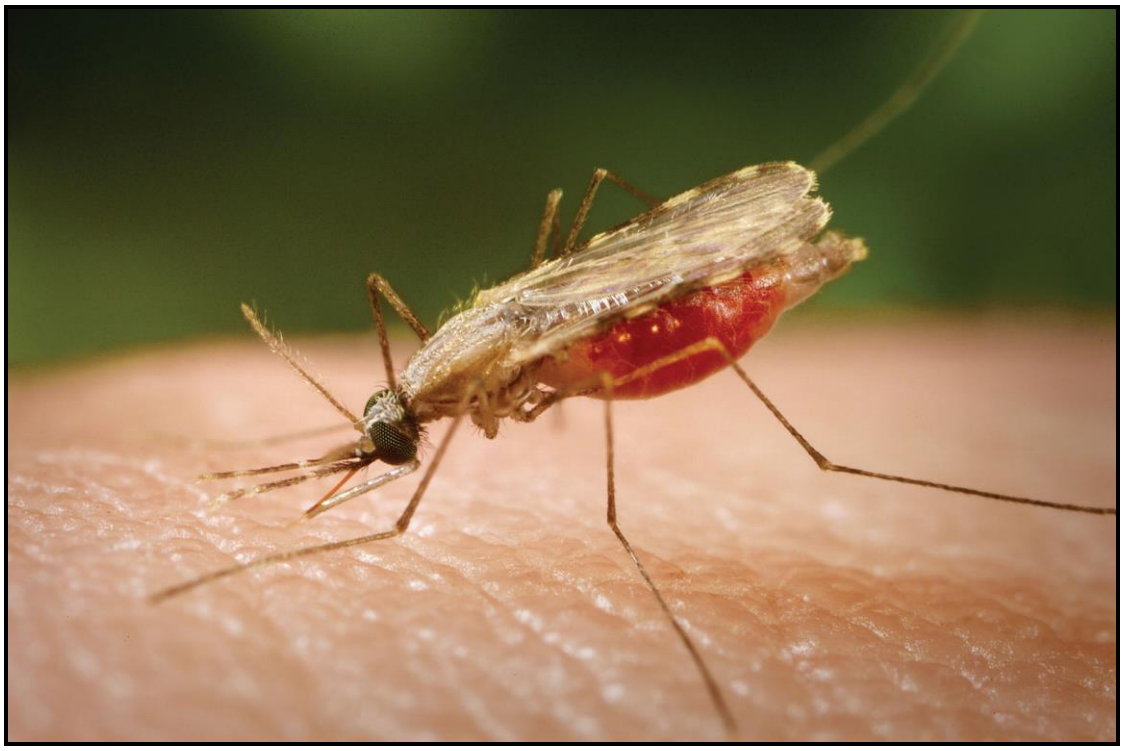

Figure 1.1: The Anopheles mosquito (Encyclopædia Britannica Online, 2013:1).

1.4 CURRENT DISTRIBUTION AND EPIDEMIOLOGY OF MALARIA

At present, malaria is mainly found in the tropical parts of the world, throughout sub-Saharan Africa and to a smaller degree in South Africa, South-East Asia, India, the Pacific islands, Central America and South America (Ashley *et al*., 2006:159). The problem is that for historical and operational reasons, most of Southern-Africa has been without any structured antimalarial vector-control campaigns (Alles *et al*., 1998:369). The global distribution of malaria is displayed in *Figure 1.2*.

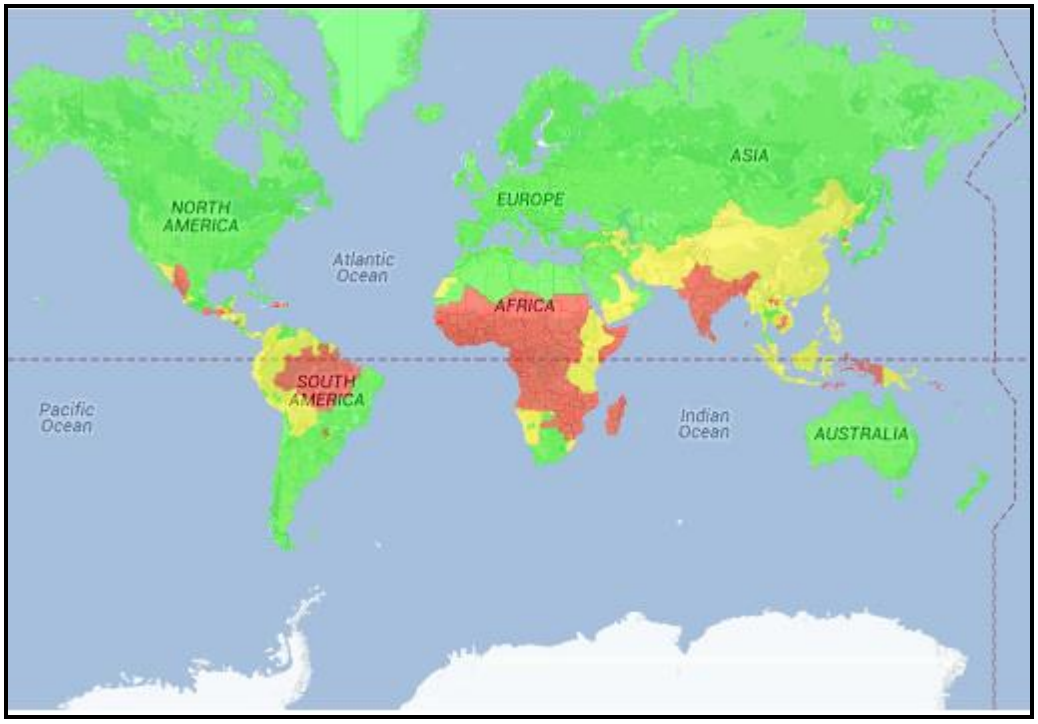

Figure 1.2: Global distribution of malaria. The red coloured areas indicate the countries at risk of malaria transmission. The yellow coloured areas indicate areas where the presence of malaria varies. The green areas indicate areas where there is no known malaria (Centre for Disease Control, 2013a:1).

Climate is a significant component in the environmental distribution and the seasonality of malaria, as it can affect all three elements of the *Plasmodium* life cycle, namely *Anopheles* mosquitoes, humans and *Plasmodium* parasites. Malaria today is confined almost exclusively to tropical and subtropical countries where climatic factors such as temperature, humidity and rainfall are ideal for the survival and multiplication of *Anopheles* mosquitoes. Temperature is particularly critical for malaria parasites to finish their development cycle or external incubation period inside the mosquito body. The surrounding warmer temperatures decrease the extent of this external cycle, thus increasing the probabilities of transmission to occur (CDC, 2013b:1).

1.5 LIFE-CYCLE OF THE MALARIA PARASITE

As illustrated in *Figure 1.3*, the natural eco system of malaria includes malaria parasites infecting the two categories of hosts, humans and female *Anopheles* mosquitoes, successfully. In the human host, the sporozoites enter the human body (Ashley *et al*., 2006:160). Subsequently, the sporozoites are directly transported by hepatic circulation to the liver, where the sporozoites penetrate the liver cells

(hepatocytes) and grow to be hepatic schizonts (Ashley *et al*., 2006:160). A hepatic schizont contains approximately 32 merozoites as it grows and multiplies in the hepatocytes. After between 1 and 2 weeks, the proliferation of thousands of merozoites causes an increase of pressure inside the hepatocytes, the cell ruptures and the merozoites are set free into the systemic blood circulation where they enter the red blood cells (erythrocytes). The pathophysiology of malaria only starts to present at this stage, where the parasite leaves the liver and starts to infect the erythrocytes (Vásquez & Tobón, 2012:106). When a certain form of the blood stage malaria parasite, (gametocytes) is consumed by the female *Anopheles* mosquito during a blood meal, the start of a new cycle of development and reproduction in the mosquito begins, as illustrated in *Figure 1.3* (CDC, 2013c:1).

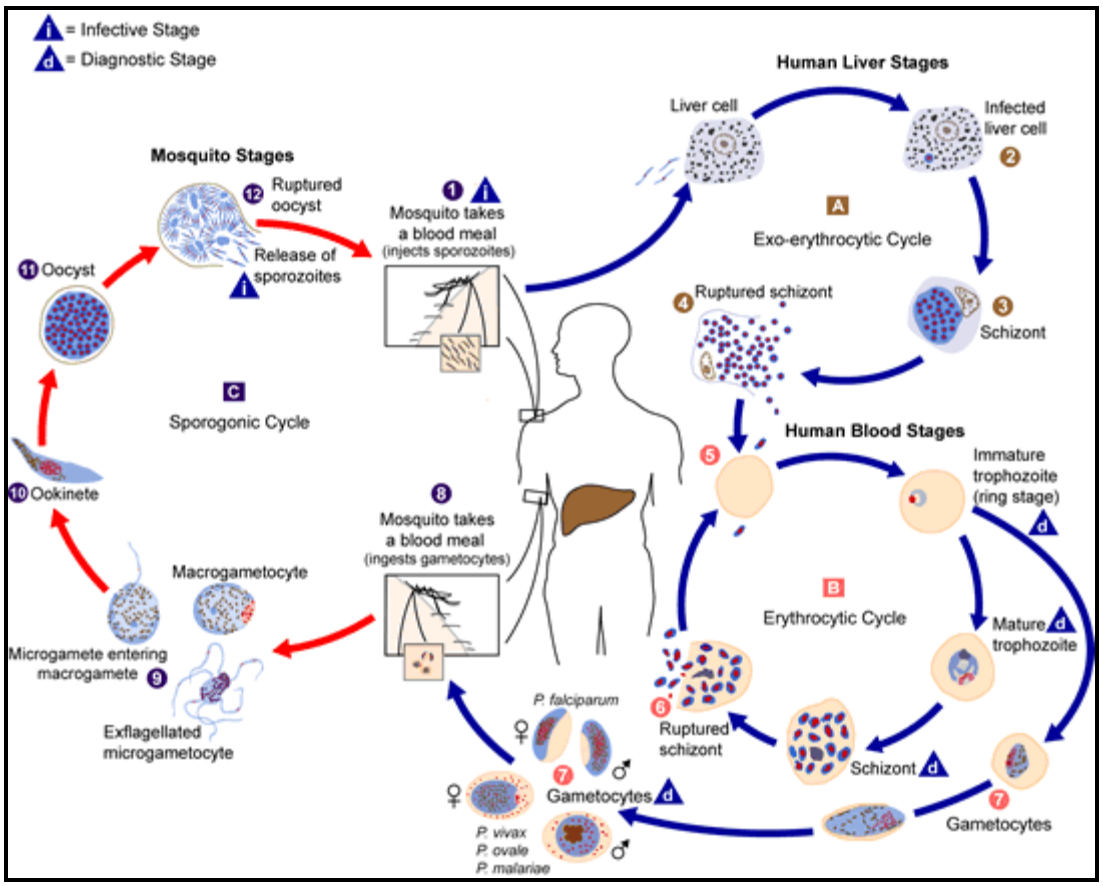

Figure 1.3: The biological life-cycle of the malaria parasite. The figure indicates the different cycles during its growth and development. (Centre for Disease Control, 2013c:1).

1.6 SIGNS AND SYMPTOMS OF MALARIA INFECTION

1.6.1 Uncomplicated malaria

The clinical appearance of an individual who contracted uncomplicated malaria can differ significantly, depending on:

- the infecting species,
- the status of parasitaemia and
- the vulnerability of the patient.

The symptoms of uncomplicated malaria observed from the patient can thus be quite common, and diagnosing the patient with malaria might be overlooked if health care practitioners are not vigilant to the probability of the patient having malaria. For the reason that untreated malaria can advance to extreme symptoms, which may be rapidly (<24 hours) fatal, malaria should always be contemplated in individuals who have had a history and probable exposure to the disease (CDC, 2013d:1).

In the beginning of infection, malaria symptoms are non-specific, almost akin to that of influenza. These symptoms include:

- fever,
- chill,
- headache,
- myalgia,
- arthralgia,
- weakness,
- vomiting,
- diarrhoea,
- loss of appetite and
- body aches (Ashley *et al*., 2006:163; CDC, 2013e:1).

Fever patterns are common in malaria infection, with fever spikes every two days in *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium ovale* malaria (tertian fever). In *Plasmodium malariae* infection, quartan fevers are more common, or fever every three days. These symptoms relate with the specific lifecycle of each *Plasmodium* species. *Plasmodium falciparum* has the ability to progress extremely quickly into severe malaria (Ashley *et al*., 2006:163).

1.6.2 Severe malaria

Symptoms and signs of severe *Plasmodium falciparum* malaria infection are mostly associated with a broad spectrum of unrousable coma and prostration. The mortality rate of cerebral malaria patients is fifteen percent in children and twenty percent in non-pregnant patients. Symptoms of cerebral malaria include:

- unrousable coma,
- convulsions,
- normocytic anaemia,
- metabolic acidosis together with respiratory distress,
- electrolyte and fluid disturbances,
- kidney failure,
- acute pulmonary oedema,
- jaundice (icterus),
- circulatory collapse,
- elevated fever,
- hyperparasitaemia,
- hypoglycaemia,
- impaired consciousness and
- prostration (Ashley *et al*., 2006:164).

In addition to the above mentioned symptoms, there is also a clinical manifestation after treatment for malaria referred to as Post Malaria Neurological Syndrome, or PMNS. Obvious symptoms include:

- confusion,
- seizures and
- tremors (Ashley *et al*., 2006:164).

The incidence of PMNS increases with mefloquine treatment and is therefore not recommended for use against severe malaria (Ashley *et al*., 2006:164).

1.7 DIAGNOSIS OF MALARIA

The diagnosis of malaria is made by means of a combination of clinical observations, case history and diagnostic laboratory tests (Bell *et al*., 2006:682).

1.7.1 Clinical (Presumptive) diagnosis

Clinical observations and physical findings are not unique and can also be a manifestation of other diseases such as seasonal influenza and general viral infections (CDC, 2013f:1). As a result, clinical diagnosis of malaria has been repeatedly shown to be unreliable. It would thus be advantageous to the patient if malaria is confirmed by a laboratory test demonstrating the malaria parasites or their components (Chandramohan *et al*., 2001:505). However, in most parts of the world affected by malaria, monetary support, as well as skilled health workforces are so rare, that reasonable clinical diagnosis is the single most meaningful prospect (Bloland, 2001:4; WHO, 2010:11).

1.7.2 Antigen detection tests

Rapid diagnostic immunochromatographic test kits, commonly referred to as Malaria Rapid Diagnostic Devices (MRDDs), frequently utilise a cassette or dipstick device and deliver results within 2 to 15 minutes (Bell *et al*., 2006). These tests detect antigens from malaria parasites in a finger-prick of blood by personnel with minimal training requirements (Bloland, 2001:4; WHO, 2010:118).

1.7.3 Microscopic observations

Analysis of Giemsa stained blood smears allows for identification of asexual forms of *Plasmodium* within red blood cells (Ashley *et al*., 2006). This technique, however, requires personnel with a high degree of training, equipment that rely on electricity and is time consuming (Bloland, 2001:4; WHO, 2010:118).

1.7.4 Molecular tests

Molecular tests are more accurate than microscopy and have the ability to distinguish between the different *Plasmodium* species, identify mixed infections and detect low-level parasitaemia (Bloland, 2001:5). A molecular diagnostic test detects parasite nucleic acids by means of a polymerase chain reaction (PCR) process. However, PCR-techniques are costly and require specialised laboratory equipment (Gkrania-Klotsas & Lever, 2007:80).

1.7.5 Serology

The process of serology identifies immunoglobulins against malaria parasites, by means of either enzyme-linked immunosorbent assays or indirect

immunofluorescence assays (CDC, 2013f:1). The process of serology does not identify a current infection, but rather measure a previous infection. The test is relatively expensive and not commonly obtainable (Bloland, 2001:5; WHO, 2010:120).

1.8 DRUGS AVAILABLE FOR THE TREATMENT OF MALARIA

A limited number of drugs exist which can be used to prevent or treat malaria. The antimalarials can be divided into five different classes:

- quinolines and arylaminoalcohols,
- antifolate combination drugs,
- antibiotics,
- artemisinin compounds and
- hydroxy-napthoquinones.

The most commonly used drugs are quinine and its related compounds, and antifolate combination drugs (Bloland, 2001:5).

1.8.1 Quinine and related compounds

Quinine, as well as its dextroisomer quinidine, has been prescribed for the treatment of malaria, especially as a last resort drug against severe malaria. Other derivatives of quinine include:

- Chloroquine, a synthetic 4-aminoquinoline related compound of quinine, was initially produced in 1934 and has since been the most commonly prescribed antimalarial drug. Traditionally, chloroquine has been the first choice drug for the treatment of uncomplicated malaria and for chemoprophylaxis, even though parasitological resistance has decreased its effectiveness.
- Amodiaquine, a comparatively commonly available derivative closely associated to chloroquine (Bloland, 2001:5).
- Primaquine, which is particularly prescribed for eliminating the exoerythrocytic forms of *Plasmodium vivax* and *Plasmodium ovale* that trigger relapses (Deen *et al*., 2008:1119), and
- Mefloquine, a quinolinemethanol derivative of quinine (Bloland, 2001:5). Mefloquine will be discussed in detail later in this chapter.

1.8.2 Antifolate combination drugs

Antifolate combination drugs are several combinations of dihydrofolate-reductase inhibitors and include:

- proguanil,
- chlorproguanil,
- pyrimethamine,
- trimethoprim and
- sulfa drugs (dapsone, sulfalene, sulfamethoxazole, and sulfadoxine (Müller & Hyde, 2013:64).

Although antifolate combination drugs possess antimalarial activity when taken alone, drug resistance can advance quickly. When antifolate drugs are used in combination, they yield a synergistic attack on the parasite and can be effective even in the manifestation of resistance to the separate drugs. Representative antifolate combinations consist of:

- sulfadoxine and pyrimethamine,
- sulfalene and pyrimethamine,
- co-trimoxazole and
- chlorproguanil and dapsone (Bloland, 2001:9).

1.8.3 Antibiotics

Potent antibiotic antimalarials, used for both treatment and prophylaxis are:

- Tetracycline and derivatives, such as doxycycline. In regions where the effectiveness of quinine has declined, tetracyclines are frequently prescribed in combination with quinine to increase recovery rates (Ejaz *et al*., 2007:502).
- Clindamycin, which has been prescribed previously, but displays inadequate benefits when likened to other existing antimalarials. The parasitological effect towards clindamycin is slow and recurrences are common (Kremsner *et al*., 1989:275). The efficacy of clindamycin amid non-immune human beings has not been entirely documented, and remains only effective as second-line treatment if used in combination with artesunate (Bloland, 2001:9; WHO, 2010:18).

1.8.4 Artemisinin compounds

Sesquiterpene lactone substances have been artificially manufactured from the *Artemisia annua* plant, and include the following:

- artemether,
- artemotil,
- arteether and
- artesunate (Bloland, 2001:9; WHO, 2010:85).

According to Bloland (2001:9), sesquiterpene lactone substances are prescribed for the treatment of severe malaria and have revealed rapid and effective parasite clearance times. Artesunate will be discussed in detail later in this chapter.

1.8.5 Miscellaneous compounds

A pair of drugs, artificially manufactured in China originally, are presently subjected to field trials, namely:

- Pyronaridine, a drug which was allegedly 100% effective during a field trial in Cameroon (Ringwald *et al*., 1996:24). However, pyronaridine was only between 63% and 88% effective during a field trial in Thailand (Looareesuwan *et al*., 1996:1189).
- Lumefantrinel, a drug which is a fluoromethanol compound. Lumefantrinel is being manufactured as a fixed-dose combination tablet with artemether (Bloland, 2001:9; WHO, 2010:86).

Other compounds are:

- Halofantrine, which is a phenanthrene-methanol compound that has activity against the erythrocytic stages of the life-cycle of the malaria parasite (Hyde, 2007:4689). The use of halofantrine has been particularly endorsed in regions with multiple drug-resistant *falciparum*. Contemporary studies have demonstrated, however, that halofantrine can instigate potentially deadly cardiac conduction defects, restricting its effectiveness (Nosten *et al*., 1993:1054).
- Atovaquone, a hydroxy-napthoquinone which is presently being prescribed most commonly for the management of opportunistic infections in patients with immunodeficiency. Atovaquone is effective against chloroquine-resistant *Plasmodium falciparum*, however, when prescribed alone, resistance

develops quickly. Atovaquone is usually given in combination with proguanil (Looareesuwan *et al*., 1996:62). An antimalarial fixed-dose combination of 100 mg proguanil and 250 mg atovaquone exists with high efficacy against *Plasmodium falciparum*, and with only mild side-effects (Wurtz *et al*., 2012:146).

1.9 COMBINATION THERAPY WITH ARTESUNATE AND MEFLOQUINE

The simultaneous use of two antimalarials, as in the case of artesunate and mefloquine, particularly while these antimalarials possess diverse action mechanisms, has the potential for preventing the advance of drug resistance to both of these drugs (Bloland, 2001:10).

1.9.1 Artemisinins

Artemisinin is a sesquiterpene lactone (Brossi *et al*., 1988:645), isolated from the plant *Artemisia annua*, a herb that has historically been taken in China for the treatment of malaria. It is an effective and rapid working blood schizontocide. Artemisinin is active against *Plasmodium vivax*, and both chloroquine-resistant and chloroquine-sensitive strains of *Plasmodium falciparum* (Baker & Burgin, 1996:372). Artemisinin and its derivatives signify a novel class of antimalarials that is effective against drug-resistant *Plasmodium falciparum* strains, and as a result are of extreme significance in the present-day antimalarial campaign (Balint, 2001:262; WHO, 2010:1).

1.9.2 Artesunate, a semi synthetic derivative of artemisinin

Artemisinin, *Figure 1.4*, has been given orally or rectally in the treatment of malaria, however, regimens were often empirical, with typical rectal dosages ranging from 10 to 40 mg/kg daily across a variable number of days. However, it has largely been replaced in practice by its derivatives such as artemether, artemotil and artesunate (Meshnick *et al*., 1996:302; WHO, 2010:37).

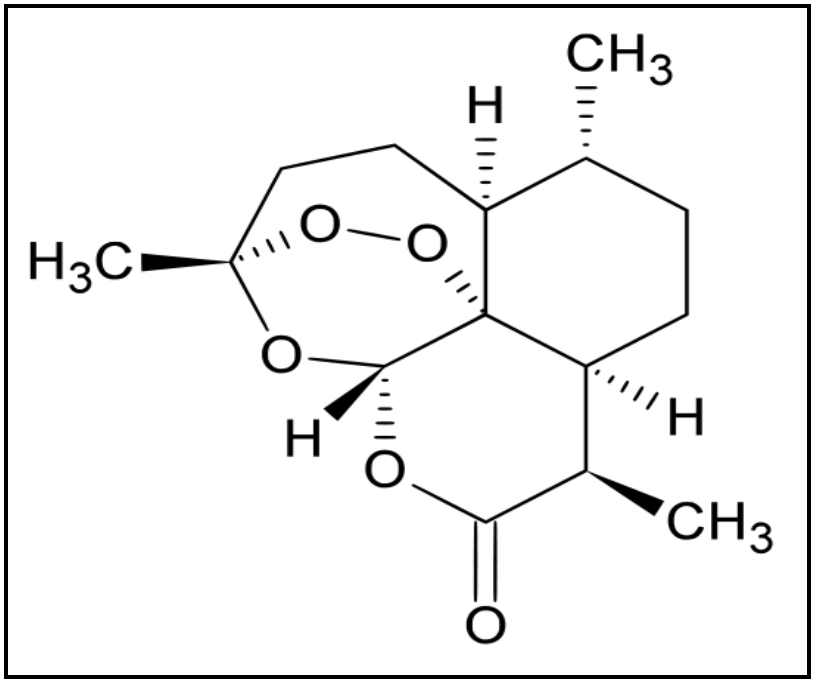

Figure 1.4: The structural formula of artemisinin (Ragimiri, 2006:1).

Artemisinins have the following properties:

- poor solubility in water and oil,
- short pharmacological half-lives,
- exhibit extensive first-pass metabolism and
- display poor oral bioavailability.

Artesunate, (*Figure 1.5*), the lactol hemiester derivative, is slightly soluble in water and soluble at a basic pH (Pogány 2006:1).

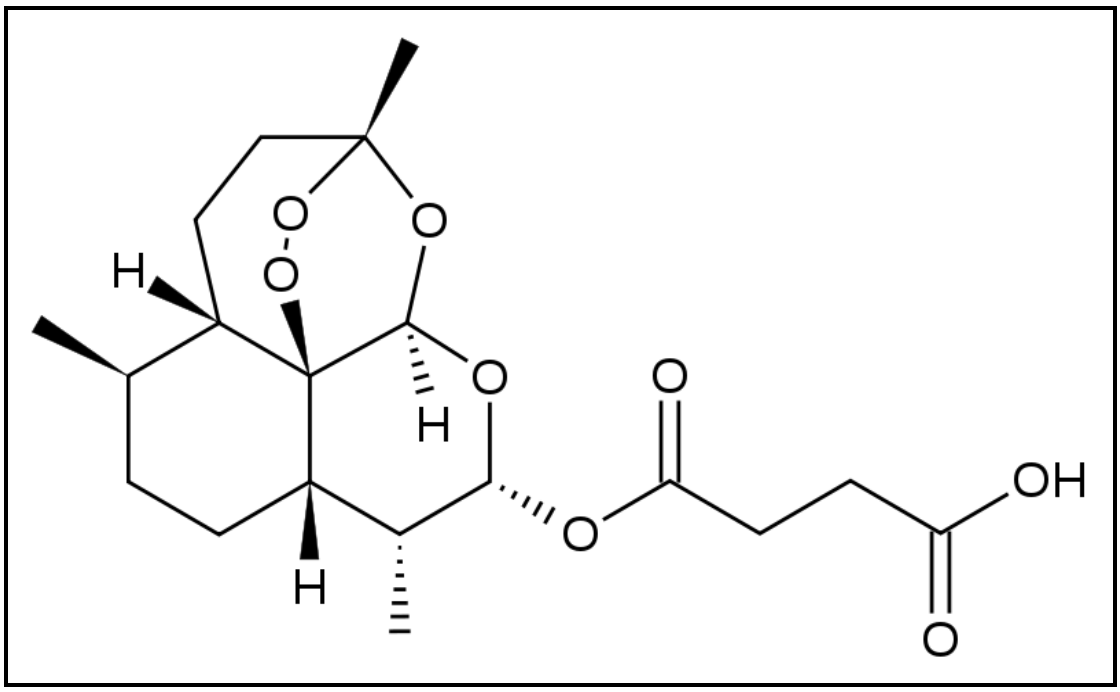

Figure 1.5: The structural formula of artesunate (Fvasconcellos, 2007:1).

With regards to mefloquine, Nosten *et al*. (2000:297) noted that rapid detection and treatment managed *Plasmodium falciparum* malaria successfully, since mefloquine monotherapy was still effective prior to 1990. However, as drug resistance to mefloquine developed, the cure rate fell to 71% in 1990. A similar tendency was observed for higher dose (25 mg/kg) mefloquine monotherapy from 1990 to 1994.

Ever since the combined widespread use of artesunate and mefloquine in 1994, the treatment rate of mefloquine improved once more to nearly 100% since 1998 onwards, and there has been a continued reduction in the prevalence of *Plasmodium falciparum* malaria in western Thailand (Nosten *et al*., 2000:297). ACTs remain thus the treatment of choice (WHO, 2010:54).

1.9.2.1 Antimalarial action

The suggested mechanism of action of artemisinin includes the splitting of endoperoxide bridges by iron generating free radicals which damage biological macromolecules, resulting in oxidative stress in the parasite cells (Meshnick *et al*., 1996:303).

Golenser *et al*. (2006:1438) noted the necessity to improve the pharmacokinetics of artesunate by changing the formulations and delivery approaches. In contrast to monotherapy, the use of combination formulations and improved pharmacokinetics should inhibit the advance of clinical parasite drug resistance. Drug resistance is a tendency which characterises all formerly established antimalarial therapies.

1.9.3 Mefloquine

Mefloquine (Lariam®) was a product of the Malaria Research Program established in 1963 by the Walter Reed Institute for Medical Research to develop promising new compounds to combat emerging strains of drug-resistant *Plasmodium falciparum*. Mefloquine is additionally used for the prophylaxis and chemotherapy of drug-resistant *Plasmodium vivax* malaria. Of many 4-quinoline methanols tested, based on their structural similarity to quinine, mefloquine *(Figure 1.6*) displayed high antimalarial activity in animal models and emerged from clinical trials as safe and highly effective against drug-resistant strains of *Plasmodium falciparum* (Schmidt *et al*., 1978:1011). Mefloquine was first used to treat chloroquine-resistant *Plasmodium falciparum* malaria in Thailand, where it was formulated with pyrimethamine-sulfadoxine to inhibit the advance of drug-resistant parasites (White, 1999:399).

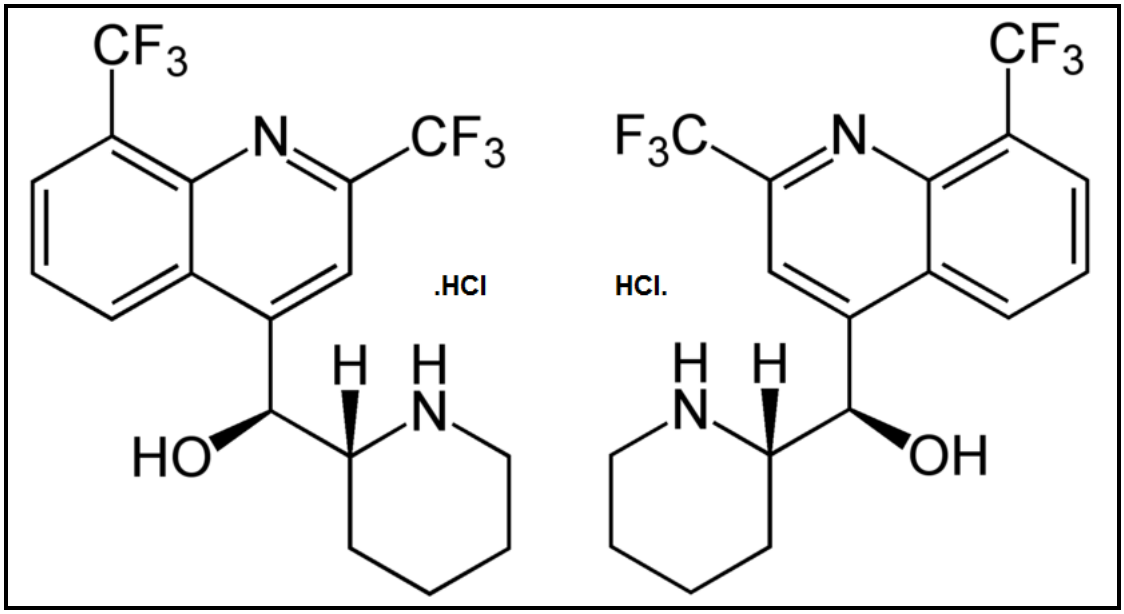

Figure 1.6: The structural formula of mefloquine hydrochloride (Ju, 2009:1).

Mefloquine is presently effective in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in South-East Asia when used in combination with artemisinin derivatives, and specifically artesunate (Na-Bangchang *et al*., 2007:146).

1.9.4 Antimalarial actions of mefloquine

The exact mechanism of action of mefloquine is unknown, but may be similar to that of chloroquine. However, mefloquine exists as a racemic mixture of four optical isomers with about the same antimalarial potency. Mefloquine has no activity against early hepatic stages and mature gametocytes of *Plasmodium falciparum* or latent tissue forms of *Plasmodium vivax*. Nevertheless, mefloquine is a highly effective blood schizontocide. The drug may have some sporontocidal activity, but is not used clinically for this purpose (Brickelmaier *et al*., 2009:1845).

1.9.4.1 Resistance to mefloquine

Certain isolates of *Plasmodium falciparum* exhibit resistance to mefloquine. The molecular basis of this resistance is not fully understood and is clearly diverse (Sidhu *et al*., 2002:210). It is proposed that the strengthening of the pfmdr1 gene is associated with resistance to quinine and mefloquine (Dorsey *et al*., 2002:2031).

1.9.4.2 Absorption, fate and excretion

Mefloquine is taken orally because parenteral preparations cause severe local reactions. The drug is well absorbed, a process enhanced by the presence of food. The drug is widely distributed, highly bound (98%) to serum proteins, and slowly eliminated with a terminal half-life of about 20 days (Karbwang & White, 1990:264).

1.9.4.3 Combination with an artemisinin compound

The development of resistance to antimalarial drugs by *Plasmodium falciparum* poses a major threat to tropical areas of the world (White & Olliaro, 1996:399). The decline in efficacy of mefloquine against *Plasmodium falciparum* since 1990 has been so rapid that the use of mefloquine alone is not indicated anymore (Price *et al*., 1995:523).

Although mefloquine is especially useful as a prophylactic agent for susceptible tourists who stay for only brief periods in areas where *Plasmodium falciparum* and *Plasmodium vivax* are endemic, the fact is that in areas where malaria is due to drug-

resistant strains of *Plasmodium falciparum*, recent evidence indicates that mefloquine is more effective when used in combination with an artemisinin compound (Hutagalung, 2005:46).

1.9.5 Simultaneous dosing of artesunate and mefloquine

The WHO stated that the use of artemisinins as monotherapy should be discouraged, since monotherapy will advance resistance to this crucially significant antimalarial compounds (2010:21). A parallel-group, randomised, double-blind study in 104 hospitalised patients with acute, uncomplicated *Plasmodium falciparum* malaria was executed in Central and Western Africa from March to July 2001. Patients from the investigational group were randomly subjected to take concurrent dosages of artesunate (200 mg per day) and mefloquine (250 mg per day), from the first to the third day of treatment. The reference group received sequential dosing of artesunate (200 mg per day for 3 days) plus mefloquine (250 mg on the second, and 500 mg on the third day of treatment). The patients were observed for 28 days, and parasitological and clinical results were evaluated. The fortnight cure rate was 100% in the experimental group and 98% in the control group, with no recurrences after 28 days. The mean times to fever and parasite clearance were comparable between both groups and tolerability was satisfactory in both groups. The number of patients who vomited was statistically and meaningfully less in the experimental group compared to the control group (3.8% against 19.2%). The researchers concluded that a three day course, taken once a day, of the combined administration of artesunate and mefloquine, offers an effective treatment for patients with uncomplicated *Plasmodium falciparum* malaria, and is well tolerated (Massougbodji *et al*., 2002:655).

In summary, the following doses are suggested by the WHO for the treatment of acute, uncomplicated *Plasmodium falciparum* malaria:

- Oral artesunate: 4 mg/kg/day once a day for three days, with mefloquine either split over two days as 15 mg/kg on day one and 10 mg/kg on day two, or over three days as 8.3 mg/kg/day, once a day for three days.
- The therapeutic dose range for artesunate is between 2 - 10 mg/kg/dose/day, and 7 - 11 mg/kg/dose/day for mefloquine (WHO, 2010:20).

To combine the dosage regimen, as recommended by the WHO, of artesunate and mefloquine into a solid oral fixed-dose combination for the treatment of acute, uncomplicated *Plasmodium falciparum* malaria, it is critical that the fixed-dose combination exhibits adequate:

- ingredient compatibility,
- stability,
- similar absorption rates and
- similar oral bioavailability to the separate tablet formulations or standard reference fixed-dose combinations (WHO, 2010:17).

1.10 PHARMACEUTICAL QUALITY BY DESIGN

Utilising quality by design principles to develop a double fixed-dose combination of artesunate and mefloquine will enhance satisfactory ingredient compatibility and stability (Liltorp *et al*., 2011:424). Drug and excipient compatibilities play a significant role during formulation (Kopelman & Augsburger, 2002:35). Regardless of the fact that excipients can influence the bioavailability and stability of active pharmaceutical ingredients, the common principles of picking appropriate excipients for dosage forms are imprecise, and excipients are frequently selected without methodical drug-excipient compatibility analysis (Yu, 2008:785). The importance of the quality by design concept is that quality ought to be manufactured into a product with a thorough understanding of the product and processes by which it is developed and produced, together with an understanding of the risks associated with the manufacturing of the product, and how to minimise those risks effectively (Yu, 2008:781).

1.10.1 Building quality into products

Process Analytical Technology (PAT) is a set of principles for designing, examining, and managing manufacturing processes. In effect, PAT applications are established by comprehensive, science-based understanding of the chemical and mechanical properties of all components of the projected product. With the intention of designing a process that delivers a reliable product, the chemical, physical, and biopharmaceutical properties of the drug and other components of the manufacturing process must be determined. Even though the skill of analysing the chemical characteristics, such as identity and purity is established, certain physical qualities,

such as the solid forms, particle sizes, and particle shapes of substances are more challenging to analyse and manage (Gujral *et al*., 2007:2).

The PAT principles are consistent with the contemporary FDA beliefs that quality cannot be verified into products, but that quality should be integrally designed into products. As stated in the draft guidance for the industry (FDA, 2004:1) the preferred state of pharmaceutical manufacturing is that:

- Product value and performance are guaranteed by the proposal of effective and efficient manufacturing processes.
- Product and process specifications are established on the systematic awareness of how formulation and process aspects affect product quality.
- Quality assurance is uninterrupted and in progress.
- Appropriate regulatory policies and procedures are custom-made to support the most contemporary level of scientific knowledge.
- Risk-based regulatory methodologies describe both the level of scientific knowledge and the proficiency of process controls linked to product quality and performance (Balboni, 2003:54).

According to the Solid State Chemical Information (2010:1), a step by step approach to achieve products with build-in quality would proceed as follows:

- Define the solid forms accessible and their relevance to manufacture and use.
- Pick the ideal solid forms of the APIs.
- Develop analytical procedures to validate the presence of, and quantify the concentration of the particular solid forms of the APIs.
- Examine the physical properties of the APIs and excipients, such as particle size, particle shape, stability, ease of drying, filterability, solubility, dissolution rate, etc.
- Organise a manufacturing process that constantly delivers the preferred form of the API possessing the sought after physical characteristics.
- Contribute in setting API specifications.
- Define excipient compatibility.
- Contribute in formulation design.
- Participate in drug product manufacturing approaches that are consistent with the solid state properties of the API.
- Participate in establishing drug product specifications.

1.11 FORMULATION APPROACH FOR ARTESUNATE AND MEFLOQUINE

The formulation properties of artesunate and mefloquine are limited in literature. Even though high treatment rates are clearly required, combination treatments, as opposed to monotherapy are expensive. The manufactured cost is about 10 USD for a course treatment of artesunate for an adult, and the price of a container of artesunate and sulfadoxine-pyrimethamine, or artesunate and amodiaquine is between 12 and 18 USD. These prices compare with 0.15 USD for chloroquine, 0.25 USD for sulfadoxine-pyrimethamine, and 24 USD for artemether-lumefantrine, which until 2007 was the only available fixed-dose combination apart from an artesunate and amodiaquine fixed-dose combination (Espié *et al*., 2012:2). There is thus a dire need for a cost effective artemisinin-based combination therapy of artesunate and mefloquine.

1.11.1 Dosing

1.11.1.1 Pharmacokinetic properties of artesunate

Artesunate performs like a prodrug that is rapidly hydrolysed into dihydroartemisinin (DHA) and has an elimination half-life of less than half an hour (Na-Bangchang, 1998:375). Metabolism and pharmacokinetic studies have shown that artesunate is hydrolysed rapidly to DHA (Tan, 2009:12). Orally administered artesunate and intravenous artesunate were compared and the absolute bioavailability of artesunate was low, but the relative bioavailability of dihydroartemisinin was high (Batty *et al*., 1998:823).

The mean weight among malaria patients in Ghana (n = 520), was found to be 49.6 kg (Asante *et al*., 2009:3), whilst the therapeutic dose range for artesunate is between 2 and 10 mg/kg per dosage per day (WHO, 2010:20). Presuming then that the average weight of an adult malaria patient is 50 kg, a double fixed-dose combination would have to contain approximately 200 mg of artesunate and 415 mg of mefloquine.

The enteric coating of artesunate particles as controlled-release technology may render the release of artesunate to be absorbed further down in the gastrointestinal tract (GIT). By modifying the release of artesunate in the GIT, the problematic short half-life of artesunate can be addressed.

1.11.1.2 Pharmacokinetic properties of mefloquine

Simpson *et al*. (1999:472) concluded that the “pharmacokinetic properties of mefloquine in malaria were relatively unaffected by demographic variables (other than body weight) or disease severity”. If it is assumed that the clearance and distribution volume of mefloquine are unaffected by the dosage regimen, then splitting the 25 mg/kg mefloquine dose increases oral bioavailability and the therapeutic reaction in the treatment of acute, uncomplicated *Plasmodium falciparum* malaria. The therapeutic dose range is between 7 and 11 mg/kg per dosage per day for mefloquine. (WHO, 2010:20).

1.11.2 Granulation

For the proposed oral double fixed-dose combination of artesunate and mefloquine to transpire, the formulator has to consider the wet granulation technique to improve the poor flow properties of the raw artesunate and mefloquine powders, in addition to improving the compressibility of the raw artesunate and mefloquine powders.

Excipients and active ingredients are processed through two major methods of precompression treatment known as granulation and slugging (Otto, 2002:2). Wet granulation employs a liquid phase to render cohesion between particles whereas slugging is a so-called dry method whereby granules are formed through compression and milling of the formed ("slugged") compacts (Milosovich, 1963:557).

Granulation prior to compression is the process of particle size enlargement and is done to improve flowability and compressibility of powder mixtures. The granulated particles are capable of being subjected to higher compression pressures and produce stronger tablets than primary powders. Granules usually have a uniform distribution of all the ingredients in the formulation and provide an effective powder mix prior to compression. In addition, granules prevent segregation during compression (Summers & Aulton, 2007:411). In modern times, there have been substantial developments in our comprehension of how wetting, nucleation, growth and break-up merge to regulate wet granulation processes. Nevertheless, the scale-up and control of wet granulation processes still remain a challenge (Muzzio *et al*., 2002:6).

It is therefore necessary to understand the important mechanisms involved during size enlargement processes and their relation to each other. Controlling granulation and coating processes and overcoming their disadvantages are highly beneficial (Hemati *et al*., 2003:19).

1.11.3 Delayed-release formulations for oral dosage forms

Possessing a pH of approximately 2, chyme emerging from the stomach is quite acidic. Artesunate, however, is soluble at a basic pH (Pogány 2006:16). To increase its pH, the duodenum discharges cholecystokinin, a hormone, which results in the gall bladder contracting and releasing alkaline bile into the duodenum. In addition, the duodenum similarly produces a hormone, secretin, to stimulate the pancreatic discharge of great amounts of sodium bicarbonate, which elevates the pH of the chyme to 7, in advance of reaching the jejunum. The basic pH of the lower GIT would thus provide a more suitable environment for artesunate to dissolve. As a result of its solubility at a basic pH and the need to protect the artesunate from premature uptake, the requirement for a double fixed-dose combination of artesunate and mefloquine and for artesunate to possess delayed release qualities is justified (Pogány, 2006:16).

Oral dosage forms can be formulated to provide controlled release of the active ingredients in the GIT. The release of actives can thus be controlled by swelling, permeable coatings and matrix structures. Generally, the polymer dispersion (coating material), is sprayed onto the solid particles in suitable equipment while the wetting agent evaporates, thus forcing the colloidal particles together (Bodmeier & Paeratakul, 1994:1519) to obtain particles with controlled release properties.

According to Degussa (2010:1), to obtain controlled release particles, the following values for spherical particles with diameters in a range of 0.5 to 1.2 mm can be used to estimate the percentage polymer weight gain applicable during coating:

- enteric coatings: 10 - 30%,
- sustained-release coatings: 5 - 20%,
- taste-masking coatings: 5 - 10% and
- moisture protection coatings: 10 - 30%.

Apart from the size of the particles, the solubility of the active, surface structure, and mechanical stability, different amounts of polymers may be needed (Guignon *et al*., 2003:194). Therefore, it is recommended to start with a coating trial in which samples at different polymer weight gains should be taken and tested in order to determine the correct amount of polymer required for the intended purpose.

1.12 ACTIVE PHARMACEUTICAL INGREDIENT RELEASE MECHANISMS

The appropriate and precise release of APIs from delivery vehicles of different categories is, for understandable reasons, of primary significance for an effective and harmless pharmacological treatment of a disease (Siepmann & Siepmann, 2008:329). Mathematical modelling demonstrates an imperative part in this framework, providing means to analyse experimental release data and to clarify the fashion in which formulation and design aspects affect the release profiles of drugs (Siepmann & Peppas, 2001:139). It is thus consequently acknowledged that considerable attempts have been allocated to developing mathematical models to describe the drug-release process (Frenning, 2011:89).

Several design factors are utilised to impart an altered drug release. As described in this section, mathematical modelling plays a significant part in this context, providing tools to analyse experimental release data and to explain the manner in which formulation and design factors affect the release profile of an oral dosage form (Frenning, 2011:89).

For this study and for all the reasons described earlier in this chapter, mefloquine needs to be dissolved rapidly, and a part of the artesunate dosage needs to be released later in the digestive track. Consequently, an opportunity exists for the manufacturing of a controlled release, double fixed-dose combination of artesunate and mefloquine.

1.12.1 The technology of controlled release dosage forms

Controlled release, oral drug delivery systems can be categorised into two groups:

- Single unit dosage forms (SUDFs), such as capsules or tablets, and
- Multiple unit dosage forms (MUDFs), such as pellets, granules or mini-tablets (Lopesa *et al*., 93:2006).

The idea of MUDFs was originally presented during the start of the 1950s. The manufacturing of MUDFs is a general approach to control the release of a drug, as exposed by the reproducibility of the release profiles when likened to the release profiles obtained with SUDFs (Lopesa *et al*., 93:2006).

Furthermore, based on the release mechanism, controlled release preparations can be categorised according to the mechanism by which drug release takes place. According to Bruck (1983:6), the preparations can be categorised as:

- mechanical systems,
- osmotic systems,
- dissolution systems and
- diffusion systems.

1.12.2 Mechanical systems

Mechanical pumps were initially developed to release insulin and heparin directly into the blood circulation (Bruck, 1983:8). With these systems a constant drug level is maintained as long as the pump is functional. Disadvantages include the requirement of a surgical procedure and the initial high costs involved (Gabbe *et al*., 2000:1287).

1.12.3 Osmotic systems

A system exploiting osmotic pressure to achieve zero-order drug release is described as an osmotic pump. The system comprises a water soluble drug and an osmotic active substance surrounded with a semi permeable membrane with a supply opening. Once exposed to water, the core absorbs water osmotically at a controlled rate, established by the membrane permeability to water, and by the osmotic pressure of the core formulation. Water moves into the system across the membrane by means of diffusion and dissolves the enclosed drug. The dissolved drug exits the system as a result of osmotic differences. The pressure formed inside the system prompts a release of the solution at a gentle but constant rate (Theeuwes, 1975:1987).

However, the rate of drug release is reduced remarkably if the osmotic active substance concentration decreases below the satisfied level (Lee & Robinson, 1978:172). The osmotic pump delivers between 60-80% of the drug at a constant rate (Theeuwes, 1984:293).

1.12.4 Systems regulated by dissolution

Encapsulated preparations and dissolution matrices demonstrate a dissolution regulated drug release (Maderuelo *et al*., 2011:3).

1.12.4.1 Encapsulated preparations

Encapsulated preparations have an enclosed active pharmaceutical ingredient coated with a slow dissolving film layer or gel. The release rate of the enclosed active pharmaceutical ingredient is determined by the film solubility and layer thickness (Maderuelo *et al*., 2011:4).

1.12.4.2 Dissolution matrices

Matrix systems can be obtained via various different microencapsulation processes. The drug is compressed together with a weak water soluble excipient. Drug release is a function of the filler solubility, tablet porosity and the presence of hydrophobic substances and tablet wettability. Compression force, dissolution medium, drug and excipient properties additionally influence dissolution (Parrot, 1981:162). The rate of dissolution from a solid is described by the amended Noyes-Whitney equation:

Equation 1.1

$$dc / dt = k_D A(C_s - C)$$

Where:

dc / dt	=	dissolution rate
k_D	=	constant (dissolution rate)
A	=	area
C_s	=	saturated solubility of drug
C	=	drug concentration in dissolution medium

The dissolution rate will remain constant as long as there is a sufficient amount of drug available to stabilise C_s, and on condition that the area, A, remains constant. The dissolution process, however, alters the surface area of the drug if exposed to the dissolution medium (Buys 1988:12).

According to Ritchel & Udeshi (1987:739), for spherical particles, the varying area can be substituted by the mass of the particles. The release of drug according to the mass of spherical particles can be given by the law of the cube roots (*Equation 1.2*):

Equation 1.2

$$W_0^{1/3} - W^{1/3} = k_D$$

Where:

$W_0^{1/3}$	=	initial drug amount in the centre
$W^{1/3}$	=	amount of drug remaining in the centre
k_D	=	cube root dissolution rate constant

1.12.5 Systems regulated by diffusion

The rate of diffusion will conform to Fick's Law of Diffusion. Fick's Law declares that the rate of variation in concentration of dissolved material through time is directly proportionate to the difference in concentration between the opposite sides of the diffusion layer. Both diffusion and dissolution is present in a specific dosage form. However, one mechanism usually dominates the other mechanism (Lee & Robinson, 1978:140).

1.12.5.1 Structures coated with a water immiscible membrane

A water immiscible polymer or a combination of water immiscible and a water soluble polymer coated upon a drug nucleus is one technique to produce a diffusion based system. *Equation 1.3* describes this particular drug release (Lee & Robinson, 1978:140):

Equation 1.3

$$dM / dt = DA\Delta C / \ell$$

Where:

A	=	area
dM / dt	=	release rate
D	=	diffusion coefficient of drug between membrane and nucleus

ℓ = membrane thickness

ΔC = concentration difference across membrane

1.12.5.2 Structures coated with a semi-soluble membrane

In this case the drug nucleus is coated with a semi-soluble membrane, containing a complex of a water soluble and water insoluble polymer. The water soluble polymer dissolves and the drug diffuse through the membrane pores (Lee & Robinson, 1978:141). *Equation 1.4* describes this particular drug release:

Equation 1.4

$$dM/dt = AD(C_1 - C_2)/\ell$$

Where:

C_1 = drug concentration inside nucleus

C_2 = drug concentration in dissolution medium

A = area

D = diffusion coefficient

ℓ = membrane thickness

1.12.6 Matrix release subject to drug diffusion

The Higuchi equation (1963:1145) explains drug release dependent on diffusion out of an inert matrix. The Higuchi equation is described as:

Equation 1.5

$$Q' = Dt \cdot (2A - Cs) \cdot Cs^{1/2}$$

According to Schwartz *et al*. (1968:275), the Higuchi equation can be transformed to *Equation 1.6*:

Equation 1.6

$$Q' = KSt^{1/2}$$

Where:

Q' = amount of drug released

S = *area*

K = $(D.\varepsilon / \tau(2A - \varepsilon.C_s)C_s)^{1/2}$

A = amount of drug present in matrix

C_s = drug solubility in matrix

ε = porosity factor

τ = crinkle factor

A linear correlation would be possible between the amount of drug released and the square root of time. Schwartz *et al*. (1968:275) differentiated the above equation to *Equation 1.7*:

Equation 1.7

$$dQ'/dt = K^2.S^2/2Q$$

Where:

dQ' / dt = release rate

The equation can logarithmically be altered to *Equation 1.8*:

Equation 1.8

$$\log Q' = \log K + \frac{1}{2}\log t$$

A graph of $log\ Q'$ versus $log\ t$ would result in a straight line with an inclination of 0.5 if the drug is released by means of Fickian diffusion (Schwartz *et al*., 1968:275).

1.13 THE MANUFACTURING OF CONTROLLED RELEASE DOSAGE FORMS

Polymeric film coatings have frequently been applied for accomplishing controlled release of an API from a pharmaceutical preparation, since a coated dosage form facilitates the prolonged and accurate release of drug with decent reproducibility (Tarvainen *et al*., 2004:179).

1.13.1 Coating

Numerous solid pharmaceutical dosage forms are manufactured with coatings, either on the exterior surface of tablets, or on materials encapsulated within gelatine capsules. According to Eurotherm (2003:1), coating assists in a number of purposes:

- coating shields the tablet (or the capsule contents) from gastric acids,
- coating safeguards the stomach lining from aggressive drugs, such as enteric coated aspirin,
- coating enables a delayed release of the medication and
- coating assists in maintaining the shape of the tablet.

The coating procedure can be designed to direct the dissolution rate in addition to controlling the site of release.

1.13.1.1 Coating techniques

Advanced and particular coating techniques were developed to facilitate the developing requirements of the pharmaceutical industry. In summary, various coating techniques include:

- dip coating,
- functional coating,
- electrostatic coating,
- magnetically assisted impact coating,
- pseudolatex coating,
- compression coating,
- laser assisted cold spray coating,
- photo curable coating,
- supercritical fluid coating and
- pan coating (Boxi & Beg, 2013:1).

The pan coating technique was employed during this study, and is subsequently described in more detail.

1.13.1.2 Coating process design and control

The coating process basically consists out of the following phases:

- loading the material to be coated into the coating pan,
- rotating the mixed tablet bed or fluidized bed,

- spraying of the coating liquid on to the rotating bed,
- drying and removal of the solvent,
- cooling the thin deposit of coating material around each tablet core and
- unloading the tablet cores (Porter, 2007:502).

The industrial coating process is graphically illustrated in *Figure 1.7* (Gendre *et al*., 2011:238). Industrial coating proceeds in a controlled atmosphere inside a perforated rotating drum. Angled mixing baffles attached to the drum, and air flow inside the drum provides means of agitating the particle bed. Consequently, the particles or tablets are pick up and rolled from the sides into the centre of the drum, exposing every particle or tablet surface to a uniform quantity of the sprayed coating agent.

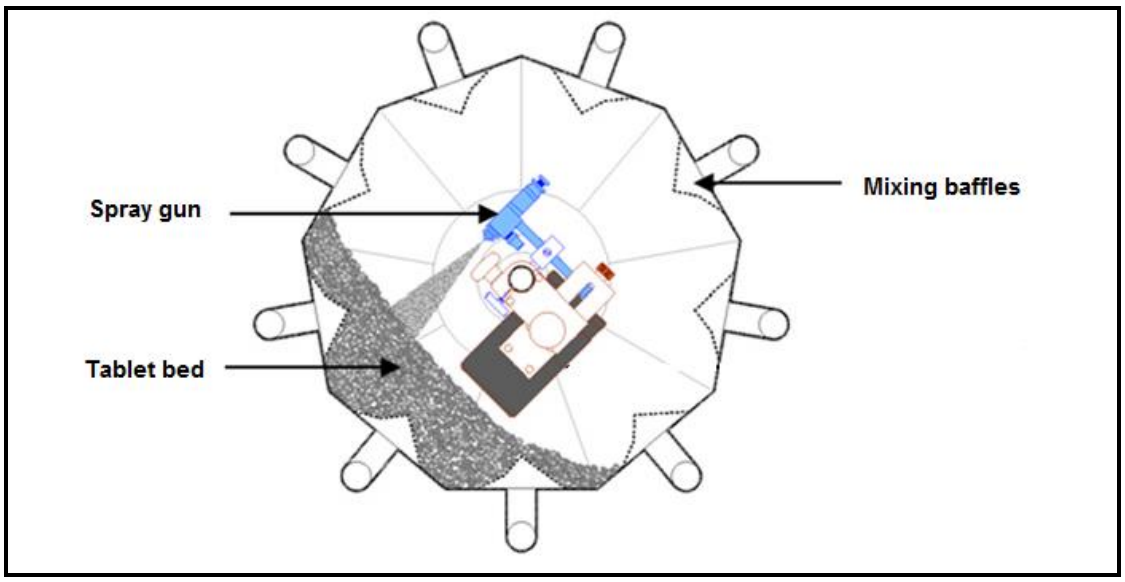

Figure 1.7: The coating process (Gendre et al., 2011:238).

The spray coating fluid is subsequently dried onto the particles or tablets by heated air drawn through the perforated rotating drum and particle bed from an inlet fan. The flow of air is controlled with regard to volumes and temperatures. To provide controlled drying and extracting rates, the inside pressure of the drum is kept slightly negative relative to the exterior environment, in order to provide a completely isolated process atmosphere for the operator (Ishrae, 2010:1).

1.13.1.3 Coated particles

Coated particles are covered with either a single layer, or several layers of mixtures of various substances. Substances include natural or synthetic resins, gums,

gelatine, inactive and insoluble fillers, sugars, plasticisers, polyols, waxes, colouring matter authorised by the competent authority, and sometimes flavouring substances and active substances. The substances used as coatings are frequently sprayed as a solution or suspension in conditions in which vaporisation of the bridging liquid occurs. When the coating is a very thin polymeric coating, the product is known to be film-coated (Ishrae, 2010:1).

Enteric polymeric coatings have been used to reduce the incidence of stomach irritation from drugs such as aspirin (Petroski, 1989:945) and to transport drugs to the duodenum, jejunum or ileum (Sherif *et al*., 1969:28). Formerly, it has been proposed imperative that malaria parasites should be exposed continually to drug levels above the inhibitory *in vivo* level for an optimal antiparasitic result. If this hypothesis is accurate, a once a day dosing with a rapidly-eliminated drug would seem to be insufficient. However, recent studies have confirmed that once-daily dosing gives results identical to those following more frequent administration. The pharmacodynamic properties of rapidly-eliminated antimalarial drugs may be more relevant to the design of dosage regimens than their pharmacokinetic properties (Bethell *et al*., 1997:197) as might be in the case of artesunate.

Acrylic polymers such as Eudragit® L and Eudragit® S can be applied as coatings to conventional solid oral dosage forms like tablets, capsules and small particles and to manufacture pellets and granules (Evonik, 2010:1). Acrylic polymers are specifically recommended for the manufacturing of delayed-release oral dosage forms in addition to spray-dried powders intended for compression (Evonik, 2010:1), and its application will be investigated in this study.

1.14 SUMMARY

The advance of fixed-dose combinations (FDCs) is becoming ever more important from a public health perception. It is being used in the treatment of a wide range of illnesses, such as in the management of HIV/AIDS, malaria and tuberculosis, which are considered among the leading infectious disease threats worldwide (WHO, 2005:95).

Artemisinin-based combination therapies decrease the threat of resistance of *Plasmodium falciparum* to monotherapies and improve medication compliance by reducing the medication burden of patients (WHO, 2010:13).

Artesunate and mefloquine are not at present available as an oral fixed-dose combination. Artesunate and mefloquine are currently presented as blister packs with separate scored tablets containing 50 mg of artesunate and 250 mg base of mefloquine, respectively (WHO, 2010:20).

One area of importance involves the use of artesunate and mefloquine for artemisinin-based combination therapy (ACT) in directly compressible tablet formulations after being subjected to size enlargement processes. Following in *Chapter* 2 are the experimental methods and apparatus employed and the materials used throughout the study.

2. CHAPTER TWO

EXPERIMENTAL METHODS, MATERIALS AND APPARATUS

The experimental materials, procedures and apparatus used in the different experiments are described in this chapter.

2.1 MATERIALS

The materials used in the study are presented in *Table 2.1*.

Table 2.1: Information about the materials used in this study.

Compound	Lot number	Manufacturer
Ac-Di-Sol®	T017C	FMC Corporation, Philadelphia, Pennsylvania, USA.
Artesunate	IF-AR-080830	IFFECT CHEMPHAR Co., Ltd., Nanjing, China.
Avicel® PH200	M939C	FMC Corp., Cork, Ireland.
Eudragit® L 100	B051203084	Röhm GmbH & Co. KG, PharmaPolymers, D-64293, Darmstadt, Germany.
Kollidon® 30	86085224UO	BASF Aktiengesellschaft, Ludwigshafen, Germany.
Kollidon® VA64	62-8826	BASF Aktiengesellschaft, Ludwigshafen, Germany.
Magnesium stearate	472131	Kirsch Pharma, Isando, Johannesburg, RSA.
Mefloquine hydrochloride	IF-ME-080830	DB Fine Chemicals, Johannesburg, RSA.

2.2 POWDER EVALUATION

2.2.1 Shape and surface structure of particles

A supplementary method of classification of the physicochemical properties of powders, granules and tablets is by scanning electron microscopic observations. The analysis of the physical powder, agglomerate and tablet properties is often the observation of an assortment of macroscopic occurrences. Electron micrographs deliver information on a micro level that might render a better understanding of the macroscopic events under which process variables exert their actions.

2.2.1.1 Experimental conditions

Powder and granule samples were fixed on double-sided conductive carbon tape which was placed in a sampling tray and dusted with an inert gas. The samples were consequently sputter-coated with gold and palladium (80:20) to form a layer of approximately 28 nm on the surface of the samples. An Eiko® ion coater (model IB-2, Eiko Engineering, Japan) was used in all coating procedures and operated under a vacuum superior than 0.06 Torr. Samples were studied using a Philips® XL 30 DX 4i SEM microscope (Eindhoven, The Netherlands).

2.2.2 Particle size and size distribution

Particle size analyses of the powders and granules were conducted by means of laser diffraction, using a Malvern® Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK), fitted with a sample suspension unit. The dispersion unit contained water and cyclohexane during the particle size distribution measurements of artesunate and mefloquine respectively.

The calculation of the span of a powder sample is made to determine its particle size distribution. The span gives an indication of the width of the distribution based on the 10^{th}, 50^{th} and 90^{th} percentile. The span can be calculated by using the following equation (Malvern Instruments Ltd, 2012:1):

Equation 2.1

$$Span = \frac{D_{v,0.9} - D_{v,0.1}}{D_{v,0.5}}$$

2.2.3 X-ray powder diffraction (XRPD)

X-ray powder diffraction is an effective method to distinguish between different solid phases in different or the same powdered samples. The advantage of using XRPD is that this method does not require large single crystals, but instead can be readily applied to any powdered sample (Brits, 2003:67).

The X-ray powder diffractogram yields information about the diffraction characteristics of the sample. These diffraction characteristics include the intensities of the maximum diffraction peaks and the angles at which they occur. The pattern of scattered radiation that is unique to each crystal structure is called the diffraction pattern. The diffraction pattern can thus also be used to determine the crystal structure of the powdered sample (Hanawalt, Rinn & Frevel 1938:457).

2.2.3.1 Experimental conditions

X-ray powder diffraction patterns were obtained at room temperature using a Bruker D8 Advance diffractometer (Bruker, Karlsruhe, Germany). Approximately 20 mg samples were weighed into aluminium sample holders. The measurement conditions were: target Cu; voltage 40 kV, current, 30 mA; divergence slit, 2 mm; antiscatter slit, 0.6 mm; monochromator; scanning speed, 2º/min (step size, 0.025º; step time, 1.0 sec.).

The XRPD traces of the powders were compared with regard to peak position and relative intensity, peak shifting and the presence or lack of peaks in certain regions of º2θ values.

2.2.4 Infrared absorption spectroscopy (IR)

Silverstein *et al*. (1991:91) stated that “A peak-by-peak correlation [of the IR spectra] is excellent evidence for identity. It is unlikely that two compounds, except enantiomers, give exactly the same IR spectrum.” Therefore, IR-analysis proves to be a suitable method to implement for the characterisation of different crystal forms, thus an appropriate procedure to determine whether the manufacturing process interfered with the artesunate and mefloquine.

2.2.4.1 Experimental conditions

IR-spectra were recorded on a Nicolet Nexus 470-FT-IR spectrometer (Nicolet instrumentation corporation, Madison, USA) over a range of 0 - 4000 cm^{-1}. The diffuse reflectance method was used. Potassium bromide was used as background material. The main absorptions in the IR-spectral results of the samples were compared to determine possible significant differences with regard to polymorphic form or polymorphic modifications.

2.3 CHARACTERISATION OF FLOW PROPERTIES

2.3.1 Flow properties

Of major importance when handling a drug is powder flow. Flow behaviour can often be described best by quantification of the process of flow. Numerous methods have been described either directly, using dynamic or kinetic methods, or indirectly, generally by measurements carried out on static powder beds (Staniforth, 2000:601). These include:

- the angle of repose,
- hopper flow rate and
- the individual tablet weight variation, and
- the critical orifice diameter.

Other methods used to determine and attach a measurement to the flow of a powder are:

- Carr's compressibility index (Carr, 1965:69) and
- Hausner's ratio (Hausner, 1967:7).

A more uniform powder particle shape will lead to a smaller angle of repose and a smaller Carr's index, both of which are indications of good powder flow properties (Staniforth & Aulton, 2007:178).

2.3.1.1 Angle of repose (AOR)

The angle of repose has been used as an indirect method of quantifying powder fluidity. Particles will start to slide when the angle of inclination is large enough to overcome the cohesive forces between particles (Staniforth, 2000:601). The flow rate of a powder is proportional to the angle of repose. Powders with angles of repose

greater than 50º have poor flowability and powders with angles close to 25º have good flow properties (Staniforth & Aulton, 2007:175).

2.3.1.2 Flow rate

Particle and process related effects can affect the flow rate of a powdery substance. Particle-related effects are affected by particle size, shape and density; and process related effects are orifice diameter, hopper width, head size (height of the powder bed) and hopper wall angle (Staniforth & Aulton, 2007:174).

The flow rate is a useful measurement for materials that have some capacity to flow, but is not useful for cohesive materials. An inappropriate orifice diameter may result in an erratic measurement of the flow rate due to funnel flow (Staniforth & Aulton, 2007:175). A mass flow hopper (*Figure 2.1*) was therefore preferred, and an orifice diameter of 8 mm was chosen. The time it takes for a predetermined mass of powder to be discharged from the hopper can be used to determine the powder flow rate through an orifice (Staniforth, 2000:601).

2.3.1.3 Critical orifice diameter (COD)

The critical orifice diameter is the smallest opening (diameter) through which a powder will flow freely without assistance or interference. The apparatus used for the critical orifice diameter is the same one used in the determination of angle of repose. This apparatus was designed by Buys (2006:40).

2.3.2 Experimental conditions

All flow experiments were conducted under ambient conditions. The apparatus utilised for the flow measurements consisted out of a set of brass discs between 5 and 10 mm thick that was stacked on top of each other. The largest brass disc opening was 32.0 mm and the smallest was 1.5 mm. The angle between the opening and the orifice of each disc was machined to a set angle. A cylinder was fitted at the top of the funnel to create a holding chamber for the measured powder fraction. Brass discs were chosen to minimise the effect of static electricity between the powder and the cylinder (*Figure 2.1*).

2.3.2.1 Angle of repose

To determine the angle of repose for a powder sample, 25 g of powder was poured into the holding chamber of the apparatus, fitted with a 10.0 mm orifice that was kept shut with a shutter disc. The shutter disc was opened and the powder discharged from a height of 15 cm onto a horizontal glass surface. The height and radius of the formed powder cones, flowing from the apparatus depicted in *Figure 2.1*, were measured and the angles of repose were trigonometrically calculated.

2.3.2.2 Flow rate

To determine the flow rate of the powders, 25 g of powder was poured into the apparatus depicted in *Figure 2.1*, and fitted with a shutter disc with an orifice diameter of 8.0 mm. The time it took for the powder to be discharged from the container was noted to determine the flow rate.

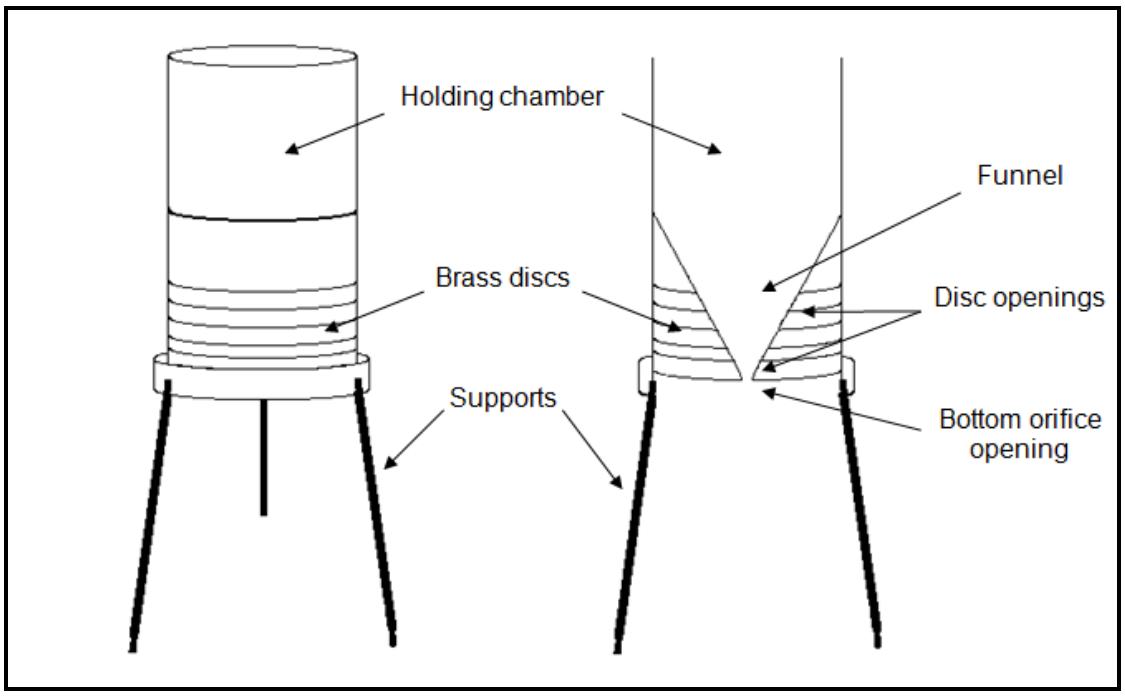

Figure 2.1: Diagram of apparatus used in determining the angle of repose, flow rate and critical orifice diameter (COD), (Horn, 2008:34).

2.3.2.3 Critical orifice diameter

A set mass of powder (25 g) was loaded into the holding chamber of the apparatus depicted in *Figure 2.1*, while the bottom orifice was kept shut with a shutter disc. Opening the orifice allowed the powder to flow through the orifice (if possible). Interchangeable brass discs allowed the orifice diameter (available range of 1 to 32

mm) to be changed. The smallest orifice diameter through which the powder flowed freely was noted as the critical orifice diameter of that specific powder. The experiment was repeated in triplicate for each powder and the average COD and the percent relative standard deviation were calculated.

The ranking index shown in *Table 2.2* was used to classify the flow characteristics of the various materials based on their respective critical orifice diameter.

Table 2.2: Ranking index used to classify flow according to the critical orifice diameter (Horn, 2008:43).

Flow description	**Critical orifice diameter (mm)**
Excellent	1 - 5
Good	6 - 9
Average	10 - 15
Poor	16 - 20
Very poor (cohesive)	> 20

2.3.2.4 Bulk and tapped density

A known quantity of each sample (25 g) was weighed on a Precisa® analytical balance (model 240A, OERLIKON AG, Zurich) and poured through a funnel into a 100 ml graduated cylinder. The cylinder was then lightly tapped twice to collect all the powder sticking on the wall of the cylinder. The volume was then read directly from the cylinder and used to calculate the bulk density. For tapped density, the cylinder was tapped from a height of 15 cm, 50 times on a wooden bench top to attain a constant volume reading from the cylinder. The bulk density was calculated using *Equation 2.2*:

Equation 2.2

$$\rho_{bulk} = \frac{mass}{volume}$$

Equation 2.3 was used to calculate the tapped density:

Equation 2.3

$$\rho_{tapped} = \frac{mass}{volume}$$

2.3.2.5 Hausner ratio and Carr's index

Carr's index and the Hausner ratio previewed the degree of densification, which could occur during tableting. The Carr's "percentage compressibility" was calculated using *Equation 2.4*:

Equation 2.4

$$\%\ \ compressibility = \frac{\rho_{\text{tapped}} - \rho_{\text{bulk}}}{\rho_{\text{tapped}}} \times 100$$

The Hausner ratio was calculated using *Equation 2.5*:

Equation 2.5

$$\frac{\rho_{\text{bulk}}}{\rho_{\text{tapped}}}$$

As a rule, the higher the Hausner ratio, the lower the powder flowability will be (Liu *et al*., 2008:112).

2.4 GRANULE PREPARATION: DESIGN OF EXPERIMENTS BY MEANS OF FRACTIONAL FACTORIAL DESIGN

A design of experiments (DOE) is a structured and organised method to determine the relationship among factors that influence outputs of a process (Huang *et al*., 2009:31). When a DOE is applied to a pharmaceutical process, factors are the raw material attributes (e.g. particle size) and process parameters (e.g. speed and time), while outputs are the critical quality attributes such as blend uniformity, tablet hardness, thickness, and friability (Verma *et al*., 2009:185).

As each unit process has many input and output variables as well as process parameters, it is impossible to experimentally investigate all of them. Scientists have to use prior knowledge and risk management to identify key input and output variables and process parameters to be investigated by a DOE (Yu, 2008:788). At times, it is not feasible or desirable to include all possible combinations of factor levels for the different factors.

A fractional factorial design should be devised to study the effects of all the different factors in various combinations. To illustrate the advantage of a fractional factorial design, suppose the following scenario: a pharmaceutical company wished to study six different powder mixtures, five levels of binder content, and four compressing settings on a tablet press. For this scenario, a complete factorial study would then involve 6 x 5 x 4 = 120 experiments. Such a study might be extremely costly and time consuming. Under these conditions, it may be possible to design a fractional factorial study containing only a fraction of the 120 factor level combinations, which will still provide information about the main effects of each of the three factors as well as about any important interactions of these factors. *Table 2.3* displays the fractional factorial design pattern for a scenario.

Table 2.3: An example of a fractional factorial design of experiments. The alphabetic letters display the factors. The factor levels are displayed either as 0 or 1.

		B_0		B_1	
		C_0	C_1	C_0	C_1
D_0	E_0		X	X	
	E_1	X			X
D_1	E_0	X			X
	E_1		X	X	

Informed trial and error experimentation is used in product development, often utilising experience obtained with similar materials and equipment. This may involve adjusting the particle size distribution of the solids, changing binder properties and selection of granulation conditions. Thus, the selection of suitable processing conditions can be time consuming. Frustratingly also, granulation processes remain difficult to control (Knight, 2004:156).

The correct amount of granulating liquid and the correct monitoring and detection of the granulation kinetics are important issues. For this study, as a result of the exploratory nature of the experiments and the large amount of factors contributing to granule formation, a fractional factorial design was used to determine optimum conditions. The optimisation tables contain the factors investigated on two levels and marks of values obtained from the experiments. Since the moist agglomeration process is a critical unit process (Leuenberger, 2001:279) and successful compression can only be obtained from an effective free-flowing mixture, percentage differences of the concentration of binders and disintegrant used were minimal.

2.5 GRANULE PREPARATION

All the raw materials for artesunate and mefloquine granules were weighed inside a 250 cm^3 glass container to obtain a mass of 100 g. The formulas are described in detail in *Chapter 3*. The glass containers were fitted with a screw cap. Parafilm® was used to seal the openings of these containers prior to mixing. All mixing procedures employed a Turbula®-mixer (model T2C W.A. Bachofen, Basel, Switzerland) at 69 rpm for 10 minutes as the standard method to ensure a proper distribution of powder particles.

2.5.1 Artesunate

The values obtained from the particle size analysis were used to estimate the surface for artesunate to be coated and granulated with Eudragit® L100. The formulas are described in detail in *Chapter 3*. The raw powders were mixed together and the artesunate was subsequently coated by spraying ethanol and simultaneously mixing the artesunate with Eudragit® L100. The granules were forced through a 4000 µm mesh stainless steel sieve, dried, and kept away from light at 25 ± 0.5 °C for 24 hours. After 24 hours, the primary granules were forced through an 841 µm mesh stainless steel sieve for a second time to produce small free-flowing granules. The granule recovery determined the most effective formulas.

2.5.2 Mefloquine

Mefloquine, Ac-Di-Sol® and Kollidon® VA64 were mixed together, wetted with ethanol and granulated. The formulas are described in detail in *Chapter 3*. The granules were forced through a 4000 µm mesh stainless steel sieve, dried, and kept away from light at 25 ± 0.5 °C for 24 hours. After 24 hours, the primary granules were forced through

an 841 µm mesh stainless steel sieve to produce small secondary granules. The granule recovery determined the most effective formulas.

2.5.3 Granulate recovery

The effectiveness of a particular experiment was described in terms of the amount of granules it could produce. Granule formation accounted for particles greater than 0.1 mm. The granule recovery is a value expressed in percentage and was calculated by determining the weight of the granules and dividing it by the weight of the powder feed, multiplied with one hundred. The equation is given as:

Equation 2.6

$$\%\ GR = \frac{W_{granules}}{Feed_{\ powder}} \times 100$$

Where:

$\%\ GR$	=	percentage granule recovery
W	=	sieved granule weight
$Feed$	=	powder amount used in granulation process

2.5.4 Evaluation of granules

The granules were evaluated in terms of flowability, particle size, shape and structure similar to the methods described in sections *2.2* and *2.3.*

2.6 MIXTURE PREPARATION OF THE DOUBLE FIXED-DOSE COMBINATION

2.6.1 Selection of excipients

Excipients were carefully selected in advance based on theoretical compatibility, theoretical stability and to eliminate disturbances during the analytical process of HPLC. The APIs and excipients were studied subsequently as mixtures and tablets employing x-ray powder diffraction and infrared absorption spectroscopy for stability and to identify excipient incompatibilities.

2.6.1.1 Lubricants

Lubricants are added to pharmaceutical powder mixtures to ensure that tablet formation and ejection can occur with low friction between the solid and the die wall,

preventing the tablets from being damaged (Alderborn, 2007:452). Typical lubricants include magnesium stearate and talc. Magnesium stearate is possibly incompatible with salts of weak bases and strong acids (e.g. Amodiaquine.2HCl) because the formed $MgCl_2$ is highly hygroscopic and, as a result, its lubricant properties also change (Pogány, 2006:16). Both magnesium stearate and talc were investigated as possible lubricants.

2.6.1.2 Fillers (or diluents)

Fillers are added to pharmaceutical powder mixtures to improve compactibility (Alderborn, 2007:450). Avicel® PH200 was selected as the preferred filler, since most other starches are hygroscopic and absorb atmospheric moisture rapidly, as displayed in *Table 2.4* (Ek *et al*., 2008:1).

Table 2.4: The approximate equilibrium moisture content values at 50% relative humidity of various starches (Ek et al., 2008:1).

Starch type	Moisture content values at 50% RH
Corn	11%
Potato	18%
Rice	14%
Wheat	13%

2.6.2 Mixture preparation

The aim of the mixture preparation was to add excipients to facilitate the manufacturing of a compressible mixture for a double fixed-dose combination of artesunate and mefloquine. The compressibility of the mixtures as a function of the optimum concentration (% w/w) of Avicel® PH200 and the lubricant concentration (% w/w) are described in the following paragraphs.

2.6.2.1 Addition of Avicel® PH200, magnesium stearate and talc

The optimum granule formulas of artesunate (A_1 B_0 C_1) and mefloquine (A_0 B_0 C_1 D_0) recovered from the fractional factorial design experiments were mixed with ten different concentrations of Avicel® PH200 as filler, with magnesium stearate or talc as lubricants. A total mass of 100.0 g of the artesunate and mefloquine granules together with the additional excipients were prepared in 250 cm^3 glass containers fitted with a screw cap. Parafilm® was used to seal the openings of these containers

prior to mixing. All mixing procedures employed a Turbula®-mixer (model T2C W.A. Bachofen, Basel, Switzerland) at 69 rpm for 10 minutes as the standard method to ensure a proper distribution of powder particles. Compression followed the mixing stage and an upper punch setting of 31.5 was used during compression.

The design of the experiment regarding the compressibility of ten mixtures of artesunate and mefloquine granules, with different concentrations of Avicel® PH200, magnesium stearate and talc is displayed in Table 2.5.

Table 2.5: The design of the compressibility experiment of ten mixtures of artesunate and mefloquine granules with different concentrations of Avicel® PH200, magnesium stearate and talc.

Formula number	Avicel® PH200 (% w/w)	Magnesium stearate (% w/w)	Formula number	Avicel® PH200 (% w/w)	Talc (% w/w)
1	33.4	1.0	**6**	33.4	1.0
2	32.4	2.0	**7**	32.4	2.0
3	31.4	3.0	**8**	31.4	3.0
4	30.4	4.0	**9**	30.4	4.0
5	29.4	5.0	**10**	29.4	5.0

2.6.2.2 Ac-Di-Sol® as disintegrant

Ac-Di-Sol® (croscarmellose sodium) is highly effective in relatively low concentrations compared to starch, and do not as a norm impart a negative effect on the process of direct compression or tablets properties (List & Muazzam, 1979:24; Gissinger & Stamm, 1980:189).

A disintegration test was performed on six tablets of three different formulations to determine the optimal amount of Ac-Di-Sol® as an internal and external disintegrant. The three different tablet formulas contained Ac-Di-Sol® concentrations of 0.50, 0.75 and 1.00% w/w. The amount of Avicel® PH200 was adjusted to 31.41%, 31.86% and 32.00% w/w respectively to accommodate for the 50:50, 75:25 and 100:0 intragranular disintegrant percentage versus extragranular disintegrant percentage.

A total mass of 100.0 g of the artesunate and mefloquine granules, together with the additional excipients, were prepared in 250 cm^3 glass containers fitted with a screw

cap. Parafilm® was used to seal the openings of these containers prior to mixing. All mixing procedures employed a Turbula®-mixer (model T2C W.A. Bachofen, Basel, Switzerland) at 69 rpm for 10 minutes as the standard method to ensure a proper distribution of powder particles. Compression followed the mixing stage and an upper punch setting of 31.5 was used during compression.

2.7 TABLET COMPRESSION OF THE POWDER MIXTURES PREPARED AFTER THE SECONDARY BLENDING STAGE

The micromeritic properties of the coated artesunate granules, the mefloquine granules and the double fixed-dose combination mixture of artesunate and mefloquine were evaluated in terms of flowability and compressibility. Furthermore, the effects of the different formulas on the tablet properties were analysed to identify possible optimum formulas. Optimum formulas were identified and tested for compressibility. Indication to compression settings was nominated as the terms, setting or settings. Upper punch settings of between 25 and 35 were applied to avoid damage to the punches and dies, rather than the higher upper punch settings which are inclined to cause damage to the compression equipment. The filling volume of the die was altered by the adjustment of the lower punch setting. Concave faced compression tooling was utilised to manufacture tablets that presented a convex surface for the reason that a large round biconvex tablet is easier to swallow than a flat round tablet. A Cadmach® eccentric press (Ahmedabad, India) was employed during all tableting procedures. After manufacturing was completed, tablets were stored in sealed glass containers away from light at room temperature for 24 hours preceding further analysis.

2.8 TABLET EVALUATION

Tablet dimensions were evaluated to establish the applicability of the manufacturing processes for the oral fixed-dose combination in terms of tablet weight variation, friability and physical proportions. Artemisinin and its derivatives represent a new class of antimalarials (Balint, 2001:262) and as a result of limited information regarding dissolution and assay procedures for artesunate, British Pharmacopoeia (2012) and United States Pharmacopoeia (2011) methods were used to evaluate the dosage form. The crushing strength, friability, diameter and thickness of the tablets are non-official pharmacopoeial tests and were conducted for data gathering purposes.

2.8.1 Tablet crushing strength, diameter and thickness

The crushing strength, diameter and thickness were determined for 20 tablets of each formulation using a Pharma Test® (model PTB-311) tablet test unit (Pharma Test, Switzerland).

2.8.2 Tablet density and tensile strength

Tablet density (g.cm^{-3}) was calculated using the following equation to determine tablet volume:

Equation 2.7

$$V_{tablet} = \frac{2}{6}\pi h\left(3a^2 + h^2\right) + \pi a^2 \cdot b$$

Where:

V_{tablet}	=	tablet volume
h	=	curvature height
a	=	radius
b	=	band height

And for tensile strength:

Equation 2.8

$$\sigma_t = \frac{2F}{\pi dt}$$

Where:

σ	=	tensile strength
F	=	crushing force
d	=	tablet diameter
t	=	tablet thickness

2.8.3 Weight variation

The weight variation test of tablets is an official British Pharmacopoeia (2012a:1) test. Twenty tablets of each batch were dusted and weighed on a Precisa® analytical

balance (model 240A, OERLIKON AG, Zurich) and were measured against the BP limits for tablet weight variation.

2.8.4 Friability

Tablet friability is a non-official pharmacopoeial test. Twenty tablets of each formulation were tested on a Roche® friabilator for a duration of four minutes at 25 rpm. *Equation 2.9* was used to calculate the percentage friability.

Equation 2.9

$$\%F = 100\frac{w_b - w_a}{w_b}$$

Where:

w_a = weight of tablets before rotation

w_b = weight of tablets after rotation

2.8.5 Disintegration

According to the British Pharmacopoeia (2012:1), where a dissolution test is prescribed, a disintegration test may not be required. However, for this study, the disintegration test was done to determine the correct compression settings and optimum mixture formulas. The disintegration equipment and method used are described in the British Pharmacopoeia (2012b:1). The disintegration times of six tablets from each formulation were determined using an Erweka® GmbH tablet disintegration test unit (Type ZT503, Heusenstamm, Germany). A set limit of 15 minutes was employed and all formulations that did not meet the standard were considered non-disintegrating. The disintegration medium was distilled water and was maintained at a temperature of 37 ± 2°C by a thermostat.

2.9 DISSOLUTION STUDIES

Dissolution testing is an essential requirement for the development and establishment of *in vitro* dissolution and possible *in vivo* performance, as well as registration and quality control of solid oral dosage forms. The official dissolution method of the USP (2011:1), for delayed-release dosage forms was used as a guideline to determine the concentration of artesunate and mefloquine dissolved in the dissolution medium as a function of time.

2.9.1 Apparatus and experimental conditions

Dissolution tests were performed in an Erweka® DT6R (Erweka, Heusenstamm, Germany) dissolution apparatus. A thermostat regulated the temperature of the medium at 37.0 ± 0.5°C. The dissolution studies were initiated in an acidic dissolution medium, consisting of 750 cm^3 of 0.1 N HCl (pH ~ 1.20). After 2 hours of operation in 0.1 N HCl, the final dissolution samples of the acidic medium stage were withdrawn prior to adjustment of the pH. A volume of 250 ml of 0.20 M tribasic sodium phosphate that has been equilibrated to 37.0 ± 0.5°C was added to the dissolution beakers within 5 minutes after the sampling of the final acid medium stage withdrawals, and adjusted if necessary to a pH of 6.8 ± 0.05 with 2 N HCl or 2 N sodium hydroxide. The standardised USP (2011:1) paddle-method was used in all studies and paddles were rotated at a constant speed of 100 rpm.

2.9.2 Method of tablet dissolution

The dissolution vessels were filled with the dissolution medium and the rods were pushed down into the vessels with the paddles reaching between 25 mm and 27 mm from the bottom of the vessel. The timekeeping of the dissolution test started when each tablet reached the dissolution medium (t = 0). Samples of 3 cm^3 were withdrawn at time (t) = 5, 10, 15, 30, 45, 90, 120, 130 and 150 minutes. Samples were withdrawn at a constant height, through in-line Pall Acrodisc® syringe filters, and the withdrawn volume was replaced immediately after sampling with new, preheated dissolution medium. A correction was made for the cumulative dilution caused by replacement of the withdrawn sample with an equal volume of preheated medium. The sampling was performed through Pall Acrodisc® syringe filters, 25 mm, 0.45 µm, GHP, Universal Polypropylene (New York, USA) and the samples were transferred to 2 cm^3 glass HPLC polytops. The samples were subsequently analysed with the HPLC method described in *Chapter* 5.

2.9.3 Standard preparation

2.9.3.1 Full-strength standards:

A mass of 20 mg of artesunate and 42 mg of mefloquine were weighed and transferred quantitatively into a 100 ml volumetric flask. The transferred artesunate and mefloquine were subsequently dissolved in ± 10 ml methanol and made up to volume (100 ml) with water. Thispreparation was filtered through in-line Pall Acrodisc

syringe filters, 25 mm, 0.45 µm, GHP, Universal Polypropylene (New York, USA), and the samples were transferred to 2 cm^3 glass polytops. The standard was analysed by means of six injections prior to each dissolution experiment.

2.9.4 Computation of dissolution data

The concentration of drug dissolved (µg.cm^{-3}) at each sampling time was calculated using *Equation 2.10* and the amount of drug lost during sampling was corrected by using *Equation 2.11*.

Equation 2.10

$$x = \frac{y^* - c}{1000m}$$

Where:

y^* is the corrected absorbency (from *Equation 2.11*); x is the drug concentration (µg.cm^{-3}) and m and c are the slope and y-axis intercept respectively obtained from the standard curve.

Equation 2.11

$$y_n^* = y_n + \frac{V_s}{V_m}\sum^{n-1} y^*$$

Where:

y^*_n is the corrected absorbency of n^{th} sample, y_n is the measured absorbency of n^{th} sample; V_s is the sampling volume; V_m is the dissolution medium volume and $\Sigma^{n-1}\, y^*$ is the sum of all the corrected absorbencies prior to the n^{th} sample.

2.9.5 Dissolution parameters

2.9.5.1 DRi and AUC

The initial slope of the dissolution curve between t_0 and t_5 was proposed to be a fair approximation of the initial dissolution rate (DRi) (%.min^{-1}) of mefloquine from the tablet formulation. The significance of this parameter is discussed in *Chapter 5* and *Chapter 6*. The area under the dissolution curve (AUC) was calculated from t_0 up to completion of the dissolution test and would give an indication of the extent of

dissolution of active ingredient during this period. The calculation of the AUC (%.min) was made by application of the trapezoidal rule and the parameter was determined for the whole extent of the dissolution experiments. The trapezoidal rule is described by *Equation 2.12*.

Equation 2.12

$$AUC = 0.5 \times \sum_{t=n}^{t=0} (t_n - t_{n-1})(c_n - c_{n-1})$$

Where:

$(t_n - t_{n-1})$ is the time difference between two consecutive sampling intervals and c_n and c_{n-1} is the corresponding concentrations of the tracer at t_n and t_{n-1}.

2.10 ASSAY OF TABLETS

2.10.1 Instrument parameters

The HPLC system was equipped with a variable wavelength UV detector and an integrator. In this instance a Hewlett Packard Agilent 1100 equipped with a variable wavelength UV detector was used. The parameters and conditions are summarised in *Table 2.6*.

Table 2.6: The instrument parameters.

Column	ODS Hypersil, 5 µm, 100 Å, 4.6 mm x 150 mm
Mobile phase	Acetonitrile / KH_2PO_4 buffer, pH 3.0
Flow rate	1.0 ml/min
Injection volume	20 µl
Wavelength	210.8 nm
Retention time (artesunate)	3 minutes
Retention time (mefloquine)	9 minutes

The peak areas of the responses obtained from the chromatograms for the withdrawn dissolution samples were measured to calculate the percentage content, with reference to the raw powder standard preparations of $C_{19}H_{28}O_8$ (artesunate) and $C_{17}H_{17}ClF_6N_2O$ (mefloquine), *Table 2.1*, respectively.

2.11 CALCULATIONS

The data are presented as the mean ± percent relative standard deviation (%RSD). All calculations were made using Microsoft® Excel™ 2003 - 2010 for Windows™ (Microsoft® Corporation, Seattle, Washington, USA).

3. CHAPTER THREE

THE PARAMETERS CONTRIBUTING TO THE SUCCESSFUL TABLETING OF A FIXED-DOSE COMBINATION OF ARTESUNATE AND MEFLOQUINE

3.1 INTRODUCTION

Chapter 1 described wet granulation and coating of a powdery substance as particle design methods that comprise several imperative factors to ultimately deliver a product with suitable flow and compressibility properties. Particle flowability and compressibility are two critical process parameters tested when a material is designed for direct compression. Artesunate and mefloquine lack flowability. The interpretations of the results obtained by the powder characterisation experiments and the size enlargement techniques are discussed in this chapter.

The objectives of this chapter were to:

- Determine the parameters that contribute to the wet granulation and coating of artesunate.
- Determine the parameters that contribute to the wet granulation of mefloquine.
- Prepare both enteric coated artesunate granules and mefloquine granules with a narrow span and mean particle size of at least 100 µm.
- Evaluate the raw API powders and assess the manipulated API particle structures that were achieved by means of wet granulation and coating.
- Analyse the effects of the binder type, binder concentration, disintegrant concentration and bridging liquid volume on granule formation.
- Design a double fixed-dose combination powder mixture with desired tableting properties, for example good flow, compressibility and handling properties.

3.2 ASSESSMENT OF THE PHYSICAL CHARACTERISTICS OF ARTESUNATE AND MEFLOQUINE RAW MATERIAL

3.2.1 The compression capabilities of artesunate and mefloquine

Both raw artesunate and mefloquine powder failed automated compression with the tablet press settings described in *Chapter 2*, section *2.7*. Additionally, all tablets compressed by selectively placing an amount of powder in the tablet die and then subsequently lowering the upper punch slowly but forcefully by hand, produced tablets with inadequate quality. The tablets were scratched, capped and damaged upon ejection. This was probably due as a result of friction between the die and powder particles (Alderborn, 2007:452).

3.2.2 Results of the assessment of the micromeritic properties of artesunate and mefloquine

The particle size, particle size distribution, flow properties, densities, the Hausner ratios and Carr's indices of the raw APIs were assessed. *Table 3.1* summarises the data obtained from the various experiments conducted using the methods described in *Chapter 2*. The results with regards to the micromeritic properties of artesunate and mefloquine are presented in *Table 3.1*.

Table 3.1: The physical characteristics of raw artesunate and mefloquine powder. Results of density measurements, mean ± %RSD, volumes ± 0.5 cm^{-3} (n = 3).

Property	Artesunate	Mefloquine
Volumetric mean particle size (µm)	53.34	93.98
Tapped density ($g.cm^{-3}$)	0.50 ± 0.23	0.23 ± 0.94
Bulk density ($g.cm^{-3}$)	0.43 ± 1.29	0.18 ± 0.67
Hausner ratio	0.86 ± 1.19	0.78 ± 0.46
Carr's index	14.49 ± 6.99	22.09 ± 1.61
Angle of repose (º)	27.3 ± 3.3	23.2 ± 3.5
Flow rate ($g.sec^{-1}$)*	Failed	Failed
COD (mm)	15 ± 0.00	20 ± 0.00

**8.0 mm orifice*

3.2.3 Discussion

Although artesunate and mefloquine failed tablet compression as a result of poor flowability and ejection friction under the conditions described in *Chapter 2*, section *2.7*, the powders had percentage Carr's indices of 14.49 ± 6.99 and 22.09 ± 1.61 respectively, thus indicating fair and good compressibility for powdered raw material, according to the generalised descriptions of powder flow and percentage compressibility by Carr (Staniforth & Aulton, 2000:177). The relationship between powder flowability and percentage compressibility is listed in *Table 3.2*.

Table 3.2: Relationship between powder flowability and % compressibility (Staniforth & Aulton, 2007:177).

Percentage compressibility range	Flow description
5-15	Excellent (free-flowing)
12-16	Good (free-flowing powdered)
18-21	Fair (powdered)
23-28	Poor (very fluid powders)
28-35	Poor (very cohesive powders)
35-38	Very poor (fluid cohesive powders)
>40	Extremely poor (cohesive powders)

The ratio $\rho_{\text{untapped}} : \rho_{\text{tapped}}$ is related to interparticulate friction and can be used to predict powder flow properties. Powders with low interparticulate friction have ratios of approximately 1.2, whereas more cohesive, less free-flowing powders have ratios greater than 1.6 (Hausner 1967:7).

Although artesunate and mefloquine had low interparticulate friction ratios according to Hausner (1967:7), with values of 0.86 ± 1.19 and 0.78 ± 0.46 respectively, the experiment for flow rate failed under the conditions described in *Chapter 2*, section 2.3.2.2. Both artesunate and mefloquine powders failed the experiment for flow rate through an orifice with a diameter of 8.0 mm. In addition, the COD for artesunate and mefloquine were 15.0 mm and 20.0 mm respectively, indicating very poor and cohesive flow according to Horn (2008:43). This powder flow data therefore explains the failure to compress tablets from the artesunate and mefloquine raw material.

3.2.4 Visual evaluation of the shape and surface structure of the raw APIs

3.2.4.1 Artesunate

Artesunate powder exhibited poor flow properties as is evident from the data in *Table 3.1*. The poor flow properties of artesunate may be the result of the uneven surface structure and size of the particles, as depicted in *Figure 3.1*. It is noted in literature that particles with different shapes can have a detrimental effect on the flowability of a powder (Staniforth & Aulton, 2007:170), in the same manner as which can be observed for artesunate, as depicted in *Figure 3.1*.

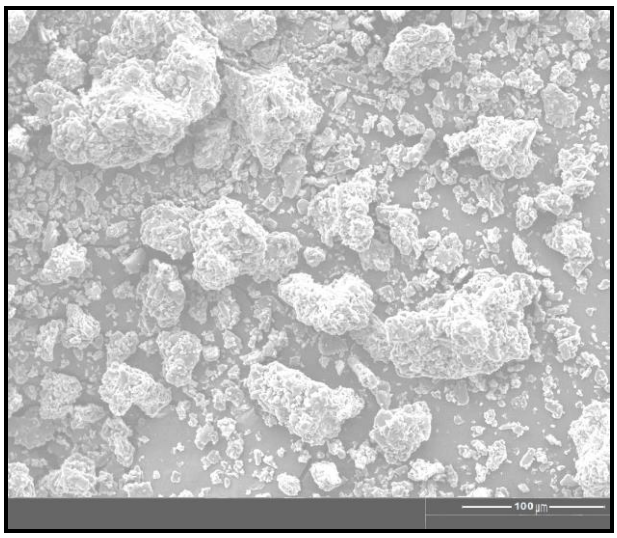

Figure 3.1: SEM micrograph of artesunate.

3.2.4.2 Mefloquine

Scanning electron microscope (SEM) images revealed that mefloquine particles contained sharp edges and uniform match-like structures. On the contrary, spherical particles exhibit superior powder flowability compared to non-spherical particles (Podczeck *et al*., 1996b:194). The match-like structure of the mefloquine particles may contribute to its poor flow, due to the cohesiveness between particles depicted in *Figure 3.2*. According to the data shown in *Table 3.1*, mefloquine can be classified as possessing relatively high cohesiveness, interparticulate friction and poor flowability. The cohesiveness and poor flow of mefloquine can be problematic, as it

can cause difficulties in material blending and transfer operations (Staniforth & Aulton, 2007:169).

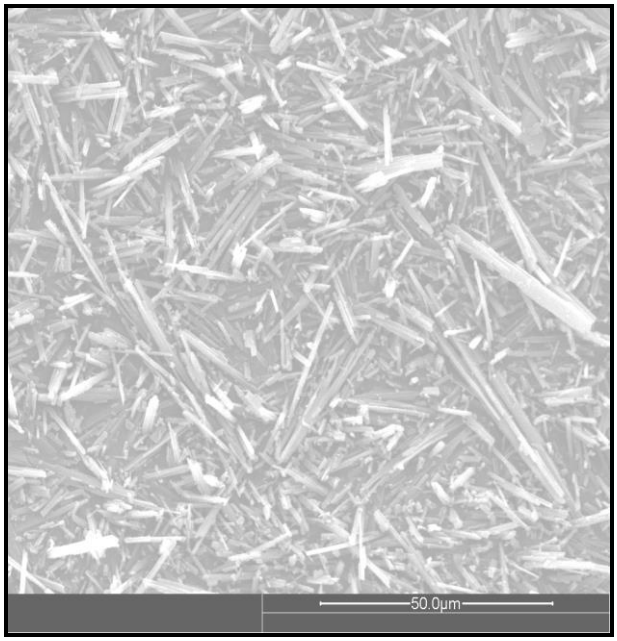

Figure 3.2: SEM micrograph of mefloquine.

3.2.5 Particle size and size distribution

In an effort to fully understand the flow properties of artesunate and mefloquine, a particle size and size distribution analysis were conducted. The particle size distribution results of artesunate and mefloquine powder are displayed in *Table 3.3.* Both APIs have a wide frequency distribution curve, as can be seen from the span values of 2.21 and 3.47 for artesunate and mefloquine respectively. The large span can be indicative of poor flowability.

Table 3.3: A summary of the size distribution of the artesunate and mefloquine powder particles.

Sample	Volumetric mean particle size (µm)	D[v,0.5] (µm)	D[v,0.9] (µm)	D[v,0.1] (µm)	$Span = \frac{D_{v,0.9} - D_{v,0.1}}{D_{v,0.5}}$
Artesunate powder	53.34	41.59	102.24	10.26	2.21
Mefloquine powder	93.98	60.81	225.42	15.02	3.47

3.2.6 Conclusion

Results showed that both artesunate and mefloquine raw material possess poor flow properties as a result of its particle structures, particle sizes, and broad size distributions. These weak flow properties necessitate the size enlargement and granulation processes for the raw APIs.

In an effort to narrow the size distribution of artesunate and mefloquine raw material, these powders were subjected to wet granulation. Besides the advantage of possessing a manufactured narrower size distribution, granulation would also enhance powder flow and ease the tableting process.

3.3 THE DEVELOPMENT OF ARTESUNATE GRANULES

3.3.1 The role of the acrylic polymers, Eudragit® L

Many pharmaceuticals irritate the stomach due to their chemical properties. Others undergo chemical changes in the acidic gastric environment and through the action of enzymes, thus becoming less effective. Eudragit® L100 dissolves at pH values above 6.0 thereby protecting the integrated particle from the low pH of stomach acid. The polymers can also be used as binders to prepare pellets, granules and sustained-release tablets (Evonik, 2010:1).

Particle properties that affect flowability include mean particle size, size distribution, particle shape and surface roughness (Liu *et al*., 2008:109). As particle size falls below 100 µm, powders become cohesive and flow problems are likely to occur (Staniforth & Aulton, 2007:170). Artesunate powder exhibited poor flow properties, and it may be the result of its rough surface structure as depicted in *Figure 3.1*, and size (volumetric mean particle size = 53.35 µm), as determined during the particle size analysis (*Table 3.3)*.

The artesunate sample had a specific surface area of 0.385 $m^2.g^{-1}$, and a volumetric mean particle size of 53.34 µm, which justify the need for it to be granulated to obtain a larger mean particle size for improved flowability (*Table 3.4*).

Table 3.4: Specific surface area and the volumetric mean particle size for artesunate powder.

Sample	Specific surface area ($m^2.g^{-1}$)	Volumetric mean particle size (µm)
Artesunate	0.385	53.34

As a guideline, to calculate the amount of Eudragit® polymer coating to be used for artesunate, theoretical spherical artesunate particles with diameters in the range of 0.5 - 1.2 mm should allow for the following polymer weight gain percentages depending on the coating purpose:

- enteric coatings: 10 - 30%.
- sustained-release coatings: 5 - 20%.
- taste-masking coatings: 5 - 10%.
- moisture protection: 10 - 30% (Degussa, 2010:1).

However, depending on the solubility of the active, surface structure, size of the particles and mechanical stability, different amounts may be needed (Guignon *et al*., 2003:194). Therefore, it was decided to conduct a fractional factorial design coating experiment in order to determine the optimum polymer and polymer amount. The factorial design in *Table 3.5* was employed to determine the optimum factors and levels for the manufacturing of enteric coated artesunate granules (ECAG).

Table 3.5: The factors and level designation for the development of enteric coated artesunate granules (ECAG).

Factor	Designation	Level	
		0	**1**
Coating agent	**A**	Kollidon® SR	Eudragit® L
Coating concentration	**B**	23% w/w	30% w/w
Liquid phase addition method (20 ml)	**C**	POURED	SPRAYED

3.3.1.1 Results and discussion

As response, the granule recovery was used. The experimental runs of the fractional factorial design are displayed in *Table 3.6*.

The optimum formula is indicated in the shaded value in *Table 3.6* ($A_1 B_0 C_1$).

Table 3.6: The enteric coated artesunate granule recovery percentages for the factorial design.

	A_{0} = Kollidon® SR		**A_{1} = Eudragit® L**	
	$B_{0=23\% w/w}$	**$B_{1=30\% w/w}$**	**$B_{0=23\% w/w}$**	**$B_{1=30\% w/w}$**
C_{0} = POURED	**7.35%**	**0.00%**	**85.19%**	**29.54%**
C_{1} = SPRAYED	**7.39%**	**5.02%**	**97.52%**	**54.75%**

Table 3.6 clearly indicates the factors and levels most likely to produce favourable results, as summarised in *Table 3.7*, for the size enlargement of artesunate ($A_1 B_0 C_1$). Further efforts were made to focus in on the factors of the cell with the highest granule recovery shown in *Table 3.6* to optimise the granulation and coating process of artesunate.

Table 3.7: The percentage granule recovery of the different formulations of the fractional factorial design of the development of enteric coated artesunate granules.

Experiment	Result
$A_0 B_1 C_0$	0.00%
$A_1 B_0 C_1$	97.52%
$A_0 B_0 C_0$	7.35%
$A_0 B_0 C_1$	7.39%
$A_0 B_1 C_1$	5.02%
$A_1 B_1 C_1$	54.75%
$A_1 B_1 C_0$	29.54%
$A_1 B_0 C_0$	85.19%

The agglomerated spherical particles of artesunate and Eudragit® L100, depicted in *Figure 3.3* after the granulation and coating process of artesunate, resulted in a free-flowing powder. The image displays an artesunate granule coated with Eudragit® L100 (23% w/w). The thin smooth layer of the acrylic polymer on the surface of the

artesunate particle should protect the particles from the acid in the stomach, but should dissolve completely in a basic medium to release the artesunate.

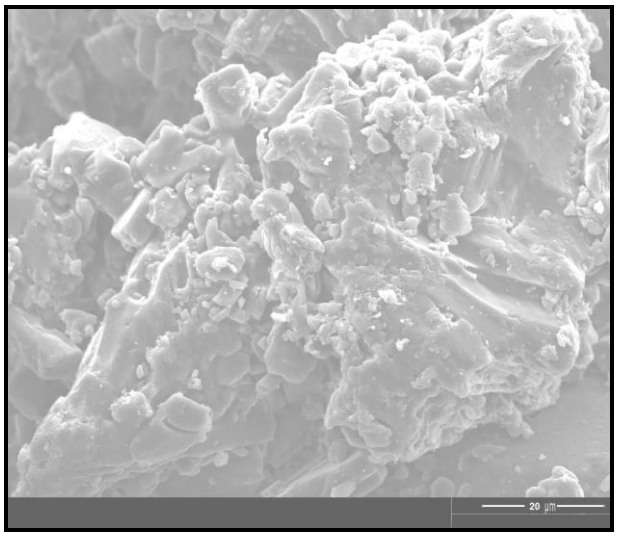

Figure 3.3: A SEM micrograph of enteric coated artesunate granules (ECAG).

3.3.2 The relationship between particle size distributions and flowability for artesunate

A histogram of the particle size distributions for artesunate powder and enteric coated artesunate granules are presented in *Figure 3.4* and *Figure 3.5* respectively. The artesunate granules on display contained artesunate (77% w/w) and Eudragit® L100 (23% w/w).

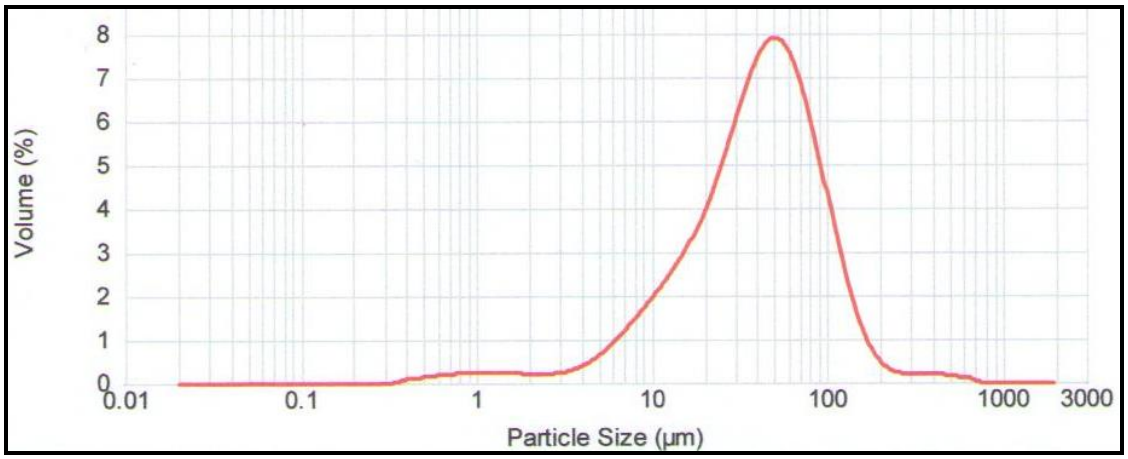

Figure 3.4: The particle size distribution for artesunate powder.

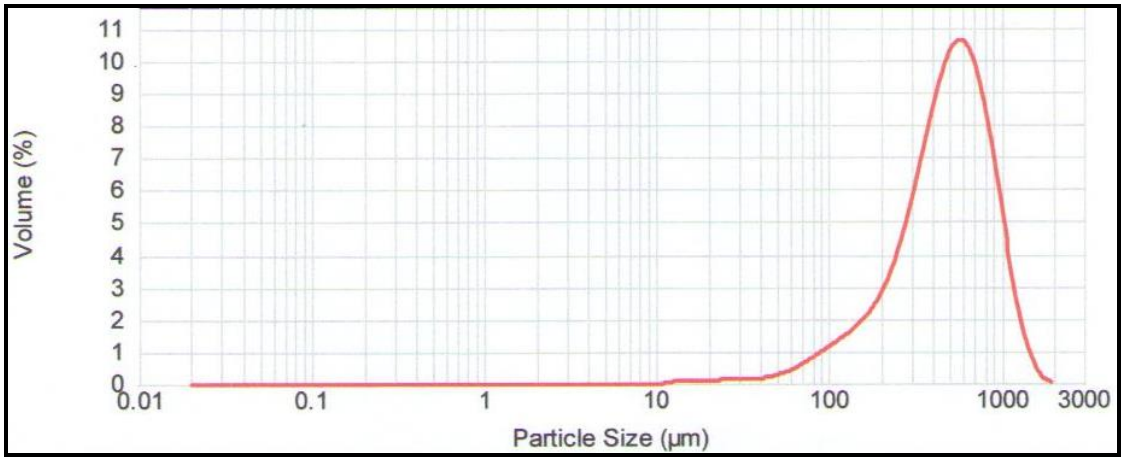

Figure 3.5: The particle size distribution for enteric coated artesunate granules.

The volumetric mean particle sizes for artesunate powder and coated artesunate as well as the span of each size distribution are listed in *Table 3.8*.

Table 3.8: Size distribution parameters of artesunate and enteric coated artesunate obtained from the Malvern® Mastersizer 2000.

Sample	Volumetric mean particle size (µm)	D[v,0.5] (µm)	D[v,0.9] (µm)	D[v,0.1] (µm)	$Span = \frac{D_{v,0.9} - D_{v,0.1}}{D_{v,0.5}}$
Artesunate powder	53.34	41.59	102.24	10.26	2.21
Coated artesunate	572.61	528.83	1002.70	192.13	1.54

Artesunate powder had a span of 2.30 prior to the coating process. The enteric coated artesunate granules had a narrower size distribution span of 1.54. The percentage improvement regarding the size distribution span for artesunate was 33.04%.

Artesunate powder had a volumetric mean particle size of 56.42 ± 7.72 µm prior to coating and granulation, and obtained a volumetric mean particle size of 572.61 ± 9.43 µm after coating and granulation.

The micromeritic properties of artesunate, in comparison to artesunate granules are displayed in *Table 3.9*.

Table 3.9: A comparison of the micromeritic properties of artesunate and enteric coated artesunate granules (ECAG). Mean ± %RSD (n = 3).

Property	Artesunate	ECAG
Mean particle size (µm)	56.42 ± 7.72	572.61 ± 9.43
Tapped density ($g.cm^{-3}$)	0.50 ± 0.23	0.43 ± 1.95
Bulk density ($g.cm^{-3}$)	0.43 ± 1.29	0.33 ± 1.33
Hausner ratio	0.86 ± 1.19	0.77 ± 1.41
Carr index	14.49 ± 6.99	22.85 ± 4.76
Angle of repose (º)	27.3 ± 3.3	22.0 ± 1.5
Flow rate ($g.sec^{-1}$)	Failed	1.84 ± 4.35

Artesunate granules generally had improved flowability properties compared to raw artesunate, with the most indicative difference being the failure of the raw artesunate flow rate experiment, compared to the flow rate value of 1.84 ± 4.35 $g.sec^{-1}$ for artesunate granules.

3.4 THE DEVELOPMENT OF MEFLOQUINE GRANULES

The manufacturing process of the mefloquine granules took place according to the methods described in *Chapter* 2. The granulation process factors and levels investigated are listed in *Table 3.10*. The main aim was to determine the most influential factors and levels in terms of percentage granule recovery that will bind mefloquine particles together for granule formation. Ac-Di-Sol® was investigated as an intragranular disintegrant as a factor on two levels, with the aim of facilitating the disintegration of the individual granules during dissolution.

Table 3.10: The factors and level designation of the wet granulation process of mefloquine.

Factor	Designation	Level	
		0	1
Kollidon® (Binder type)	**A**	VA64	K30
Kollidon® (% w/w)	**B**	5% w/w	6% w/w
Ac-Di-Sol® (% w/w)	**C**	0.5%	1.0%
Bridging liquid volume	**D**	20 ml	40 ml

3.4.1.1 Results and discussion

The granule recoveries for each of the experimental runs for the fractional factorial design are displayed in *Table 3.11*. The granule recoveries are summarised in *Table 3.12*.

Table 3.11: The mefloquine granule recovery percentages for the different formulations.

		A_0 = Kollidon® VA64		A_1 = Kollidon® 30	
		B_0= Kollidon 5% w/w	B_1= Kollidon 6% w/w	B_0= Kollidon 5% w/w	B_1= Kollidon 6% w/w
C_0 = 0.5% w/w	D_0= 20 mL		95.21%	96.21%	
	D_1= 40 mL	0.61%			0.42%
C_1 = 1.0% w/w	D_0= 20 mL	96.50%			95.81%
	D_1= 40 mL		1.82%	1.96%	

The results obtained from *Table 3.11* and *Table 3.12* indicated that the factors and levels in the lower left quadrant of *Table 3.11* and $A_0\,B_0\,C_1\,D_0$ of *Table 3.12* are the most influential factors and levels in terms of granule formation (96.50%) and granule recovery.

Table 3.12: A summary of the percentage recovery of the different formulations of the fractional factorial design of the wet granulation of mefloquine.

Experiment	Result
$A_1 B_0 C_0 D_0$	96.21%
$A_1 B_1 C_0 D_1$	0.42%
$A_1 B_1 C_1 D_0$	95.81%
$A_1 B_0 C_1 D_1$	1.96%
$A_0 B_1 C_0 D_0$	95.21%
$A_0 B_1 C_1 D_1$	1.82%
$A_0 B_0 C_1 D_0$	96.50%
$A_0 B_0 C_0 D_1$	0.61%

3.4.2 Kollidon® VA64 and Kollidon® 30 as binders for the preparation of mefloquine granules

The granulation process was necessary to obtain a free-flowing powder mixture. A crucial ingredient of any granule is the binder. Literature studies of various binders illustrated that Kollidon® VA64 (Castellanos Gil *et al*., 2008:310) and Kollidon® 30 were most likely to be suitable as a result of its solubility in ethanol and consequently decreasing the risk of the APIs being exposed to hydrolysis. The optimum binder between Kollidon® VA64 and Kollidon® 30 and quantity of binder were investigated.

3.4.2.1 Results and discussion

The results for the comparative study between Kollidon® VA64 and Kollidon® 30 as binders for mefloquine granules are displayed in *Table 3.13.*

Table 3.13: The comparison of the compressibility of Kollidon® VA64 and Kollidon® 30 as binders for mefloquine granules. Granules contained Ac-di-sol® (1.0% w/w).

Kollidon® VA64 (% w/w)	Result	Kollidon® 30 (% w/w)	Result
1	Failed	1	Failed
2	Failed	2	Failed
3	Failed	3	Failed
4	Compressed	4	Compressed*
5	Compressed	5	Compressed*
6	Failed	6	Failed

**compression was detrimentally influenced by granules and tablets being sticky.*

There were no significant differences in granule formation, however, it was found that the same concentration of Kollidon® VA64 (5% w/w) were less sticky during the second granulation step and compression. Since it is less hygroscopic than Kollidon® 30, Kollidon® VA64 gives granules that have a lower tendency to stick to the punches of the tableting machine (Bühler, 2008:98).

To minimise the possibility of artesunate being hydrolysed in the presence of mefloquine granules, (Tan, 2009:12) ethanol instead of water was used as the binder vehicle for the granulation of mefloquine. Kollidon® VA64 and Kollidon® 30 are both soluble in ethanol as well as water, and was selected based on the solubility in ethanol.

Mefloquine granules are displayed in the SEM micrograph in *Figure 3.6*. Note the improved spherical particles of the granules consisting of mefloquine, Kollidon® VA64 (5% w/w) and Ac-Di-Sol® (1% w/w) depicted in *Figure 3.6* after the granulation process.

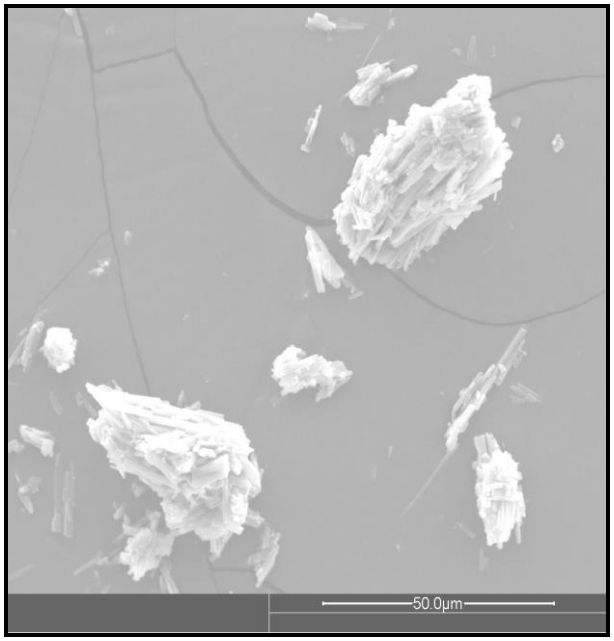

Figure 3.6: A SEM micrograph of granulated mefloquine.

The granules that contained mefloquine (94% w/w), Kollidon® VA64 (5% w/w) or Kollidon® 30 (5% w/w) and Ac-di-sol® (1% w/w) were compared in terms of micromeritic properties and the results are displayed in *Table 3.14*.

Table 3.14: The properties of mefloquine granules. Granules contained mefloquine (94% w/w), Kollidon® VA64 (5% w/w) or Kollidon® 30 (5% w/w) and Ac-di-sol® (1% w/w). Mean ± %RSD (n = 3).

Property	**Mefloquine and Kollidon® VA64**	**Mefloquine and Kollidon® 30**
Mean particle size (µm)	454.8	485.6
Tapped density ($g.cm^{-3}$)	0.60 ± 0.24	0.59 ± 1.38
Bulk density ($g.cm^{-3}$)	0.50 ± 0.61	0.49 ± 1.12
Hausner ratio	0.83 ± 0.70	0.82 ± 1.00
Carr's index	17.26 ± 3.35	17.57 ± 4.67
Angle of repose (º)	20.5 ± 5.0	21.1 ± 5.5
Flow rate ($g.sec^{-1}$)	2.29 ± 4.16	1.00 ± 5.00
COD (mm)	2	2

3.4.2.2 The relationship between particle size distributions and flowability for mefloquine

A histogram of the particle size distributions of mefloquine powder and mefloquine granules are presented in *Figure 3.7* and *Figure 3.8* respectively. The mefloquine granules on display contained mefloquine (94% w/w), Kollidon® VA64 (5% w/w) and Ac-di-sol® (1% w/w).

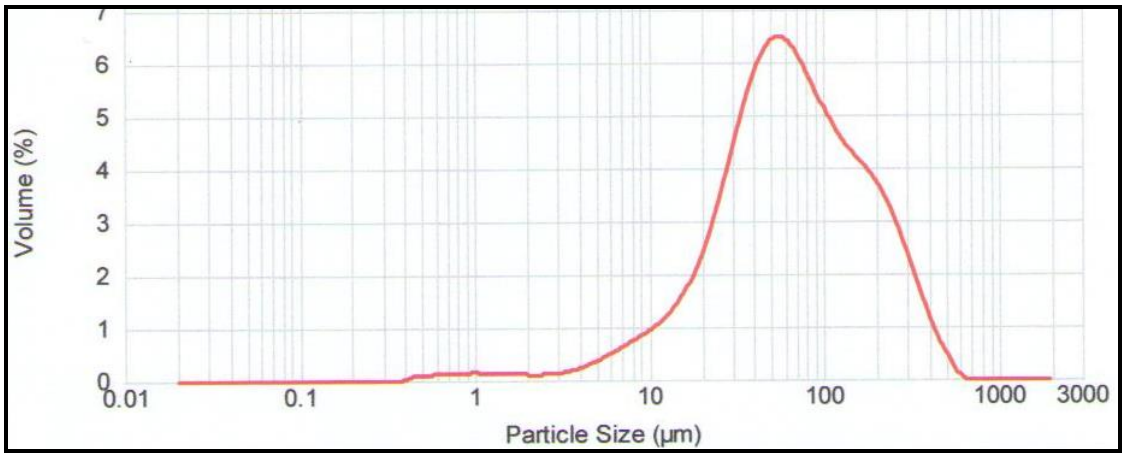

Figure 3.7: The particle size distribution for mefloquine powder.

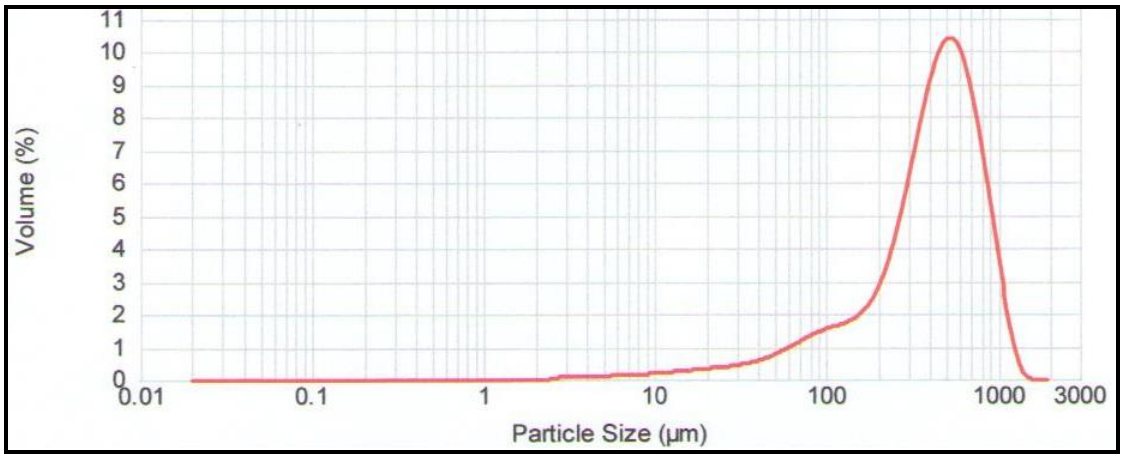

Figure 3.8: The particle size distribution for mefloquine granules.

The volumetric mean particle sizes for mefloquine powder and mefloquine granules as well as the span of each size distribution are compared and listed in *Table 3.15*.

Table 3.15: Size distribution parameters of mefloquine and granulated mefloquine samples obtained from the Malvern® Mastersizer 2000.

Sample	Volumetric mean particle size (µm)	D[v,0.5] (µm)	D[v,0.9] (µm)	D[v,0.1] (µm)	$Span = \frac{D_{v,0.9} - D_{v,0.1}}{D_{v,0.5}}$
Mefloquine powder	93.98	60.81	225.42	15.02	3.47
Mefloquine granules	470.23	445.83	845.38	107.71	1.66

The micromeritic properties of mefloquine granules, in comparison to mefloquine powder are displayed in *Table 3.16*.

Table 3.16: A comparison of the micromeritic properties of mefloquine and granulated mefloquine with Kollidon® VA64 (GMKVA64). Mean ± %RSD (n = 3).

Property	Mefloquine	GMKVA64
Mean particle size (µm)	93.98 ± 5.29	470.23 ± 4.63
Tapped density ($g.cm^{-3}$)	0.23 ± 0.94	0.60 ± 0.24
Bulk density ($g.cm^{-3}$)	0.18 ± 0.67	0.50 ± 0.61
Hausner ratio	0.78 ± 0.46	0.83 ± 0.70
Carr index	22.09 ± 1.61	17.26 ± 3.35
Angle of repose (º)	23.2 ± 3.5	20.5 ± 5.0
Flow rate ($g.sec^{-1}$)	Failed	2.29 ± 4.16

Mefloquine powder had a span of 3.47 prior to the granulation process and the mefloquine granules had a narrower size distribution span of 1.66. The percentage improvement regarding the size distribution span for mefloquine was 52.16%.

Mefloquine powder had a volumetric mean particle size of 93.98 ± 5.29 µm prior to coating and granulation and obtained a volumetric mean particle size of 470.23 ± 4.63 µm after coating and granulation.

It can be concluded that the Kollidon® VA64 and Kollidon® 30 mefloquine granules exhibited similar micromeritic properties, although Kollidon® VA64 was elected as the most suitable binder for having a less sticky texture than the Kollidon® 30 mefloquine granules and for being less hygroscopic.

3.4.3 Conclusion

For powders with the same median size, the narrower the size distribution, the better the flowability. For powders with narrow size distributions, the flowability increases significantly with the increase in particle size (Liu *et al*., 2008:109). It can be concluded that the increase in flowability for artesunate and mefloquine can be attributed to the particle size increase of 914.91% and 400.35% respectively and the narrowing of the size distribution span for artesunate and mefloquine of 30.32% and 52.16% respectively.

The angle of repose of enteric coated artesunate and granulated mefloquine improved by 25.7% and 11.6% respectively compared to the angle of repose for artesunate and mefloquine powder. These improved angles of repose values motivate the size enlargement procedures since compression will benefit from improved flow properties. Typically, the lower the angle of repose of a dry material, the more free-flowing the material is (Carr, 1965:163).

The micromeritic properties, especially the flowability and the span of the particle size distribution of both of artesunate and mefloquine were improved. The improvement was obtained by granulation and coating for mefloquine and artesunate respectively.

3.5 EXCIPIENT COMPATIBILITY

3.5.1 Infrared absorption spectroscopy (IR)

3.5.1.1 Results and discussion

The IR spectrum of the double fixed-dose combination mixture was compared to the IR spectrum of the double fixed-dose combination tablets and the spectra displayed a position and intensity correlation. The data obtained from the IR spectra are displayed in *Table 3.17*.

The mean %RSD of the peak positions of both samples were 2.83 and the mean %RSD of the intensities of the peaks of both samples was 10.70. Infrared absorption spectroscopy revealed a peak-by-peak correlation of the IR spectra of artesunate and mefloquine mixtures prior to granulation and tableting and of tablets after being exposed to the conditions under which wet granulation and tableting took place. A

peak-by-peak correlation is excellent evidence for identity and proved the process to be safe to ensure drug stability. IR-spectra results of the samples are given in *Figure 3.9* and *Figure 3.10*.

Tue Oct 12 12:35:07 2010 (GMT+02:00)
FIND PEAKS:
Spectrum: Placebo Herman 05.10.2010
Region: 4000.0 400.0
Absolute threshold: 88.850
Sensitivity: 50
Peak list:

Position:	448.6	Intensity:	72.344
Position:	492.0	Intensity:	73.797
Position:	513.6	Intensity:	68.401
Position:	529.3	Intensity:	68.790
Position:	595.1	Intensity:	70.842
Position:	616.7	Intensity:	71.145
Position:	669.1	Intensity:	67.435
Position:	715.3	Intensity:	64.883
Position:	737.3	Intensity:	66.000
Position:	756.1	Intensity:	74.311
Position:	780.5	Intensity:	56.153
Position:	835.0	Intensity:	63.555
Position:	865.4	Intensity:	68.254
Position:	873.2	Intensity:	68.303
Position:	897.8	Intensity:	65.614
Position:	925.2	Intensity:	66.268
Position:	942.4	Intensity:	68.806
Position:	957.2	Intensity:	69.376
Position:	1008.7	Intensity:	56.111
Position:	1028.9	Intensity:	65.113
Position:	1054.9	Intensity:	62.159
Position:	1110.1	Intensity:	40.917
Position:	1126.5	Intensity:	44.042
Position:	1140.6	Intensity:	42.590
Position:	1171.6	Intensity:	50.284
Position:	1188.2	Intensity:	52.422
Position:	1199.1	Intensity:	57.178
Position:	1213.0	Intensity:	58.504
Position:	1265.7	Intensity:	60.481
Position:	1316.4	Intensity:	44.019
Position:	1375.7	Intensity:	57.163
Position:	1434.8	Intensity:	60.120
Position:	1445.9	Intensity:	60.921
Position:	1517.8	Intensity:	73.298
Position:	1567.9	Intensity:	66.716
Position:	1755.7	Intensity:	66.302
Position:	2715.6	Intensity:	72.182
Position:	2850.2	Intensity:	63.984
Position:	2954.4	Intensity:	58.816
Position:	3233.9	Intensity:	62.581

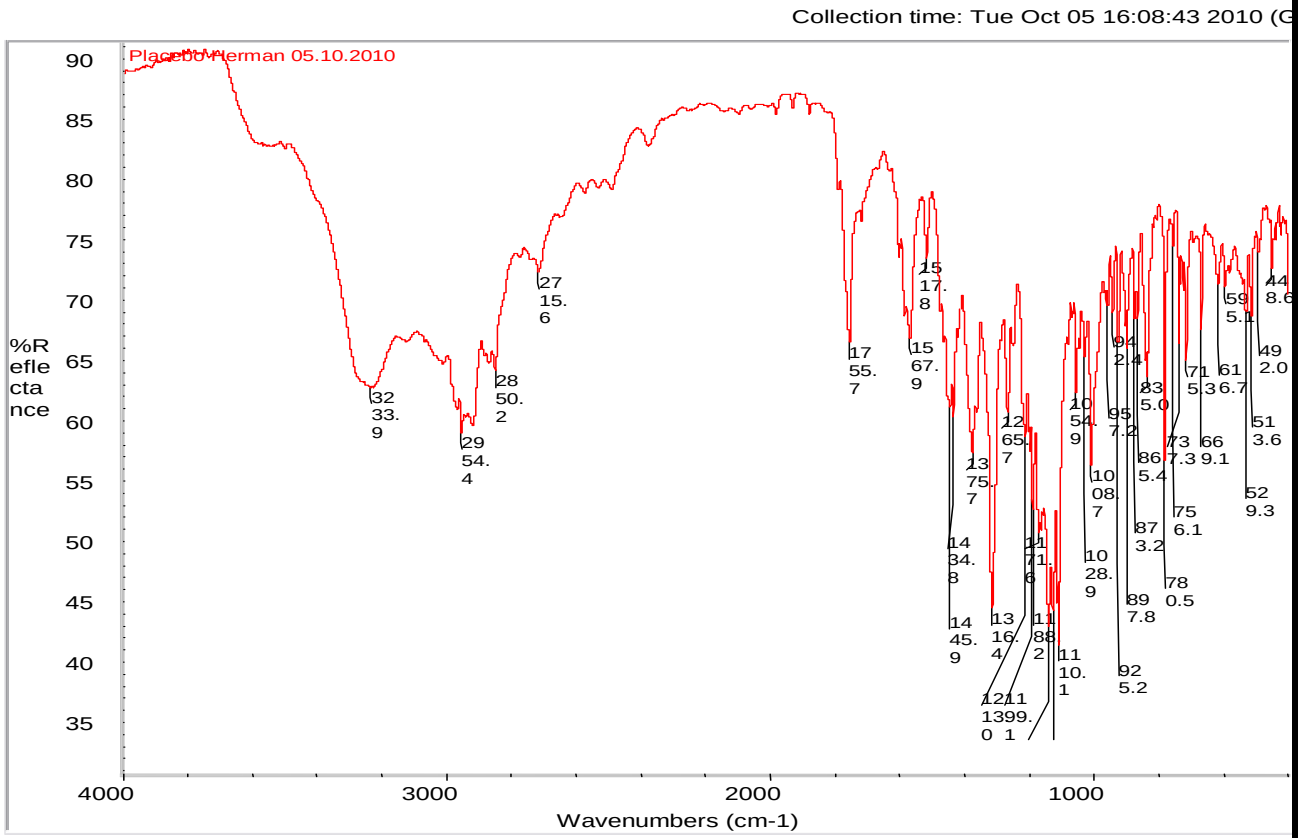

Figure 3.9: The IR spectra of the double fixed-dose combination powder formula.

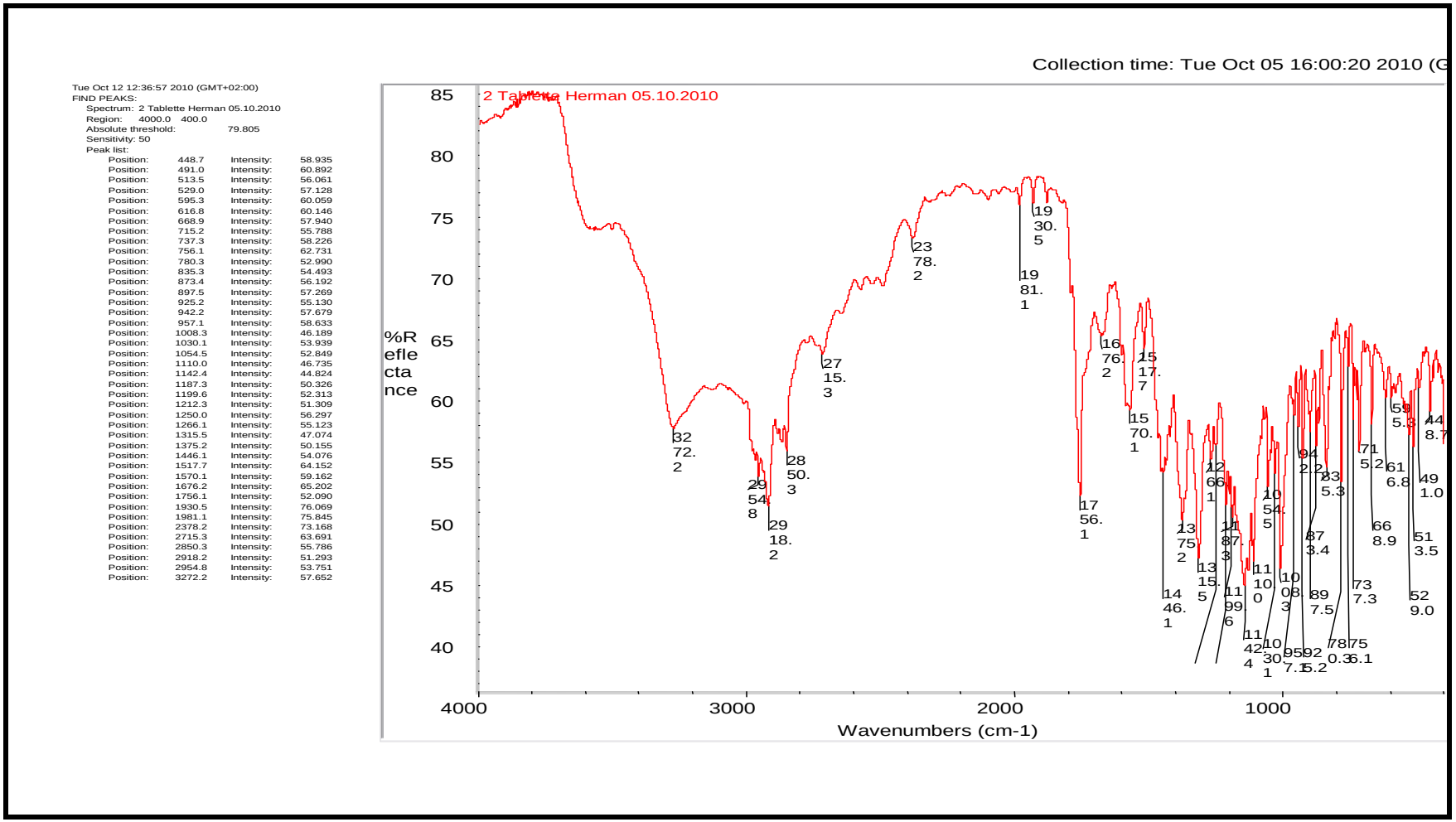

Figure 3.10: The IR spectra of the double fixed-dose combination tablets.

Table 3.17: The position and intensity of the peaks of the IR spectra of the double fixed-dose combination powder formula compared to the position and intensity of the IR spectra of the double fixed-dose combination tablets.

Tablets	Mixture	Position			Tablets	Mixture	Intensity		
Position	Position	STDEV	AVE	%RSD	Intensity	Intensity	STDEV	AVE	%RSD
448.7	448.6	0.07	448.65	0.02	58.935	72.344	9.48	65.64	14.44
491.0	492.0	0.71	491.5	0.14	60.892	73.797	9.13	67.34	13.55
513.5	513.6	0.07	513.55	0.01	56.061	68.401	8.73	62.23	14.02
529.0	529.3	0.21	529.15	0.04	57.128	68.79	8.25	62.96	13.10
595.3	595.1	0.14	595.2	0.02	60.059	70.842	7.62	65.45	11.65
616.8	616.7	0.07	616.75	0.01	60.146	71.145	7.78	65.65	11.85
668.9	669.1	0.14	669	0.02	57.94	67.435	6.71	62.69	10.71
715.2	715.3	0.07	715.25	0.01	55.788	64.883	6.43	60.34	10.66
737.3	737.3	0.00	737.3	0.00	58.226	66	5.50	62.11	8.85
756.1	756.1	0.00	756.1	0.00	62.731	74.311	8.19	68.52	11.95
780.3	780.5	0.14	780.4	0.02	52.99	56.153	2.24	54.57	4.10
835.3	835.0	0.21	835.15	0.03	54.493	63.555	6.41	59.02	10.86
873.4	865.4	5.66	869.4	0.65	56.192	68.251	8.53	62.22	13.70
897.5	873.2	17.18	885.35	1.94	57.269	68.303	7.80	62.79	12.43
925.2	897.8	19.37	911.5	2.13	55.13	65.614	7.41	60.37	12.28
942.2	925.2	12.02	933.7	1.29	57.679	66.268	6.07	61.97	9.80
957.1	942.4	10.39	949.75	1.09	58.633	68.806	7.19	63.72	11.29
1008.3	957.2	36.13	982.75	3.68	46.189	69.376	16.40	57.78	28.37
1030.1	1008.7	15.13	1019.4	1.48	53.939	56.111	1.54	55.03	2.79
1054.5	1028.9	18.10	1041.7	1.74	52.849	65.113	8.67	58.98	14.70
1110.0	1054.9	38.96	1082.45	3.60	46.735	62.159	10.91	54.45	20.03
1142.4	1110.1	22.84	1126.25	2.03	44.824	40.917	2.76	42.87	6.44
1187.3	1126.5	42.99	1156.9	3.72	50.326	44.042	4.44	47.18	9.42
1199.6	1140.6	41.72	1170.1	3.57	52.313	42.59	6.88	47.45	14.49
1212.3	1171.6	28.78	1191.95	2.41	51.309	50.284	0.72	50.80	1.43
1250.0	1188.2	43.70	1219.1	3.58	56.297	52.422	2.74	54.36	5.04
1266.1	1199.1	47.38	1232.6	3.84	55.123	57.178	1.45	56.15	2.59
1315.5	1213.0	72.48	1264.25	5.73	49.074	58.504	6.67	53.79	12.40
1375.2	1265.7	77.43	1320.45	5.86	50.155	60.481	7.30	55.32	13.20
1446.1	1316.4	91.71	1381.25	6.64	54.076	44.019	7.11	49.05	14.50
1517.7	1375.7	100.41	1446.7	6.94	64.152	57.163	4.94	60.66	8.15
1570.1	1434.8	95.67	1502.45	6.37	59.162	60.12	0.68	59.64	1.14
1676.2	1445.9	162.85	1561.05	10.43	65.202	60.921	3.03	63.06	4.80
1756.1	1517.8	168.50	1636.95	10.29	52.09	73.298	15.00	62.69	23.92
1930.5	1567.9	256.40	1749.2	14.66	76.069	66.716	6.61	71.39	9.26
1981.1	1755.7	159.38	1868.4	8.53	75.834	66.302	6.74	71.07	9.48
2715.3	2715.6	0.21	2715.45	0.01	63.691	72.182	6.00	67.94	8.84
2850.3	2850.2	0.07	2850.25	0.00	55.786	63.984	5.80	59.89	9.68
2954.8	2954.4	0.28	2954.6	0.01	53.751	58.816	3.58	56.28	6.36
3272.2	3233.9	27.08	3253.05	0.83	57.652	62.581	3.49	60.12	5.80
Average %RSD of position				**2.83**	**Average %RSD of intensity**				**10.70**

3.5.2 X-ray powder diffraction (XRPD)

3.5.2.1 Results and discussion

The XRPD traces of the artesunate and mefloquine mixture prior to granulation and tableting, and of the tablets after being exposed to the conditions under which granulation and tableting occurred, were identical with regard to peak position and relative intensity, peak shifting and the presence or lack of peaks in certain regions of ºθ values. The X-ray powder diffractogram of the mixture before and after tableting is illustrated in *Figure 3.11*.

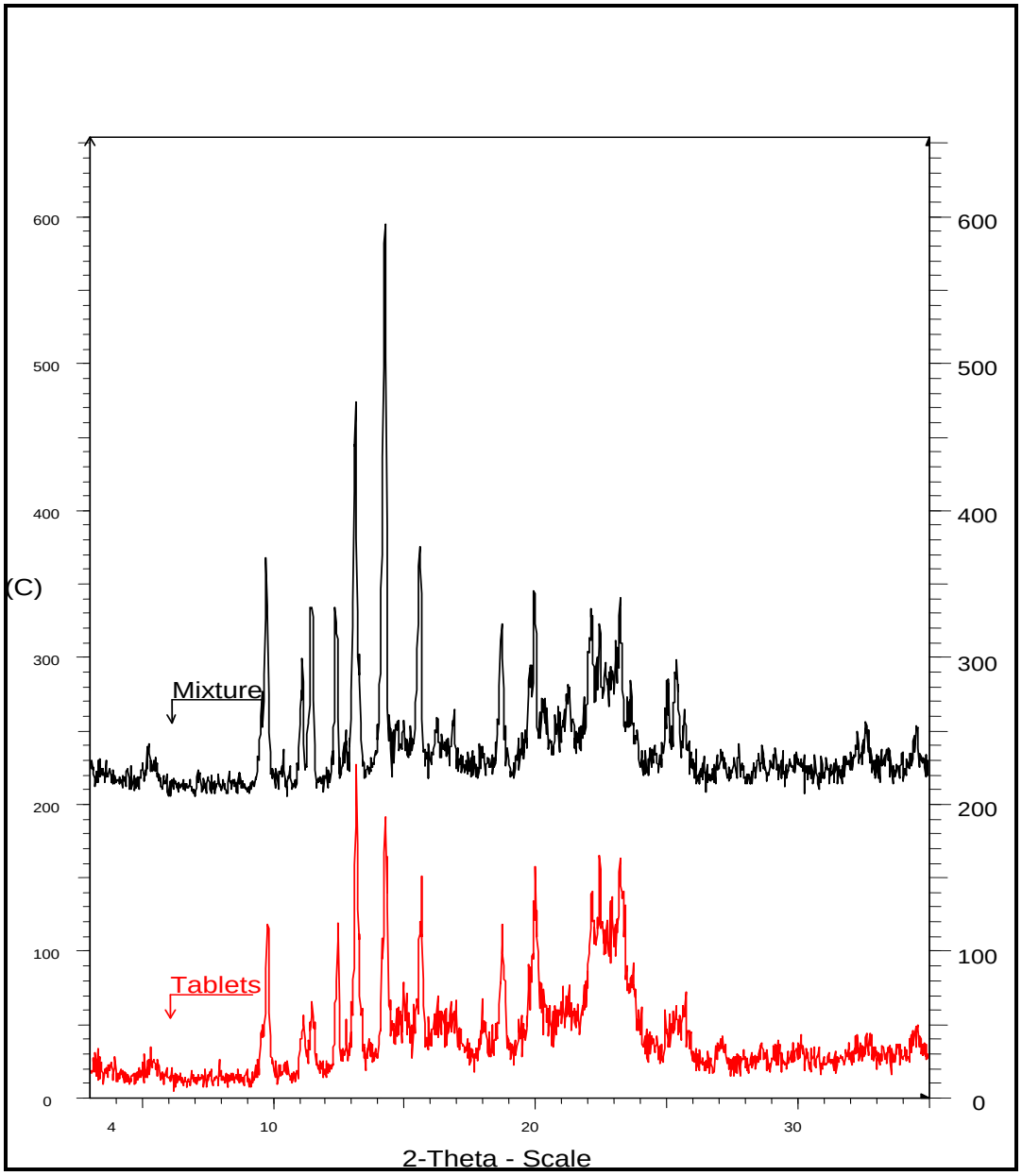

Figure 3.11: The XRPD patterns of the double fixed-dose combination tablets (displayed in red) and the double fixed-dose combination powder formula (displayed in black).

3.5.3 Conclusion

It can be concluded that a marked improvement of the micromeritic properties of the APIs was obtained and that the size enlargement process was a critical step in the manufacturing of the double fixed-dose combination. In addition the processes proved to be non-detrimental towards the APIs in terms of x-ray powder diffraction and infrared absorption spectroscopy results.

3.6 MIXTURE PREPARATION OF THE DOUBLE FIXED-DOSE COMBINATION

The development of a compressible and disintegrating tablet from an artesunate and mefloquine granule mixture necessitated the addition of compatible tableting excipients to the API mixture.

The granule formulas of artesunate and mefloquine could not be compressed successfully and required additions of a filler, lubricant and disintegrant to facilitate compression and disintegration. Subsequently, the effects of the additions of Avicel® PH200, magnesium stearate, talc and Ac-Di-Sol® were investigated.

A secondary blending stage was necessary to mix the artesunate and mefloquine granules together with the filler, lubricant and external disintegrant.

3.6.1 Filler (or diluent)

Diluents provide powder volume and weight to tablets. Lactose is the most common diluent in tablets. However, its main drawback is that some individuals have intolerance towards lactose. Apart from lactose, the most widely used fillers are powdered celluloses. Celluloses, like microcrystalline cellulose (Avicel® PH200) are biocompatible, chemically inert and have good tableting and disintegrating properties (Alderborn, 2007:450).

3.6.1.1 Avicel® PH200

Avicel® PH200, a microcrystalline cellulose, was considered as a diluent as a result of the compatibility of microcrystalline cellulose towards the other excipients and APIs, as proved by the IR spectra (*Figure 3.10*) and XRPD pattern results (*Figure 3.11*).

Additionally, compacts of lactose are weaker than compacts of microcrystalline cellulose and, whereas microcrystalline cellulose is inert and harmless to humans when taken orally as opposed to lactose. Furthermore, ethical problems may be associated with lactose. Examples of such problems include lactose being a potential bovine spongiform encephalopathy (BSE) virus carrier and lactose being a non-vegetarian dairy product (Ek *et al*., 2008:1).

As a result of the potential drawbacks associated with lactose, Avicel® PH200 was selected instead as a filler and the concentrations investigated are displayed in *Table 3.18*. The low crystallinity cellulose is advantageous for use in the manufacture of dry compositions comprising moisture-sensitive drugs. In particular, it has been found that by producing mixtures of moisture-sensitive drugs with low crystallinity cellulose, undesired hydrolysis of the drug can be effectively avoided. The low crystallinity cellulose is stable and is an excellent tableting powder and excellent granulating additive (Ek *et al*., 2008:1), justifying its potential usage as a filler for this study.

3.6.2 Lubricants

3.6.2.1 Magnesium stearate and talc

The artesunate and mefloquine granule mixtures were unable to be compressed successfully without the use of a lubricant and the correct concentration of filler. Tablets contained scratch marks and capping occurred without a lubricating agent. The most efficient and compatible lubricant between magnesium stearate and talc, as well as the optimum concentration of lubricant were investigated and the results obtained are displayed in *Table 3.18*.

Table 3.18: The compression results of the concentration and type of lubricant used for the double fixed-dose combination.

Formula number	Avicel® PH200 (% w/w)	Magnesium stearate (% w/w)	Result	Formula number	Avicel® PH200 (% w/w)	Talc (% w/w)	Result
1	33.4	**1.0**	Failed	**6**	33.4	**1.0**	Failed
2	32.4	**2.0**	Failed	**7**	32.4	**2.0**	Failed
3	31.4	**3.0**	Compressed	**8**	31.4	**3.0**	Failed
4	30.4	**4.0**	Compressed	**9**	30.4	**4.0**	Failed
5	29.4	**5.0**	Compressed	**10**	29.4	**5.0**	Compressed

It was found that 1 - 4% w/w talc failed and only a 5% w/w talc formula was tableted successfully. Tablets containing a minimum of 3% w/w magnesium stearate were compressed successfully. The possibility exists that mefloquine could be incompatible with magnesium stearate, but no disturbances were found as proved by the data obtained from the IR spectra (*Figure 3.10*) and XRPD patterns (*Figure 3.11*). The mixture containing a minimum of 3% w/w magnesium stearate was identified as

the optimum formula as opposed to the 5% w/w talc formula, as shown from the compression experiments.

3.6.3 Disintegrant

Ac-Di-Sol® (croscarmellose sodium) was subsequently chosen as a suitable disintegrant as a result of its good disintegrating abilities and effectiveness at relatively low concentrations as described in literature. Finally, Ac-Di-Sol® was also chosen as a result of the compatibility of croscarmellose sodium towards the other excipients and APIs, as proved by the IR spectra (*Figure 3.10*) and XRPD pattern results (*Figure 3.11*).

3.7 OPTIMIZED COMPRESSION CONDITIONS

Compression of the powder mixtures was conducted as described in *Chapter 2*, section *2.7*.

3.7.1 Compressibility

3.7.1.1 Results and discussion

The double fixed-dose combination mixtures exhibited free-flowing properties as presented in *Table 3.9* for artesunate granules and *Table 3.14* for mefloquine granules, and was successfully compressed on a Cadmach® tablet press. No sticking and lamination occurred. An average expansion of 0.81 ± 0.05% in tablet diameter was observed for all the compressed tablets. The tablet surfaces exhibited a satisfactory appearance with no indication of frictional damage on tablet edges. It was found that the mixtures with Avicel® PH200 = 31.4% w/w, magnesium stearate = 3.0% w/w and Ac-Di-Sol® = 1.0% w/w could be compressed into disintegrating tablets, in accordance with USP and BP specifications, with the correct artesunate and mefloquine dosage per tablet, by means of an upper punch setting of between 31 and 32. The significance of the tablet punch setting in relation to the disintegration time and disintegrating agent concentration per tablet will be discussed in sections *3.7.2* and *4.2.6.*

3.7.2 The effect of the tablet punch setting on the tablet disintegration time

3.7.2.1 Results and discussion

The double fixed-dose combination mixture (artesunate and mefloquine granules combined = 64.6% w/w, Avicel® PH200 = 31.4% w/w, magnesium stearate = 3.0% w/w and Ac-Di-Sol® = 1.0% w/w) was successfully compressed with an upper punch setting of 32. *Figure 3.12* displays the effect of upper punch setting on tablet disintegration times. The data was compiled from six tablets compressed at each upper punch setting. All eighteen tablets possessed a total concentration of 1.0% w/w Ac-Di-Sol® per tablet, split into a concentration of 0.5% intragranular and 0.5% extragranular per tablet.

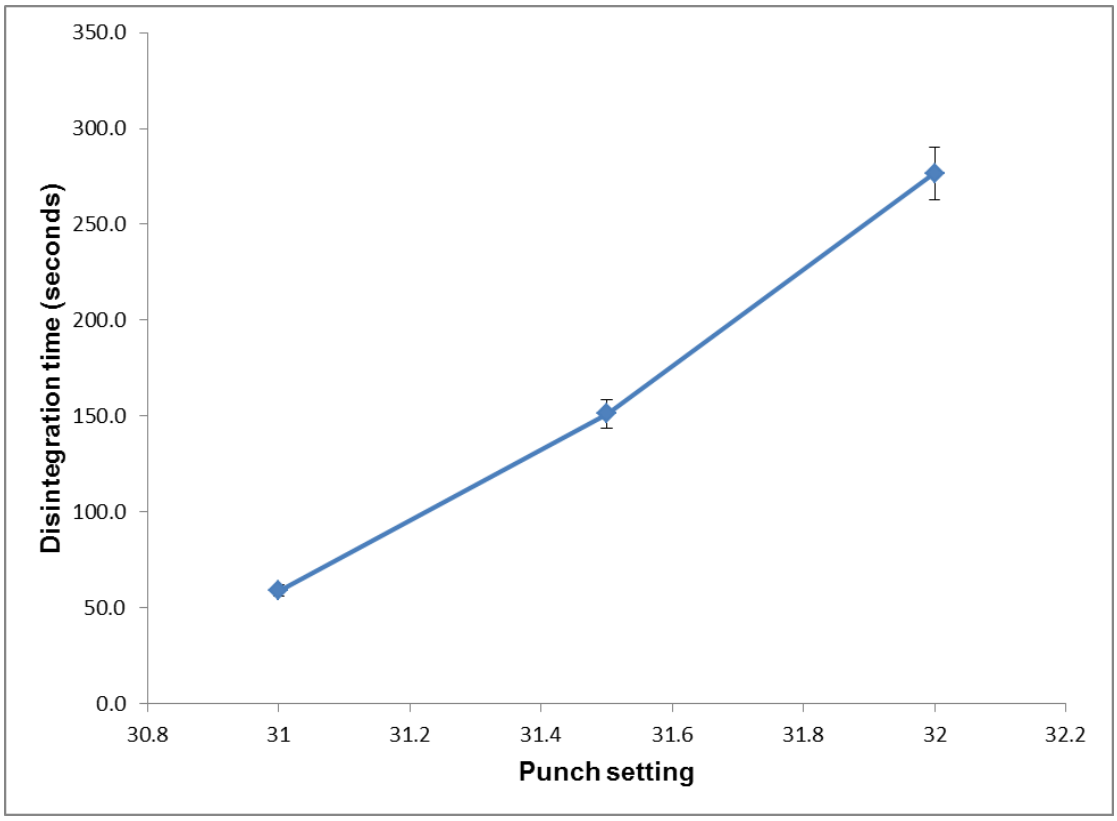

Figure 3.12: The average disintegration time of the double fixed-dose combination tablets as a function of tablet press punch setting.

Tablets compressed with an upper punch setting of less than 30 disintegrated rapidly. The tablets compressed with an upper punch setting of less than 30 also exhibited a relatively low average crushing strength of 31.1 ± 65.5 Newton. Tablets compressed with an upper punch setting of 32 exhibited an increase in crushing

strength (242.75 ± 8.30 Newton) and it was accompanied by longer disintegration times. *Figure 3.12* displayed an R^2 value of 0.9921 and the relationship was described by the following equation of *y = 217.6x - 6692.2*. It can be concluded that the concentration of disintegrating agent (1.0% w/w) in the formula was sufficient up until an upper punch setting of 32. A higher total percentage of Ac-Di-Sol® will be excessive, since an upper punch setting of 32 and a 1.0% Ac-Di-Sol® w/w powder mixture produced tablets with the ability to disintegrate within the 15 minute time limit for six tablets set by the BP (2012:1a). An upper punch setting of 32 would thus still be satisfactory within the set parameters. The compaction profile of the mixture at various upper punch settings is displayed in *Figure 3.13*.

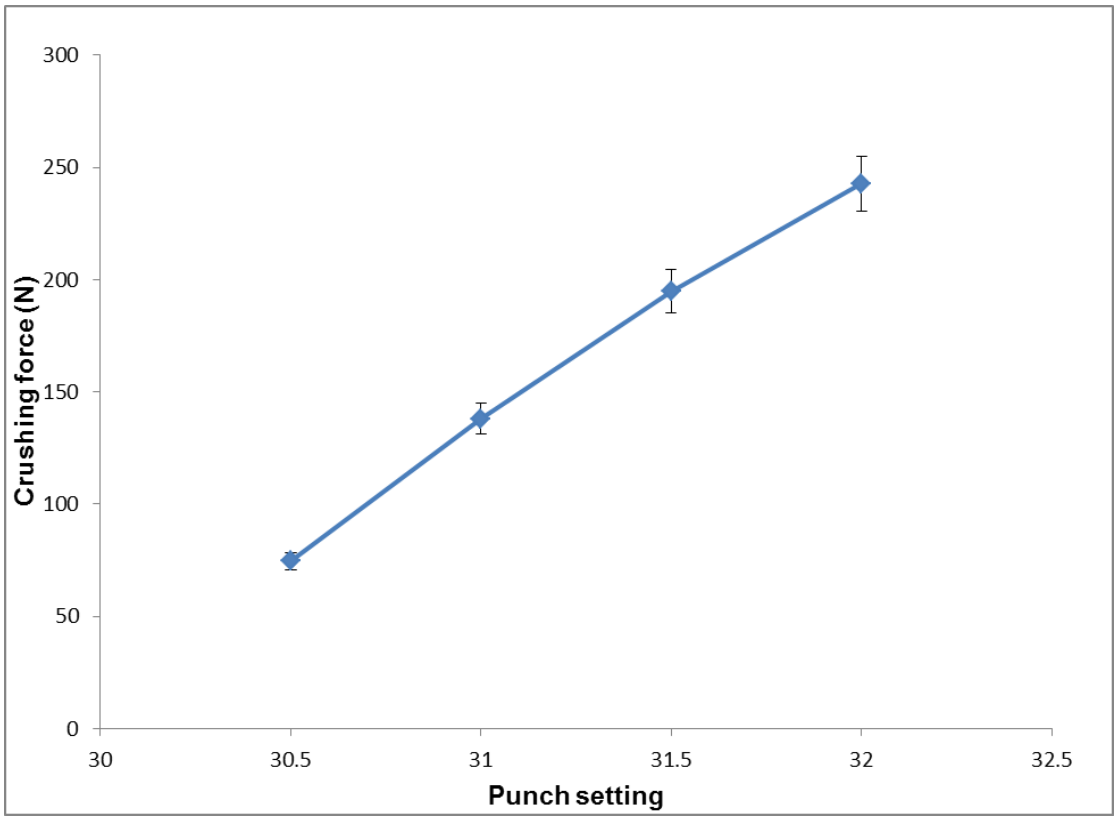

Figure 3.13: The compaction profile of the double fixed-dose combination mixtures.

3.7.2.2 Conclusion

Compression occurred successfully and an almost linear relationship between punch setting and crushing force was detected between a setting of 30.5 and 32. The linear relationship between punch setting and crushing force is a good indicator of the

range of upper punch settings that can be used to compress tablets from the double fixed-dose combination mixture.

3.8 SUMMARY

A double fixed-dose combination powder mixture, presented in *Table 9.14* in the annexure, with good flow, compressibility and handling properties was developed. Tablets were compressed at punch settings that produced sufficiently hard tablets, however, suitable to facilitate disintegration.

Following in *Chapter* 4 is an analysis of the physical properties of the double fixed-dose combination oral solid dosage form of artesunate and mefloquine.

4. CHAPTER FOUR

EVALUATION OF THE TABLETS OF THE FIXED-DOSE COMBINATION OF ARTESUNATE AND MEFLOQUINE

4.1 INTRODUCTION

The previous chapter provided information on the factors that provided wet granulated mefloquine particles and artesunate granules with enhanced micromeritic properties. The process utilised uncomplicated equipment, altering the API powders into a dust free, compressible and a free-flowing powder mixture.

In this chapter the results of the evaluation of the physical properties of the tablets of the oral double fixed-dose combination of artesunate and mefloquine will be presented. The tablets were evaluated in terms of physical dimensions, weight variation, friability, and disintegration to establish the applicability of the manufacturing process. The quality of the compressed tablets was evaluated according to standards set by the British Pharmacopoeia.

4.2 PHYSICAL EVALUATION OF THE ORAL DOSAGE FORM

Tablets were evaluated to establish the applicability of the process in terms of tablet weight variation, friability and physical dimensions as described in *Chapter 2*.

The experiments done in the previous chapter identified a specific double fixed-dose combination tablet formulation as the double fixed-dose combination formula with the most promising properties. The physical evaluations were conducted on the tablets with the double fixed-dose combination formulation as presented in *Table 4.1*.

Table 4.1: Tablet composition of the 2FDC.

Compound	Amount per tablet (% w/w)
Ac-Di-Sol®	1.0
Artesunate	18.4
Avicel® PH200	31.4
Eudragit® L 100	5.5
Kollidon® VA64	2.1
Magnesium stearate	3.0
Mefloquine hydrochloride	38.6
Total	100.0

4.2.1 Tablet dimensions

4.2.1.1 Results and discussion

It was hypothesized that round biconvex tablets would ease swallowing and consequently aid in patient compliance. The physical measurements of the double fixed-dose combination tablet are displayed in *Table 4.2*.

Table 4.2: The physical dimensions of the double fixed-dose combination tablets. Mean ± %RSD (n = 3).

Dimension	Measurement*
Diameter (mm)	12.74 ± 0.06
Radius (mm)	6.37 ± 0.22
Curvature height (mm)	0.67 ± 1.39
Band thickness / band height (mm)	5.79 ± 0.07
Overall height / tablet thickness (mm)	7.13 ± 0.27

4.2.2 Crushing strength

4.2.2.1 Results and discussion

Table 4.3: Crushing strength results of the double fixed-dose combination tablet formulation.

Tablet	Mass (g)	Crushing Force (N)
1	1.041	211
2	1.043	227
3	1.043	267
4	1.044	266
5	1.045	235
6	1.045	235
7	1.045	253
8	1.045	256
9	1.046	267
10	1.047	266
11	1.048	248
12	1.048	235
13	1.052	253
14	1.053	256
15	1.055	199
16	1.060	267
17	1.063	235
18	1.064	242
19	1.064	222
20	1.068	214
Total	**21.018**	**4854**
Average	**1.051**	**242.756**
STDEV	**0.008**	**20.7**
%RSD	**0.803**	**8.5**

The tablets exhibited an average crushing strength of 242.75 ± 8.30 Newton. This value would serve as a reference during future tests and future formulations.

4.2.3 Tensile strength

4.2.3.1 Results and discussion

The force required to fracture a tablet depends on the dimensions of the tablet (Alderborn, 2007:466). The average tensile strength of the double fixed-dose combination was 1.89 ± 14.67 $N.mm^{-2}$. The tensile strength of the double fixed-dose combination tablet was adequate in comparison to a headache tablet available on the market, since the tablets must possess a minimum mechanical strength to sustain potential loading encountered during processing and handling. The dimension of the face curvature and the cylinder length of the tablets proved to be sufficient.

4.2.4 Friability

4.2.4.1 Results and discussion

A friability test revealed that the final formulation of the double fixed-dose combination tablets could withstand normal handling. No loss of weight was recorded after 4 minutes, and a small loss in weight of less than 0.001% resulted after being tested for an additional 6 minutes with a Roche friabilator. Small values in friability imply much less abrasion during transportation, which is favourable.

4.2.5 Weight variation

4.2.5.1 Results and discussion

The weight variation of tablets is an indirect measurement of powder fluidity. Not more than two of the individual weights of each of the tablet formula as displayed in *Table 4.4* deviated from the average weight by more than the 5% deviation as shown in *Table 4.5*, and none deviated by more than twice that percentage. The average mass of the 20 tablets were 1.0509 ± 0.803 gram.

Table 4.4: Mass variation test results of the double fixed-dose combination tablet formulation.

Tablet	Mass (g)	% Variation	Result
1	1.041	0.952	Complied
2	1.043	0.752	Complied
3	1.043	0.752	Complied
4	1.044	0.657	Complied
5	1.045	0.609	Complied
6	1.045	0.562	Complied
7	1.045	0.562	Complied
8	1.045	0.562	Complied
9	1.046	0.467	Complied
10	1.047	0.419	Complied
11	1.048	0.286	Complied
12	1.048	0.257	Complied
13	1.052	-0.085	Complied
14	1.053	-0.199	Complied
15	1.055	-0.418	Complied
16	1.060	-0.865	Complied
17	1.063	-1.151	Complied
18	1.064	-1.246	Complied
19	1.064	-1.246	Complied
20	1.068	-1.627	Complied
Total	**21.0181**		
Average	**1.0509**		
STDEV	**0.008**		
%RSD	**0.803**		

Table 4.5: British Pharmacopoeia (2012:1a) limits for tablet weight variation.

Average weight of tablet	Percentage deviation
80 mg or less	10
More than 80 mg and less than 250 mg	7.5
250 mg or more	5

4.2.6 Disintegration

4.2.6.1 Results and discussion

The effect of the upper punch setting on the tablet disintegration time was investigated. A disintegration test was performed on six tablets of three different formulations to determine the optimal amount of internal and external disintegrant [Intragranular disintegrant percentage: Extragranular disintegrant (%)]. Three different tablet formulas contained Ac-Di-Sol® concentrations of 0.50, 0.75 and 1.00% w/w. The amount of Avicel® PH 200 was adjusted to 31.41%, 31.86%, 32.00% w/w for the 50:50, 75:25 and 100:0 formulas respectively. The results are displayed in *Table 4.6* and graphically presented in *Figure 4.1*

Table 4.6: ***The ratio of the internal granular disintegrant percentage against the external percentage Ac-Di-Sol® per tablet mixture against disintegration time. (Ac-Di-Sol® at 0.50, 0.75 and 1.00% w/w of total powder mixture).***

Intragranular disintegrant percentage: Extragranular disintegrant (%)	Disintegration time (minutes)
50:50	4.54 ± 2.36
75:25	15.13 ± 0.90
100:0	20.90 ± 3.52

It is evident that the tablets needed to contain at least a 50:50 distribution on a weight basis for a total Ac-Di-Sol® concentration of 1.00% w/w to render rapid tablet disintegration.

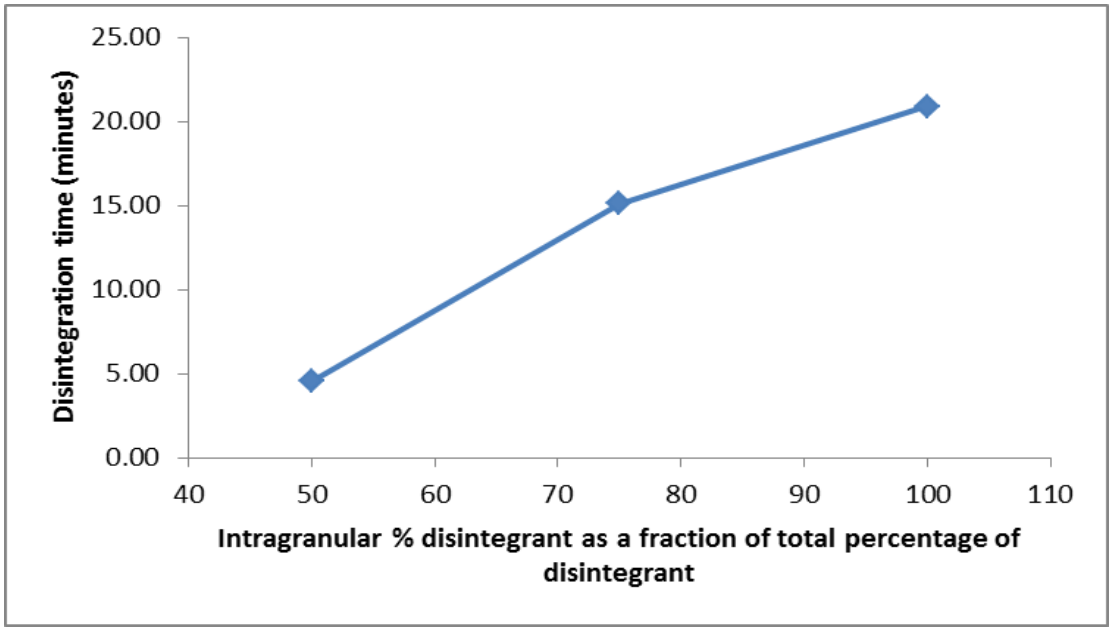

Figure 4.1: The internal fraction of the total percentage of Ac-Di-Sol® per tablet mixture against disintegration time.

Table 4.7 presents a summary of the physical properties and compliances obtained from the various experiments.

Table 4.7: The summary of the physical properties of the tablets of the double fixed-dose combination.

Property	Value
Tablet density (mg.mm^{-3})	1.275
Crushing strength (Newton)	242.75
Tensile strength (N.mm^{-2})	2.385
Friability (%)	<0.001 (Complied with BP recommendations)
Weight variation	Complied with BP standards
Disintegration time (seconds)	276.6 (Complied with BP recommendations)

4.3 SUMMARY

The physical tablet properties of the double fixed-dose combination of artesunate and mefloquine proved to be in accordance with the pharmacopoeial standards. The %RSD for the individual mass of twenty tablets for the test for tablet weight variation of the formula was 0.803%, which confirmed excellent powder flow into the tablet die.

In addition the tablets resisted abrasion with a loss of mass of less the 0.001% during friability testing.

The physical properties of the double fixed-dose combination of artesunate and mefloquine tablets proved to be viable for manufacturing purposes. However, the establishment of feasible physical properties are worthless unless the incorporated drug can carry out is therapeutic function. In the majority of cases, this can only occur when the drug substance has dissolved in the fluids of the gastrointestinal tract. Following in *Chapter* 5 is the evaluation of the dissolution profiles of the double fixed-dose combination.

5. CHAPTER FIVE

DISSOLUTION STUDIES OF THE ORAL FIXED-DOSE COMBINATION OF ARTESUNATE AND MEFLOQUINE

The antimalarial function of the double fixed-dose combination can only be accomplished when the drug substance has dissolved in the fluids of the gastrointestinal tract and absorbed into the systemic circulation. It is therefore important to determine the pharmaceutical availability of the two APIs in the double fixed-dose combination. Assay procedures are intended to measure the analyte present in a given sample. In this chapter, the dissolution profiles of the artesunate and mefloquine will be presented and discussed as well as the HPLC method that was employed to quantify the concentrations of the two APIs in the dissolution samples. In this chapter, the following will be discussed:

- Development of a validated HPLC method for analysing artesunate and mefloquine in dissolution samples.
- Dissolution profiles of artesunate and mefloquine from the double fixed-dose combination tablet.

5.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

A validated HPLC method for the analysis of artesunate and mefloquine was used to quantify the concentration of artesunate and mefloquine in the dissolution samples.

5.1.1 Validation of the HPLC method for artesunate and mefloquine

This method was developed and validated at the Analytical Technology Laboratory, North-West University, Potchefstroom, South Africa.

5.1.1.1 Linearity

The linearity for artesunate was determined by performing linear regression analysis on the plot. Standard solutions were prepared in double distilled water to obtain concentrations ranging from 0.0 µg/ml to 250.3 µg/ml for artesunate as displayed in *Figure 5.1*.

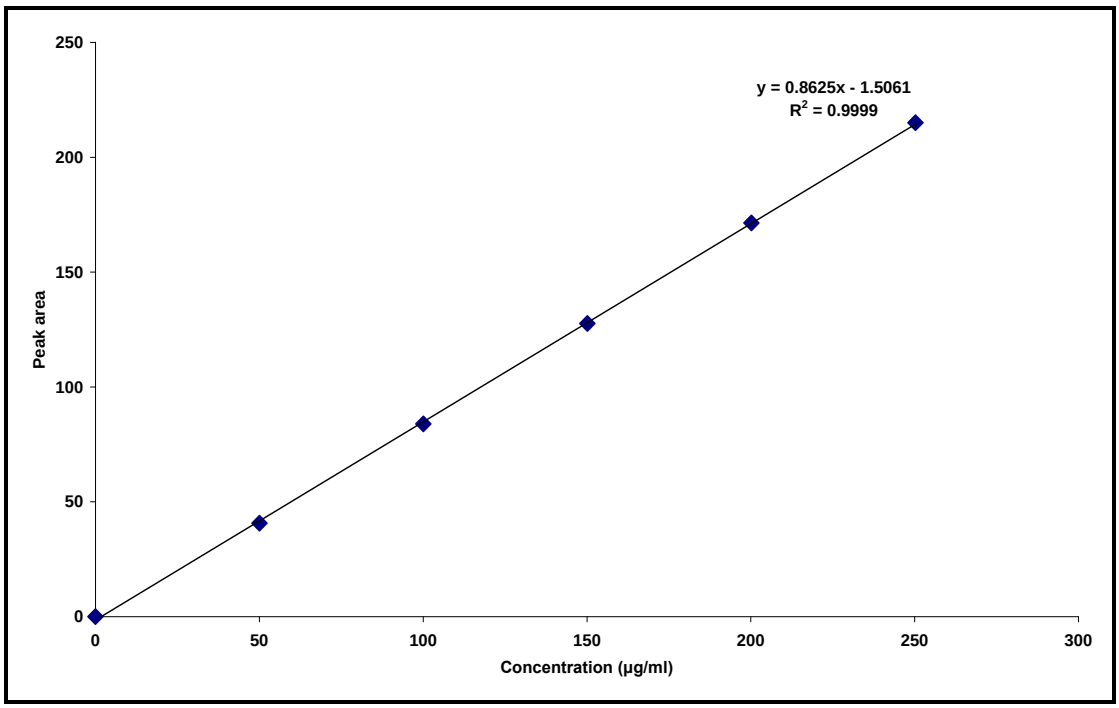

Figure 5.1: The linear regression graph for artesunate to determine linearity and range.

The regression value (R^2) was greater than 0.999 and the Y-intercept was 1.506 for artesunate.

The linearity for mefloquine was determined by performing linear regression analysis on the plot. Standard solutions were prepared in water to obtain concentrations ranging from 1 µg/ml to 403 µg/ml for mefloquine. The regression value (R^2) was greater than 0.999 and the Y-intercept was 0.042 for mefloquine as displayed in *Figure 5.2*.

5.1.1.2 Specificity

For specificity a solution from the placebo powder similar to the sample solution was prepared in water. The placebo did not generate any peaks that interfered with the determination of the active ingredients.

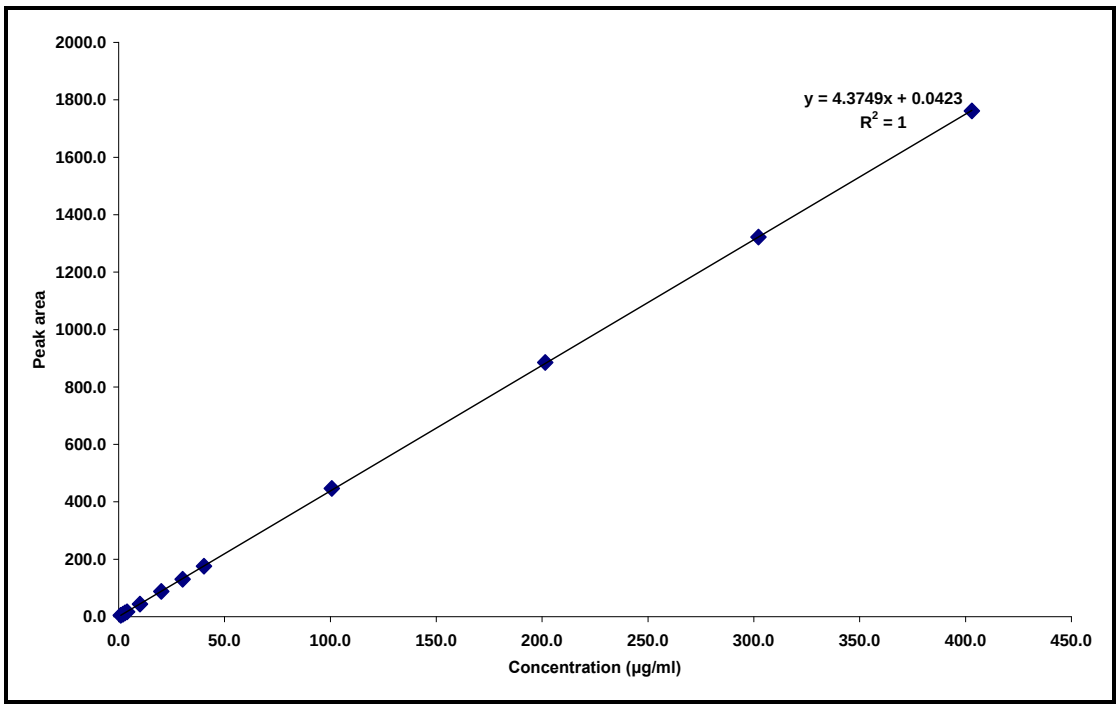

Figure 5.2: The linear regression graph for mefloquine to determine linearity and range.

From *Figure 5.1* and *Figure 5.2* it is evident that the peak area as a function of concentration followed a linear relationship. This is confirmed by a R^2 value of greater than 0.999 for both artesunate and mefloquine.

5.1.1.3 Precision and Accuracy

Precision (repeatability) was determined by performing HPLC analysis of a low, medium and high concentration sample for both artesunate and mefloquine.

To determine accuracy, amounts of the placebo equivalent to the amount of sample powder that would contain 80%, 100% and 120% of 100 mg of artesunate and mefloquine were weighed. Quantities of the active ingredients at concentrations of approximately 80%, 100% and 120% respectively of the expected sample concentration, known as spiking, were made up to volume and filtered. The samples were analysed in duplicate by means of HPLC.

The results for accuracy are displayed in *Table 5.1* and *Table 5.2* for artesunate and mefloquine respectively. The results for precision are displayed in *Table 5.3* and *Table 5.4* for artesunate and mefloquine respectively.

Table 5.1: Results for artesunate to determine accuracy.

Artesunate					
Conc. spiked	**Area**		**Mean**	**Recovery**	
µg/ml				**µg/ml**	**%**
160.4	145.951	136.179	141.0650	**165.5**	103.2
160.4	137.204	134.587	135.8955	**159.5**	99.5
160.4	139.959	140.336	140.1475	**164.4**	102.5
200.5	174.055	173.932	173.9935	**203.3**	101.4
200.5	175.107	175.745	175.4260	**204.9**	102.2
200.5	173.953	173.496	173.7245	**202.9**	101.2
240.6	207.057	206.379	206.7180	**240.8**	100.1
240.6	207.959	207.981	207.9700	**242.2**	100.7
240.6	205.835	205.341	205.5880	**239.5**	99.6

Statistical analysis	
Mean	101.1
SD	1.2
%RSD	1.2

Table 5.2: Results for mefloquine to determine accuracy.

Mefloquine					
Conc. spiked	**Area**		**Mean**	**Recovery**	
µg/ml				**µg/ml**	**%**
320.4	1371.145	1369.714	1370.4295	313.2	97.8
320.4	1356.156	1353.988	1355.0720	309.7	96.7
320.4	1395.698	1396.454	1396.0760	319.1	99.6
400.6	1713.281	1717.320	1715.3005	392.1	97.9
400.6	1704.605	1704.698	1704.6515	389.6	97.3
400.6	1701.503	1701.699	1701.6010	388.9	97.1
480.7	2039.459	2040.496	2039.9775	466.3	97.0
480.7	2061.732	2060.242	2060.9870	471.1	98.0
480.7	2049.143	2048.835	2048.9890	468.3	97.4

Statistical analysis	
Mean	97.6
SD	0.8
%RSD	0.8

Table 5.3: The mean percentage of recovered, standard deviation and percent relative standard deviation (%RSD) for mefloquine by analysing three sets of samples on the same day to determine intra-day precision.

Mefloquine concentrations (µg/ml)	Mean % recovered	Standard deviation	%RSD
320.4	98.0	1.48	1.51
400.6	97.4	0.41	0.42
480.7	97.5	0.50	0.51

The acceptance criterion for %RSD was set at 2.0% or less. The intra-day precision for mefloquine was acceptable with an RSD of 1.51% or less.

Table 5.4: The mean, percentage of recovered, standard deviation and percent relative standard deviation (%RSD) for artesunate by analysing three sets of samples on the same day to determine intra-day precision.

Artesunate concentrations (µg/ml)	Mean % recovered	Standard deviation	%RSD
160.4	101.7	1.97	1.94
200.5	101.6	0.52	0.52
240.6	100.1	0.60	0.57

The acceptance criterion for %RSD was set at 2.0% or less. The intra-day precision for artesunate was acceptable with an RSD of 1.94% or less.

The HPLC method was validated and was therefore suitable to analyse artesunate and mefloquine in tablets for stability testing, quality control and batch release purposes. No interference was encountered from samples, thus the method can be regarded as being stability indicating. A chromatogram of the reference standards is presented in the annexure, *Figure 9.1.*

5.2 THE DISSOLUTION PROFILES OF ARTESUNATE AND MEFLOQUINE

A dissolution study of six of the double fixed-dose combination tablets was performed for the establishment of *in vitro* dissolution behaviour. The dissolution conditions are described in section *2.9*.

5.2.1 Results and discussion

5.2.1.1 Artesunate

Artesunate particles were transformed into enteric coated granules. As the larger granules were granulated again, artesunate particle surfaces might have been exposed due to breakage. This was not entirely undesirable, as an immediate release of a part of the artesunate dose was desirable. That might explain the rapid DRi of 29.4% of the total artesunate dose in 0.1 N HCl. After two hours of operation in 0.1 N HCl, the medium was adjusted to reach a pH of 6.8 ± 0.05. The remainder of the artesunate granules dissolved rapidly as expected from 120 minutes onwards as is evident from *Figure 5.3*. After 150 minutes, an average of 54.8 ± 8.7% (n = 6) of the artesunate content dissolved. Complete artesunate dissolution data was limited

after 150 minutes. This is due to disturbances, possibly due to the fact that artesunate is rapidly converted to dihydroartemisinin (Tan, 2009:10), and therefore could not be detected by the analytical method. As a future investigation, it would be advantageous if dihydroartemisinin could be determined with the HPLC methods used for assay. However, the methods require further development to accommodate related substances.

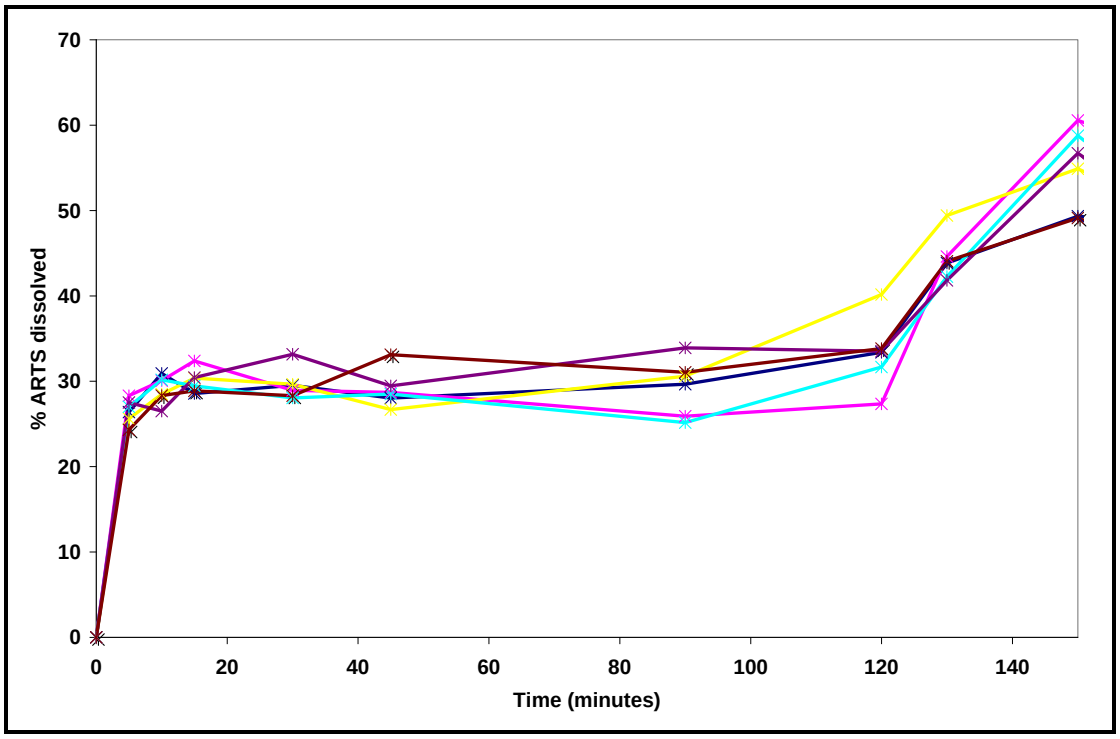

Figure 5.3: The dissolution profile of artesunate from six double fixed-dose combination tablets.

In conclusion, the dissolution profiles of artesunate from the double fixed-dose combination tablets corresponded to what was expected. The profiles exhibited a rapid initial dissolution rate followed by a period of no release up until two hours of simulated gastric residence time. After two hours a rapid secondary initial dissolution rate was observed at a pH of 6.8. The secondary release might maintain the therapeutic concentration of artesunate in the body briefly longer and thus prolonging the antimalarial action of the API without the administration of a second dose. The possibility of prolonging the release of artesunate at a pH of 6.8 even longer should be investigated in order to enhance the formulation. *Figure 5.4* displays the average dissolution profile of six of the double fixed-dose combination tablets, and indicates

the rapid initial release curve of artesunate, followed by a period of no release, and a secondary release of artesunate after 2 hours from a dissolution medium with a pH of 6.8.

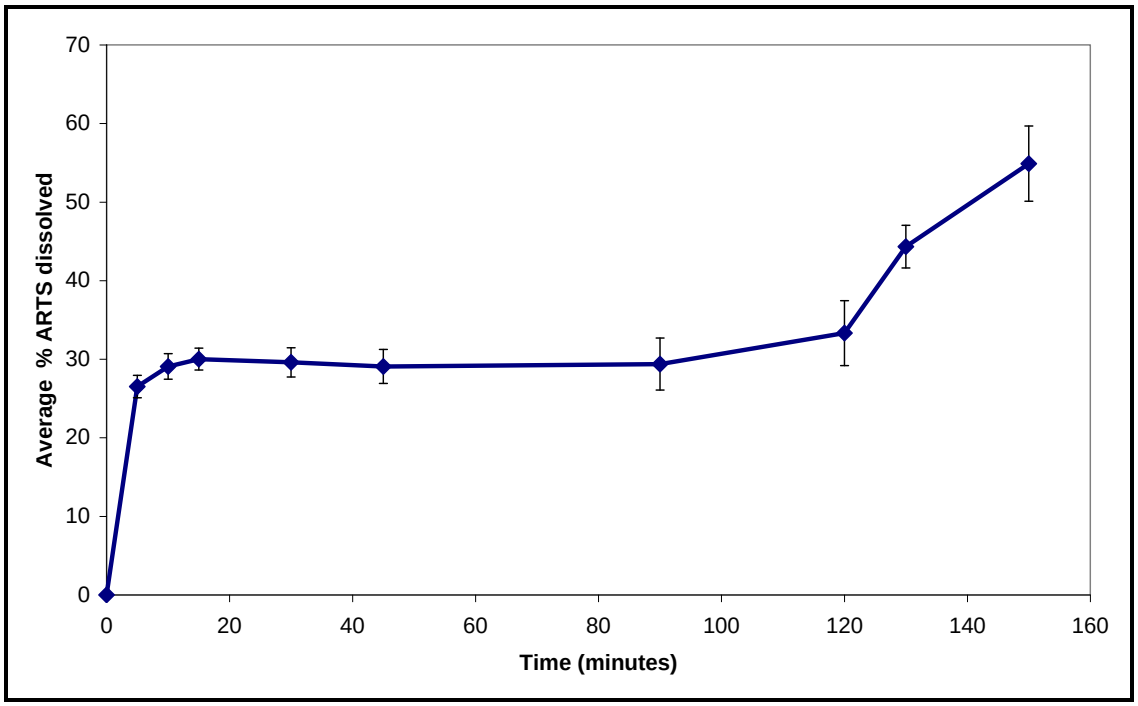

Figure 5.4: Mean percentage of artesunate dissolved as a function of time from the double fixed-dose combination tablets.

The average initial dissolution rate (DRi) of artesunate was 0.547 %.min^{-1} and the AUC was recorded as 7174.45 %.min. The average %RSD of the samples for the duration of the test was 6.87.

5.2.1.2 Mefloquine

The dissolution profiles of mefloquine from the double fixed-dose combination are depicted in *Figure 5.5*.

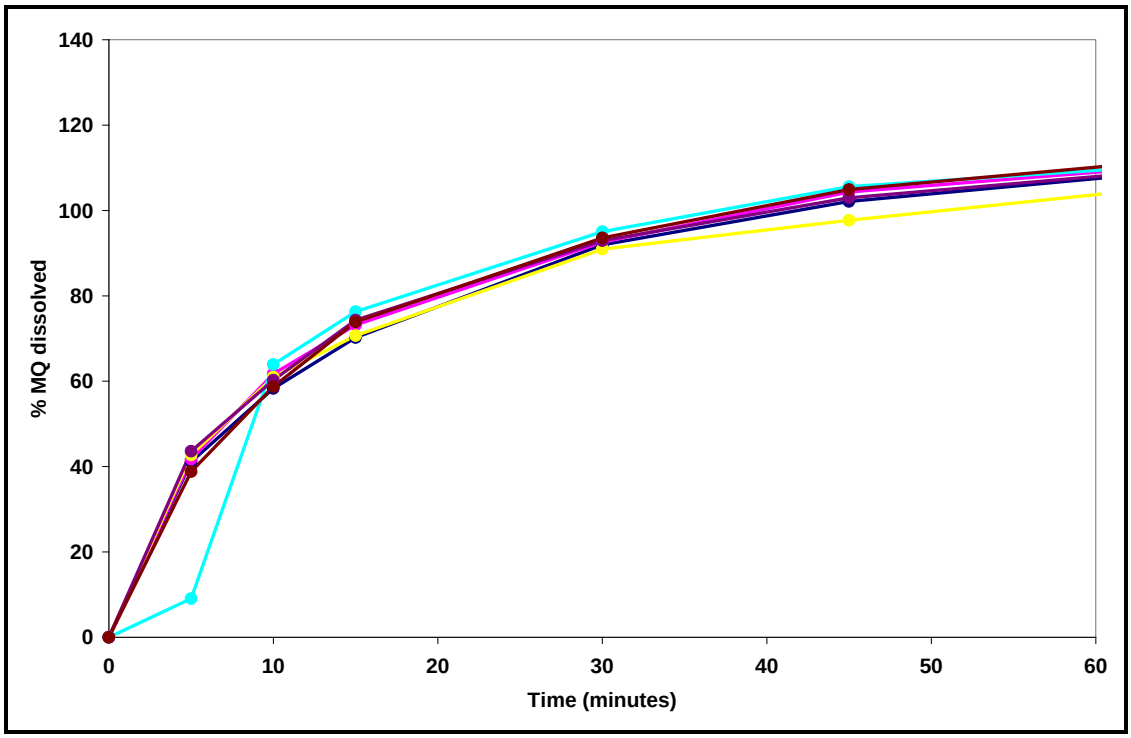

Figure 5.5: Dissolution profile of mefloquine from six double fixed-dose combination tablets.

The average initial dissolution rate (DRi) of mefloquine was 0.305 %.min^{-1}. It was 45.8% slower than the DRi of artesunate. The uncoated artesunate particles may possibly have dissolved through a system regulated by dissolution in comparison to the mefloquine granules resulting in a higher DRi value. It might be concluded from the preparation technique utilised and from the spherical granules that were obtained, that mefloquine release took place passing through a porous system and as a result of a combination of diffusion through a polymer (Kollidon® VA64) and diffusion through pores in the system (Peppas, 1985:111; Siepmann & Göpferich, 2001:231). The AUC of mefloquine was recorded as 11999.76 %.min, as displayed in *Table 5.5*.

Table 5.5: The DRi and AUC of artesunate and mefloquine.

Artesunate		Mefloquine	
***DRi* (%.min^{-1})**	0.547	***DRi* (%.min^{-1})**	0.305
***AUC* (%.min)**	7174.45	***AUC* (%.min)**	11999.76

The mean percentage mefloquine dissolved from the double fixed-dose combination tablets as a function of time is displayed in *Figure 5.6*.

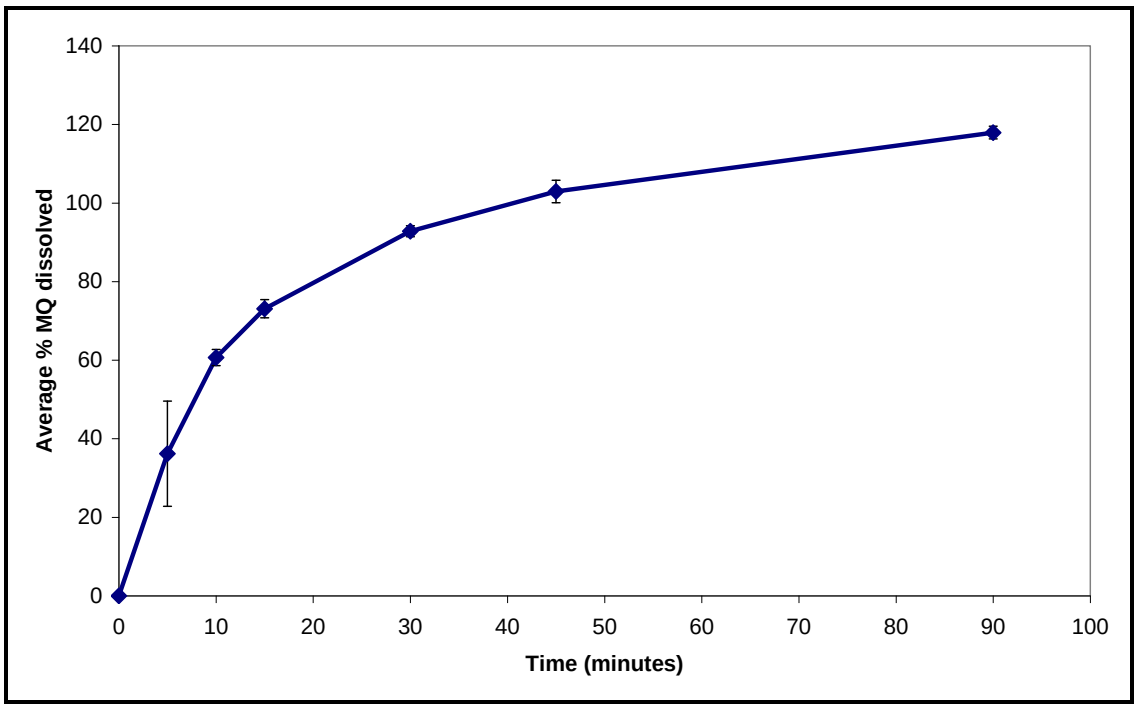

Figure 5.6: Average percentage mefloquine dissolved as a function of time from the double fixed-dose combination tablets.

An immediate release of mefloquine in 0.1 N HCl was required and was achieved. All the mefloquine granules disintegrated and an average of 102.9 ± 2.8% mefloquine dissolved completely in the acidic medium during the first 45 minutes.

5.3 SUMMARY

The dissolution profiles of artesunate and mefloquine from the tablets displayed a rapid initial release of both the APIs, followed by a time of no release for artesunate, and a secondary release stage for artesunate again. The secondary release stage of artesunate is a result of the coating polymer around the artesunate particles

dissolving at a basic pH, thus exposing the artesunate particles to the basic solution for dissolution of artesunate at a basic pH.

The dissolution profile of mefloquine in 0.1 N HCl revealed complete dissolution within 45 minutes.

An analytical procedure was developed and validated to measure the concentrations of artesunate and mefloquine from the double fixed-dose combination released during dissolution. The analytical results presented evidence that the pharmaceutical quality by design concept maximise satisfactory ingredient compatibility, stability and expected dissolution performance.

The method performed well and should be suitable to analyse artesunate and mefloquine in products for stability testing, quality control and batch release purposes. To develop a double fixed-dose combination with improved potential absorption rates and oral bioavailability necessitated the study of the dissolution model of the APIs. Following in *Chapter 6* is an analysis of the type of release artesunate and mefloquine exhibited.

6. CHAPTER SIX

DETERMINING THE DRUG RELEASE MECHANISM OF MEFLOQUINE HYDROCHLORIDE AND ARTESUNATE FROM A FIXED-DOSE COMBINATION SOLID ORAL DOSAGE FORM

6.1 INTRODUCTION

When designing delayed-release dosage forms, the solubility characteristics of the active must be taken into account as it can strongly influence the designing methods and the overall release profile of a drug. In fact, mefloquine is only slightly soluble in water. As in the case of a possible double fixed-dose combination of artesunate and mefloquine, it is also necessary to delay the dissolution of artesunate in the stomach.

Artesunate is an artemisinin with a high clinical efficacy but has a problematic short elimination half-life. As a result, the effective antimalarial half-life in the blood is therefore problematic short (Price *et al.,* 1995:526), and a secondary release of artesunate lower in the gastrointestinal tract might be beneficial to prolong the antimalarial effect of artesunate

The release mechanism of mefloquine and artesunate was determined from the dissolution profiles presented in *Chapter 5*.

Dissolution studies were conducted in 0.1 N HCl (pH ~ 1.20), in accordance with the dissolution method described in *Chapter 2*. The zero-order model, first order model and matrix models are available to describe drug release mechanisms. The dissolution data obtained from the dissolution studies were examined to determine the release kinetics of artesunate and mefloquine obtained from compressed granulated mefloquine and enteric coated artesunate mixtures. The functions attained from each of the models were plotted. Linearity was determined from the calculation of the correlation coefficient.

6.1.1 Zero-order release

6.1.1.1 Results and discussion

The zero-order release model is a highly sought-after immediate release model for the formulator and the release kinetics obtained of mefloquine from the double fixed-dose combination tablets will provide insight in improving the double fixed-dose combination formula. A zero-order release mechanism ensures that a steady amount of drug is released over time, reducing potential peak or trough fluctuations and side effects, while enhancing the amount of time the drug concentrations remain within the therapeutic window (Colorcon 2012:1). The following equation describes zero-order kinetics (Donbrow & Samuelov, 1980:):

Equation 6.1

$$\frac{Mt}{M\infty} = Kt$$

Where:

Mt	=	accumulated amount of drug released in time
M_∞	=	accumulated amount of drug in time ∞
K	=	zero-order release constant
t	=	time

A linear relationship between the amount of drug released and time is an indication of zero-order drug release. The experimental dissolution data is given in *Table 9.4* (d) of the annexure, and is graphically represented in *Figure 6.1* (R^2 = 0.7047).

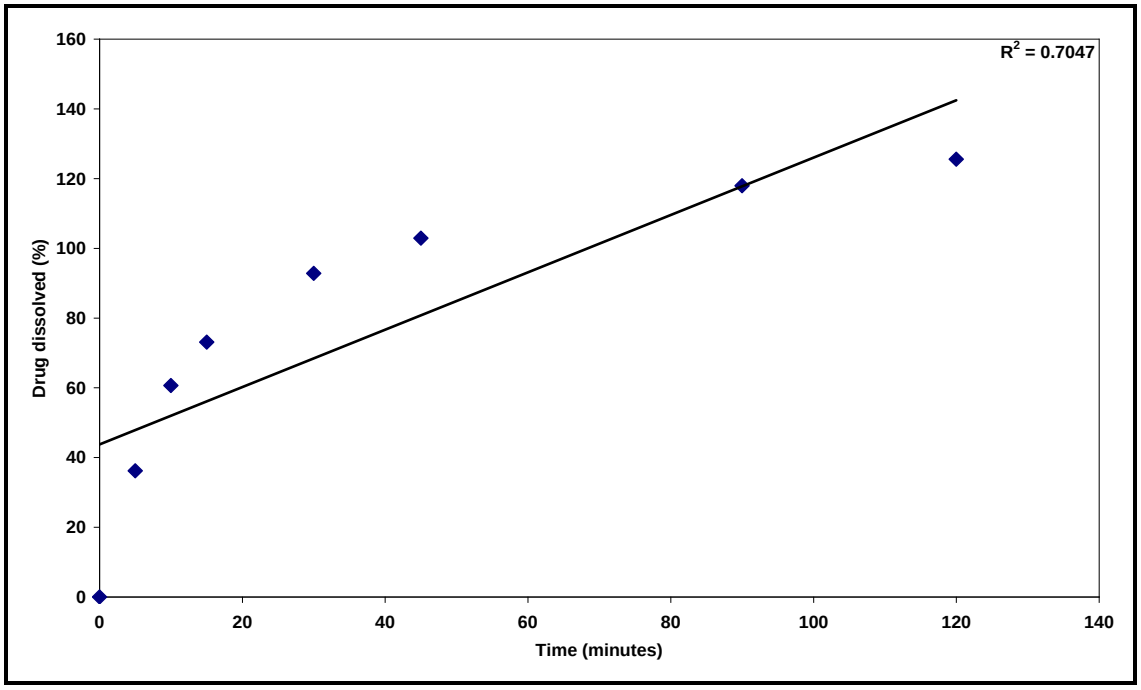

Figure 6.1: A plot of the percentage drug dissolved as a function of time.

No linear relationship existed between the rate of mefloquine dissolved against time (R^2 = 0.7047), as presented in *Figure 6.1*.

6.1.2 First order release

6.1.2.1 Results and discussion

First order release dosage forms, such as those containing water-soluble drugs in porous matrices (Mulye & Turco, 1995:943), release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminishes.

The following equation describes first order kinetics:

Equation 6.2

$$\log W = \log W_0 + kt/2.303$$

Where:

W	=	remaining drug concentration
W_0	=	initial amount of drug present

k = first order rate constant

t = time

A linear relationship between the logarithm of the amount of drug remaining and time would indicate first order drug release. The processed experimental dissolution data is given in the annexure, *Table 9.4* (a), and is graphically represented in *Figure 6.2*.

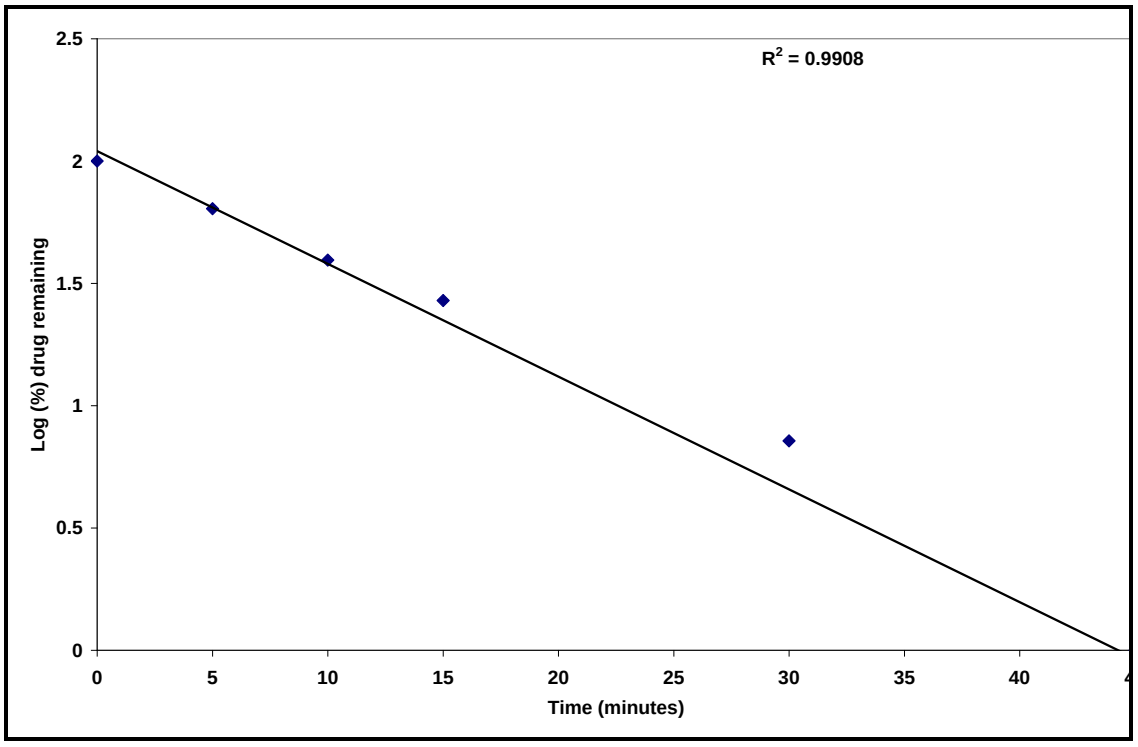

Figure 6.2: A plot of the logarithm of the percentage drug remaining as a function of time.

The plot of the logarithm of the percentage drug remaining as a function of time exhibited a correlation coefficient R^2, of 0.9908. An additional test to verify first order release was done to establish a linear relationship between the rate of drug release and the amount of drug released (Donbrow & Benita, 1982:548). The rate of drug release, dQ'/dt, according to *Equation 6.2* can be derived to *Equation 6.3*:

Equation 6.3

$$dQ'/dt = kW_0 - kQ'$$

The rate of drug release was calculated from the processed experimental dissolution data given in the annexure, *Table 9.4* (b), and is graphically represented in *Figure 6.3*.

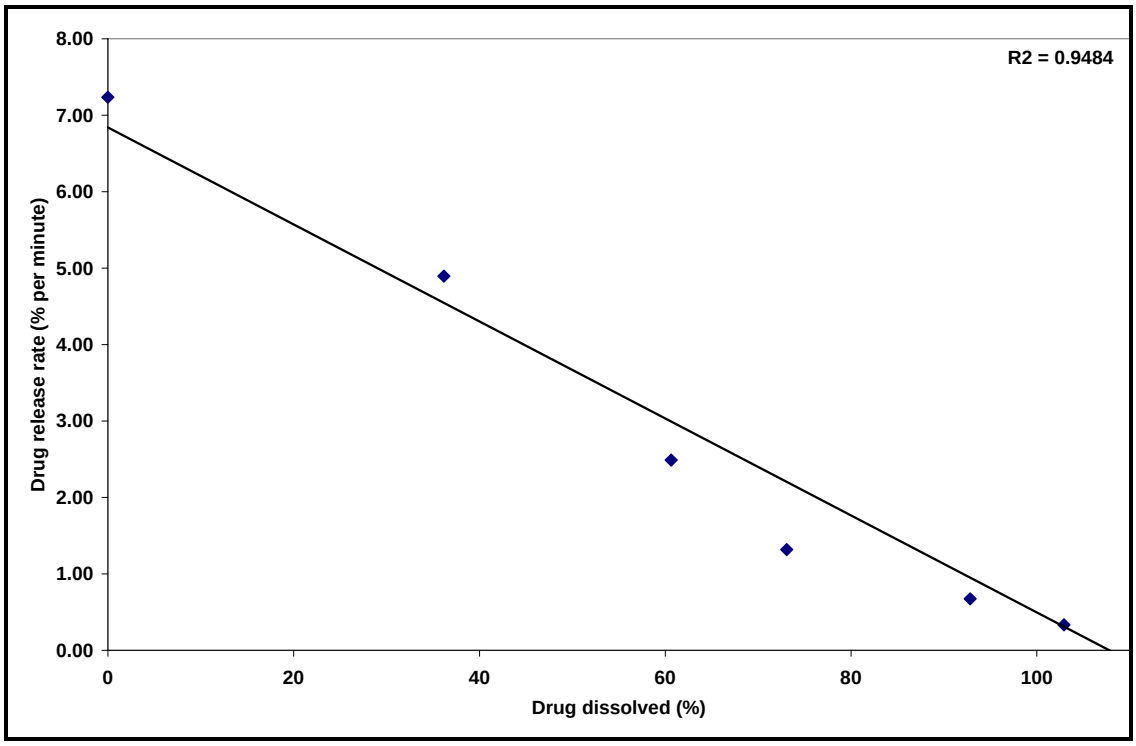

Figure 6.3: A plot of the rate of drug release as a function of the percentage of drug dissolved.

The plot of the rate of drug release as a function of the percentage of drug dissolved exhibited a correlation coefficient R^2, of 0.9484.

6.1.3 Matrix: Dissolution

The differences between the initial mefloquine amount in the centre and the amount of mefloquine remaining in the centre ($W_0^{1/3} - W^{1/3}$) and time were investigated to determine if the release of mefloquine was subjected to the dissolution of drug particles in the dissolution medium.

6.1.3.1 Results and discussion

According to the law of the cube roots, a linear relationship would exist between $W_0^{1/3} - W^{1/3}$ and time if drug release is subjected to the dissolution of drug particles in the dissolution medium (Ritchel & Udeshi, 1987:739). For spherical particles,

$W_0^{1/3}$ is the initial drug amount in the centre, and $W^{1/3}$ is the amount of drug remaining in the centre. The processed experimental dissolution data is given in the annexure, *Table 9.4* (c) and is graphically represented in *Figure 6.4*.

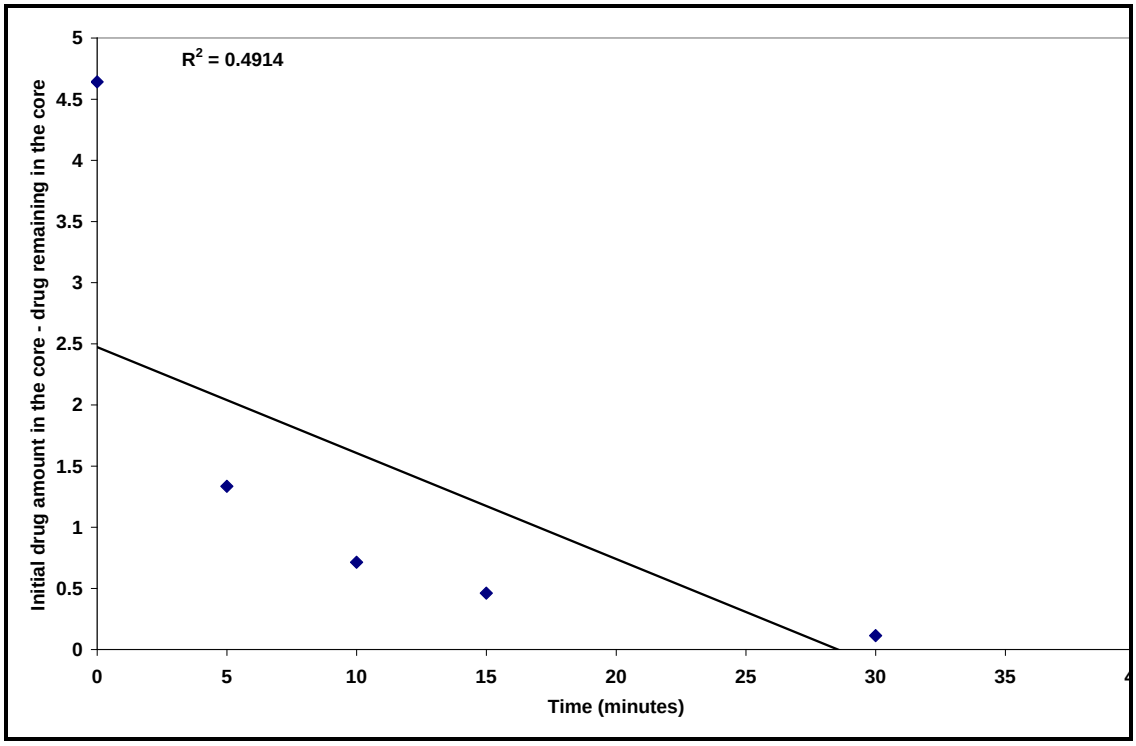

Figure 6.4: $W_0^{1/3} - W^{1/3}$ ***as a function of time.***

The correlation coefficient, R^2, of the graph presented in *Figure 6.4*, of the relationship between $W_0^{1/3} - W^{1/3}$ and time was 0.4914.

6.1.4 Matrix: Diffusion

The data obtained from the dissolution of mefloquine was utilised to determine whether the release of mefloquine was subjected to diffusion from an inert matrix.

6.1.4.1 Results and discussion

The Higuchi equation (1963:1145) explains drug release, dependent on diffusion, out of an inert matrix. The Higuchi equation can be described as:

Equation 6.4

$$q = \{(2C_o - C_s)C_s Dt\}^{1/2}$$

Where:

q	=	amount of drug released
C_o	=	initial concentration of drug present in base
C_s	=	solubility of drug in base
D	=	diffusion coefficient of drug in base
t	=	time

The Higuchi equation can be transformed to *Equation 6.5* (Schwartz *et al*., 1968:275):

Equation 6.5

$$Q' = KSt^{1/2}$$

A linear relationship would exist between the percentage of drug dissolved and the square root of time if the drug release is subjected to diffusion from an inert matrix. The processed experimental dissolution data is given in the annexure, *Table 9.4* (e) and is graphically represented in *Figure 6.5*.

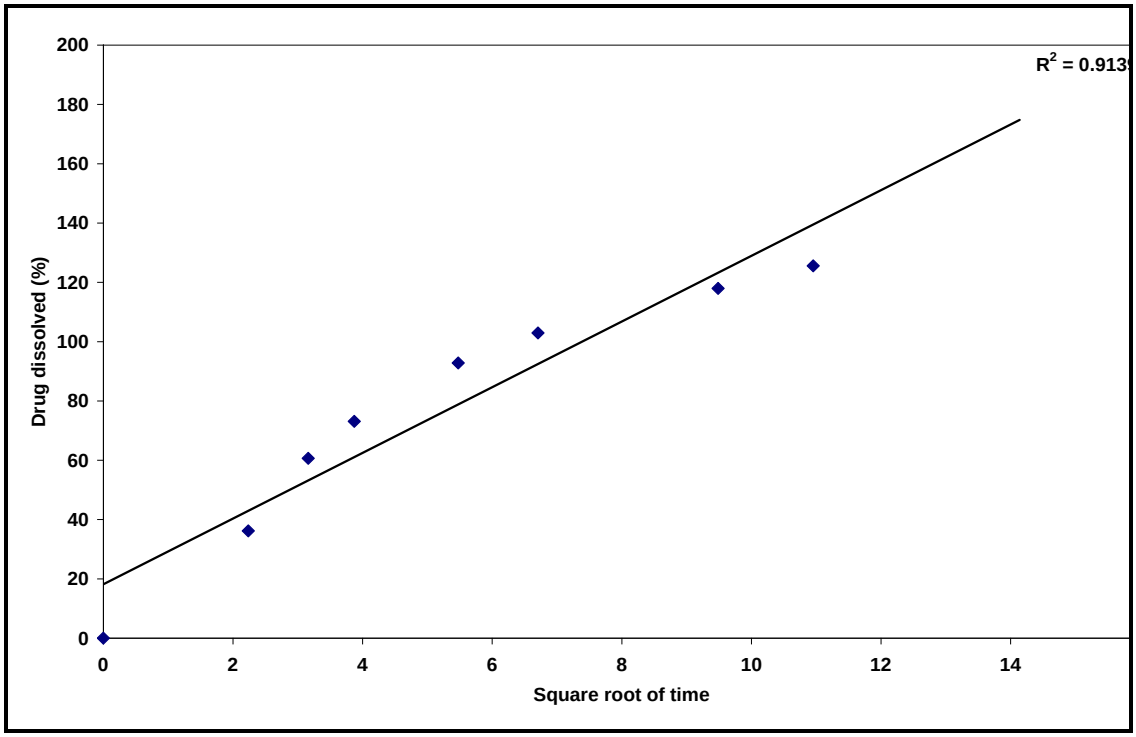

Figure 6.5: The percentage of drug dissolved as a function of the square root of time.

Equation 6.5 can logarithmically be presented as *Equation 6.6*:

Equation 6.6

$$\log Q' = \log K + \frac{1}{2}\log t$$

To provide an additional test for drug diffusion derived from a matrix a graphic representation of *log Q'* as a function of *log t* from the processed experimental dissolution data is given in the annexure, *Table 9.4* (f). The data is graphically represented in *Figure 6.6* (R^2 = 0.7680).

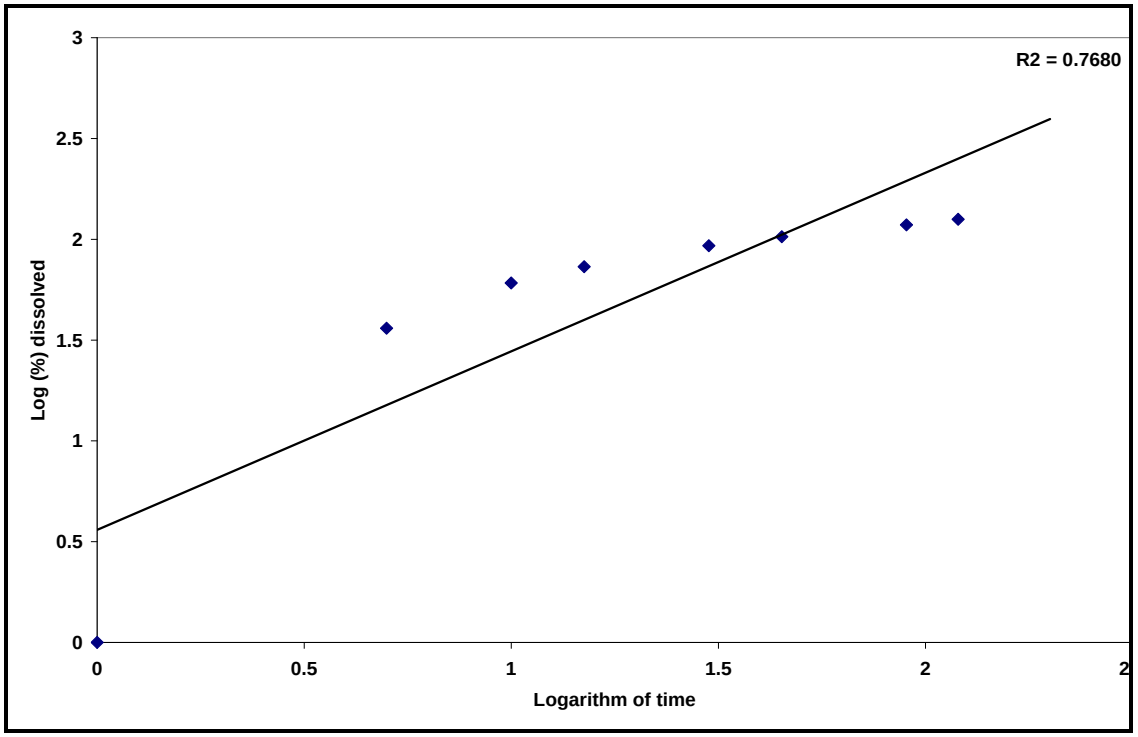

Figure 6.6: The logarithm of percentage of drug dissolved as a function of the logarithm of time.

The graph of the percentage of drug dissolved as a function of the square root of time, exhibited a correlation coefficient, R^2, of 0.9139. The graph of the $log\ Q'$ as a function of the logarithm of time, exhibited a correlation coefficient, R^2, of 0.7680. The R^2 value of 0.7680 is indicative of a weak correlation for the diffusion of mefloquine from an inert matrix.

6.1.5 Artesunate

6.1.5.1 Results and discussion

The dissolution profile of artesunate was fitted to the various models described in this chapter. The dissolution profile of artesunate, under the same conditions as mefloquine, had no linearity with any of the various models, and can indicative of artesunate having two different release stages during the dissolution tests.

6.2 SUMMARY

The R^2-values are summarised in *Table 6.1*. No linear dependency for zero-order drug release was observed from *Figure 6.1* for the percentage of mefloquine released against time (R^2 = 0.7047).

The linearity between the logarithm of the amount of drug remaining and time was investigated to determine whether drug release was subjected to first order kinetics. The R^2 value obtained from the dissolution data was 0.9908. The linearity of the release profile of a drug diffusing from an inert matrix was tested (R^2 = 0.9139). It is clear that there is a legitimate linear relationship for both first order kinetics and the matrix model based on diffusion for the release of mefloquine from the double fixed-dose combination tablets.

Additional tests were done to differentiate the two matrix models from each other (Donbrow & Benita, 1982:79). For first order release the rate of drug release is proportionate to the percentage of drug dissolved. Linearity was present between the rate of release and the percentage of drug released (R^2 = 0.9484).

Table 6.1: Summary of R^2-values.

Release model	R^2	Data points
Zero-order	0.7047	8
First order		
$\log W = \log W_0 + kt/2.303$	0.9908	8
$dQ'/dt = kW_0 - kQ'$	0.9484	6
Matrix: diffusion		
$Q' = KSt^{1/2}$	0.9139	8
$\log Q' = \log K + \frac{1}{2}\log t$	0.7680	8
Matrix: dissolution		
$W_0^{1/3} - W^{1/3}$	0.4914	8

A test was conducted to establish whether drug dissolution were the rate limiting factor in the dissolution medium, according to the law of the cube roots. A weak linear relationship existed between $W_0^{1/3} - W^{1/3}$ and time (R^2 = 0.4914). Thus the dissolution of mefloquine is not the release rate limiting factor from the double fixed-dose combination tablets.

The correlation coefficients were generally closer to R^2 = 0.999 for the tests conducted to identify matrix models subjected to diffusion release. A linear correlation is present between the percentage of drug released and the square root of time (R^2 = 0.9139). However, a less linear relationship was found between the logarithm of the amount of drug released and the logarithm of time (R^2 = 0.7680). It can be concluded from the preparation technique utilised and from the spherical granules that were obtained, that mefloquine release took place passing through a porous system and as a result of a combination of diffusion through a polymer and diffusion through pores in the system (Peppas, 1985:111; Siepmann & Göpferich, 2001:231). The presence of the acrylic polymer Eudragit® L100 might explain the above mentioned fact.

It can be concluded that the release of mefloquine from the double fixed-dose combination tablets is in accordance with the first order model where diffusion is the rate limiting factor. The influence of the physical properties of the mefloquine granule on drug release would be of value for future investigations. The dissolution profile of artesunate failed to fit any of the described models and can be the result of the enteric coated artesunate granules and the secondary release of artesunate from a medium with a pH of 6.8.

7. CHAPTER SEVEN

SUMMARY

More than 250 million people worldwide experience a malarial illness annually. Additionally, the development of resistance to antimalarial drugs by *Plasmodium falciparum* poses a major threat to the tropical areas of the world. Recent evidence indicates that mefloquine is more effective when used in combination with artesunate. Although fixed-dose combination formulations are technically difficult to design, a fixed-dose combination of artesunate and mefloquine may improve medication compliance by reducing the treatment burden of patients.

Artesunate and mefloquine raw material lacked the desired micromeritic properties of pharmaceutical excipients intended for direct compression and therefore granulation was included in the manufacturing process. A method for the manufacturing of an original compressible ACT powder mixture was developed. The parameters contributing to successful granulation were evaluated and the influences of the various factors on the granulation of artesunate and mefloquine were described. Optimum formulations and processes were identified. An HPLC method was developed to verify the presence of, and quantify the concentration of artesunate and mefloquine during dissolution studies.

The wet granulation technique, used for the size enlargement of particles, rendered spherical artesunate and mefloquine granules that could be compressed, unlike the unprocessed raw APIs. During a control experiment, artesunate and mefloquine raw powder mixtures failed to be compressed into tablets with the use of a Cadmach® eccentric press. However, it was proved that a granulated artesunate and mefloquine mixture, blended with specific excipients, had free-flowing and dust free properties. Round biconvex tablets with an average hardness of greater than 240 Newton were compressed, which resulted in tablets with weight variations within pharmacopoeial limits.

SEM micrographs provided explanations for the inferior flowability results obtained of the investigated raw APIs. Particle size analysis and SEM micrographs confirmed that larger, more consistently sized particles of the manufactured granulated APIs

contributed to enhanced micromeritic properties. The mixture was compressed successfully with the addition of Avicel® PH200 and magnesium stearate.

The tablets complied with various tests required by the BP and USP. Tablet friability of less than the recommended 1% of the BP was observed to withstand every day handling. In addition, the tablets had adequate weight variation with a %RSD of 0.803 and well within the limits prescribed by the pharmacopoeias. The tablets had sufficient hardness and tensile strength to ultimately withstand normal handling procedures. Furthermore, the tablets passed the BP test for disintegration by disintegrating in 37 ± 0.5°C distilled water in an average time of 276.6 seconds and well within the set 15 minute time limit.

The APIs were stable during and after the manufacturing process. The granulation and tableting processes proved safe and compatible for both the APIs, given the unchanged peaks obtained from the X-ray powder diffraction and infrared absorption spectroscopy prior to and after the manufacturing processes. No peaks of degradation were observed, indicating compatibility between all the APIs and excipients during and after the manufacturing processes of the double fixed-dose combination.

An HPLC method from available literature was adapted and validated for analytical procedures. Dissolution studies according to a USP method were done to verify and quantify the release of the APIs in the double fixed-dose combination.

The initial drug dissolution rates of the tablets were satisfactory. A total of 29.4% of artesunate dissolved within the first two hours of simulated gastric residence time and the rest of the observable concentration after two hours in pH 6.8. Mefloquine revealed complete dissolution within 45 minutes. Complete artesunate dissolution data was limited in these studies due to the fact that artesunate is rapidly converted to dihydroartemisinin (Tan, 2009:10). As a future investigation, it would be advantageous if dihydroartemisinin could be determined with the same methods used for assay. However, the methods require further development to accommodate related substances.

Various tests were conducted to establish the type of mefloquine and artesunate release from the fixed-dose combination as it was necessary to ensure a rapid release of mefloquine and to refine the manufacturing process for future formulations. The correlation coefficients were acceptable for the tests conducted to identify first order release with regards to mefloquine. A linear correlation was present between the percentage of drug released and the square root of time (R^2 = 0.943). Additionally, a linear relationship was found between the logarithm of the amount of drug released and the logarithm of time (R^2 = 0.917). With a slope of 0.659, it can be concluded that from the preparation technique utilised and from the spherical granules that were obtained, that drug release took place passing through a porous system and as a result of a combination of diffusion through a polymer and diffusion through pores in the system (Peppas, 1985:111; Siepmann & Göpferich, 2001:231).

It can be concluded that the release of mefloquine from the tablets is in accordance with the matrix first order model where diffusion is the rate limiting factor, presenting key information for the refinement of future double fixed-dose combination formulations.

A concept pre-clinical phase, double fixed-dose combination solid oral dosage form for artesunate and mefloquine was developed considering that the world is in dire need of new double fixed-dose combinations against malaria. In view of the amount of excipients and manufacturing processes used, a practical ACT concept formulation was developed. A concept double fixed-dose combination of artesunate and mefloquine was designed to be taken once daily for three days for the treatment of uncomplicated malaria with the additional purpose to ease patient compliance and prohibit the errant use of artemisinin monotherapy.

The pharmaceutical quality by design concept and the fractional factorial design served as useful guides, resulting in effective manufacturing processes and an end product.

The granules resulting from the size enlargement and coating techniques could be predicted to some extent and, in the event of future experiments be controlled. Streamlining the double fixed-dose combination tablet formula, accelerated stability

testing, clinical studies, and espesecially the improvement of the HPLC assay method for artesunate would be essential future scenarios.

7.1 RECOMMENDATIONS

This study proposes the following recommendations for future investigations:

- The utilisation of the differential scanning calorimetry (DSC) technique in order to complement the IR and XRPD data obtained from the 2FDC of artesunate and mefloquine.

- An investigation in order to determine the *in vivo* efficacy of the artesunate and mefloquine 2FDC.

- An investigation to design other oral fixed-dose combinations of antimalarials utilising PAT.

- An investigation to design oral fixed-dose combinations for other diseases utilising PAT.

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9. ANNEXURES

9.1 DISSOLUTION DATA

Table 9.1: The dissolution data for artesunate from the double fixed-dose combination tablet.

Time	Artesunate % Dissolved					
(min)	TABLET 1	TABLET 2	TABLET 3	TABLET 4	TABLET 5	TABLET 6
0	0	0	0	0	0	0
5	26.389	28.307	25.607	27.007	27.502	24.300
10	30.918	30.064	28.488	30.176	26.506	28.310
15	28.559	32.383	30.359	29.450	30.437	28.872
30	29.512	28.931	29.648	28.043	33.160	28.307
45	28.029	28.695	26.680	28.504	29.458	33.101
90	29.653	25.900	30.596	25.160	33.903	31.045
120	33.379	27.340	40.162	31.667	33.518	33.830
130	43.838	44.610	49.409	42.246	41.812	44.047
150	49.334	60.545	54.877	58.757	56.709	49.119
AVE DRi[1]	**0.547**					
AVE AUC[2]	**7174.453**					

[1] *DRi = initial dissolution rate (%.min^{-1})*

[2] *AUC = area under curve (%.min)*

Table 9.2: The dissolution data for mefloquine from the double fixed-dose combination tablet.

Time	Mefloquine % Dissolved					
(min)	TABLET 1	TABLET 2	TABLET 3	TABLET 4	TABLET 5	TABLET 6
0	0	0	0	0	0	0
5	41.127	41.741	42.719	9.067	43.590	38.797
10	58.307	61.698	60.961	63.914	60.248	58.724
15	70.199	73.178	70.668	76.277	74.301	73.885
30	91.939	92.596	90.914	95.033	92.899	93.560
45	102.109	104.321	97.696	105.579	102.937	104.879
90	118.144	118.215	115.718	116.971	117.882	120.573
120	125.286	125.716	N/A	125.910	124.283	126.467
AVE *DRi*[1]	**0.305**					
AVE *AUC*[2]	**11999.76**					

[1]*DRi = initial dissolution rate (%.min^{-1})*

[2]*AUC = area under curve (%.min)*

Table 9.3: Standard deviations of the dissolution test results.

	Artesunate	Mefloquine	Artesunate	Mefloquine
Time (min)	**STDEV**	**STDEV**	**%RSD**	**%RSD**
0	0	0	0	0
5	1.43	13.38	5.38	36.99
10	1.62	2.06	5.57	3.40
15	1.39	2.30	4.63	3.15
30	1.86	1.41	6.27	1.52
45	2.18	2.86	7.48	2.78
90	3.31	1.61	11.26	1.36
120	4.14	0.82	12.42	0.65
130	2.72	3.54	6.13	5.67
150	4.78	2.26	8.72	6.76

9.2 DRUG RELEASE MECHANISM DATA

Table 9.4: Determining the drug release mechanism of mefloquine hydrochloride from a fixed-dose combination.

a)	
First order I	
Time (minutes)	**Log % Dissolved**
0	0.000
5	8.189
10	11.031
15	12.400
30	14.326
45	39.660
90	63.657
120	69.429

b)	
First order II	
$kW_0 - kQ'$	dQ'/dt
0	7.23
36.174	4.89
60.642	2.49
73.085	1.32
92.923	0.67
102.920	0.33
117.917	0.25
125.532	N/d

c)	
Matrix dissolution	
Time (minutes)	$W_0^{1/3} - W^{1/3}$
0	0.000
5	3.307
10	3.929
15	4.180
30	4.528
45	4.687
90	4.903
120	5.007

d)	
Zero-order	
Time (minutes)	**Dissolved (%)**
0	0
5	36.174
10	60.642
15	73.085
30	92.823
45	102.920
90	102.571
120	102.292

e)	
Matrix diffusion I	
$\sqrt{\text{Time}}$	**Dissolved (%)**
0	0
2.236	36.174
3.162	60.642
3.872	73.085
5.477	92.823
6.708	102.920
9.486	102.571
10.954	102.292

f)	
Matrix diffusion II	
Log Time	**Log % Dissolved**
0	0.000
0.699	8.189
1.000	11.031
1.176	12.400
1.477	14.326
1.653	39.660
1.954	63.657
2.079	69.429

9.3 MASS VARIATION

Table 9.5: Mass variation test results of the double fixed-dose combination tablet formulation.

Tablet	Mass (g)	% Variation	Result
1	1.041	0.952	Complied
2	1.043	0.752	Complied
3	1.043	0.752	Complied
4	1.044	0.657	Complied
5	1.045	0.609	Complied
6	1.045	0.562	Complied
7	1.045	0.562	Complied
8	1.045	0.562	Complied
9	1.046	0.467	Complied
10	1.047	0.419	Complied
11	1.048	0.286	Complied
12	1.048	0.257	Complied
13	1.052	-0.085	Complied
14	1.053	-0.199	Complied
15	1.055	-0.418	Complied
16	1.060	-0.865	Complied
17	1.063	-1.151	Complied
18	1.064	-1.246	Complied
19	1.064	-1.246	Complied
20	1.068	-1.627	Complied
Total	**21.0181**		
Average	**1.0509**		
STDEV	**0.008**		
%RSD	**0.803**		

9.4 VALIDATION OF AN HPLC ASSAY METHOD:

9.4.1 Determination of artesunate and mefloquine hydrochloride content

The aim was to determine the content in a double fixed-dose combination product containing artesunate and mefloquine hydrochloride.

9.4.2 Specification

The tablet must contain the following:

- Artesunate 200 mg (180 - 220 mg).
- Mefloquine hydrochloride 400 mg (360 - 440 mg)

9.4.3 Summary

In *Table 9.6* a summary of the validation results is given.

Table 9.6: Summary of validation results.

Parameter	Acceptance criteria	Results
Specificity	The placebo should not generate any peaks that will interfere with the determination of the active ingredients. Extra peaks formed under stress conditions should be noticeable from those of the active ingredients.	Complies
Linearity and Range	The method is linear over the range: 20 - 150 % of the expected sample concentration. R^2 is not less than 0.99.	artesunate: Linear from 50 - 250 µg/ml (25 - 125 % of expected sample concentration) R^2: 0.9999 Complies mefloquine: Linear 1 - 403 µg/ml (1 - 150 % of expected sample concentration) R^2: 0.9999
Accuracy (Recovery)	Recovery must be between 98%- 102%.	artesunate: 101% mefloquine hydrochloride: 98%
Precision (Repeatability)	RSD of 2.0% or less.	Complies

9.5 THE EXPERIMENTAL DATA OF THE MICROMERITIC PROPERTIES OF THE POWDERS BEFORE THE SIZE ENLARGEMENT PROCESS.

Table 9.7: Artesunate.

	CARR's index	Hausner ratio	Angle of repose (º)	Flow rate ($g.sec^{-1}$)	Bulk volume (cm^{-3})	Tapped volume (cm^{-3})	Tapped density ($g.cm^{-3}$)	Bulk density ($g.cm^{-3}$)
Sample 1	14.00	0.86	27.3	Fail	58.5	50.1	0.50	0.43
Sample 2	15.66	0.84	26.4	Fail	59.4	50.1	0.50	0.42
Sample 3	13.82	0.86	28.2	Fail	57.9	49.9	0.50	0.43
AVE	14.49	0.86	27.3	Fail	58.60	50.03	0.50	0.43
STDEV	1.01	0.01	0.9	Fail	0.75	0.12	0.00	0.01
%RSD	6.99	1.19	3.3	Fail	1.29	0.23	0.23	1.29

Table 9.8: Mefloquine

	CARR's index	Hausner ratio	Angle of repose (º)	Flow rate ($g.sec^{-1}$)	Bulk volume (cm^{-3})	Tapped volume (cm^{-3})	Tapped density ($g.cm^{-3}$)	Bulk density ($g.cm^{-3}$)
Sample 1	21.74	0.78	23.1	Fail	142.1	110	0.23	0.18
Sample 2	22.08	0.78	22.5	Fail	143.1	111.5	0.22	0.17
Sample 3	22.45	0.78	24.1	Fail	141.2	109.5	0.23	0.18
AVE	22.09	0.78	23.2	Fail	142.13	110.33	0.23	0.18
STDEV	0.36	0.00	0.8	Fail	0.95	1.04	0.00	0.00
%RSD	1.61	0.46	3.5	Fail	0.67	0.94	0.94	0.67

9.6 THE EXPERIMENTAL DATA OF THE MICROMERITIC PROPERTIES OF AVICEL PH200.

Table 9.9: Avicel PH200.

	CARR's index	Hausner ratio	Angle of repose (º)	Flow rate ($g.sec^{-1}$)	Bulk volume (cm^{-3})	Tapped volume (cm^{-3})	Tapped density ($g.cm^{-3}$)	Bulk density ($g.cm^{-3}$)
Sample 1	32.61	0.67	23.0	6.15	80.0	54.5	0.46	0.31
Sample 2	32.21	0.68	23.9	5.95	79.8	54.1	0.46	0.31
Sample 3	30.80	0.69	24.4	5.97	80.2	55.5	0.45	0.31
AVE	31.87	0.68	23.8	6.02	80.00	54.70	0.46	0.31
STDEV	0.95	0.01	0.7	0.11	0.20	0.72	0.01	0.00
%RSD	2.98	1.40	3.0	1.83	0.25	1.32	1.31	0.25

9.7 THE EXPERIMENTAL DATA OF THE MICROMERITIC PROPERTIES OF THE RAW POWDERS AFTER THE SIZE ENLARGEMENT PROCESS.

Table 9.10: Enteric coated artesunate. Artesunate (77.0% w/w) and Eudragit® L100 (23.0% w/w).

	CARR's index	Hausner ratio	Angle of repose (º)	Flow rate ($g.sec^{-1}$)	Bulk volume (cm^{-3})	Tapped volume (cm^{-3})	Tapped density ($g.cm^{-3}$)	Bulk density ($g.cm^{-3}$)
Sample 1	23.26	0.77	22.2	1.76	75.0	60.0	0.42	0.33
Sample 2	23.68	0.76	22.1	1.85	76.0	58.0	0.43	0.33
Sample 3	21.62	0.78	21.6	1.92	74.0	58.0	0.43	0.34
AVE	22.85	0.77	22.0	1.84	75.00	58.67	0.43	0.33
STDEV	1.09	0.01	0.3	0.08	1.00	1.15	0.01	0.00
%RSD	4.76	1.41	1.5	4.35	1.33	1.97	1.95	1.33

Table 9.11: Mefloquine granules. The granules contained mefloquine (94.0% w/w), Kollidon® VA64 (5.0% w/w) and Ac-Di-Sol® (1.0% w/w).

	CARR's index	Hausner ratio	Angle of repose (º)	Flow rate ($g.sec^{-1}$)	Bulk volume (cm^{-3})	Tapped volume (cm^{-3})	Tapped density ($g.cm^{-3}$)	Bulk density ($g.cm^{-3}$)
Sample 1	17.77	0.82	19.6	2.29	50.5	41.5	0.60	0.50
Sample 2	17.37	0.83	20.2	2.38	50.1	41.4	0.60	0.50
Sample 3	16.63	0.83	21.6	2.19	49.9	41.6	0.60	0.50
AVE	17.26	0.83	20.5	2.29	50.17	41.50	0.60	0.50
STDEV	0.58	0.01	1.0	0.10	0.31	0.10	0.00	0.00
%RSD	3.35	0.70	5.0	4.16	0.61	0.24	0.24	0.61

Table 9.12: Mefloquine granules. The granules contained mefloquine (94.0% w/w), Kollidon® 30 (5.0% w/w) and Ac-Di-Sol® (1.0% w/w).

	CARR's index	Hausner ratio	Angle of repose (º)	Flow rate ($g.sec^{-1}$)	Bulk volume (cm^{-3})	Tapped volume (cm^{-3})	Tapped density ($g.cm^{-3}$)	Bulk density ($g.cm^{-3}$)
Sample 1	17.77	0.82	21.1	1.00	51.0	41.5	0.60	0.49
Sample 2	16.67	0.83	22.2	0.95	51.0	42.5	0.59	0.49
Sample 3	18.27	0.82	19.9	1.05	52.0	42.5	0.59	0.48
AVE	17.57	0.82	21.1	1.00	51.33	42.17	0.59	0.49
STDEV	0.82	0.01	1.2	0.05	0.58	0.58	0.01	0.01
%RSD	4.67	1.00	5.5	5.00	1.12	1.37	1.38	1.12

9.8 HPLC DATA

Data File C:\HPCHEM\1\DATAH1\HERMAN\14MEI001.D Sample Name: STD

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Sample Name     : STD                          Location : Vial 1
Acq. Operator   : HERMAN                          Inj :   1
                                               Inj Volume : 20 µl
Acq. Method     : C:\HPCHEM\1\METHODS1\ART1.M
Last changed    : 5/7/2010 8:04:13 AM by KRISTIN
Analysis Method : C:\HPCHEM\1\METHODS1\ART1.M
Last changed    : 9/9/2010 9:07:01 AM by MONIQUE
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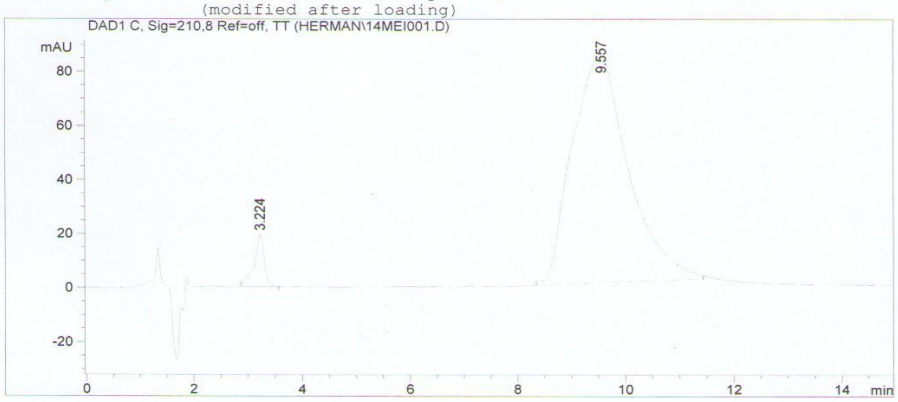


```
                          Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=210,8 Ref=off, TT

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.224 BB    0.1785  246.17348   19.34454   3.8847
   2   9.557 BB    0.9538 6090.80176   84.01260  96.1153

Totals :                  6336.97523  103.35715

 Results obtained with enhanced integrator!

                          *** End of Report ***
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H 1 9/9/2010 9:07:05 AM MONIQUE Page 1 of 1

Figure 9.1: The chromatogram of the reference standards of artesunate (small peak on the left-hand side of the chromatogram) and mefloquine (larger peak on the right-hand side of the chromatogram).

Table 9.13: Tablet composition of the 2FDC.

Compound	Amount per tablet (% w/w)
Ac-Di-Sol®	1.0
Artesunate	18.4
Avicel® PH200	31.4
Eudragit® L 100	5.5
Kollidon® VA64	2.1
Magnesium stearate	3.0
Mefloquine hydrochloride	38.6
Total	100.0

10. PRESENTATION AT THE 9th WORLD MEETING ON PHARMACEUTICS, BIOPHARMACEUTICS AND PHARMACEUTICAL TECHNOLOGY, 31st MARCH to 3rd APRIL 2014

FORMULATION OF A DOUBLE-FIXED DOSE COMBINATION (2-FDC) SOLID ORAL DOSAGE FORM CONTAINING ARTESUNATE AND MEFLOQUINE

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INTRODUCTION

More than 250 million people worldwide experience a malarial illness annually. Furthermore, between one and three million deaths are caused by malaria in sub-Saharan Africa (Balint, 2001:261). It is also important to keep in mind that parasite resistance to existing antimalarial drugs has reached alarming levels in Africa and Southeast Asia. Recent evidence indicates that mefloquine is more effective when used in combination with an artemisinin compound. Artemisinin and its derivatives represent a class of antimalarials that is effective against drug-resistant *Plasmodium falciparum* strains and, therefore, they are of utmost importance in the current antimalarial campaign (WHO, 2010:17). Artesunate, an artemisinin derivative with a problematic short half-life, is effective against drug-resistant *Plasmodium falciparum* strains and is of significance in the current antimalarial campaign. A fixed-dose combination of mefloquine and artesunate may improve medication compliance by reducing the treatment burden of patients. Although fixed-dose combination formulations are technically difficult to design, they are strongly preferred and recommended over blistered co-packaged or loose tablet combinations to promote adherence to treatment and to reduce the potential selective use of the medicines as monotherapy (WHO, 2010:17).

In this paper, the possibility of formulating a double fixed-dose combination (2-FDC) tablet of artesunate and mefloquine that exhibited immediate release of mefloquine and a dual release of artesunate was investigated.

EXPERIMENTAL METHODS

Materials

Mefloquine hydrochloride (Lot: IF-ME-080830, DB Fine Chemicals, South Africa), Artesunate (Lot: IF-AR-080830, IFFECT CHEMPHAR Co., China), Avicel® PH200 (Lot: M939C, FMC Corproration, Ireland), Magnesium stearate (Lot: 472131, Kirsch Pharma, South Africa), Kollidon® VA64 (Lot: 62-8826, BASF, South Afrca), Ac-Di-Sol® (Lot: T017C, FMC Corproration, Ireland), Eudragit® L100 (Lot: B051203084, Röhm GmbH & Co, Germany). Al other reagents were of analytical grade and used as received.

Tablet formulation

Artesunate

An artesunate and Eudragit® L100 mixture was prepared by mixing and subsequently coated by spraying ethanol and simultaneously mixing the mixture with a pestle in a mortar. The obtained granules were forced through a 5 mesh stainless steel sieve, dried, and kept away from light at 25 ± 0.5°C for 24 hours. The primary granules were forced through a 5 mesh stainless steel sieve for a second time to produce small free-flowing granules.

Mefloquine

Mefloquine, Ac-Di-Sol® and Kollidon® VA64 were mixed and subsequently sprayed with ethanol in a mortar and granulated through a 5 mesh stainless steel sieve. The primary granules were dried and kept away from light at 25 ± 0.5°C for 24 hours. The granules were forced through a 5 mesh stainless steel sieve to produce secondary granules.

Mixing procedures

All mixing procedures employed a Turbula®-mixer (model T2B, W.A. Bachofen, Switzerland) at 69 rpm for 10 minutes.

Combination

The double fixed-dose combination mixture of mefloquine, artesunate and the relevant excipients were compressed immediately after mixing using a Cadmach® eccentric press (Ahmedabad, India). After manufacturing was completed, tablets were stored in sealed glass containers away from light at room temperature for 24 hours preceding further analysis.

X-ray powder diffraction

X-ray powder diffraction patterns were obtained at room temperature using a Bruker D8 Advance diffractometer (Bruker, Karlsruhe, Germany).

The XRPD traces of the powders were compared with regard to peak position and relative intensity, peak shifting and the presence or lack of peaks in certain regions of º2θ values.

Infrared spectroscopy

IR-spectra were recorded on a Nicolet Nexus 470-FT-IR spectrometer (Nicolet instrumentation corporation, Madison, USA) over a range of 0 – 4000 cm^{-1}. Potassium bromide was used as background material. The main absorptions in the IR–spectral results of the samples were compared to determine possible

significant differences with regard to polymorphic form or polymorphic modifications.

Dissolution studies

The USP dissolution method (2011) for delayed-release dosage forms was used as a guideline.

Dissolution tests were performed in an Erweka DT6R (Erweka, Heusenstamm, Germany) dissolution apparatus. A thermostat regulated the temperature of the medium at 37.0 ± 0.5°C. The dissolution studies were initiated in an acid dissolution medium, consisting of 750 cm^3 of 0.1 N HCl (pH ~ 1.20). After 2 hours of operation in 0.1 N HCl, the final dissolution samples of the acidic medium stage were withdrawn prior to adjustment of the pH. A volume of 250 ml of 0.20 M tribasic sodium phosphate that was equilibrated to 37.0 ± 0.5°C was added to the dissolution beaker within 5 minutes and adjusted if necessary to a pH of 6.8 ± 0.05 with 2 N HCl or 2 N sodium hydroxide. The standardised USP (2011) paddle-method was used in all studies and paddles were rotated at a constant speed of 100 rpm. Samples of 3 cm^3 were withdrawn at t = 5, 10, 15, 30, 45, 90, 120, 130 and 150 minutes. A validated HPLC method for the analysis of mefloquine and artesunate was used to quantify the concentration of mefloquine and artesunate in the dissolution samples.

RESULTS AND DISCUSSION

Tablet formulation

The composition of the 2-FDC tablet are given in table 1.

Compound	Quantity per tablet (g)
Ac-Di-Sol®	0.01
Artesunate	0.200
Avicel® PH200	0.342
Eudragit® L 100	0.060
Kollidon® VA64	0.022
Magnesium stearate	0.033
Mefloquine hydrochloride	0.415
Tablet weight	**1.082**

Table 1: Composition of the 2-FDC tablet

X-ray powder diffraction

The XRPD traces of the mefloquine and artesunate mixture prior to granulation and tableting, and of the tablets after being exposed to the conditions under which granulation and tableting occurred, were identical with regard to peak position and relative intensity, peak shifting and the presence or lack of peaks in certain regions of º2θ values.

Infrared spectroscopy

The IR spectra of the double fixed-dose combination tablets was compared to the IR spectra of the double fixed-dose combination mixture and the spectrums displayed a position and intensity correlation.

Dissolution studies

The mean dissolution profile of the artesunate and mefloquine from the 2-FDC tablets are presented in figures 1 and 2 respectively.

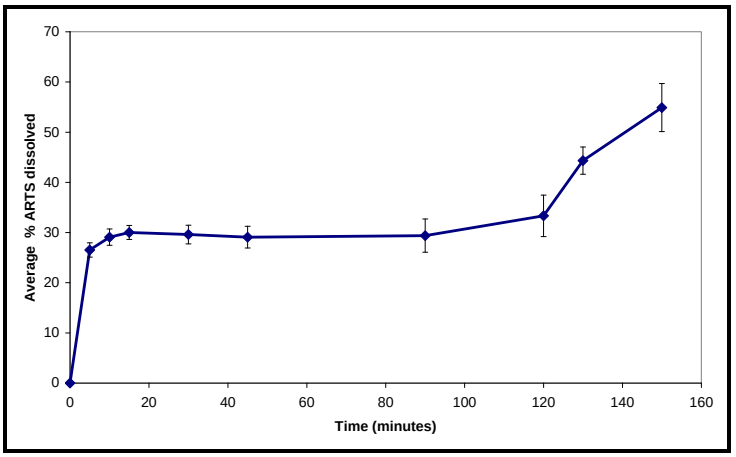

Figure 1: Mean dissolution profile of 2-FDC tablets indicating artesunate (ARTS) release.

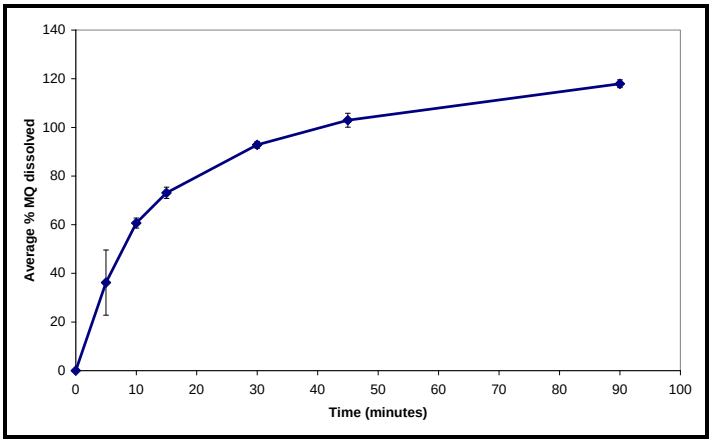

Figure 2: Dissolution profiles of 2-FDC tablets indicating mefloquine (MQ) release.

CONCLUSION

A 2-FDC tablet containing mefloquine and artesunate was successfully formulated exhibiting excipient and API compatibility. Immediate release of the total mefloquine dose in an acidic environment was illustrated and a dual release for artesunate was illustrated.

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3. (WHO) World Health Organisation. Guidelines for the treatment of malaria - 2nd ed. Geneva: WHO Press. [Web:] http://whqlibdoc.who.int/publications/2010/9789241547925_eng.pdf (2010) [Date of access: 17 November 2010]