Development and evaluation of an oral fixed-dose triple combination dosage form for artesunate, dapsone and proguanil

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This dissertation is dedicated to my parents,

Gawie and Emily van der Merwe

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
BP	British Pharmacopoeia
CDC	Center for Disease Control and Prevention
COD	Critical Orifice Diameter
CRT	Chloroquine resistance transporter
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
DNA	Deoxyribonucleic acid
DSC	Differential Scanning Calorimetry
FA	Formulation containing Avicel® PH 101 as filler
FG	Formulation containing Granulac® 200 as filler
HPLC	High Pressure Liquid Chromatography
IP	International Pharmacopoeia
IPT	Intermittent preventive treatment
IPTp	Intermittent preventive treatment during pregnancy
mAu	Milli absorption units
TB	Tuberculosis
USP	United States Pharmacopoeia
WHO	World Health Organization

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ABSTRACT

Malaria is a life-threatening disease caused by *Plasmodium spp* and causes over one million deaths annually. The complex life cycle of the malaria parasite offers several points of attack for the antimalarial drugs. The rapid spread of resistance against antimalarial drugs, especially chloroquine and pyrimethamine-sulphadoxine, emphasises the need for new alternatives or modification of existing drugs. Artemisinin-based combination therapies (ACT's) with different targets prevent or delay the development of drug resistance and therefore have been adopted as first-line therapy by all endemic countries. Proguanil-dapsone, an antifolate combination is more active than pyrimethamine-sulphadoxine and is being considered as an alternative to pyrimethamine-sulphadoxine. Artesunate-proguanil-dapsone is a new ACT that has well-matched pharmacokinetics and is relatively rapidly eliminated; therefore there is a reduced risk of exposure to any single compound and potentially a decreasing risk of resistance. A few studies have been done on a triple fixed-dose combination therapy for malaria treatment and such a combination for artesunate, proguanil and dapsone are not currently investigated, manufactured or distributed. The aim of this study was to develop a triple fixed-dose combination for artesunate, proguanil and dapsone.

The formulation was developed in three phases; basic formulation development, employing factorial design to obtain two possible optimised formulations and evaluating the optimised formulations. During the formulation development the most suitable manufacturing procedure and excipients were selected. A full 2^4 factorial design (four factors at two levels) was used to obtain the optimised formulations. As end-points to identify the optimised formulations, weight variation, friability, crushing strength and disintegration of the tablets, were used. Statistical analysis (one way ANOVA) was used to identify optimal formulations. To identify any interaction between the active pharmaceutical ingredients (API's) and the API's and excipients, differential scanning calorimetry was done. Flow properties of the powder mixtures (of the optimised formulations) were characterised by means of angle of repose; critical orifice diameter (COD); bulk density and tapped density; and flow rate. Tablets of the two optimised powder formulations were compressed. The tablets were evaluated and characterised in terms of weight variation, friability, crushing strength, disintegration and dissolution behaviour. Initial formulation development indicated that wet granulation was the most suitable manufacturing

method. The results from the factorial design indicated that different amounts (% w/w) of the lubricant and binder as well as two different fillers influenced the weight variation, crushing strength and disintegration statistically significant. Two formulations containing two different fillers (microcrystalline cellulose or Avicel® PH 101, and lactose or Granulac® 200) were found to be within specifications and ideal for manufacturing.

Tablets prepared from the FA formulation (formulation containing Avicel® PH 101) complied with the standards and guidelines for weight variation, friability, crushing strength and disintegration as set by the British Pharmacopoeia (BP). Tablets had an average crushing strength of 121.56 ± 0.022 N. Tablets disintegrated within 52.00 seconds and a maximum weight loss of 0.68% occurred during the friability test. Weight variation of the tablets prepared from the FG formulation (formulation containing Granulac® 200) complied with the standards. Average crushing strength was 91.99 ± 6.008 N and the tablets disintegrated within 140.00 seconds. Percentage friability (1.024%) did not comply with the guideline of a percentage friability of less than 1%, however, no cracked or broken tablets were seen.

Dissolution showed that 98, 93 and 94% of artesunate, proguanil and dapsone were respectively released (of the label value) within 15 minutes for the FA formulations. Release of artesunate, proguanil and dapsone for the FG formulation was 62, 85 and 92% for the same time period. The release of the three API's (the FG formulation) increased to 78, 89 and 92%, respectively, after 45 minutes.

Keywords: artemisinin-based combination therapy, artesunate, dapsone, malaria, proguanil, tableting, triple fixed-dose combination therapy, wet granulation

UITTREKSEL

Malaria is 'n lewensbedreigende siekte wat veroorsaak word deur *Plasmodium spp*. Malaria veroorsaak meer as een miljoen sterftes per jaar. Die komplekse lewensiklus van die malaria parasiet bied verskeie setels vir geneesmiddelwerking. Die vinnige verspreiding van weerstand teen malaria-geneesmiddels, veral chlorokien en pirimetamien-sulfadoksien, beklemtoon die noodsaaklikheid vir 'n nuwe alternatief of modifisering van die bestaande middels. Artemisinin-gebaseerde kombinasie terapie (AKT) wat verskillende setels van werking het, voorkom of vertraag die ontwikkeling van weerstand teen die medikasie en daarom is AKT deur al die endemiese lande aanvaar as die eerste linie terapie. Proguanil-dapsoon as antifolaat-kombinasie is meer aktief as pirimetamien-sulfadoksien en word beskou as 'n alternatief vir pirimetamien-sulfadoksien. Artesunaat-proguanil-dapsoon is 'n nuwe AKT wat goed ooreenstem in farmakokinetika en al drie geneesmiddels word redelik vinnig uitgeskei. Gevolglik is daar 'n vermindering in die risiko van blootstelling aan enige enkele bestanddeel en moontlik 'n dalende risiko in weerstand. 'n Paar studies is gedoen op 'n driedubbele vaste-dosis kombinasie terapie vir malaria behandeling en die kombinasie van artesunaat, proguanil en dapsoon is tans nog nie ondersoek, vervaardig of versprei nie. Die doel van hierdie studie was om 'n driedubbele vaste-dosis kombinasie van artesunaat, proguanil en dapsoon te ontwikkel.

Die formulering is in drie fases ontwikkel; basiese formulering, die gebruik van 'n faktoriaal ontwerp om twee optimale formules te identifiseer en die evaluering van die optimale formules. Tydens die ontwikkeling van die formulering is die mees geskikte vervaardigingsmetode en hulpstowwe gekies. 'n 2^4-Faktoriaalontwerp (4 faktore op 2 vlakke) is gebruik om die optimale formules te bepaal. Om die optimale formules te identifiseer is die massavariasie, afsplyting, breeksterkte en disintegrasietyd van die tablette geëvalueer. Statistiese ontleding (een-rigting ANOVA) van die resultate is toegepas in die identifisering van die optimale formules. Differensiële skandeer kalorimetrie is gebruik om enige interaksie tussen die aktiewe farmaseutiese bestanddele (AFB); en die AFB en hulpstowwe te identifiseer. Poeier-vloei eienskappe van die optimale mengsels bestem vir tablettering is gekarakteriseer deur gebruik te maak van die rushoek; kritiese openingsdeursnee; pakkingsdigtheid en skynbare digtheid; asook vloeitempo. Tablette is vervolgens vervaardig van die twee optimale poeier formules. Die eienskappe van die tablette van die twee optimale formules is geëvauleer en ontleed.

Die aanvanklike formuleringstudie het getoon dat nat-granulering die mees geskikte vervaardigings metode was. Die resultate van die faktoriaal-ontwerp het aangedui dat verskillende hoeveelhede (% m/m) van die smeermiddel en bindmiddel sowel as die twee verskillende vulstowwe die massavariasie, breeksterkte en disintegrasietyd statisties betekenisvol beïnvloed het. Resultate het getoon dat twee formules met verskillende vulstowwe (mikrokristallyne sellulose of Avicel® PH 101 en laktose of Granulac® 200) geskik is vir vervaardiging.

Die tablette wat vervaardig is van die FA formulering (formulering wat Avicel® PH101 bevat) het voldoen aan die spesifikasies vir massavariasie, afsplyting en disintegrasie soos gestel in die Britse Farmakopie (BP). Tablette van hierdie formulering het ’n gemiddelde breeksterkte van 121.56 ± 0.022 N gehad. Al die tablette het binne 52.00 sekondes gedisintegreer en ’n maksimum massa-verlies van 0.68% tydens die verbrokkelingstoets getoon. Die massavariasie van die tablette wat berei is van die FG formule (formulering wat Granulac® 200 bevat) het ook aan die aan die spesifikasies vir massavariasie voldoen. Die gemiddelde breeksterkte was 91.99 ± 6.008 N en die tablette het gedisintegreer binne 140.00 sekondes. Die persentasie verbrokkeling (1.024%) het nie aan die gestelde riglyne van minder as 1% voldoen nie, maar aangesien die tablette nie ooglopend gekraak, gebreek of versplinter was nie, kan die persentasie verbrokkeling as aanvaarbaar beskou word indien die breeksterkte-resultate ook in aanmerking geneem word.

Dissolusietoetsing het getoon dat artesunaat, proguanil en dapsoon onderskeidelik 98, 93 en 94% vrystelling (van die etiketwaarde) binne 15 minute vir die FA formulering getoon het. Vrystelling van artesunaat, proguanil en dapsoon vir die FG formulering was onderskeidelik 62, 85 en 92% vir dieselfde tydgleuf. Die vrystelling van die drie aktiewe bestanddele (FG formule) het verhoog na onderskeidelik 78, 89 en 92% na 45 minute.

Sleutelwoorde: artemisinin-gebaseerde kombinasie terapie, artesunaat, dapsoon, malaria, proguanil, tablettering, driedubbele vaste-dosis kombinasie terapie, nat granulering

INTRODUCTION AND AIM OF STUDY

Malaria is considered as the most prevalent parasitic disease in the world and it remains, with AIDS and tuberculosis, one of the three major infective diseases (Santos-Magalhães & Mosqueria, 2010; Lewison & Srivastava, 2008). Malaria is caused by a parasite, *Plasmodium spp,* which commonly infects a certain type of mosquito, *Anopheles*, that feeds on humans. Almost all deaths and severe infections are caused by *Plasmodim falciparum* (CDC, 2011; Ashley *et al.*, 2006). Malaria chemotherapy remains one of the most important measures to reduce malaria disease and mortality.

The rapid spread of *P. falciparum* strains that are resistant to chloroquine and pyrimethamine-sulphadoxine emphasise the need for pharmacological initiatives to counter the resulting increases in malaria mortality and morbidity rates. New antimalarial agents in such initiatives may be derived as novel compounds or as a modification of existing drugs (Dondorp *et al.*, 2010; WHO, 2010; Murambiwa *et al.*, 2011). According to the World Health Organization (WHO, 2010) the recommendation for treatment of uncomplicated *P. falciparum* malaria are an artemisinin-based combination therapy (ACT). All malaria endemic countries have adopted artemisinin-based combination treatment as first-line treatment.

Pyrimethamine-sulphadoxine is still promoted by the WHO as the safest option for preventing malaria during pregnancy (Briand *et al*., 2007; WHO, 2011a) and in infants (Odhiambo *et al*., 2010). Unfortunately, resistance to pyrimethamine-sulphadoxine develops readily and thus there is a pressing need for effective, safe, feasible and affordable drugs that a have lower selection pressure for resistance (Sulo *et al*., 2002). Proguanil-dapsone is an antifolate combination similar to pyrimethamine-sulphadoxine, which is active against the pyrimethamine-sulphadoxine-resistant forms of parasites (Ogunfowokan *et al*., 2009). It is also more potent than pyrimethamine-sulphadoxine (Krudsood *et al.*, 2005). Combination therapy with different targets can prevent or delay the development of drug resistance (White, 1999). The combination of proguanil and dapsone provide sequential inhibition of folate biosynthesis and show synergy in antimalarial activity (Sulo *et al.*, 2002; Luzzatto, 2010). The active metabolite of proguanil, cycloguanil, selectively inhibits the dihydrofolate reductase (DHFR) of sensitive parasites, causing inhibition of DNA synthesis and depletion of folate cofactors. Dapsone inhibits dihydropteroate synthase (DHPS) in the folate synthesis pathway (Shapiro & Goldberg,

2007). Proguanil-dapsone combination exerts lower resistance pressure on *P. falciparum* than pyrimethamine-sulphadoxine does, because it is rapidly eliminated (Sulo *et al.*, 2002).

Artemisinin and its derivatives (artesunate, artemether, and dihydroartemisinin) are the most potent and rapidly acting antimalarial drugs (White *et al.,* 1999). The short half-life of artesunate limits the possibility of selection for resistance (Rosenthal, 2008). The rational for using ACT is that artemisinin leaves a much smaller amount of parasites for the partner drug to kill while its concentration in plasma remains high (Ashley *et al.*, 2006). Artemisinin and its derivatives are well tolerated (White *et al.*, 1999). According to Dondorp *et al*. (2010) resistance to artemisinin and its derivatives has emerged in western Cambodia, but has not spread to different parts of the region.

Initially only individually formulated antimalarial compounds were available, subsequently products were co-blistered. The development of a triple fixed-dose combination became a priority. The development of fixed-dose combinations is especially challenging (Lacaze *et al.*, 2011). Few studies have been published describing the formulation of triple fixed-dose combinations for malaria (Lacaze *et al.*, 2011; Dondorp *et al.*, 2010; WHO, 2010). Fixed-dose combination formulations are preferred and recommended over blistered and co-packaged combinations. In some parts of Africa there are still low levels of resistance to amodiaquine and pyrimethamine-sulphadoxine monotherapy; and artesunate plus amodiaquine or pyrimethamine-sulphadoxine remains effective options, but the drugs are also used as monotherapies providing continued selection pressure that may lead to resistance (WHO, 2010). Further research into combination therapy is therefore essential.

The combination of artesunate-proguanil-dapsone has not been evaluated in clinical trials and there is not a triple fixed-dose combination product available. This remains an important combination therapy to investigate because it could be used as an alternative to pyrimethamine-sulphdoxine, especially in preventing malaria during pregnancy and in infants.

Aim of study

The aim of this study was to develop an oral triple fixed-dose combination tablet containing artesunate, proguanil and dapsone; and to characterise and evaluate the features of the tablets.

Objectives

The specific objectives of the study were to:

- conduct a literature study on malaria and malaria treatment,
- conduct a literature study on direct compression and wet granulation as possible manufacturing methods for a fixed-dose triple combination tablet containing artesunate, proguanil and dapsone,
- use a full factorial design to investigate the effects of different excipients on tableting and to identify the most suitable formulation composition,
- prepare an oral fixed-dose triple combination tablet based on the most suitable formulation composition,
- evaluate the features of the tablets using weight variation, friability, crushing strength and disintegration,
- evaluate the *in vitro* release properties of the fixed-dose triple combination tablets with dissolution testing.

CHAPTER 1

MALARIA

1.1 Introduction

Malaria is a life-threatening disease caused by a parasite, *Plasmodium*. There are four types of human malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. Recently, some human cases of malaria have also occurred with *Plasmodium knowlesi*, a monkey malaria. Among the above mentioned, *P. falciparum* is the deadliest. Malaria is transmitted through the bite of the female mosquito, *Anopheles*. Transmission is more intense in places where the mosquito is relatively long-lived, so that the parasite has time to complete its development inside the mosquito, and where it prefers to bite humans rather than other animals (Breman *et al.*, 2006; CDC, 2011; WHO, 2011b).

Malaria is mainly confined to Africa, Asia and Latin America. The problem of malaria control in tropical countries is aggravated by inadequate health infrastructures and poor socio-economic conditions. Moreover, in the last few decades the parasites has shown resistance to the drugs normally used to combat it. The *Anopheles* mosquito has become resistant to some of the insecticides used to control it. This has lead to a significant increase in the incidence of malaria (Ashley *et* al., 2006; Lewison & Srivastava, 2008).

Historically malaria was thought to be caused by the offensive vapour emanating from the Tiberian marshes and therefore the word “malaria” came from Italian, which means “bad air” (White, 2009). Systematic control of malaria really began with the discovery of the malaria parasite by Charles Louis Alphonse Laveran in 1880 (Lewison & Srivastava, 2008). Laveran was examining the fresh blood of a patient and observed moving bodies which he identified as parasites of the red blood cells (White, 2009; CDC, 2011). Giovanni Batista Grassi and Raimondo Filettie introduced the names *P. vivax* and *P. malariae* for two of the malaria parasites that affected humans in 1890. Laveran believed that there was only one species, *Oscillaria malariae*. William H. Welch reviewed the subject and in 1897 he named the malignant tertian malaria parasite *P. falciparum*. In 1922, John William Watson Stephens described the fourth human malaria parasite, *P. ovale* (CDC, 2011). Ronald Ross demonstrated the transmission of human malaria by the *Anopheles* mosquito in 1897 (Lewison & Srivastava,

2008). Ross reported the presence of pigmented bodies in the gut of the mosquito which fed on patients with malaria (White, 2009; CDC, 2011).

In the 1950's, attempts to eradicate the disease from most parts of the world failed, primarily because of the development of resistance to insectides and antimalarial drugs. Since 1960, transmission of malaria has risen in most regions where the infection is endemic (Shapiro & Goldberg, 2007). According to the World Health Organization (2010) the number of cases of malaria rose from 233 million in 2000 to 244 million in 2005 and 247 million in 2008, but decreased to 225 million in 2009. The number of deaths due to malaria is estimated to have decreased from 985 000 in 2000 to 781 000 in 2009. While progress in reducing the malaria burden in all WHO Regions has been remarkable, there was evidence of an increase in malaria in four countries in 2009 which included Rwanda, Sao Tome, Principe and Zambia (WHO, 2010).

In Africa a child dies every 45 seconds of malaria and the disease accounts for 20% of all childhood deaths (CDC, 2011; WHO, 2011b). Infections with *P. falciparum* cause much of these mortality rates which affects children less than 5 years, pregnant women and non-immune individuals (Shapiro & Goldberg, 2007). The mortality rates vary significantly under different circumstances. Intense malaria transmission is found in many parts of tropical Africa, the much lower malaria inoculation in areas of southern Asia and the epidemic outbreaks occasionally on both continents (Alles *et al.*, 1998).

1.2 Distribution and epidemiology

Malaria has a worldwide distribution, being found in tropical areas, throughout sub-Saharan Africa and to a lesser extent in South-Africa, South East Asia, the Pacific islands, India and Central and South America (Ashley *et al.* 2006). The distribution includes 109 countries (Figure 1.1) (White, 2009). As a vector-borne infectious disease, malaria's distribution is limited to the regions of the world that are hospitable to the *Anopheles* mosquito. Currently, malaria zones are restricted to tropical and subtropical biomes of the world, but the *Anopheles* mosquito has been known to survive in cooler climates as well. However, this type of mosquito has been systematically eliminated from the world's cooler regions through the use of insecticides, but the

threat of re-introduction remains, even in these areas. Cases of malaria still occur in non-endemic countries, mostly in returning travellers or immigrants (CDC, 2011).

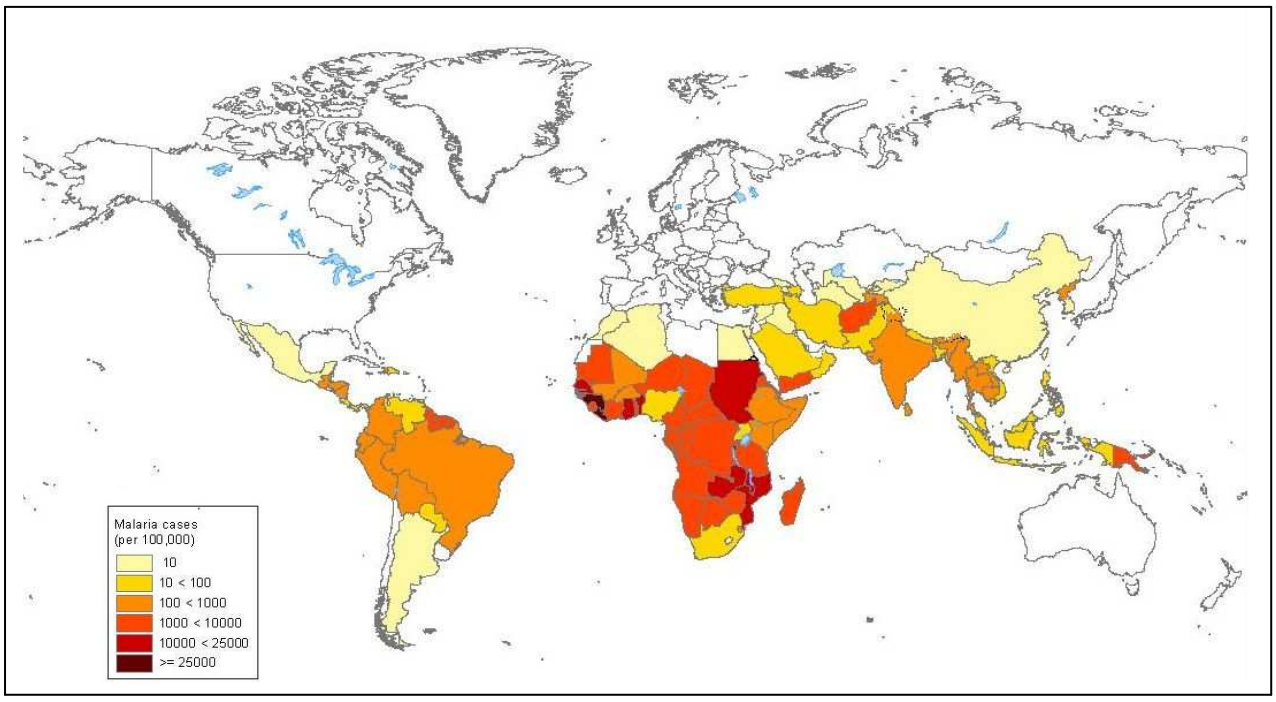


Figure 1.1: Global distribution of malaria. The dark coloured areas indicate the countries at risk of malaria transmission and the number of cases per 100 000. (Adapted from WHO 2011c.)

Approximately half of the world's population is at risk of contracting malaria. Most malaria cases and deaths occur in sub-Saharan Africa. However, Asia, Latin America, and to a lesser extent the Middle East and parts of Europe are also affected (WHO, 2011b). Malaria transmission does not occur at temperatures below 16 ºC or above 33 ºC and at altitudes more than 2000 m, because development in the mosquito cannot take place. The optimum conditions for transmission are high humidity and an ambient temperature between 20 ºC and 30 ºC. Although rainfall provides breeding sites for the mosquito, extreme rainfall may wash away mosquito larvae and pupae (White, 2009). The epidemiology of malaria is complex and may vary considerably even within small geographical areas (Breman *et al.*, 2006; White, 2009). Malaria transmission intensities vary from very low to extremely high (Figure 1.1). The

behaviour of man also plays an important role in the epidemiology of malaria. To transmit the infection there must be a human reservoir of viable gametocytes (White, 2009).

1.3 Life-cycle

The natural ecology of malaria involves malaria parasites infecting successively two types of hosts: humans and female Anopheles mosquitoes (Figure 1.2). In humans, the parasites grow and multiply first in the liver cells and then the red blood cells; and destroy them, releasing daughter parasites (merozoites) that continue the cycle by invading other red blood cells. When a certain form of blood stage parasites (gametocytes) are picked up by a female *Anopheles* mosquito during a blood meal, they start another, different cycle of growth and multiplication in the mosquito (CDC, 2011).

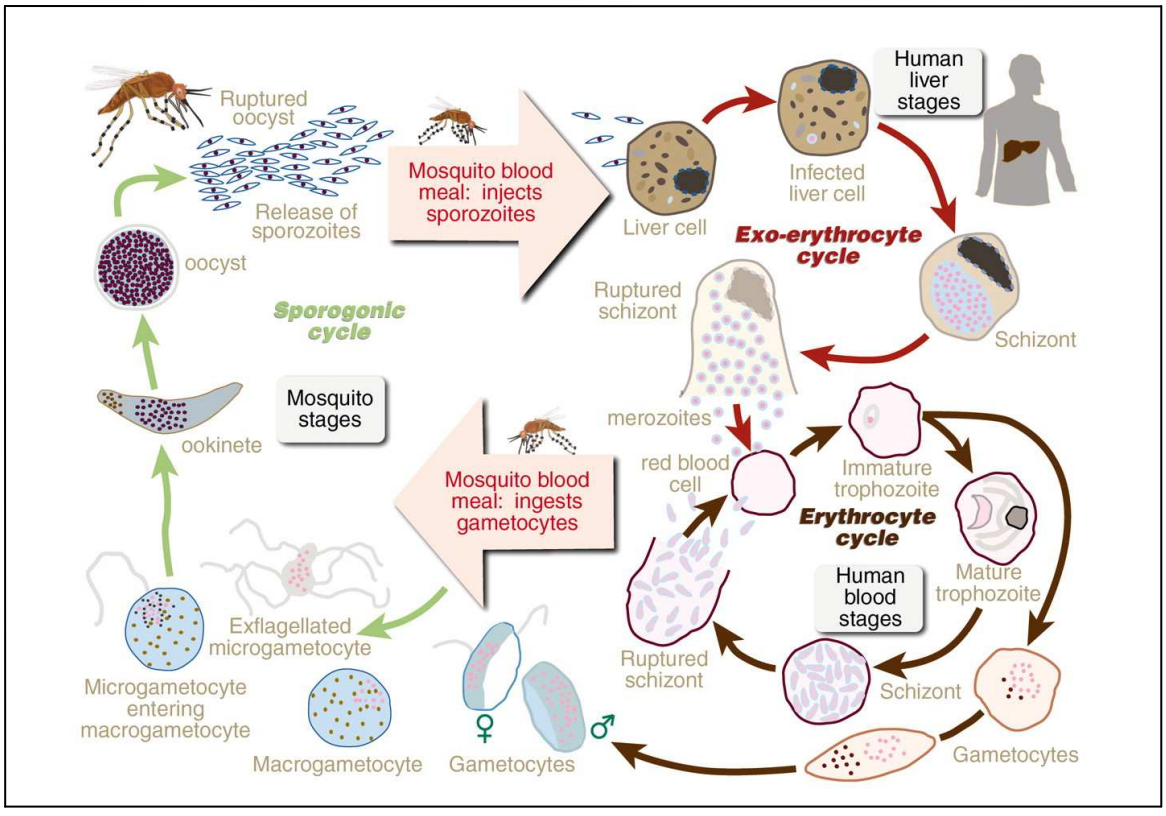


Figure 1.2: Life cycle of the malaria parasite. The figure indicates the three different cycles during the *Plasmodium* spp. growth and development. (Adapted from Scott, 2007).

During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and exoerythrocytic stage tissue schizonts mature in the liver, which rupture and release merozoites. After this initial replication in the liver, the parasite undergoes asexual multiplication in the erythrocytes during the erythrocytic cycle. Merozoites infect red blood cells. The ring stage trophozoites mature into shizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestation of the disease (Rosenthal, 2004; Breman *et al.,* 2006; CDC, 2011).

The gametocytes, male (microgametocyte) and female (macrogametocyte), are ingested by an *Anopheles* mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. In the mosquito's stomach, the microgamete penetrate the macrogamete generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture and release sporozoites which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (CDC, 2011). This complex life cycle offers several points of attack for drugs (White, 2008b; Doerig *et al.*, 2010).

1.4 Antimalarial drugs

In general, the antimalarial drugs are more toxic than antibacterial drugs, i.e. the therapeutic ratio is narrower, but serious adverse effects are rare (White, 2009). Antimalarial drugs can be categorised by the stage of the parasite that they affect and the clinical indication for their use for either prophylaxis or treatment (Bruce-Chwatt, 1962; Shapiro & Goldberg, 2007). Many drugs have been developed and used against malaria and they target different stages of the parasite life cycle. Table 1.1 gives a list of the drugs used in prophylaxis and treatment.

Antimalarial drugs play a central role in the control and ultimate elimination of malaria, but, in most circumstances, they cannot do the job alone, because of the resistance of *P. falciparum* against the drugs. If the parasite becomes resistant to the current classes of effective antimalarial drugs (notable the artemisinin derivatives), then effective control and elimination will not be possible (White, 2008b).

Table 1.1: A list of the drugs used in prophylaxis and treatment of malaria (Adapted from Rosenthal, 2004).

Prophylaxis	Treatment
Chloroquine	Chloroquine
Mefloquine	Amodiaquine
Proguanil	Quinine
	Quinidine
	Mefloquine
	Primaquine
	Pyrimethamine-sulphadoxine
	Doxycycline
	Halofantrine
	Lumefantrine
	Artemisinins
	Atovaquone-proguanil

Drugs that act on asexual erythrocytic stages of the parasites life cycle are called blood schizonticides; and those that act against forms of *Plasmodium* (sexual stages) and prevent transmission to mosquitoes, are gametocides. Sporontocides prevent transmission of malaria by preventing or inhibiting formation of malarial oocysts and sporozoites in infected mosquitoes (Tracy & Webster, 2001; Rosenthal, 2004). Table 1.2 gives a list of the drugs according to the mechanism of action. Antimalarial drugs can be classified in five classes: the quinolines and

arylaminoalcohols, antifolates, the artemisinin derivatives, the hydroxynaphtaquinones and antibacterial agents (Table 1.2) (Ashley *et al.,* 2006).

Table 1.2: Classification of antimalarial drugs (Adapted from Ashley *et al.,* 2006).

Class	Drugs
Quinolines and arylaminoalcohols	Chloroquine, Amodiaquine, Quinine, Quinidine, Mefloquine, Halofantrine, Primaquine, Lumefantrine, Piperaquine
Folate biosynthesis inhibitors (Antifolates)	Pyrimethamine, Proguanil, Chlorproguanil, Trimethoprim
Artemisinin derivates	Artemisinin, Dihydroartemisinin, Artemether, Artesunate
Hydroxynaphtaquinones	Atovaquone
Antibacterial drugs	Clindamycin, Tetracyclines, Sulphadoxine, Dapsone

1.4.1 Quinolines and arylaminoalcohols

Chloroquine has been the drug of choice in treatment and prophylaxis of malaria since 1940, but its utility against *P. falciparum* has been comprised by drug resistance. Chloroquine is a highly effective blood schizonticide against *P. falciparum* (Rosenthal, 2004) with gametocytocidal activity against asexual erythrocytic form of *P. malariae, P. ovale* and *P. vivax* (Murambiwa *et al*., 2011). The mechanism of action remains controversial. It probably acts by concentrating in parasite food vacuoles, preventing the polymerisation of the haemoglobin breakdown product, heme, into hemozoin and thus eliciting parasite toxicity due to the buildup of free heme (Rosenthal, 2004).

Quinine, derived from shrubs of various species of Rubiaceous genera, Cinchona and Remijia, was the first successful chemical used against malaria (Murambiwa *et al*., 2011). It is an alkaloid of cinchona, the powdered bark of the cinchona tree (Shapiro & Goldberg, 2007). Quinine and quinidine remain first-line therapies for severe *P. falciparum* malaria, though toxicity concerns complicate therapy. It is highly effective against blood schizonticide of all four species of human malaria parasites (Rosenthal, 2004) and is also a gametocytocidal for *P. malariae* and *P. vivax*, but has no direct activity against the gametocytes of *P. falciparum*. Resistance to quinine is rare, but cases have been reported (Murambiwa *et al*., 2011).

Mefloquine is effective against many chloroquine-resistant strains of *P. falciparum* and against other species. It has strong blood schizonticidal activity, but is not active against hepatic stages or gametocytes (Rosenthal, 2004; Murambiwa *et al*., 2011). It is usually reserved for the prevention and treatment of malaria caused by drug-resistant *P. falciparum* (Shapiro & Goldberg, 2007).

Primaquine is an 8-aminoquinoline and is a schizonticide used to eradicate the pre-erythrocytic liver latent tissue forms of *P. vivax* and *P. ovale*. Whilst primaquine remains the drug of choice to eradicate and control hypnozoites, the drug may precipitate haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD) deficient patients (Murambiwa *et al*., 2011).

1.4.2 Folate biosynthesis inhibitors

The folate synthesis inhibitors, pyrimethamine, proguanil, chlorproguanil and trimethoprim, are used in combination regimens (Rosenthal, 2004. The inhibition of *falciparum* folate metabolism remain an attractive target for malaria treatment (Murambiwa *et al*., 2011). Pyrimethamine is a slow-acting blood schizonticide with antimalarial effects similar to those of proguanil. However, pyrimethamine has more significant antimalarial potency and its half-life is longer than that of cycloguanil, the active metabolite of proguanil. Pyrimethamine and proguanil inhibit dihydrofolate reductase (DHFR) and are mostly used in combination with sulphonamines, including sulphadoxine, and antibacterial drugs including dapsone, that inhibit dihydropteroate synthase (DHPS) in the folate synthesises pathway of *P. falciparum* (Shapiro & Goldberg, 2007). A simplified version of the folate synthesis pathway is shown in Figure 1.3. Combinations of the two classes therefore provide synergistic inhibition of folate biosynthesis.

This synergy is important for the efficacy of the drug. Combinations of pyrimethamine with sulphadoxine or of proguanil with dapsone are used to treat chloroquine-resistant *P. falciparum* malaria (White, 1999).

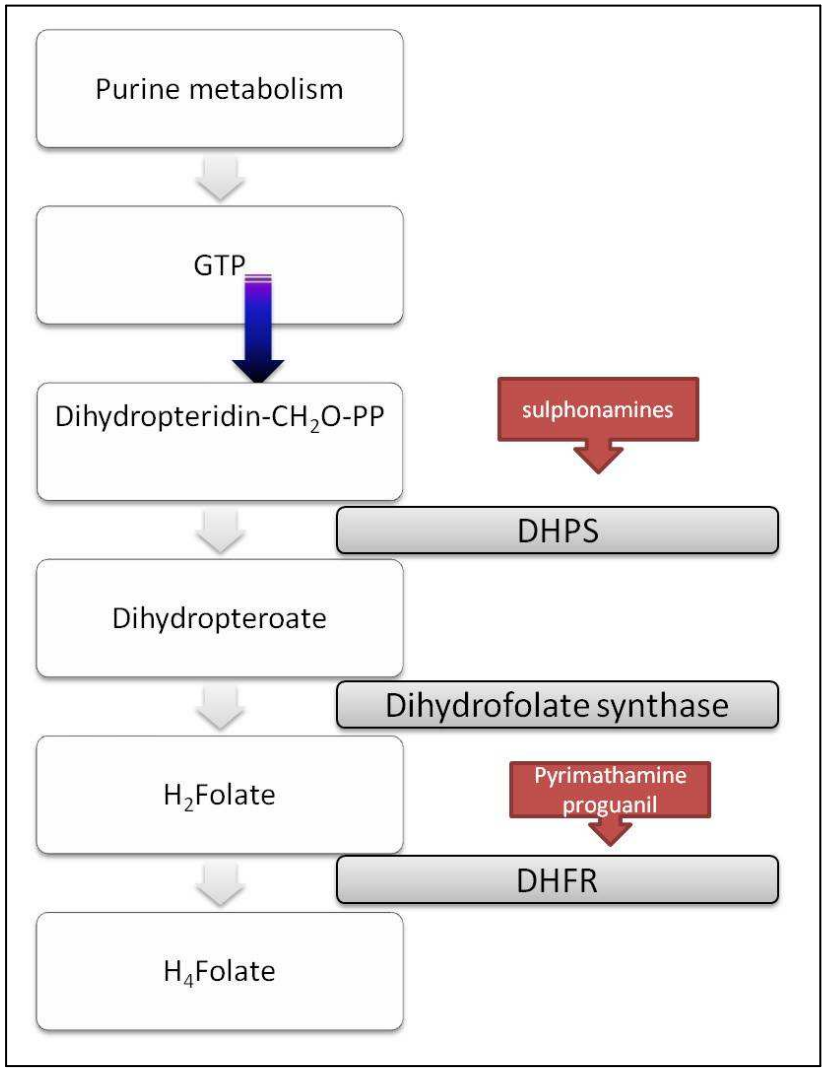


Figure 1.3: A simplified version of *P. falciparum* folate biosynthesis pathway. The blue arrow represents the shortened pathway. The enzymes are shown in the grey shaded boxes and the drugs inhibiting the enzymes in the red boxes. (Adapted from http://sites.huji.ac.il/malaria/maps/folatebiopath.html).

1.4.3 Artemisinin derivates

Artemisinin is a sesquiterpene lactone endoperoxide derived from the weed *qing hao* (*Artemisia annua*), also called sweet wormwood or annual wormwood (Shapiro & Goldberg, 2007). Artemisinin and analogs are rapidly acting blood schizonticides against all human malaria parasites. The mechanism of action is not completely explained but the antimalarial activity probably results from the production of free radicals that induce the iron-catalysed cleavage of the artemisinin endoperoxide bridge in the parasite food vacuole. Artesunate and artemether are two of the most important artemisinin derivatives that play an essential role in the treatment of multidrug-resistant *P. falciparum* malaria (Rosenthal, 2004; Shapiro & Goldberg, 2007).

Of all the antimalarial drugs, the artemisinin derivatives have the broadest time window of action on the asexual malarial parasites, from young rings to early schizonts. This explains why they produce the most rapid therapeutic responses. The rapid clearence of parasites reflects killing and removal of ring stages parasites (White, 2009). Additionally they have the ability to kill gametocytes and therefore interrupt malaria transmission (Ashley *et al*., 2006).

1.4.4 Hydroxynaphtaquinones

Atovaquone was developed as a promising synthetic derivative with potent activity against malaria. Atovaquone in combination with proguanil induce high cure rates with few relapses and minimal toxicity and is used for treatment and prophylaxis (Shapiro & Goldberg, 2007).

1.4.5 Antibacterial drugs

The antibacterial drugs used in malaria treatment include sulphonamides, sulfones and tetracyclines. Sulphonamides and sulfones are used together with pyrimethamine and often with quinine to treat chloroquine-resistant *P. falciparum*. The sulphonamides and sulfones are slow acting blood schizontocides active on DHPS in the parasite folate biosynthesis pathway (Figure 1.3). As already mentioned in section 1.4.2 the sulphonamides are used together with an inhibitor of parasite DHFR to enhance their antiplasmodial action and work synergistically. Tetracyclines are slow acting blood schizontocides that are used alone for short-term

prophylaxis in areas with chloroquine and mefloquine resistance. They are useful for the treatment of acute malaria owing to multidrug-resistant strains of *P. falciparum*. Doxycycline and tetracycline are two tetracyclines that are usually recommended and clindamycin is an alternative (Shapiro & Goldberg, 2007).

Dapsone in combination with other antimalarials provide a valuable alternative for both treatment and chemoprophylaxis. Dapsone acts against bacteria and protozoa in the same way as sulphonamides, by inhibiting the synthesis of dihydropteroate (Figure 1.3) through competition with dihydropteridin-CH_2O-PP for the active site of DHPS (Brabin *et al.*, 2004).

1.5 Resistance

Resistance is the ability of the parasite to survive or multiply in the presence of antimalarial drug concentrations that normally destroy parasites or control their multiplication (WHO, 2005b). Growing resistance to antimalarial medicines has spread rapidly, undermining malaria control efforts (WHO, 2011b). The problem with resistance is particularly severe in developing countries, where the burden of infectious diseases is relatively greater and where patients with a resistant infection are less likely to have access to, or be able to afford, expensive second-line treatments, which typically have more complex regimens than first-line drugs (Laxminarayan *et al*., 2006).

The evolution of drug resistance is facilitated by a number of factors, including increasing use of antibiotics and antimalarial drugs; insufficient controls on drug prescribing; inadequate compliance with treatment regimens; poor dosing (sub-therapeutic drug levels); lack of infection control and increasing frequency; and speed of travel. These all lead to the rapid spread of resistant organisms (Laxminarayan *et al*., 2006). Antimalarial drug resistance develops when spontaneously occurring parasite mutants with reduced susceptibility are selected and are then transmitted (White, 1999). *P. falciparum* has developed resistance to all classes of antimalarial drugs with the general exception of the artemisinin derivates (White, 2009). *P. falciparum* depicts multidrug resistance to chloroquine, pyrimethamine-sulphadoxine and mefloquine monotherapies and quinine is slowly losing its potency (Ashley *et al.*, 2006).

1.5.1 Resistance to folate biosynthesis inhibitors and sulphonamides

Resistance to proguanil and pyrimethamine were reported within a few years of the introduction as monotherapies (White, 2009). Pyrimethamine-sulphadoxine is eliminated slowly, providing prophylaxis after treatment, but also favouring the selection of resistance. The mechanism of clinical failure is not known: resistance to both drugs individually has been reported, but their respective importance remains unclear. Rapidly eliminated antifolate drugs (such as proguanil) are very likely to exert less resistance selection pressure. *P. falciparum* resistance to pyrimethamine retains sensitivity to other DHFR inhibitors (Amukoye *et al.*, 1997).

Resistance to antifolate drugs is caused by single point mutations in the genes encoding the target enzymes. For the DHFR inhibitors the initial mutation conferring resistance is usually at position 108 in the gene encoding DHFR (Peterson *et al.*, 1988; White, 1999). Multiple mutations at position 51, 59 and 108 are relatively resistant to pyrimethamine-sulphadoxine (Watkins *et al.*, 1997; White, 1999). Interestingly, mutations conferring moderate pyrimethamine resistance do not necessarily confer cycloguanil resistance, and vice versa. For example, mutations at position 16 and 108 present high level resistance to cycloguanil but not pyrimethamine. In general, proguanil are more active than pyrimethamine against the resistant mutants and are more effective clinically too, but are ineffective against parasites with a 164 mutation (White, 2009).

Sulphonamide and sulphone resistance also develop by progressive acquisition of mutations in the gene encoding the target enzyme DHPS. Specifically in *P. falciparum,* changed amino acid residue associated with reduced antifolate susceptibility, have been found at positions 436, 437, 540, 581, and 613 in the DHPS domain. Parasites with DHPS mutations nearly always have DHFR mutations as well. The addition of the 540 to the 437 mutation is associated with particularly high failure rates. *P. falciparum* parasites with “quintuple” mutations are now widespread in tropical countries and are associated with high pyrimethamine-sulphadoxine treatment failure rates, as well as poor responses to the artesunate-sulphadoxine-pyrimethamine combination (White, 2009).

1.5.2 Artemisinin and derivatives

The mechanism of action of the artemisinin drugs remains a subject of considerable debate. Initially it was thought to involve generation of carbon centred free radicals which alkylate critical proteins. Parasiticidal activity is dependent on the integrity of the peroxide bridge. However, artemisinin has recently been shown to be a potent inhibitor of a sarcoplasmic endoplasmic reticulum calcium transporting ATPase, and it has been proposed that this is the target. However, the synthetic peroxide RBX11160, which has similar pharmacodynamic properties to the artemisinins, is only a very weak inhibitor of ATPase. Therefore, clearly other mechanisms of action are involved. In general, multi-drug resistant parasites are more resistant to artemisinin derivates, and moderate reduction in susceptibility can experimentally be induced (White, 2009).

1.5.3 Quinolines and related drugs

Chloroquine resistance is associated with reduced concentrations of drug in the acid food or digestive vacuole. Both reduced influx and increased efflux have been implicated. The resistant parasites lose chloroquine from the digestive vacuole 40 – 50 times faster than drug-sensitive parasites. This efflux mechanism is similar to that found in multi-drug resistant mammalian tumour cells. The first efflux mechanism to be characterised was the ATP-requiring transmembrane pump, P glycoprotein. Gene encoding these multi-drug resistant proteins have been identified in *P. falciparum*. These unmutated multi-drug resistant genes are found in increased copy numbers in most quinine and mefloquine resistant parasites; and point mutations are associated with chloroquine resistance. Amplification of *P. falciparum* multi-drug resistant proteins is the main contributor to mefloquine resistance. The critical discovery has been the association of point mutations in chloroquine resistance transporter (CRT, a food vacuolar membrane protein thought to have a transporter function), with chloroquine resistance. The central role of a CRT mutation resulting in a change in coding at position 76 genes in mediation chloroquine resistance has been shown unequivocally in the laboratory by transfection studies and in epidemiological studies where therapeutic responses are predicted by this single polymorphism. CRT may also play an important role in amodiaquine and quinine resistance (White, 2009).

The chloroquine efflux mechanism in resistant parasites can be inhibited by a number of structurally unrelated drugs: calcium channel blockers, tricyclic antidepressants, phenothiazines, cyproheptadine, antihistamines, etc. whereas mefloquine resistance is reversed by penfluridol, which does not reduce chloroquine efflux (White, 2009).

1.5.4 Atovaquone

Atovaquone interferes with parasite mitochondrial electron transport, and it depolarises the parasite mitochondria; thereby blocking cellular respiration. High levels of resistance result from single point mutation in the gene encoding cytochrome *b.* This is one of three genes encoded in the 6 kilobase (kb) extra chromosomal mitochondrial DNA (White, 2009).

1.5.5 Prevention of resistance using combinations of antimalarial drugs

Low clearance and a shallow concentration-effect relationship increase the chance of selection. Use of combinations of antimalarial drugs that do not share the same resistance mechanisms will reduce the chance of selection, because the chance of a resistant mutant surviving is the product of the per parasite mutation rate for the individual drugs, multiplied by the number of parasites in an infection that are exposed to the drugs (White, 1999; White, 2009). According to Watkins & Mosobo (1993) drugs which are rapidly eliminated are very likely to exert less resistance selection pressure.

1.6 Combination treatment

The rapid spread of strains resistant to chloroquine and pyrimethamine/sulphadoxine highlight the need to defy the resulting increases in malaria mortality and morbidity rates. New and effective antimalarial agents may be derived as novel compounds or modifications of existing drugs (Fidock *et al.,* 1998). There is a continuing need for new and improved treatments for malaria (WHO, 2010).

Early diagnosis and treatment of malaria reduce disease and prevent deaths. Effective drug treatment contributes to reducing malaria transmission (Breman *et al*., 2006; WHO, 2011b) and can prevent uncomplicated malaria from developing into more severe illness (Bukirwa *et al*., 2004). Combination therapy is required to combat the resistant strains and preserve antimalarial effectiveness. It is important that new combinations include agents with different mechanisms of action used at appropriate doses to ensure maximal and rapid parasite killing (Wootton *et al.*, 2008; WHO, 2011a). The best available treatment, particularly for *P. falciparum* malaria, is artemisinin-based combination therapy (ACT) (WHO, 2011a; WHO, 2011b). Uncomplicated malaria can be treated with oral drugs whereas severe infections will be hospitalised and treated with injectables (Ashley *et al.*, 2006).

The rationale for combining drugs with independent modes of action to prevent the emergence of resistance was first developed in anti-tuberculosis chemotherapy and the same principle applies to the treatment of malaria (White *et al*., 1999; Ashley *et al.*, 2006; WHO, 2011a). The rationale for antimalarial combination therapy is two-fold:

i) the combination is often more effective; and

ii) in the very rare event that the parasite is resistant to one of the medicines, this resistant parasite will be killed by the other antimalarial medicines (WHO, 2011a).

Current combination therapies include artemether and lumefantrine; artesunate and amodiaquine; artesunate and mefloquine; artesunate and pyrimethamine-sulphadoxine; dihydroartemisinin and piperaquine; and artesunate and tetracycline, doxycycline or clindamycin. Recurrence of *P. falciparum* malaria can be due to re-infection or recrudescence. Treatment failures often result from drug resistance, poor adherence or inadequate drug exposure; and lead to recurrence of infection. The choice of ACT is based on the level of resistance of the partner medicine in combination determined by clinical treatment failures. ACT have proven to be more effective in the clinical setting by reducing recurrence, but is also more costly (Davis *et al.*, 2011).

1.7 Intermitted preventative treatment

Important risk groups raising concern due to limiting treatment options are pregnant woman and children under the age of five. Pregnant woman, or woman attempting to get pregnant, should always be strongly discouraged to visit malaria endemic areas; mainly because they are more at risk to succumb to severe malaria. Furthermore, should they survive an episode of malaria in pregnancy and go on to deliver, the adverse effects on the infant are likely to be permanent. Spontaneous abortion, preterm delivery, low birth weight, stillbirth, congenital infection and maternal death can occur when infected with malaria during pregnancy. Intermitted preventative treatment (IPT) during pregnancy (IPTp) was implemented in 2004 and consist of the administration of a single curative dose of an efficacious antimalarial drug at least twice during pregnancy regardless of whether the woman is infected or not. The goal of this initiative is to promote the use of antimalarial drugs given in standard treatment dosages at predefined intervals after the first movement of the foetus is noted. Pyrimethamine-sulphadoxine is currently considered the most effective and safest in areas with stable *P. falciparum* transmission and where resistance against this drug is low. The WHO recommends the use of chloroquine in uncomplicated cases of malaria where chloroquine-sensitive strains are prevalent and pyrimethamine-sulphadoxine in chloroquine-resistant areas (Briand *et al.*, 2007).

1.8 The importance of artesunate, proguanil and dapsone in malaria treatment

1.8.1 Artesunate

Artesunate is an artemisinin derivative (section 1.4.3) and is the sodium salt of the hemisuccinate ester of artemisinin (Figure 1.4). The empirical formula is $C_{19}H_{28}O_8$ and artesunate have a molecular mass of 384.41 g/mol (Lisgarten *et al*., 2002). It is soluble in water and has extremely poor stability in aqueous solutions at acid or neutral pH (Rosenthal, 2004).

Artesunate are useful for oral, intravenous, intramuscular and rectal administration. Artesunate is commonly used in combination with mefloquine to treat highly resistant falciparium malaria (Rosenthal, 2004). Formulations available include tablets containing 50 mg or 200 mg of sodium artesunate; ampoules for intramuscular or intravenous injection containing 60 mg

anhydrous artesunaic acid; and rectal capsules with 100 mg or 400 mg sodium artesunate (WHO, 2006).

Artesunate is rapidly absorbed after oral administration with peak plasma levels after 1.5 h. It is converted to dihydroartemisinin and the antimalarial activity is determined by dihydroartemisinin elimination (WHO, 2006). The bioavailability of artesunate is 82% (Clarke, 2011).

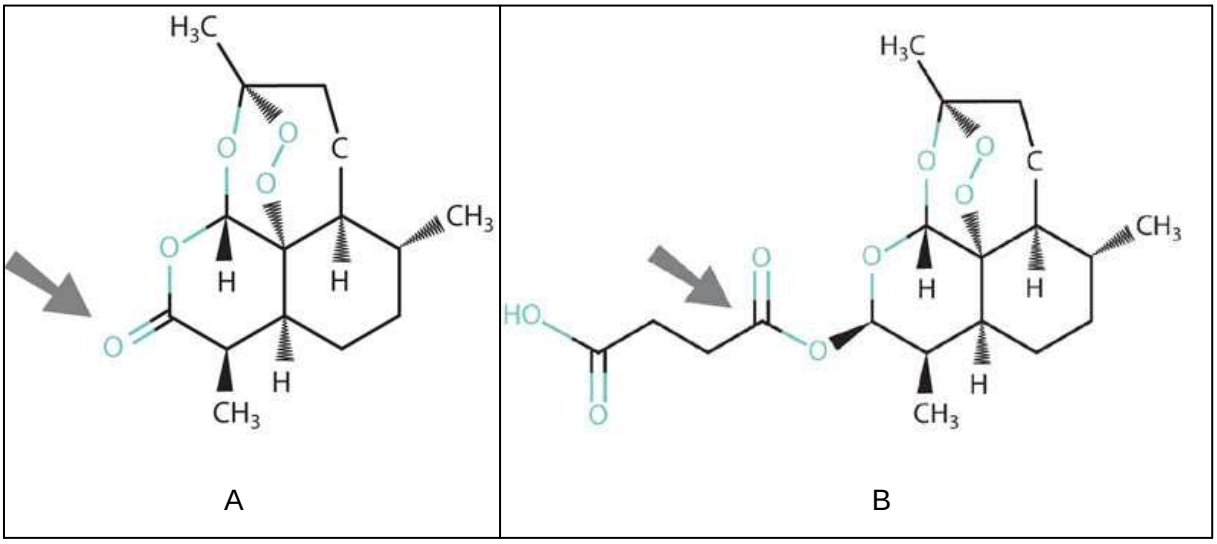


Figure 1.4: Structure of artemisinin (A) and artesunate (B). The arrow indicate the hemisuccinate ester group (Gaudin *et al*., 2007).

Effective monotherapy with artesunate requires seven days of treatment, increasing the risk of resistance developing through poor compliance. It is therefore combined with other antimalarial drugs given in a shorter 3-day course (Angus *et al.*, 2002; Wootton *et al.*, 2008; WHO, 2011a). Several treatment regimens have been examined with the optimal regimen (which has been adopted as standard) being 4 mg/kg artesunate daily for 3 days in combination with other antimalarial drugs (Gibbon, 2005; Olliaro *et al.*, 2010).

Artesunate is well tolerated and generally safe. The most common toxic effects that have been identified are nausea, vomiting, anorexia and dizziness; these are probably due, in many patients, to acute malaria, rather than to the drugs. More serious toxic effects, including

neutropenia, anaemia, haemolysis and elevated levels of liver enzymes, have been seldom noted. Neurotoxicity is the most important concern regarding artemisinins. Studies showed that intramuscular dosing was more toxic than oral dosing and that fat-soluble artemisinins were more toxic than artesunate. Another concern about artemisinins is embryotoxic effects, which have been demonstrated in animals. Studies from Asia and Africa, including 44 treatments during the first trimesters, showed similar levels of congenital abnormalities, stillbirths and abortion in patients who received, and those who did not receive, artesunate during pregnancy (Rosenthal, 2008). There are no known drug interactions with artesunate (WHO, 2006).

1.8.2 Proguanil

One prophylactic drug that undergoes a resurgence of interest for use against malaria is proguanil, an antifolate (section 1.4.2). Proguanil acts as an antimalarial agent in its native form as well as the active metabolite, cycloguanil. This explains why a proguanil combination provides 100% efficacy in treating *P. falciparum* (Fidock *et al.,* 1998). Proguanil is considered as the safest of all the antimalarial drugs. It has been used as a prophylactic drug in malaria, but has been evaluated in combination with dapsone as a treatment of uncomplicated chloroquine-resistant *falciparum* malaria. Proguanil and dapsone are more rapidly eliminated than pyrimethamine and sulphadoxine. The use of this combination provides less selective pressure for the emergence of resistance (White, 2009). Proguanil act slowly against erythrocytic forms of susceptible strains of all four human malaria species. It also has depicts some activity against hepatic forms. Proguanil are not used alone as antimalarial, but are effective in combination with atovaquone (Rosenthal, 2004).

Proguanil is a synthetic biguanide derivative of pyrimidine (Figure 1.5) (Rosenthal, 2004). The empirical formula is $C_{11}H_{16}ClN_5$ and it has a molecular mass of 253.7 g/mol (Clarke, 2011). Proguanil is slightly soluble in water, sparingly soluble in ethanol and practically insoluble in methylene chloride (BP, 2011).

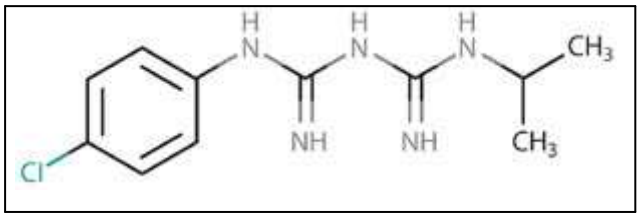


Figure 1.5: The structure of proguanil (Rosenthal, 2004).

Proguanil reach peak plasma levels approximately 5 hours after an oral dose and has an elimination half-life of approximately 16 hours (Rosenthal, 2004). It is only available in oral formulation (100mg) in combination with atovaquone (250 mg) for prophylaxis (Gibbon, 2005). Common side effects of proguanil include gastric intolerance, mouth ulcers and stomatitis. Skin rash; hair loss; anaemia and neutropenia; hyponatraemia; elevated liver enzymes and amylase; headache; insomnia; fever; and angioedema have been reported (Gibbon, 2005).

1.8.3 Dapsone

Dapsone was initially used in the treatment of leprosy when it was noted that patients, though living in an endemic area, showed lower incidence of malaria than the general population. Dapsone is a folic acid synthesis inhibitor (Section 1.4.5) with an empirical formula of $C_{12}H_{12}N_2O_2S$ (Figure 1.6) and a molecular mass of 248.3 g/mol (BP, 2011; Clarke, 2011). Dapsone is very slightly soluble in water, freely soluble in acetone and sparingly soluble in alcohol. It dissolves freely in dilute mineral acids (BP, 2011). After oral ingestion dapsone produces a 70 – 80% bioavailability (Gibbon, 2003).

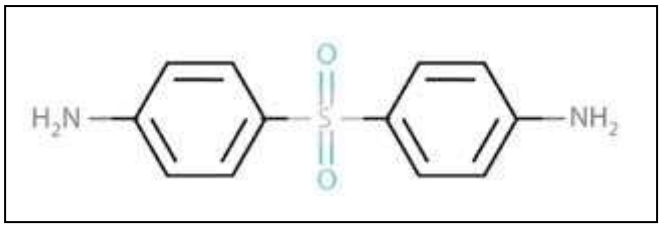


Figure 1.6: The structure of dapsone (Clarke, 2011).

Dapsone is available in tablets for oral administration that contains 100 mg dapsone for the treatment of leprosy, *Pneumocystis carinii*, pneumonia, toxoplasmosis and dermatitis herpetiformis (Gibbon, 2005). One of dapsone's side-effects, haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD) deficiency, is a major problem (Wolf *et al.*, 2002). This side-effect is predictable and much more severe in people who have an inherited G6PD deficiency. Drug-induced haemolytic anaemia is dose dependent (Degowin *et al.*, 1966; Mobacken, 2008; Luzzatto, 2010) and is caused when a daily dose of 100 mg or more is given as long-term treatment in G6PD deficiency patients (Degowin *et al.*, 1966; Wolf *et al*., 2002). Haemolytic anaemia in any patient is caused at a daily dose of 200 mg or more (Degowin *et al.*, 1966). Other side effects include methaemoglobinaemia, haematological adverse effects (bone marrow depression) and cutaneous reactions (range from minor skin rashes to toxic epidermal necrolysis). The sulphone syndrome, characterised by fever, malaise, jaundice with hepatic necrosis and anaemia, is a hypersensitivity reaction which may occur after five to six week's therapy (Gibbon, 2005).

Drug interactions include probenecid, pyrimethamine (increases the risk of haematological disorders), trimethoprim and rifampicin. Probenecid reduces the renal excretion of dapsone and enhances the risk of untoward effects. Trimetoprim increases plasma concentrations whereas rifampicin may cause a significant reduction in dapsone concentrations (Gibbon, 2005).

1.8.4 Combination therapy with artesunate, dapsone and proguanil

Due to the rapid spread of resistance to pyrimethamine-sulphadoxine, the WHO identified a need to develop a new antifolate combination therapy that could be used to replace pyrimethamine-sulphadoxine. Due to clinical success of proguanil-dapsone in the treatment

and prophylaxis of malaria, initial studies focused on the combination of chlorproguanil-dapsone combined with artesunate. The triple combination proved to be clinically more effective than chlorproguanil-dapsone (Winstanley, 2001; Krudsood *et al.*, 2005; Wootton *et al*., 2008). Chlorproguanil is a chemical derivate of proguanil with similar clinical efficacy at smaller dosages (Krudsood *et al.*, 2005). It is administered as a prodrug and is metabolised in the liver to chlorocycloguanil with an extra meta-chloride group compared to cycloguanil (Anderson, 2005).

A follow-up study showed that artesunate-chlorproguanil-dapsone appeared to be clinically more effective (89.1% versus 83%) than chlorproguanil-dapsone in specific African countries (Burkina Faso, Ghana, Kenya, and Nigeria). Both the combinations caused haemolysis in patients with a Glucose-6-phosphate dehydrogenase (G6PD) deficiency (30% versus 29%) but not in normal patients (<1% versus 3%) (Tiono *et al*., 2009). The addition of artesunate to chlorproguanil-dapsone had no clinically effect on the pharmacokinetics of chlorproguanil and dapsone (Miller *et al*., 2009).

The combination was evaluated in various clinical trials (Fanello *et al.*, 2008; Owusu-Agyei *et al.,* 2008; Premji *et al.,* 2009) together with other combinations including artesunate-amodiaquine, artemether-lumefantrine and amodiaquine-sulphadoxine. These studies were done in various African countries including Rwanda (Fanello *et al.,* 2008), Ghana, Mozambique, Tanzania, Gabon, Kenya (Mutabingwa *et al.,* 2009; Conteh *et al*., 2010; Odhiambo *et al.,* 2010) and Cameroon (Whegang *et al*., 2010). Haemolytic anaemia was observed in patients with G6PD-defiency with chlorproguanil-dapsone combined with artesunate, but also with amodiaquine combined with pyrimethamine-sulphadoxine (Fanello *et al*., 2008). Although the combination of artesunate-chlorproguanil-dapsone proved effective, parasitaemia was only reduced by ~ 70 – 72% (Fanello *et al.,* 2008; Whegang *et al*., 2010). A recent study confirmed that the artesunate-chlorproguanil-dapsone combination is also not effective in Rwanda where the population has a high resistance to antifolate drugs (Karema *et al.,* 2010). A combination product endorsed by the WHO must prove to be clinically effective by reducing parasitaemia by 90% (WHO, 2010). There may be other reasons for clinical failure including imposed immune status of the patients, poor absorption and bioavailability of the drug, suboptimal dose schedule during the trails and interaction with other drugs (Fanello *et al*., 2008). There are certain advantages over using chlorproguanil-dapsone combined with artesunate. The triple combination has a simple treatment regime (once daily for three days) which favours

compliance and is much less expensive than any other marketed antimalarial drug combination (Fanello *et al*., 2008; Wootton *et al*., 2008). The potential for artesunate and dapsone (oxidant drugs) to cause clinically relevant haemolysis in malaria patients with G6PD-defiency has been identified, but was well characterised (WHO, 2005a). The triple combination therefore still remains a viable option for normal malaria patients and in countries with low resistance to antifolate drugs.

Proguanil-dapsone is more active than pyrimethamine-sulphadoxine against resistant *P. falciparum*, in particularly the triple DHFR mutants. The treatment doses of proguanil used are 5 – 8 mg/kg per day in combination with dapsone 2.5 mg/kg (White, 2009) for three consecutive days (Bell *et al.*, 2009). Mutabingwa *et al*. (2001) confirmed that a three daily dose of a proguanil-dapsone combination was very effective in treating *P. falciparum* malaria compared to a standard single-dose pyrimethamine-sulphadoxine. The combination of proguanil and dapsone is being considered as an alternative antimalarial to pyrimethamine-sulphadoxine, because of its more significant efficacy and its shorter half-lives (Krudsood *et al*, 2005).

Artesunate-proguanil-dapsone (APD) is an interesting combination for investigation as the three compounds have well-matched pharmacokinetics, i.e. all are relatively rapidly eliminated. Thus, there is a reduced risk of parasite exposure to any single compound after elimination of the partner drug, potentially decreasing the risk of resistance to emergence (Wootton *et al.,* 2008). The artemisinin combination treatments (ACT) are generally reliable treatments and are now the treatment of choice for *P. falciparum* malaria (White, 2009; WHO, 2011a). Proguanil has already been proven to be effective in IPT and is more cost effective than chlorproguanil. It remains important to investigate a possible triple fixed-dose combination as an alternative to pyrimethamine-sulphadoxine (WHO, 2010).

Conclusion

Malaria is a life-threatening disease and is with AIDS and TB the three major diseases in Africa. Malaria is extended throughout the world, but is more severe in tropical areas with a lesser extent in South Africa. Malaria control is aggravated due to poor health infrastructure and poor socio-economic conditions. The WHO recommended ACT as first line treatment, because of resistance of the parasite to pyrimethamine-sulphadoxine and chloroquine. The rational for using combination therapy is to prevent the appearance of resistance; and combination proved to be more effective. Requirements for combination therapy are that i) the agent must have a different mechanism of action and ii) appropriate doses must be used.

Artesunate, a derivate of artemisinin, is very potent and kill the greatest fraction of parasites and therefore leaves a small fraction of the parasite for the partner drug to kill. The combination of proguanil-dapsone is a new combination that has the same mechanism of action as pyrimethamine-sulphadoxine, but is less resistant and more effective.

CHAPTER 2

DOSAGE FORM DESIGN: TABLETS

2.1 Introduction

Active pharmaceutical ingredients (API's) are rarely administered as pure chemical substances alone and are almost always given as formulated preparations or medicines. These can vary from relatively simple solutions or conventional tablets to complex drug delivery systems. Various excipients, and relatively simple or complex manufacturing processes can be used during formulation. The excipients provide varied and specialised pharmaceutical functions (York, 2002).

The route that is most frequently used for drug administration is the oral route. Oral dosage forms are usually intended for systemic effects. Compared to other routes, the oral route is the simplest, most convenient and safest means of drug administration. The most popular oral dosage forms are tablets, capsules, suspensions, solutions and emulsions (York, 2002).

Tablets are prepared by compression, and besides an active ingredient, also contain excipients (York, 2002). Powder flow properties of powder mixtures intended for tableting, are essential in the production of tablets, allowing uniform filling of the die to ensure weight uniformity (Staniforth, 2002). Properties such as particle shape and size can influence the flow properties of powders (Staniforth, 2002; Allen *et al*., 2011). The flow properties of typical tablet and capsule formulation excipients, active ingredients and representative formulation blends can be described with parameters such as angle of repose; critical orifice diameter; bulk density and tapped density; and flow rate (Taylor *et al.*, 2000). Direct compression is a faster, simpler and easier technique for tablet manufacturing than wet or dry granulation. A failure of powder flow often leads to the abandonment of direct compression and adopting the formulation to granulation during formulation and process development trails. Flow has a direct impact on the decision of whether to start developing direct compression. Inadequate powder flow is a fundamental and serious restriction to be eliminated first, if direct compression is to be used (Abe *et al*., 2009).

Glidants (silica) are usually incorporated in direct compression formulations to improve powder flow and control tablet weight. Flow properties of powders can also be improved using wet granulation (Abe *et al.*, 2009).

2.2 Powder blend compatibility

It is important that the API and the excipients are compatible before starting the tableting process. It is especially important during the preformulation process where two or more different API's are used to ensure that there is no interaction between each other. One method for determining compatibility is differential scanning calorimetry (DSC).

Differential scanning calorimetry (DSC) is a technique which measurer the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material, as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate. There are two types of DSC systems: heat-flux DSC and power-compensation DSC (Bhadeshia, 2002).

In power-compensation DSC, the temperatures of the sample and reference are controlled independently using separate, identical furnaces. The temperatures of the sample and reference are made identical by varying the power input to the two furnaces; the energy required to do this is a measure of the enthalpy or heat capacity changes in the sample relative to the reference (Bhadeshia, 2002).

In heat-flux DSC, the sample and reference are connected by a low-resistance heat-flow path. The assembly is enclosed in a single furnace. Enthalpy or heat capacity changes in the sample cause a difference in its temperature relative to the reference; the resulting heat flow is small compared to that in differential thermal analysis because the sample and reference are in good thermal contact. The temperature difference is recorded and related to enthalpy change in the sample using calibration experiments (Bhadeshia, 2002).

2.3 Physical characterisation of powder flow

Powder flow is a key requirement for the pharmaceutical manufacturing process. Tablets are manufactured on a tablet press by filling the tablet die with powders or granules based on volume. Thus, the flow of powder from the hopper into the die often determines weight, hardness and content uniformity of tablets. It is therefore essential to measure the flow properties of the materials prior to tableting or capsule filling (Shah *et al*., 2008; Staniforth, 2002). Uneven powder flow can result in capping or lamination of the tablet and can increase particle-die-wall friction, causing lubrication problems and increase dust contamination risks (Staniforth, 2002). Poor powder flow properties can also be improved using granulation (Abe *et al*., 2009).

Powder flows when the forces acting on the powder bed cause the resulting shear force to exceed the shear strength of the bed (Lindberg *et al.*, 2002). A number of factors determine the flow properties of powders, including particle shape, size and size distribution (Lindberg *et al*., 2002; Allen *et al.,* 2011). Spherical particles flow better than needles and very fine particles do not flow as freely as large particles (Allen *et al.*, 2011). However all factors acting on and between the particles can influence the flowability, for example, the humidity and state of compaction of the powder (Lindberg *et al*., 2002).

2.3.1 Angle of repose

Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles (USP, 2011). A static heap of powder, with only gravity acting upon it, will tend to form a tapering heap. One limitation exists: the angle to the horizon cannot exceed a certain value; and this is known as the angle of repose (θ). If any particle momentarily lies outside this limiting angle, it will slide down the adjacent surface under the influence of gravity until the gravitational pull is balanced by the friction caused by inter-particulate forces. Accordingly, there is a relationship between the θ and the ability of the powder to flow (Wells, 2002).

Determining the angle of repose is a relatively simple technique. It can easily be determined by allowing a powder to flow through a funnel and fall freely onto a surface. The height and

diameter (radius) of the resulting cone are measured and the angle of repose is calculated with an equation (Equation 2.1) (Allen *et al.*, 2011). Figure 2.1 illustrates the angle of repose.

$$\tan \theta = {}^{h}/_{r} \qquad \textbf{Equation 2.1}$$

Where:

h = height (cm) of the powder cone and

r = radius (cm) of the powder cone

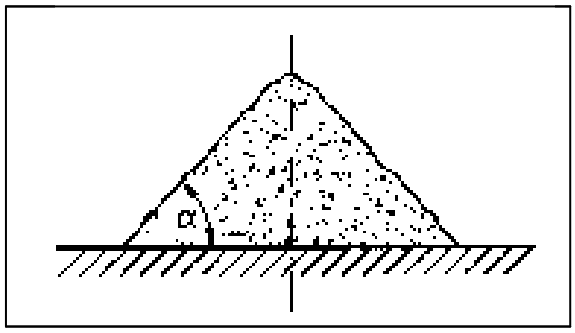


Figure 2.1: Illustration of the angle of repose. Adapted from http://www.physicsarchives.com (accessed 10 August 2011).

There is some variation in the descriptions of powder flow using the angle of repose and the values are shown in Table 2.1 (USP, 2011). Powders with a low angle of repose flow freely whereas powders with a high angle of repose flow poorly (Allen *et al.*, 2011).

Table 2.1: Flow character and corresponding angle of repose (USP, 2011).

Flow character	Angle of repose (degrees)
Excellent	25 – 30
Good	31 – 35
Fair – aid not needed	36 – 40
Passable – may hang up	41 – 45
Poor – must agitate, vibrate	46 – 55
Very poor	56 – 65
Very, very poor	>66

2.3.2 Critical orifice diameter

The critical orifice diameter (COD) is the size of the smallest hole through which powder discharges when the shutter is removed. In some cases, repetition of the experiment produces different critical orifice diameters; and in these cases maximum and minimum values are quoted. To determine the orifice diameter, a cylinder with a series of interchangeable base plate discs having orifices with different diameters are used (Staniforth, 2002).

2.3.3 Bulk density and tapped density

A simple test to evaluate the flowability of a powder is by comparing the bulk density (ρ_{Bmin}) to the tapped density (ρ_{Bmax}) of a powder. A useful empirical guide is given by Carr's compressibility index (Equation 2.2). Hausner has defined a similar index (Equation 2.3) (Wells, 2002).

$$\text{Carr's index (\%)} = \frac{\text{Tapped density} - \text{Poured density}}{\text{Tapped density}} \text{ x } 100$$

Equation 2.2

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Poured density}}$$

Equation 2.3

Table 2.2 shows the scale of flowability using Carr's index and the Hausner ratio. Values less than 1.25 indicate good flow (= 20% Carr), whereas greater than 1.25 indicates poor flow (= 33% Carr). Rapid consolidation is essential for uniform filling of the die during tablet manufacturing (Wells, 2002).

2.3.4 Flow rate

The simplest method to determine the flow rate is to measure the rate at which a powder discharges from a hopper/cylinder. A simple shutter is placed over the cylinder outlet and the cylinder is filled with powder. The shutter is then removed and the time taken for the powder to discharge is recorded. Flow rate can be calculated by dividing the powder weight discharged by the time (Staniforth, 2002). However, monitoring the rate of flow of materials through an orifice has been proposed as a better measure of powder flowability (USP, 2011).

Table 2.2: Scale of flowability (USP, 2011).

Flow character	Carr's index (%)	Hausner ratio
Excellent	≤10	1.0 - 1.11
Good	11 - 15	1.12 - 1.18
Fair	16 - 20	1.19 - 1.25
Passable	21 - 25	1.26 - 1.34
Poor	26 - 31	1.35 - 1.45
Very poor	32 - 37	1.46 - 1.59
Very, very poor	>38	>1.60

2.4 Granulation

Most powdered active ingredients require the addition of excipients such as diluents, binders, disintegrants and lubricants to provide the desired characteristics for tablet manufacturing and efficacious use. As already established (section 2.3), one important requirement during tablet manufacturing, is that the powder mixture should flow freely from the hopper of the tablet press into the die to enable high-speed compression into tablets. Granulation of powders provides free flow (Allen *et al*., 2011).

It is often necessary to improve the compaction and flow properties of powders in order to obtain uniform die-filling and to produce tablets of adequate quality. These properties are enhanced by converting fine powders into larger agglomerates by the process of wet or dry granulation. Wet granulation is traditionally used. This process consists of distributing the liquid binder in a powder blend to obtain a wetted powder mass. The wetted powder mass is subsequently screened through a sieve to obtain large granules. These granules are then dried and again screened through a sieve to obtain smaller sized granules suitable for tableting (Šantl *et al*., 2011).

Granulation remains one of the most popular approaches to improve the flow behaviour of pharmaceutical powders. Wet granulation provides particles with superior processing characteristics (Williams *et al.*, 2004). Wet granulation is a process still widely used in the pharmaceutical industry. It has not been completely replaced by direct compression technology, partly because of development considerations and habits, and partly because it remains in some cases an attractive technique. It provides better control of drug content uniformity at low drug concentrations, as well as better control of product bulk density and ultimately compactibility (Faure *et al*., 2001). Dry granulation produces irregular granules with higher densities than wet granulation. Wet processed granules are more compressible and compactable compared to granules prepared by dry granulation (Bacher *et al*., 2008). According to studies done by Šantl *et al*. (2011), wet granulation resulted in the formation of larger granules with a narrower particle size distribution, superior flow properties and lower friability, but disintegration times were longer than that of powder used for direct compression.

Wet granulation can be either aqueous or non-aqueous. Non-aqueous granulation is less common today because of potential health, safety and environmental concerns depending on the solvent used. Aqueous granulation is very commonly used in tablet manufacture. Today, most wet granulations are water based (Moreton, 2008).

Wet granulation uses a solution of the granulating agent to stick the particles of the formulation components together such that, after drying and subsequent final blend preparation, the resulting granulate has the necessary properties that allow the tablet to be formed; and the tablet produced has sufficient mechanical strength to retain its integrity through any subsequent further processing, packaging and eventual administration to the patient. Water and heat can be detrimental to the disintegrating agent and the API. It can also promote degradation of the API (Moreton, 2008).

The disintegrant can be added to the formulation at both the wet massing step and the post-granulation blending stage, just prior to compaction into tablets. This is the difference between intra- and extragranular addition. It is important to remember that both the tablet and the granules should disintegrate to give the best opportunity for the release of the API and thereby the dissolution of the active (Moreton, 2008).

The steps required in wet granulation are therefore sequentially:

- weighing and blending the ingredients,
- preparing the dampened powder or a damp mass,
- screening the dampened powder or damp mass into pellets or granules,
- drying the granulation,
- sizing the granulation by dry screening,
- adding lubricant and blending, and
- forming tablets by compression (Allen *et al*., 2011).

Each of these processes will be discussed in the following sections.

2.4.1 Weighing and blending

Specified quantities of the active ingredient, diluents or fillers, and disintegrating agent (intra-granular) are mixed with a mechanical powder blender or mixer until uniform (Allen *et al*., 2011). Fillers include lactose, microcrystalline cellulose, starch, powdered sucrose and calcium phosphate. The choice of filler is usually based on the experience of the manufacturer with the material, its relative cost, and its compatibility with the other formulation ingredients. Among the fillers most preferred are lactose, because of its solubility and compatibility, and microcrystalline cellulose, because of its easy compaction, compatibility and consistent uniformity of supply (Shangraw & Demarest, 1993).

Disintegrating agents include croscarmellose, corn and potato starches, sodium starch glycolate, sodium carboxymethylcellulose, polyvinylpyrrolidone (PVP), crospovidone, cation exchange resins, alginic acid and other materials that swell or expand on exposure to moisture and effect the rupture or breakup of the tablet. Croscarmellose (1 – 2%) and sodium starch glycolate (1 – 5%) are often preferred because of their high water uptake and rapid action. The total amount of disintegrant used is not always added prior to granulation. Often a portion

(sometimes half) is reserved and added to the finished granulation prior to tablet formation. The rationale behind this is that one portion assists the disintegration of the tablet into granules and the other portion assists in the disintegration of the granules into fine particles (Ali, 2011; Allen *et al*., 2011).

2.4.2 Preparing the damp powder mass

A liquid binder is added to the powder mixture to facilitate adhesion of the powder particles. Upon addition of the granulation fluid, either a dampened powder or a damp mass resembling dough, is formed and used to prepare granules. A good binder results in appropriate tablet hardness and does not obstruct the release of the drug from the tablet (Summers & Aulton, 2002; Ali, 2011; Allen *et al*., 2011).

Binding agents that are frequently used, include solutions of povidone. If the drug substance is adversely affected by an aqueous binder, a non-aqueous solution, or dry binder, may be used. The amount of binding agent used is part of the operator's preference; however, the resulting binder-powder mixture should compact when squeezed in the hand. The binding agent contributes to adhesion of the granules to one another and maintains the integrity of the tablet after compression. However, care must be exercised not to overwet or underwet the powder. Overwetting can result in granules that are too hard for proper tablet formation and underwetting can result in tablets that are too soft and tend to crumble. When desired, a colorant or flavorant may be added to the binding agent to prepare a granulate with an added feature (Allen *et al*., 2011).

2.4.3 Screening the damp mass into pellets or granules

The damp powder granules are screened, or the wet mass is pressed through a screen to prepare the granules. This may be done by hand or with special equipment that prepares the granules by extrusion through perforations in the apparatus. The resultant granules are spread evenly on large lined trays and dried to consistent weight or constant moisture content (Summers & Aulton, 2002; Ali, 2011; Allen *et al*., 2011).

2.4.4 Drying the granulate

Granules may be dried in thermostatically controlled ovens that constantly record the time, temperature, and humidity (Summers & Aulton, 2002; Ali, 2011; Allen *et al*., 2011).

2.4.5 Sizing the granulate by dry screening

After drying, the granules are passed through a screen of a smaller mesh than that used to prepare the original granulate. The degree to which the granules are reduced depends on the size of the punches. Screens of 12- to 20-mesh size are generally used for this purpose. Sizing of the granules is necessary so that the die cavities for tablet compression may be completely and rapidly filled by the free-flowing granulate (Ali, 2011; Allen *et al*., 2011).

2.4.6 Adding lubricant and blending

After dry screening, a dry lubricant is dusted over the spread-out granulate through a fine-mesh screen. Lubricants contribute to the preparation of compressed tablets in several ways:

- it improves the flow of the granulate from the hopper into the die cavity,
- it prevents adhesion of the tablet to the punches and dies during compression,
- it reduces the friction between the tablets and the machine and
- it gives a sheen to the finished tablet (Allen *et al*., 2011).

Among the more commonly used lubricants are magnesium stearate, calcium stearate, stearic acid and sodium stearyl fumarate. Magnesium stearate is most commonly used (Shangraw & Demarest, 1993). The quantity of lubricant used varies from one operation to another, but usually ranges from approximately 0.1% to 5% of the weight of the granulate (Allen *et al*., 2011). Together with the lubricant, the external disintegrating agent are also blended.

2.5 Tablet compression

There are various types of tablet presses or tableting machines, each varying in productivity, but similar in basic function and operation. They all compress a tablet formulation within a steel die cavity by the pressure exerted by the movement of two steel punches, a lower punch and an upper punch (Figure. 2.2) (Allen *et al*., 2011).

Operation of a single-punch tablet press describes the basic mechanical process. As the lower punch drops, the feed shoe filled with granulate from the hopper is positioned over and fills the die cavity. Feed shoe retracts, scrapes away the excessive granulate and levels the fill in the die cavity. Upper punch lowers and compresses the fill, forming the tablet. Upper punch retracts as the lower punch rises with the formed tablet to the precise level of the stage. Feed shoe moves over the die cavity, shoves the tablet aside, and once again fills the cavity with granulate to repeat the process. Samples of tablets are assayed and tested for various quality standards described in the pharmacopoeias (BP and USP) (Allen *et al*., 2011).

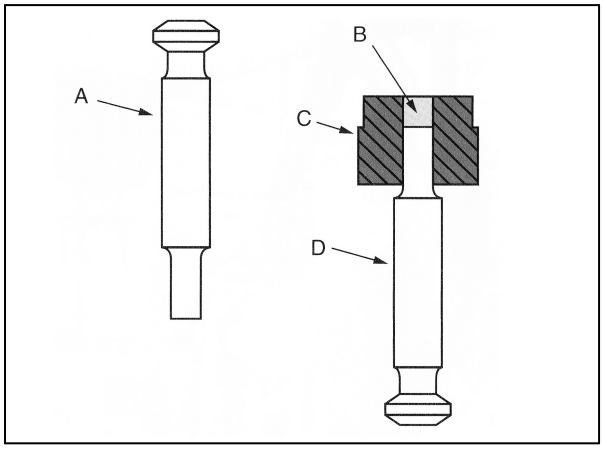


Figure 2.2: Punch and die set. *A.* Upper punch. *B.* Die cavity. *C.* Die. *D.* Lower punch (Allen *et al*., 2011).

2.6 Testing features of tablets

The physical features of compressed tablets are well known: shape; size, engraved; coated or uncoated; and coloured or uncoloured. In addition to the apparent features of tablets, tablets must meet other physical specifications and quality standards. These include: criteria for weight; weight variation; content uniformity; thickness; hardness; disintegration; and dissolution. The factors must be controlled during production and verified after the production of each batch to ensure that the established product quality standards are met (Allen *et al*., 2011).

2.6.1 Weight variation

The fill volume in the die of a tablet press determines the weight of the tablet. The fill volume is adjusted with the first few tablets to yield the desired weight and content. For example, if a tablet is to contain 20 mg of an active and if 100 000 tablets are to be produced; 2000 g of active is included in the formula. After the addition of the excipients, such as the diluents, disintegrant, lubricant and binder, the formulation may weigh 20 kg, which means that each tablet must weigh 200 mg for 20 mg of active to be present. Thus, the die cavity must be adjusted to hold a volume of granulate weighing 200 mg. During production, sample tablets are periodically removed for visual inspection and automated physical measurement (Allen *et al*., 2011).

The United States Pharmacopoeia (USP) and British Pharmacopoeia (BP) contain a test for determination of dosage form uniformity by means of weight variation. In the test, 20 tablets are weighted individually and the average weight is calculated. The tablets are assayed and the content of active ingredient in each of the 20 tablets is calculated assuming homogeneous drug distribution (BP, 2011; USP, 2011). Not more than two of the individual weights may deviate from the average weight by more than the percentage deviation shown in Table 2.3 and none may deviated by more than twice that percentage (BP, 2011).

Table 2.3: Requirements for uniformity of weight indicating the percentage deviation on average weight (BP, 2011).

Pharmaceutical form	Average Weight	Percentage deviation
Tablets (uncoated and film-coated)	80 mg or less	10
	More than 80 mg and less than 250 mg	7.5
	250 mg or more	5

2.6.2 Hardness and friability

During tablet manufacture, the punches exert force on the tablets. Generally, the higher the pressure applied, the harder the tablets, although the characteristics of the granulate also have a bearing on hardness. Certain tablets, such as lozenges and buccal tablets, which are intended to dissolve slowly, are intentionally made relatively hard. Other tablets, such as those for immediate drug release, are made relatively soft. In general, tablets should be sufficiently hard to resist breaking during normal handling and yet soft enough to disintegrate properly after swallowing (Allen *et al*., 2011).

Special dedicated hardness testers of multifunctional systems are used to measure the degree of force required to break a tablet. Multifunctional automated equipment can determine the weight, hardness, thickness and diameter of the tablet (Allen *et al*., 2011).

A tablet's durability may be determined through the use of a friabilator. This apparatus determines the tablet's friability, or tendency to crumble by allowing the tablets to roll and fall within a drum. The tablets are weighed before and after a specified number of rotations and any weight loss is determined. Resistance to loss of weight indicates the tablet's ability to withstand abrasion during handling, packaging and shipment. A maximum weight loss of not more than 1% is generally considered acceptable for most products (Allen *et al*., 2011, BP, 2011).

2.6.3 Disintegration

For the API in a tablet to become fully available for absorption, the tablet must first disintegrate and discharge the drug to the gastrointestinal fluids for dissolution. Tablet disintegration is also important for tablets containing agents that are not intended to be absorbed but rather act locally within the gastrointestinal tract. In these instances, tablet disintegration provides drug particles with an increased surface area for activity within the gastrointestinal tract (Allen *et al*., 2011).

All tablets must pass a test for disintegration, which is conducted *in vitro* using a disintegration apparatus. The apparatus consists of a basket and rack assembly containing six open-ended transparent tubes of USP-specified dimensions, held vertically upon a 10-mesh stainless steel wire screen. During testing, a tablet is placed in each of the six tubes of the basket and through the use of a mechanical device, the basket is raised and lowered in the immersion fluid at 29 to 32 cycles per minute, the wire screen always below the level of the fluid. For uncoated tablets, buccal tablets, and sublingual tablets, water at 37 ºC serves as the immersion fluid unless another fluid is specified in the individual monograph. For these tests, complete disintegration is defined as "that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus, is a soft mass having no palpably firm core" (Allen *et al*., 2011; USP, 2011). Tablets must disintegrate within 15 minutes. If one or more tablets fail to disintegrate, additional tests must be performed (BP, 2011).

2.6.4 Dissolution

Dissolution plays an important role in the availability of the drug to the organism. Dissolution rate can be a rate-limiting step in absorption and factors that affect it might also affect absorption. Dissolution rates are determined by two methods: the constant-surface method, which provides the intrinsic dissolution rate of the agent and particulate dissolution. Particulate dissolution is when a weighed amount (tablet) is added to the dissolution medium in a constant agitation system (Allen *et al*., 2011).

The goal of *in vitro* dissolution testing is to provide a reasonable prediction of, or correlation with, the product's *in vivo* bio-availability. The system relates combinations of a drug's solubility

and its intestinal permeability as a possible basis for predicting the likelihood of achieving a successful *in vivo-in vitro* correlation (Dressman *et al.*, 1998; Cardot *et al*., 2007).

As noted previously, tablet disintegration is the important first step to the dissolution of the active in a tablet. A number of formulation and manufacturing factors can affect the disintegration and dissolution of a tablet, including:

- particle size of the active;
- solubility and hygroscopicity of the formulation;
- type and concentration of the disintegrant, binder and lubricant;
- manufacturing method, particularly the compactness of the granulate and compression force used in tableting;
- and any in-process variables (Chowhan, 1994).

Together, these factors present a set of complex interrelated conditions that have a bearing on a product's dissolution characteristics. Therefore, batch-to-batch consistency is vitally important to establish dissolution test standards and controls for both materials and processes; and to implement them during production and in final testing (Allen *et al*., 2011).

There are many designs for dissolution apparatus, but the equipment consists of a variable speed stirrer motor, a cylindrical stainless steel basket on a stirrer shaft or a paddle as the stirring element, a 1000 ml vessel of glass or other inert transparent material fitted with a cover, and a water bath to maintain the temperature (37 ºC ± 0.5 ºC) of the dissolution medium in the vessel. In each test, a volume of the dissolution medium (as specified in the monographs) is placed in the vessel. The stirrer is rotated at the speed specified and at stated intervals, samples of the medium are withdrawn for chemical analysis of the proportion of active dissolved. The tablet must meet the stated monograph requirement for rate of dissolution (Allen *et al*., 2011).

In general, an aqueous medium is used. The composition of the medium is chosen on the basis of the physico-chemical characteristics of the API and the excipients within the range of conditions to which the dosage form is likely to be exposed after its administration. This applies in particular to the pH and the ionic strength of the dissolution medium. The pH of the dissolution medium is usually set between pH 1 and pH 8. For the lower pH values in the acidic range, 0.1 M hydrochloric acid is normally used. Water is only used as dissolution medium

when it is proven that the pH variation does not have an influence on the dissolution characteristics. In specific cases, dissolution media may contain enzymes, surfactants, inorganic substances and organic substances. The dissolution medium is normally 500 – 1000 ml and a stirring speed of between 50 rpm and 100 rpm is normally chosen (BP, 2011).

Requirements are met if the quantities of the active ingredient dissolved from the dosage units tested conform to the criteria specified in Table 2.4. Continued testing through the three stages (S) are done unless the results conform at either S_1 or S_2. The quantity, Q, is the amount of dissolved active ingredient specified in the monograph, expressed as a percentage of the labelled content of the dosage unit (BP, 2011; USP, 2011).

Table 2.4: Acceptance criteria during the three different stages of dissolution testing (BP, 2011).

Stage	Number tested	Acceptance criteria
S_1	6	Each unit is not less than Q + 5%
S_2	6	Average of 12 units (S_1 + S_2) is equal to or greater than Q, and no unit is less than Q - 15%
S_3	12	Average of 24 units (S_1 + S_2 + S_3) is equal to or greater than Q, not more than 2 units are less than Q - 15%, and no unit is less than Q - 25%

The quantity of API in the dissolution media can be determined by various methods but the use of high performance liquid chromatography (HPLC) enables the dissection of complex mixtures into their individual components. This can be achieved by making use of different interactions of compounds in solution, with a stationary phase. By selection of a particular combination of a mobile and stationary phase, the mode of separation can be chosen and optimised (Primer, 2001). HPLC is essentially column chromatography which perform separation under pressure.

HPLC methods can be divided into normal-phase HPLC and reverse-phase HPLC (Wells, 2002).

A hydrophobic stationary phase is used and compounds are loaded under aqueous conditions. Consequently, hydrophobic compounds preferably interact with the stationary phase, rather than remaining dissolved in the aqueous phase. After loading, the conditions in the liquid phase are slowly changed from aqueous to organic. This result in the elution of compounds from the stationary phase and detection of compounds yields an output and is given in a chromatogram. If needed, quantification of compounds can be performed by calculating the area under the curve of the peaks in the chromatogram. Depending on the types of the stationary phase, the interaction strength in the reversed phase can be varied. Several other types of columns are available, differing in interaction type and/or strength. Moreover, chromatographic modes can be used in sequence, enabling the separation of complex mixtures into individual compounds based on a variety of characteristics (Primer, 2001).

2.7 Requirement for a triple fixed-dose combination product

Fixed-dose combination products consist of two or more drugs co-formulated into a single dosage form. The use of fixed-dose combinations was first described in the 1980's with the combination of isoniazid, rifampicin and pyrazinimide for the treatment of tuberculosis and implemented by the World Health Organization (WHO) in 2001. The fixed-dose combination was proven to simplify treatment and improve patient compliance. In the case of tuberculosis it also prevented drug resistance (Blomberg *et al*., 2001). Later, triple fixed-dose combination products was developed for cancer (Ychou *et al*., 2003), pain management (Diener *et al*., 2005), antiretroviral therapy (Willig *et al*., 2008), hypertension (Calhoun *et al*., 2009) and diabetes (Bell *et al*., 2011). Commercially combination products lower administration cost by simplified packaging, less dispensing and less co-payment on the product (Blomberg *et al*., 2001).

Pharmaceutical development studies are especially important for fixed-dose combination products because they are technically more demanding than single-component products (WHO, 2005c). Issues that are specific to the development of fixed-dose combination products are summarised in Table 2.5.

Table 2.5: Important studies to consider during the development of a fixed-dose combination product (Adapted from WHO, 2005c).

Issue	How to test/Important notes
Chemical and physicochemical compatibility of the API's in an FDC with one another as well as with possible excipients.	Thermal analysis, differential scanning calorimetry (DSC).
The degradability of each API under stress conditions in the presence of the others.	Degradation of the API over time, decomposition kinetics.
Uniformity of content of each active prior to compression (tablets) or filling (for instance capsules, sachets and suspension dosage forms).	Weight variation determines whether mixing during manufacture is adequate
Validated analytical procedures.	Methods should be validated for each active in the presence of the others during development of analytical methods for quality control of the finished product, stability testing and dissolution testing*.
The dissolution rate of each active in pilot formulations.	Multipoint limits should normally be established for routine quality control of each active. For some products, different dissolution media may be acceptable for the different actives.
Different assay procedures may be necessary for the different actives in the finished product and for different purposes.	For example dissolution testing may be needed rather than stability testing.
For solid dosage forms a test and limit for content uniformity should be applied to any active that is present at a weight of 25 mg or when the API comprises 25% or less of a dosage unit.	If a solid dosage form is not subject to content uniformity testing (actives are present at < 25 mg and < 25% of the weight of a dosage unit), there should be a test (content uniformity) and limit for weight variation.

*Validation should be conducted for each active in the presence of the others and in the presence of related synthesis (process) impurities and potential degradation products. In the case of high-performance liquid chromatography (HPLC), possible interference by degradation products in the assay of the active can usually be controlled by peak purity testing.

Conclusion

Manufacturing of tablets involves an extensive investigation into various factors. Before tableting commences, the interaction between API's and excipients must be investigated first. Powder flow of the excipients and the mixtures must then be determined. Powder flow is important in the tableting process to obtain tablets with uniform weights.

There are two different methods of tableting, namely direct compression and granulation. Direct compression is the easiest and fastest method, but if the powder flow is poor, granulation should be used. Two different granulation processes exist, wet granulation and dry granulation. Wet granulation consists of a few steps, namely: weighing and blending; preparing a damp mass; screening of the damp mass into pellets or granules; drying of the granules; resizing the granules by dry screening and adding a lubricant; and blending.

Tablets that are manufactured should comply with specific standards. These standards includes weight variation, friability, crushing strength, disintegration and dissolution standards. Weight variation is important to ensure that each tablet contain the same amount of API. The friability and hardness of the tablets are important in normal handling, packing and shipment. For the active to be available for absorption, the tablets must first disintegrate and then dissolution of the active should take place.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

Pharmaceutical development, and more specifically processes used in the production of tablets are complex. Oral solid dosage forms, especially tablets, are the preferred dosage form for many drugs. The physical properties of the tablet, and consequently the dissolution thereof, are influenced by process parameters of the tablet press, type of excipient and the method of preparation (Ring et al., 2011). The development of a solid oral fixed-dose combination dosage form is especially challenging, because of the extra considerations during manufacture (Blomberg *et al*., 2001; Wechsler, 2005; Sanz & Fuster, 2009).

This chapter describes the study design and selection of processes and excipients for an oral triple fixed-dose combination tablet. The various materials used in the study together with the methods used to evaluate the properties of the tablets, are described.

3.2 Study background

Due to the effectiveness, simplicity and relative shorter duration of direct compression compared to wet granulation, it was considered as manufacturing process. However, studies have indicated that artesunate is a powder exhibiting poor flow and compression properties. A study with artesunate/amodiaquine indicated that wet granulation was essential in the formulation of tablets with artesunate (Lacaze *et al*., 2011). Furthermore, powder flow studies conducted in this study showed that direct compression was not a feasible manufacturing process. It was therefore decided to employ wet granulation during the formulation of artesunate-proguanil-dapsone tablets. It was important to select the most suitable excipients for wet granulation and various excipients were investigated to find a suitable prototype formulation that was compressible. Design of experiments using fractional factorial design was used in the second part of this study. A factorial design was used to determine the most appropriate tablet formulation for artesunate, proguanil and dapsone. In using a factorial design it was possible to select the most appropriate filler, binder and lubricant as well as the concentrations thereof.

Powder flow was done on the two optimal formulations. Powder flow studies on powder formulations producing unsatisfactory tablets were considered unnecessary. The flow properties of the different powder mixtures as well as the interaction between the different excipients and the three drugs were determined. Tablets of the 16 formulations were compressed and evaluated. Tablets were compressed using a Cadmach® single punch eccentric press (Ahmadabad, India) and the properties of the tablets were evaluated using the standards according to the BP (2011). The tablets were evaluated in terms of weight variation, friability, crushing strength and disintegration as end point to identify the two optimal formulations. Tests were conducted according to the methods and standards of the British Pharmacopoeia (BP). Dissolution testing was conducted on the two optimal formulations and HPLC analysis was used to determine the amount of the active pharmaceutical ingredients (API's) in the dissolution samples.

3.3 Materials

The API's and excipients together with their batch numbers and manufacturers used in this study are given in Table 3.1. The chemicals used in the dissolution studies were either of analytical grade or HPLC grade. These chemicals are specified in section 3.10.

Table 3.1: Information on API's and excipients used.

Compound	Batch number	Manufacturer
Ac-Di-Sol® (Croscarmellose sodium)	T017C	FMC Corporation, Philadelphia, Pennsylvania, USA
Aerosil® (Colloidal silicon dioxide)	1016102009	Degussa Corporation, Parsippany, USA
Artesunate	ASE0910030	DB Fine Chemicals, South Africa
Avicel® PH 101 (Microcrystalline cellulose)	60839C	FMC Corporation, Cork, Ireland
Avicel® PH 200	M939C	FMC Corporation, Cork, Ireland
Dapsone	20100128	DB Fine Chemicals, South Africa
Granulac® 200 (α-lactose monohydrate)	4990	Molkeri Meggle, Wasserburg, Germany
Kollidon® 30	86085224UO	BASF SE, Ludwigshafen, Germany
Kollidon® VA 64 (Co-povidone)	93520356P0	BASF SE, Ludwigshafen, Germany
Magnesium stearate	472131	Kirsch Pharma, South Africa
Proguanil	PDG/0911B/RD03	DB Fine Chemicals, South Africa
PRUV® (Sodium stearyl fumarate)	3000J103	Penwest, Reigate, Surrey, England

3.4 Formulation development

3.4.1 Direct compression

The API's (artesunate, proguanil and dapsone), lubricant (magnesium stearate, 0.5% w/w), disintegrant (Ac-di-sol®, 2% w/w), glidant (Aerosil®, 1% w/w) and filler (Avicel® PH 200, 28.27% w/w) were mixed in a Turbula®-mixer (model T2C W.A. Bachofen, Basel, Switzerland) at 69 rpm for 10 minutes. A 40 kg patient was used to calculate the amount of API. Adult malaria patients have an average weight range of 40-76 kg and the dose was based on the minimum amount of drug that will be needed (Miller et al., 2009). The choice of the amounts of API's and the excipients were based on literature. Amounts used are given in Table 3.2. The amount (% w/w) of the excipients was used according to the values given by Rowe *et al*. (2006).

Table 3.2: Amount of API's and excipients used in the direct compression formulation.

API of excipient	**Amount**
Artesunate	160 mg/tablet (4 mg/kg) (Gibbon, 2005; Olliaro *et al*., 2010)
Proguanil	320 mg/tablet (8 mg/kg) (White, 2009)
Dapsone	100 mg/tablet (2.5 mg/kg) (White, 2009)
Magnesium stearate	4.25 mg/tablet (0.5% w/w) (Rowe *et al*. 2006)
Ac-di-sol®	17 mg/tablet (2% w/w) (Rowe *et al*. 2006)
Aerosil®	8.5 mg/tablet (1% w/w) (Rowe *et al*. 2006)
Avicel® PH 200	609.75 mg/tablet (28.27% w/w)
Total	850 mg/tablet

3.4.2 Wet granulation

The artesunate, proguanil and dapsone; Avicel® PH 101 or Granulac® 200 (fillers); and intern disintegrant Ac-di-Sol® (1%), were mixed in a 250 cm^3 glass container 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. The Kollidon® VA 64 or Kollidon® 30 (depending on the formula) was weighed and dissolved in distilled water. The powder mixtures were wetted using the Kollidon® VA 64 or Kollidon® 30 solution until a damped powder mass was obtained. Granulation took placed using a 10-mesh (1700 µm) sieve. The granules were dried at 75 ºC for approximately 30 minutes until dry. The granules were subsequently forced through a 20-mesh sieve (850 µm) to produce free-flowing granules. The extragranular component of the disintegrant (Ac-di-Sol®, 1%), the lubricant (Pruv®) and glidant (Aerosil®) were weighed and mixed with the granules for 5 minutes at 69 rpm.

The independent factors used for the factorial design were the type of filler (X1), quantity of binder (X2), quantity of lubricant (X3) and quantity of glidant (X4). The dependent factors were the measured responses (Y) from the powder mixtures after tableting. These factors were weight variation, crushing strength, friability and disintegration. A four factor, two level (2^4) factorial design (a total of 16 formulations) (see Table 3.3) was used to investigate the effects of the independent factors (excipients) on the measured responses. The levels of the excipients were selected with the intention that their relative difference were big enough to present a measurable effect on the response. These levels were based on a pilot study (data not shown).

Table 3.3: Factors and levels with the assigned formulation numbers of the 2^4 factorial design.

				Diluent			
				Avicel PH® 101		Granulac 200	
				Lubricant			
				Pruv® 2%	Pruv® 5%	Pruv® 2%	Pruv® 5%
Glidant	Aerosil® 2%	Binder	Kollidon® VA 64 6%	1	2	3	4
			Kollidon® VA 64 3%	5	6	7	8
	Aerosil® 1%		Kollidon® VA 64 6%	9	10	11	12
			Kollidon® VA 64 3%	13	14	15	16

3.5 Interaction of materials

To investigate the presence of possible interaction between the different API's and excipients in the formulations, differential scanning calorimetry (DSC) was used. Thermograms were recorded with a Mettler Toledo DSC823^e (Mettler Toledo AG, South Africa) instrument. The measurement conditions were given in table 3.4.

Table 3.4.: Measurement conditions for DSC.

Sample weight	Sampler holder	Gas flow	Heat rate
1 – 5 mg	Aluminium crimp cell	Nitrogen at 80 ml/min	10 ℃ per minute

3.6 Flow properties

Characterisation of the flow properties was used during pre-formulation to investigate whether direct compression could be considered as a manufacturing method. Furthermore, flow properties of the granules of the two optimal formulations were also characterised. These properties were characterised in terms of angle of repose, critical orifice diameter (COD), flow rate, Carr's index and the Hausner ratio. Experiments were done in triplicate.

3.6.1 Angle of repose

Angle of repose was determined by using a stainless steel container with an orifice fitted with a shutter. The different powder mixtures (100 g) were poured into the container and the shutter was opened to let the powder flow through. Figure 3.1 shows the apparatus used. Photos were taken and the angle of repose was determined using the height (cm) and radius (cm) of the heap as described in section 2.3.1.

Figure 3.1: The apparatus used to determine the angle of repose. The powder heap used to determine the height and radius can be seen at the bottom of the photo.

3.6.2 Critical orifice diameter (COD)

The measurements were determined similar to the method for angle of repose described in section 2.3.2 (see Figure 3.2). The COD was determined by starting with the smallest orifice and enlarging the orifice diameter to determine the orifice with the smallest diameter through which the powder was able to flow.

Figure 3.2: The apparatus used to determine the COD.

3.6.3 Flow rate

Flow rate was determined using a stainless steel container with an orifice of 10 mm and the time taken for the powder sample (100 g) to discharge from the container was determined using a stopwatch.

3.6.4 Bulk density and tapped density

A known amount of the sample (100 g) was poured through a funnel into a 100 ml graduated measuring cylinder. The cylinder was then lightly tapped to collect all the powder sticking on the wall of the cylinder. The volume was read directly from the cylinder and used to calculate the bulk density (see Equation 3.1). The cylinder was tapped on a wooden bench top to attain a constant volume reading. This constant volume was noted and used to calculate the tapped density (see Equation 3.1). Carr's Index and the Hausner ratio were calculated using the equation given in section 2.3.3.

$$\text{Density} = \frac{\text{Weight}}{\text{Volume}} \qquad \textbf{Equation 3.1}$$

3.7 Tablet compression

Tablets were compressed using a Cadmach® single punch eccentric press (Ahmadabad, India). The upper punch settings were used to determine the mechanical strength of the tablets and the lower punch settings were used to adjust the filling volume of the die. After manufacturing the tablets were stored for 24 hours before analysis.

3.8 Evaluation of tablet properties

3.8.1 Weight variation

Twenty tablets of each batch were dusted and weighed individually on a Precisa® analytical balance (model 240A, OERLIKON AG, Zurich) according the guidelines given by the BP, 2011.

3.8.2 Friability

Ten tablets of each formulation were dusted and weighed simultaneously. The tablets were placed in a Roche® friabilator and rotated for 4 minutes at 25 rpm. The tablets were removed, dusted and weighed again. Broken tablets were not included in the weighing process. Equation 3.2 was used to calculate the percentage friability.

$$\% \text{ Friability} = \frac{w_b - w_a}{w_b} \text{ x } 100 \qquad \textbf{Equation 3.2}$$

Where:

w_a = weight of tablets after rotation

w_b = weight of tablets prior to rotation

3.8.3 Tablet crushing strength, diameter and thickness

Ten tablets of each formulation were used to determine the crushing strength, diameter and thickness using a Pharma Test® (model PTB-311) tablet test unit (Pharma Test, Switzerland).

3.8.4 Disintegration

The disintegration times of six tablets of each formulation were determined using an Erweka® tablet disintegration unit (Erweka, Heusenstamm, Germany). The disintegration medium was distilled water and was maintained at a temperature of 37 ± 0.5ºC by a thermostat. Tablets must disintegrate within 15 minutes (BP, 2011).

3.9 Dissolution

3.9.1 Assay

The assay was done by weighing 20 tablets. The tablets were crushed and the average weight was calculated. For the artesunate assay, 300 mg powder was dissolved in acetonitrile (4 mg/ml). For the proguanil assay, 187 mg powder was dissolved in acetonitrile (0.94 mg/ml) and for the dapsone assay, 60 mg powder was dissolved in acetonitrile (0.4 mg/ml).

3.9.2 Dissolution

Dissolution tests were performed in an Erweka D700 and/or an Erweka DT6R (Erweka, Heustenstamm, Germany) six station paddle- or basket-method dissolution apparatus. The paddles or baskets were rotated at a speed of 100 rpm for the dapsone dissolution, 50 rpm for the proguanil dissolution and 75 rpm for the artesunate dissolution. The temperature was regulated with a thermostat at 37 ± 0.5ºC. The dissolution studies were done according to the individual monographs and the condition of each method is given in Table 3.4. Samples were withdrawn (3 ml) and filtered using in-line Millipore 0.45 µm filters (Microsep, Sandton, South Africa). Immediately after sampling, the withdrawn volume was replaced with new dissolution

medium with the same temperature. Samples were transferred to glass HPLC-vials to be analysed using an HPLC method that is describe in section 3.10.

Table 3.5: Dissolution conditions for the different dissolution tests according to the individual monographs.

	Artesunate (Ph.Int., 2011)	**Proguanil** (BP, 2011)	**Dapsone** (USP, 2011)
Assembly	Paddles	Paddles	Baskets
Rotation speed	75rpm	50rpm	100rpm
Withdrawal times	15, 30, 45 min*	15, 30, 45, 60, 90 min	15, 30, 45, 60, 90 min
Medium	Phosphate buffer pH6.8	0.2M HCl, 0.2% NaCl	0.2M HCl
Medium volume	900ml	900ml	1000ml

* the maximum time allowed for dissolution of artesunate was limited to 45 minutes. Artesunate being extremely susceptible towards degradation during analysis.

3.10 HPLC methods

An HPLC technique based on the artesunate tablets assay method (A) of the 2011 International Pharmacopeia (Ph.Int., 2011) was adapted and used to quantitatively determine artesunate, proguanil and dapsone from assay and dissolution samples.

Analytical chromatographic separations were achieved using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a Novapak C18, 3 µm, 4.6 mm x 100 mm column (Phenomenex, Torrance, CA) held at 30 ºC. Software that was used is Rev. B 02.01-SR2 [260] Chemstation for LC 3D (Agilent Technologies, Santa Clara, CA). The mobile

phase consisted of 440 ml acetonitrile and 560 ml buffer (pH 3.0). The buffer contained potassium dihydrogen phosphate (1.36 g in 900 ml water) and the pH was adjusted to 3.0 with phosphoric acid and diluted to 1000 ml with distilled water. The flow was set at 1 ml/min for all the analytical tests except for dissolution of artesunate (2 ml/min) and the wavelength was set as 216 nm. The injection volume for artesunate was 20 µl, and for proguanil and dapsone, it was 1 µl. The solvent consisted of acetonitrile (assay) and the relevant dissolution medium (dissolution).

3.10.1 Validation of HPLC methods

The evaluation of specificity, linearity, range, accuracy, repeatability, limit of detection and limit of quantitation was based on the ICH guideline on validation of analytical procedures (ICH:Q2 (R1), 1997).

3.10.1.1 Specificity

Three standard solutions containing either artesunate (solution 1), proguanil (solution 2) or dapsone (solution 3) and a fourth standard solution containing a combination of all the actives (solution 4) were prepared. These standard solutions together with a mobile phase, solvent, dissolution medium and placebo were injected and analysed separately using the HPLC conditions specified above. Analyses of solution 1 – 3 identified the retention time of each active ingredient. The analyses of the solvent, mobile phase dissolution medium and placebo identified the retention time of any of these contributors. Figure 3.3 depicts a diagram of a typical HPLC chromatogram of the sample matrix. The solvent was identified at 0.7 and 0.9 minutes (C1), whereas the placebo was identified at 2.3 minutes (C2). Dapsone (D) was identified at 1.3 minutes, proguanil (P) at 2.8 minutes and artesunate (A) at 6.7 minutes. The resolution factor between D-C1, D-C2, and P-C2 was greater than 2.0, indicating sufficient separation between the contributors and the actives. The method was regarded as specific, as 1) all peaks were identified and 2) adequate resolution was obtained between the contributors and actives.

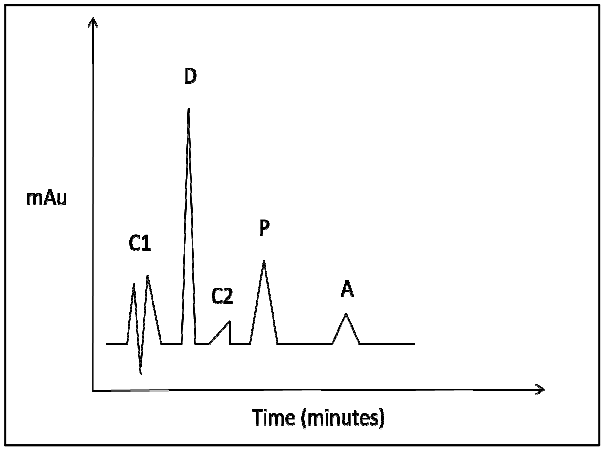


Figure 3.3: A diagram of a typical HPLC chromatogram of the analytical matrix. Time in minutes is on the x-axis and milli absorption units (mAu) on the y-axis. C1 – solvent; C2 – placebo; D – dapsone; P – proguanil; A – artesunate.

3.10.1.2 Linearity, range, repeatability and accuracy

Linearity was established for each active ingredient using five concentrations across the intended analytical concentration range (the range in which the sample was to be analysed). Artesunate standard solutions were prepared with concentrations ranging between 1.0 – 6.0 mg/ml for the assay and 34.0 – 204.0 µg/ml for dissolution. Proguanil standard solutions were prepared with concentrations ranging between 100.3 – 602.0 µg/ml for the assay and dissolution. Dapsone standard solutions were prepared with concentrations ranging between 375.1 – 1125.4 µg/ml for the assay and dissolution. A correlation coefficient (R^2) for each calibration curve (each medium) proved *linearity* across the intended analytical concentration range (Table 3.5) as the R^2 exceeded or equalled 0.99.

Table 3.6: Linearity across the analytical range.

Analysis	Regression line obtained*	Recovery (%RSD)
Artesunate assay	$y = 0.70x + 7.13$ ($R^2 = 0.99$)	98.7% (0.2%)
Artesunate dissolution	$y = 0.72x + 3.07$ ($R^2 = 0.99$)	101.8% (1.5%)
Dapsone assay and dissolution	$y = 3.83x + 293.55$ ($R^2 = 0.99$)	100.9% (0.8%)
Proguanil assay and dissolution	$y = 2.88x – 7.54$ ($R^2 = 0.99$)	98.6% (1.9%)

*Where y = HPLC response (mAu) and x = concentration (µg/ml)

The accuracy and repeatability were established across the specified range for each respective active ingredient. *Accuracy* was reported as percent (%) recovery and was determined by dividing the theoretical known concentration with the concentration derived from the linear function, multiplied by 100 (percentage). *Repeatability* of the method was assessed by calculating the relative standard deviation (%RSD) for the respective recovery values determined across the range. The recovery values obtained were found to be within 98 – 102% and the relative standard deviations of these determinations were less than 2.0% in each case, proving the capability of the method to produce results that are accurate and repeatable in the specified range.

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

The response of all the sample solutions was within the established analytical range for each respective active ingredient. No sample was analysed outside the minimum response of the standard calibration curve. The LOD and LOQ values fell below the minimum of the established concentration range, and with all sample responses above this threshold there was no need to elaborate on the LOD and LOQ as it did not pose a risk to the integrity of the results obtained.

3.11 Data analysis of factorial design

The data was analysed by Prism version 5 (Graphpad). All data were tested for normality and depending on the results was analysed by parametric (normally distributed) or non-parametric (not normally distributed) statistics. Parametric data were analysed with two way ANOVA and the Bonferroni post hoc test for significance between the variables. Non-parametrically data were analysed by one way ANOVA (Kruskal-Wallis test) with Dunns multiple comparison rank test as post hoc analysis. The significance of both cases was set as $p < 0.05$. Data were presented as mean ± standard deviation (SD) or % relative SD (%RSD). The coefficient of variation of RSD (%) equals the SD divided by the mean and can be expressed as a fraction of a percentage.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

The aim of the study was to develop an oral triple fixed-dose combination solid dosage form containing artesunate, proguanil and dapsone. Formulation of a triple fixed-dose combination product is extremely challenging due to possible incompatibilities between the different API's (Okwelogu *et al.*, 2010). This is of particular importance when artesunate is used in ACT's. Artemisinin derivatives are thermally labile and chemically reactive. It is also not stable as it is highly soluble in water (Haynes *et al.*, 2007). Combining two or more API's may also require strict adherence to regulatory requirements as described in section 2.7.

Direct compression is the preferred method for tableting and would be ideal for an antimalarial dosage form, because of its low manufacturing cost. To allow for successful direct compression during tablet manufacture the ingredients must flow freely into the die of the tablet press (Allen *et al*., 2011). The selection of various excipients to improve the physical properties of tableting mixtures is an important part of the formulation development process. Optimisation of formulations entails the evaluation of the effect of various variables in the presence of excipients (York, 2002; McCarty, 2011; Yuan, 2011). The first part of the chapter describes the selection of tableting mixtures intended for direct compression and the second part wet granulation. A factorial design was used to determine the effect of different concentrations of excipients on tablet properties. Two formulations rendering tablets with the best physical properties based on weight variation, friability, crushing strength and disintegration data were selected and the interaction of the materials, flow properties and dissolution behaviour were determined.

4.2 Formulation development

Any formulation consists of not only the active ingredients, but also various excipients with specific functions. A basic formulation consists of an API, a filler and a lubricant (York, 2002). In the initial formulation development of this study a formulation for direct compression was firstly chosen. This formulation consisted of artesunate, proguanil and dapsone and it was

decided to add a superdisintegrant (Ac-di-sol®), a filler (Avicel® PH 200) and a lubricant (magnesium stearate). Ac-di-sol®, Avicel® PH 200 and magnesium stearate are normally used in the development of tablets (Jivraj, *et al*, 2000; Yüksel *et al.*, 2007). The different amounts of the API's and excipients in the formulation DC1, are shown in Table 4.1. This formulation exhibited very poor flow properties and could not be compressed. This confirmed previous results which concluded that artesunate could not be directly compressed (Lacaze *et al*., 2011).

Flow properties of materials are enhanced by using a glidant such as Aerosil® (Abe *et al.*, 2009). Aerosil® is a colloidal silicon dioxide proven to enhance powder flow and was added to the first mixture to enhance the flow properties. It was decided to add Aerosil® to the CD1 formulation. The composition of this formulation (DC2) is given in Table 4.1. It still exhibited poor powder flow and therefore it was decided to use wet granulation as the manufacturing process.

Table 4.1: Composition of the formulations intended for direct compression (DC).

Ingredient (mg/tablet)	DC1		DC2	
	Weight per tablet (mg)	% (w/w)	Weight per tablet (mg)	% (w/w)
Artesunate	160	18.8	160	18.8
Proguanil	320	37.6	320	37.6
Dapsone	100	11.8	100	11.8
Magnesium stearate	4.25	0.5	4.25	0.5
Ac-di-sol®	17	2	17	2
Aerosil®			8.5	1
Avicel® PH 200	248.75	29.3	240.25	28.3
Total	850		850	

In the second part of the initial formulation development of this study, excipients for tableting mixtures were selected. During wet granulation various processes are followed (section 2.4) and various excipients are used. The selection of excipients is important because they have an influence on the biopharmaceutical and technological factors of the dosage form (York, 2002; Yuan, 2011). Table 4.2 gives a summary of the different processes used during the granulation and the choice of excipient.

Table 4.2: Different processes during granulation with the choice of excipient for initial formulation.

Wet granulation step	Choice of excipient
Mixing and blending of API's and excipients	API's (Artesunate, proguanil & dapsone) Filler (Microcrystalline cellulose, Avicel® PH 101) Disintegrant (Croscarmellose sodium, Ac-di-sol®)
Preparation of binder solution and massing of binder solution with powder mixture	Kollidon® 30 Distilled water
Wet screening of damp mass and drying of granules	--
Rescreening of dried granules, adding lubricant and blending with excipients	Lubricant (Magnesium stearate) Croscarmellose sodium, Ac-di-sol®

The first formulation for granulation (G1) is shown in table 4.3 and consisted of the API's, magnesium stearate, Ac-di-sol®, Kollidon® 30 and Avicel® PH 101. Water was used as granulating fluid. A study by Lacaze *et al*. (2011) indicated that artesunate undergoes a rapid breakdown in water and therefore alcoholic granulation should be used. Okwelogu *et al*. (2010), however proved that granulation with ethanol caused poor stability of the artesunate after four weeks. The different amounts of the ingredients are given in Table 4.3. Tablets were manufactured, but the lubrication was very poor. This was due to the grooves that formed on the outer surfaces of the tablets. To improve the lubrication, the concentration of the magnesium stearate was increased to 3% (G2, Table 4.3) and the amount of filler decreased to

21.7%, but the lubrication did not improve. Magnesium stearate is the most common lubricant used, but other lubricators such as Pruv® can also be used.

Pruv® consists of sodium stearyl fumarate and is a fine, white powder with agglomerates of flat, circular-shaped particles (Rowe *et al*., 2006). Results according to Shah *et al*. (1986) and Hölzer & Sjögren (1979) indicated that where magnesium stearate cannot be used due to problems of lubrication, sodium stearyl fumarate can be employed as the tablet lubricant of choice. Magnesium stearate was therefore replaced with Pruv® 3% (G3, Table 4.3) and the lubrication improved, but was still insufficient.

Aerosil® 1% (glidant) was added to Pruv® 3% (G4, Table 4.3), but lubrication still remained unsatisfactory. The Pruv® concentration was increased to 4% (G5, Table 4.3) and lubrication was sufficient, but delivered tablets with poor friability. To improve the friability of tablets a different binder can be used. Therefore Kollidon® 30 was replaced with Kollidon® VA 64. Kollidon® 30 consists of povidone and Kollidon® VA 64 consists of copovidone. Copovidone provides good adhesion, elasticity and hardness to tablets (Rowe *et al.*, 2006) and consequently the friability improved. The combination of these excipients used in granulation delivered acceptable tablets, but the formulation was not optimal. The different excipients showed good flow properties, but the tablets were still brittle and depicted low crushing strengths (test were done, but no results were obtain). It was therefore decided to investigate whether different concentration of the excipients could increase the friability and crushing strength. As the filler constituted the largest part of the excipients in the formulation it was also decided to use another filler to evaluate if the tablet properties could improve.

Table 4.3: Composition of the formulations intended for granulation (G). The manner in which the formulation was changed each time is bolded.

Ingredient (mg/tablet)	G1		G2		G3		G4		G5	
	Weight per tablet (mg)	**% (w/w)**	**Weight per tablet (mg)**	**% (w/w)**	**Weight per tablet (mg)**	**% (w/w)**	**Weight per tablet (mg)**	**% (w/w)**	**Weight per tablet (mg)**	**% (w/w)**
Artesunate	160	18.8	160	18.8	160	18.8	160	18.8	160	18.8
Proguanil	320	37.6	320	37.6	320	37.6	320	37.6	320	37.6
Dapsone	100	11.8	100	11.8	100	11.8	100	11.8	100	11.8
Magnesium stearate	4,25	0.5	**25.5**	**3**	--	--	--	--	--	--
Pruv®	--	--	--	--	**25.5**	**3**	25.5	3	25.5	3
Ac-di-sol®	17	2	17	2	17	2	17	2	17	2
Kollidon® 30	42.5	5	42.5	5	42.5	5	42.5	5	--	--
Kollidon® VA 64	--	--	--	--	--	--	--	--	**42.5**	**5**
Aerosil®	--	--	--	--	--	--	**8.5**	**1**	8.5	1
Avicel® PH 101	206.25	24.3	**185**	**21.7**	185	21.7	**176.5**	**20.8**	176.5	20.8

4.3 Evaluation of trail batches – factorial design

Factorial design is used to determine the relationship among factors that influence outputs of a process. In this study the excipients were the factors and the weight variation, friability, crushing strength and disintegration were the outputs. A 2^4 factorial design was used with four independent variables. The variables included: two different fillers 20.8% (Avicel® PH 101 and

Granulac® 200), two different concentrations (2 and 5%) of lubricant (Pruv®), two different concentrations (1 and 2%) of glidant (Aerosil®) and two different concentrations (3 and 6%) of binder (Kollidon® VA 64). In each formulation a disintegrant (Ac-di-sol®) was added in a concentration of 2%. The concentration of Ac-di-sol® was used according to literature (Rowe *et al.*, 2006).

Avicel® PH 101 consists of microcrystalline cellulose and Granulac® 200 of lactose. Microcrystalline cellulose can be used as a binder or a diluent and in both wet-granulation (PH 101) and direct compression (PH 200) formulations. Microcrystalline cellulose also has lubricant and disintegrant properties (Rowe *et al.*, 2006). It is compatible with most drugs (Jivraj *et al*., 2000), but is incompatible with strong oxidising agents (Rowe *et al*., 2006). Lactose is used as a filler or diluent and to a more limited extent in lyophilised products and infant formulations (Rowe *et al*., 2006). It is usually used in wet granulation formulations (Jivraj *et al*, 2000). Adverse reactions to lactose are largely attributed to lactose intolerance (Rowe *et al.*, 2006).

The 16 formulations were tableted. Two of the formulations (3 and 7) did not deliver acceptable tablets due to capping during the tableting process as a consequence of insufficient lubrication. Tableting was successful, but because of the lubrication problems, the tablets of certain formulations presented with small grooves on the outer surfaces. These grooves could be attributed to insufficient lubrication. The higher the concentration Pruv®, the better the lubrication was and the grooves disappeared.

Statistically, the results were analysed with one way ANOVA. Firstly, the 16 formulations were compared with each other to determine variance between them. Secondly a one way ANOVA was performed to determine the effect that the different amounts of lubricant, glidant and binder; and the two different fillers, had on the weight variation, crushing strength, friability and disintegration. Therefore (see section 3.4.2, Table 3.3) in the case of the two different filler formulations: formulations 1, 2, 5, 6, 9, 10, 13, 14, containing Avicel® PH 101, were compared to formulations 3, 4, 7, 8, 11, 12, 15, 16, containing Granulac® 200.

4.3.1 Weight variation

The weight variation was determined by accurately weighing 20 tablets of each of the 16 formulations individually. The results are summarised in Figure 4.1. See Annexure A for the values of each tablet.

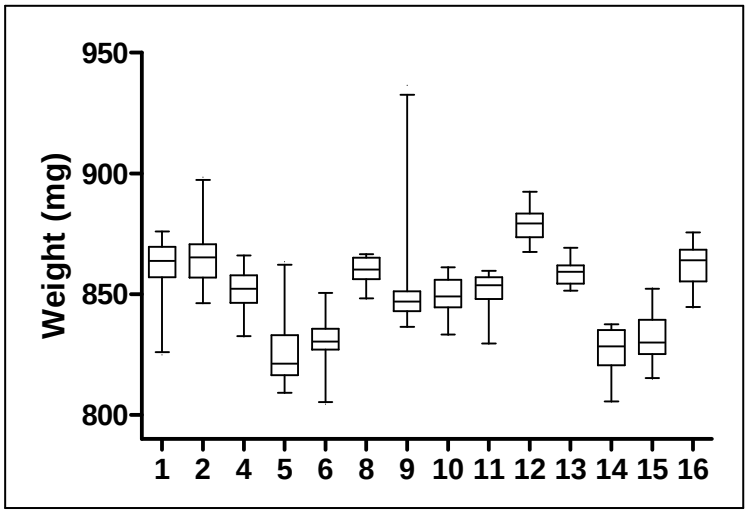


Figure 4.1: A box and whisker plot of the average weight (mg) of the different formulations (1-16) to show the weight variation. The results are presented as the mean (the horizontal line), the 25th and 75th% percentiles (the box) and the minimum and maximum values (the vertical lines or whiskers). Note that formulations 3 and 7 are not plotted.

Acceptable tablets could not be prepared from formulations 3 and 7. Therefore, the test for weight variation could not be conducted on these formulations. All the tablets evaluated (for all the formulations except for formulation 9) were within the ± 5% range (see Table 4.4) as specified by the BP. It can also be described as a measure of dispersion. Distributions with low coefficients of variation have low variance and vice versa. All of the formulations except formulations 5 and 9 had low %RSD. These two formulations had high outliers as is evident form Figure 4.1. One tablet from formulation 9 fell outside the ±10% range (Table 4.4) and therefore formulation 9 did not comply with the standard as described in section 2.6.1. (see Annexure A for the values of each tablet). The different formulations (n=14) were non-

parametrically evaluated by one way ANOVA with Dunns multiple comparison test as a post hoc test. There were variance between the formulations ($p \leq 0.05$) with formulations 5, 6, 9, 14 and 15 being significantly different from all the other formulations.

To determine the effect of the different concentrations of the excipients on the weight variation the formulations were statistically evaluated (one way ANOVA, $p \leq 0.05$). The two different fillers significantly influenced the weight variation. Formulations containing Granulac® 200 (filler) and Kollidon® VA 64 (binder) resulted in tablets with significantly higher weight variation ($p \leq 0.05$). The incorporation of a glidant and lubricant also showed a significant influence on the weight variation. Weight variation is an indirect measurement of powder fluidity. Uniform powder flow allows consistent filling and particle arrangement in the die cavity and therefore produces tablets of uniform weight and active ingredient content (Steckel & Midnermann-Nogly, 2004). The formulation with the least weight variation is therefore expected to have good flow properties. Formulations 8, 10, 12 and 13 depicted the least amount of variance.

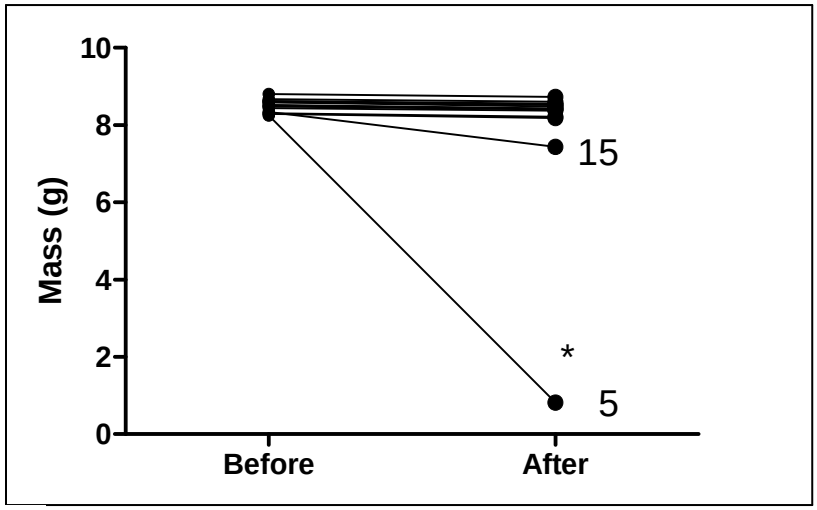
Table 4.4: The ± 5% range of the 16 different formulations of the factorial design.

Formulation	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
- 5% range (mg)	819	821		809	784	790		817	809	806	809	835	816	785	790	819
+ 5% range (mg)	905	908		894	867	873		903	894	891	894	923	902	868	873	906

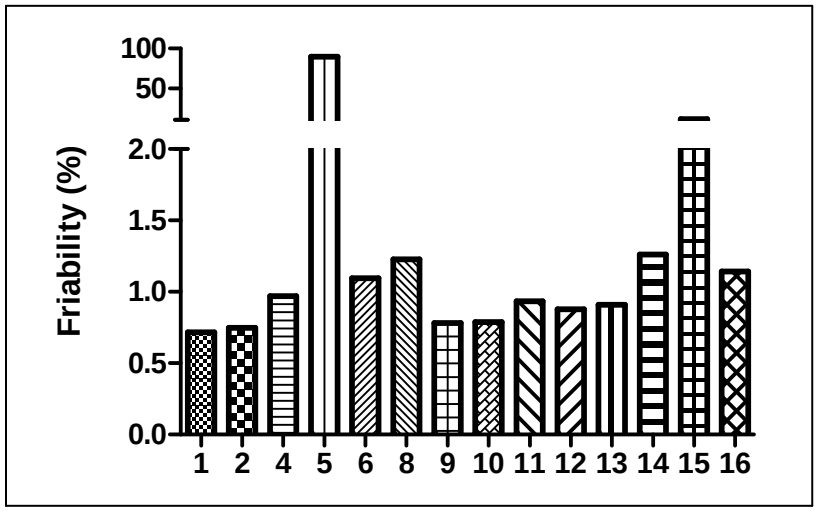
4.3.2 Friability

Friability determines the tendency of a tablet to crumble and resistance to weight loss indicates the tablet's ability to withstand abrasion. The tablets were weighed before and after friability. Results from the friability test are summarised in Figure 4.2A and it could be seen that most of the formulations had similar weight before and after with less than 1% weight loss (Figure 4.2B). Formulation 5 and 15 had large decreases in the weight after the friability test and the decrease

in the weight of these formulations were statistically significantly ($p \leq 0.05$). Eight of the formulations (1, 2, 4, 9, 10, 11, 12 and 13) had friability values of less than 1% and complied with the standard given by the BP (see Annexure B). All these formulations, except formulation 13, contained 6% Kollidon® VA 64, i.e. the highest concentration of binder used in this study. Six of the formulations (5, 6, 8, 14, 15 & 16) had friability values that were between 1.10% and 90.02%, and therefore did not comply with BP standards. These formulations contained lower concentrations of the binder (3%). Results indicated that the binder concentration had a significant influence on the friability of the tablets. According to the results formulations 1, 2, 9, 10 and 12 with the lowest friability values, were the most suitable formulations. The overall effect of the excipients on the friability statistically indicated that none of the other excipients had any influence. However, Granulac® 200 and the higher concentration of Granulac® 200 resulted in low friability values, whereas high concentrations of the glidant resulted in high friability values.



A



B

Figure 4.2: Friability (%) of the different formulations. A) the weight before and after used in the friability determination. B) the friability (%) of the formulation. Formulations 5 and 15 had high values (Note the break in the axis) and the graph was modified to visually improve the results. Formulations 3 and 7 are not plotted.

4.3.3 Crushing strength, diameter and thickness

Tablets should be hard to resist breakage, but soft enough to disperse. Tablet crushing strength or hardness is described as the resistance of tablets to capping, abrasion or breakage. The crushing strengths (N) of the formulations are shown in Figure 4.3. The mean crushing

strength of the formulations varied between 59.29 Newton and 113.47 Newton (see Annexure C). Distribution of the data indicated large variation in the crushing strength between the formulations. Formulations 1, 2, 4, 11, 12 and 13 all depicted crushing strengths above 100 N and formulations 9, 10, 12, 13 and 16 depicted crushing strengths above 90 N. Formulations 6, 8 and 14 depicted low crushing strengths of 79.24, 87.19 and 71.65 N, respectively. Formulation 5 depicted a very low crushing strength of 59.29 N. One way ANOVA of the 16 formulations indicated variance between the formulations with formulations 5, 6, 8 and 14 being significantly different from the other formulations ($p \leq 0.05$). Formulations 5, 6 and 14 did not comply with the guidelines given by the BP. Formulations 4, 6, 10, 13 and 14 all depicted low variation and formulations 4, 10 and 13 all depicted high crushing strengths and were considered the most suitable formulations. Overall, the formulations containing Avicel® PH 101 rendered tablets that were harder than the formulations containing Granulac® 200. The higher concentration binder also resulted in harder tablets. These differences were statistically significant ($p \leq 0.05$). Higher concentrations of the lubricant and binder decreased the crushing strength, but not significantly.

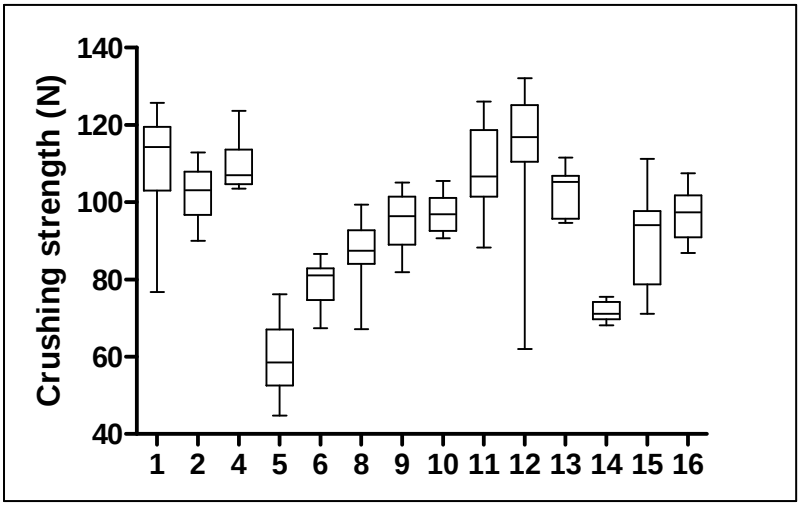


Figure 4.3: A box and whisker plot of the average crushing strength (Newton) of the different formulations (1-16). The results are presented as the mean (the horizontal line), the 25th and 75th% percentiles (the box) and the minimum and maximum values (the vertical lines or whiskers). Note that formulation 3 and 7 are not plotted.

The average diameter differed between 14.06 mm and 14.13 mm (Figure 4.4). The variation of formulations 4, 8, 11, 14 and 15 were relatively low. Formulations 1 and 5 produced tablets that were relatively wider than the other tablets. The upper punch setting during tableting were set lower for formulation 1 and 5, because the lubrication was poor and therefore the binding properties were lost. The average thickness of the different formulations differed between 5.54 ± 0.113 mm and 5.98 ± 0.113 mm. Formulation 1 and 5 produced tablets that were relatively thicker than the other formulations (Figure 4.5), because of the poor lubrication as describe above. Formulations 2, 6, 9, 13 and 14 had the least amount of variance.

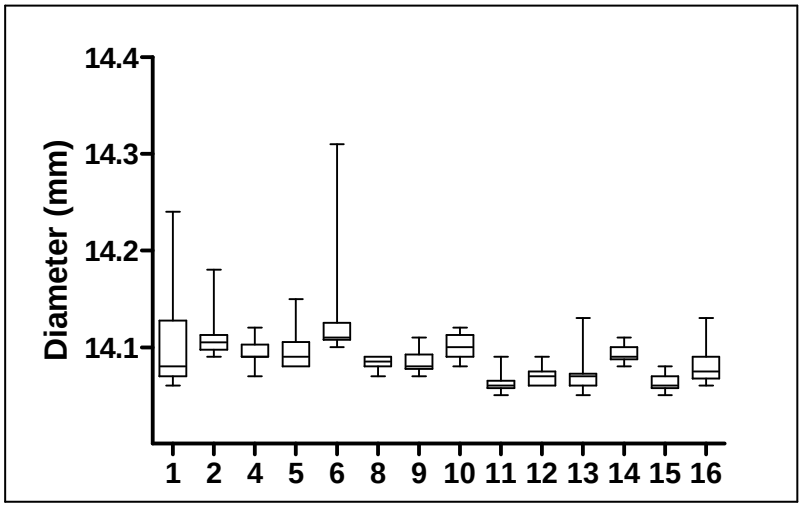


Figure 4.4: A box and whisker plot of the average diameter (mm) of the different formulations (1-16). The results are presented as the mean (the horizontal line), the 25th and 75th% percentiles (the box) and the minimum and maximum values (the vertical lines or whiskers). Note that formulation 3 and 7 are not plotted.

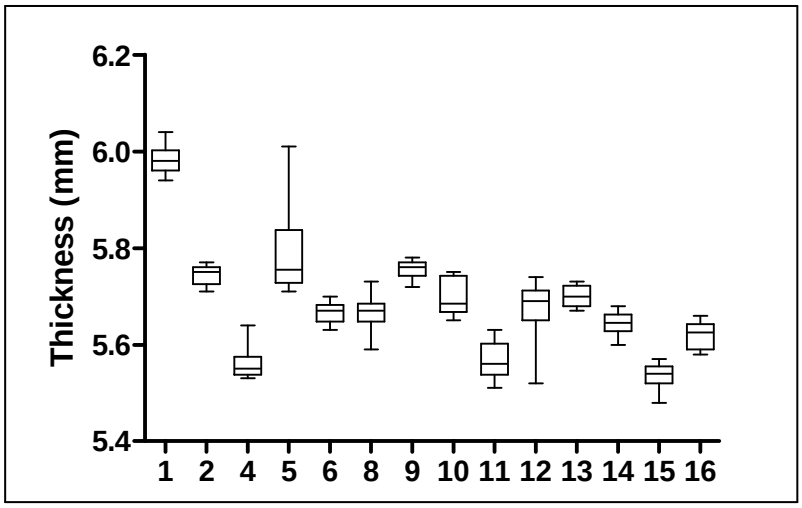


Figure 4.5: A box and whisker plot of the average thickness (mm) of the different formulations (1-16). The results are presented as the mean (the horizontal line), the 25th and 75th% percentiles (the box) and the minimum and maximum values (the vertical lines or whiskers). Note that formulation 3 and 7 are not plotted.

4.3.4 Disintegration

For the API in a tablet to become fully available for absorption, the tablet must first disintegrate and discharge the drug to the gastrointestinal fluids for dissolution. The disintegration of the 16 formulations are summarised in Figure 4.6. Formulations 4 and 12 had long disintegration times (see Annexure D), because of the high concentration of Pruv® (5%) and the high concentration of Kollidon® VA 64 (6%). Formulations 4, 12 and 16 had large coefficients of variation and differed statistically significantly from the other formulations. Disintegration of all the formulations complied with the standards given in the BP. The overall statistics indicated that the formulations that contained Avicel® PH 101 disintegrated faster than the formulations containing Granulac® 200, except for formulation 15 that contained Granulac® 200, but it also contained a low concentration (3%) Kollidon® VA 64. Formulation 10 (containing Avicel® PH 101, a high concentration (6%) of Kollidon® VA 64 and a high concentration (5%) of Pruv®) disintegrated a bit slower (41.7 seconds) than the other formulation containing Avicel PH® 101. The Aerosil® concentration is lower and therefore the binding properties of the Kollidon® is better.

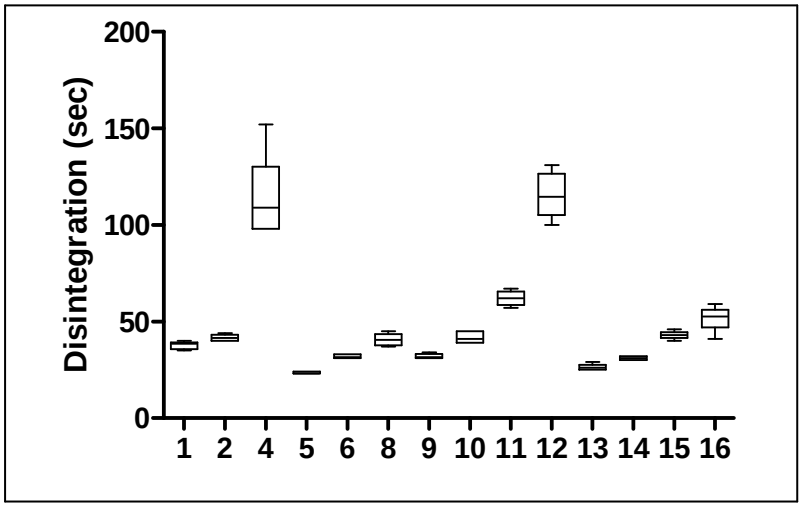


Figure 4.6: A box and whisker plot of the average disintegration (seconds) of the different formulations (1-16). The results are presented as the mean (the horizontal line), the 25th and 75th% percentiles (the box) and the minimum and maximum values (the vertical lines or whiskers). Note that formulation 3 and 7 are not plotted.

4.3.5 Selection of optimal formulations

A factorial design was used to determine the optimum tablet formulation for artesunate, proguanil and dapsone triple fixed-dose combination formulation. According to the results describe above and the data analysed by statistica (see Annexure I), it was decided on formulation 10 (FA) and formulation 12 (FG) as the optimum formulations. The formulations included Avicel® PH 101 (FA) 20.8% or Granulac® 200 (FG) 20.8%; Pruv® 5%; Aerosil® 1%; Kollidon® VA64 6%; and Ac-di-Sol® 2%.

Table 4.5: Summary of the p-values obtained with the one way ANOVA of the overall effects that the two different fillers and the different concentrations of lubricant, glidant and binder had on the weight variation, friability, crushing strength and disintegration

Excipient	p-value			
	Weight variation	**Friability**	**Crushing strength**	**Disintegration**
Filler	0.000012	0.783415	0.000014	0.000000
Lubricant	0.011759	0.183283	0.734321	0.000098
Glidant	0.460682	0.240434	0.098238	0.685521
Binder	0.000000	0.269470	0.000000	0.000000

Based on the results of the different tests obtained for the different formulations, the following conclusions were drawn (see Table 4.5):

- The lubricant and glidant had no significant influence on the crushing strength of the tablets.
- The filler and binder concentration had a significant influence on the crushing strength. The higher the concentration (6% w/w) Kollidon® VA 64 rendered tablets with a higher crushing strength and therefore tablets that complied with the BP standards regarding friability.
- Pruv® (lubricant) at a concentration of 5% w/w rendered tablets with no grooves, indicating sufficient lubrication and were therefore the suitable choice as lubricant.
- In terms of glidant, Aerosil® at a concentration of 2% w/w appeared to impart no additional benefit and therefore Aerosil® at a concentration of 1% w/w was considered suitable.

It was decided that formulation 10 and formulation 12 was the optimal formulations. Weight variation of formulation 10 and 12 was 0.0074 g and 0.0069 g, respectively. The friability of formulation 10 and 12 was 0.79% and 0.88%, respectively; and the crushing strength of formulation 10 was 97.47 N whereas formulation 12 depicted crushing strength of 113.5 N. Formulation 10 depicted an average disintegration time of 41.7 seconds and the average disintegration time of formulation 12 was 115 seconds. Formulation 10 contained Avicel® PH

101 (microcrystalline cellulose) that also function as a disintegrant, whereas formulation 12 contained Granulac® 200 (lactose) that also function as a binder (Rowe *et al*., 2006).

4.4 Interaction of materials

Differential scanning calorimetry (DSC) was used to investigate the physico-chemical compatibility between the API's and between the API's and excipients. DSC has shown to be an important tool to obtain information on interaction between substances according to the appearance, shift or disappearance of endothermic or exothermic peaks. Variations in the corresponding enthalpy values in thermal curves of API's and excipients mixtures can also be important. In this study, the thermograms of the API excipient mixtures were compared to the different API's alone. The DSC thermograms of the pure API's and the two optimal formulations, the FA and FG formulations are shown in Figure 4.7 – 4.11.

The DSC thermogram of artesunate (Figure 4.7) showed a sharp melting endotherm at 147.25 ºC corresponding to its melting point of 144 – 147 ºC and an exotherm (decomposition) at 171.97 ºC. The DSC thermogram of proguanil (Figure 4.8) showed a sharp melting endotherm at 257.31 ºC corresponding to its melting temperature of 243 – 244 ºC. The DSC thermogram of dapsone (Figure 4.9) showed a sharp melting endotherm at 177.55 ºC corresponding to its melting temperature at 175 – 176 ºC. In Figure 4.10 (FA) and Figure 4.11 (FG) there is a reduction in melting points or an endotherm reaction. This could indicate possible incompatibilities between the API's and excipients due to interactions between the API's leading to impurities. The main principle of drug interaction studies with DSC is that any impurity reduces the melting temperature of the drug. When substances are mixed, the purity of each may be reduced and generally slightly lower melting points endotherms may result and may not necessarily indicate potential incompatibility (Botha & Lötter, 1990). Therefore an accelerated or a full stability study must be done to indicate any interactions between the API's and also between the API's and the excipients.

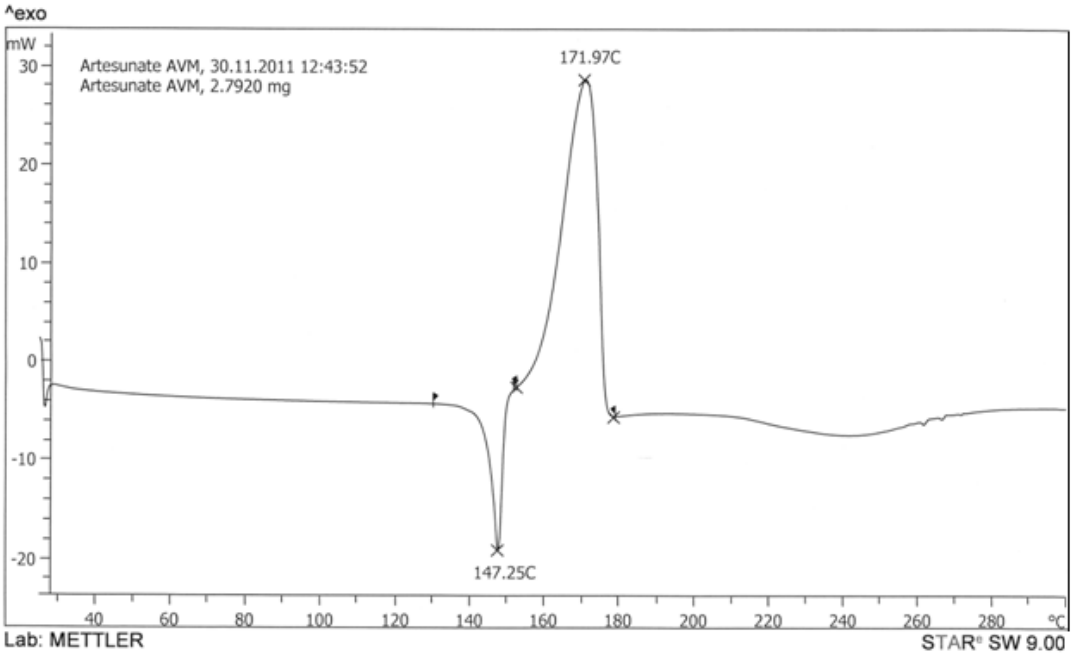


Figure 4.7: DSC thermogram of artesunate. A sharp endotherm can be observed at 147.25 ºC and a sharp exotherm as 171.97 ºC

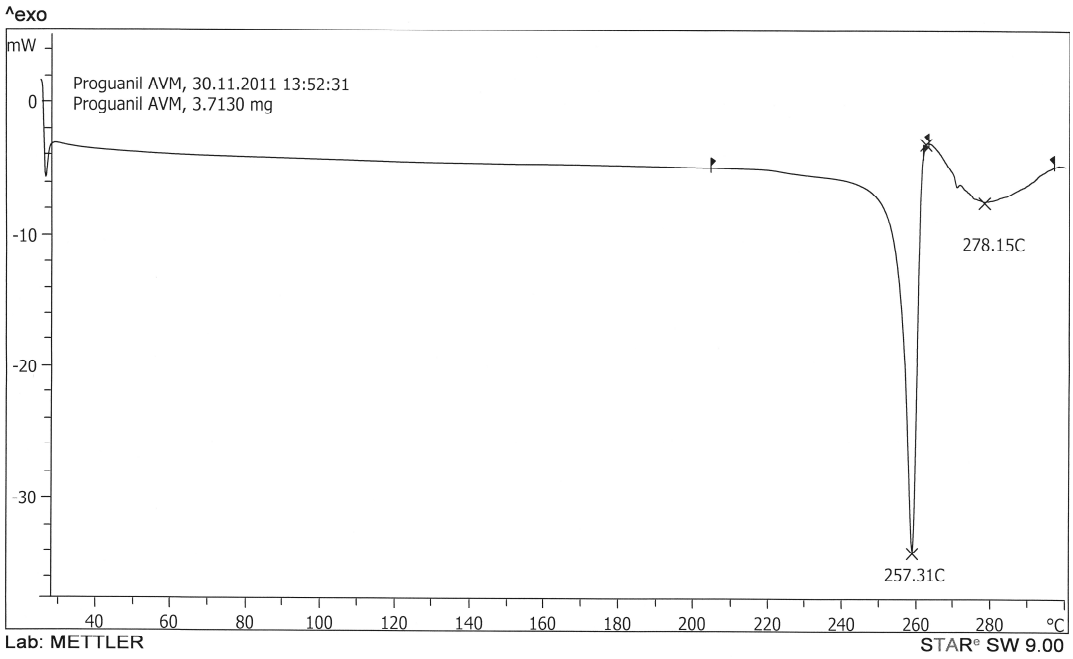


Figure 4.8: DSC thermogram of proguanil. A sharp endotherm can be seen at 257.31 ºC.

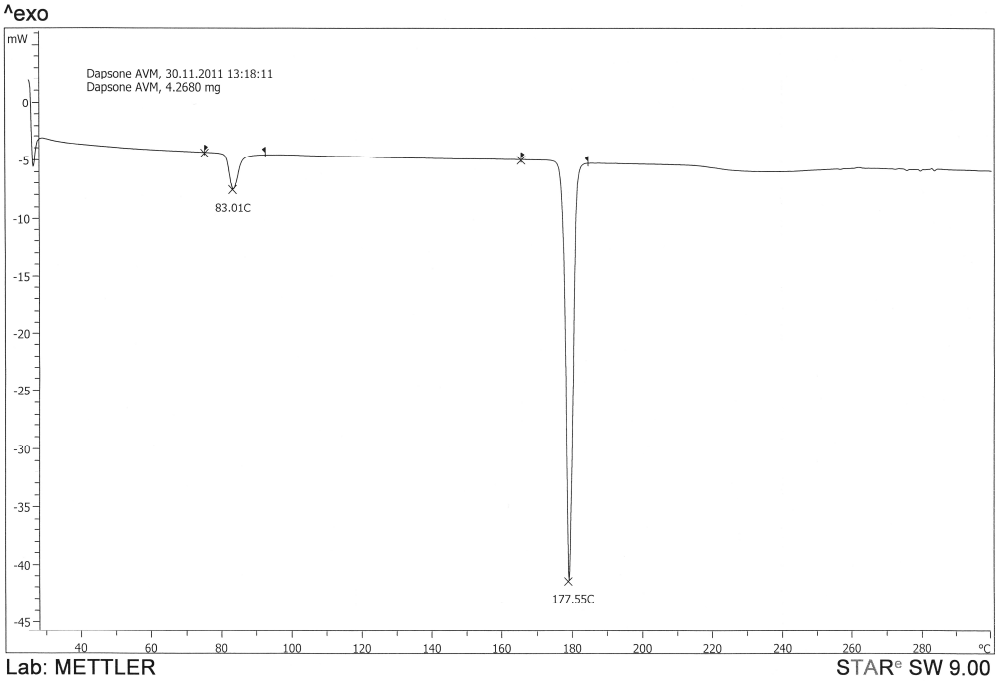


Figure 4.9: DSC thermogram of dapsone. A sharp endotherm can be seen at 177.55 ºC.

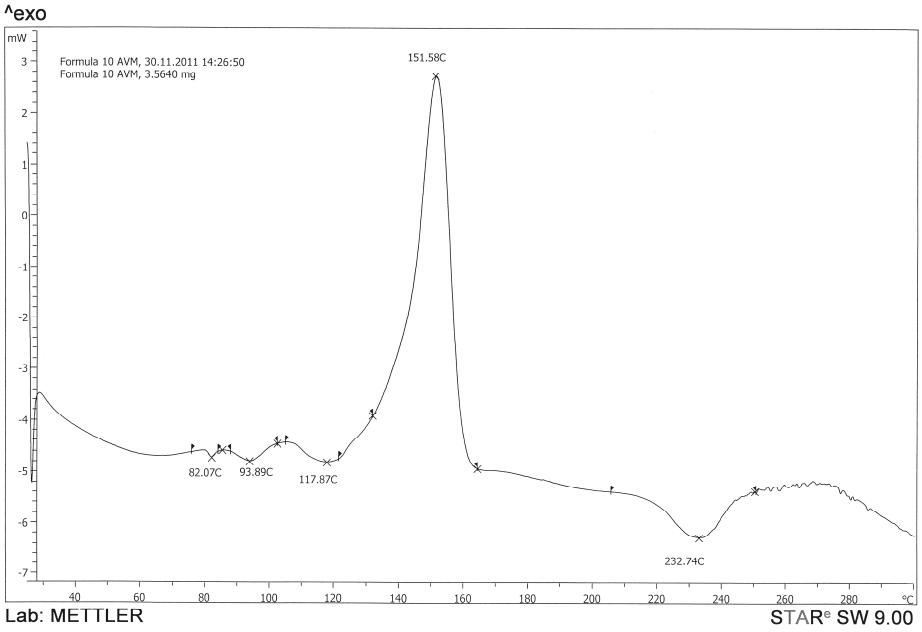


Figure 4.10: DSC thermogram of the FA formulation. Endotherms can be seen at 82.07, 93.89, 117.87 and 232.74 ºC. A sharp exotherm can be seen at 151.58 ºC.

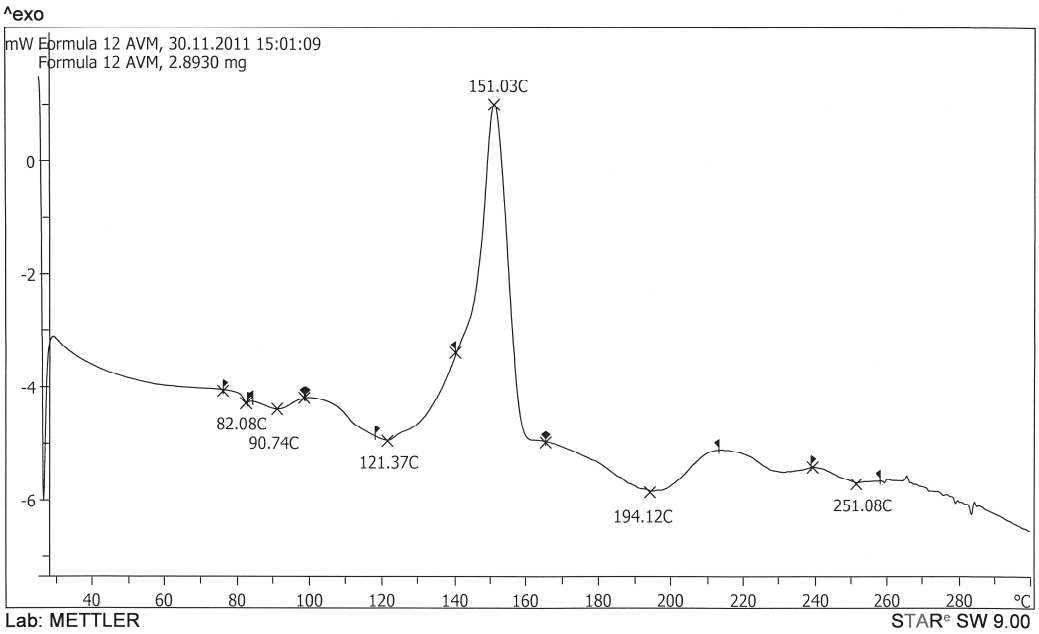


Figure 4.11: DSC thermogram of the FG formulation. Endotherm peaks can be seen at 82.08, 90.74, 121.37, 194.12 and 251.08 ºC. A sharp exotherm can be seen at 151.03 ºC.

4.5 Flow properties of two optimal formulations

Flow properties of powders are usually determined prior to tableting as the flow of the powder from the hopper into the die determines the weight, hardness and uniformity of the tablets. The flow properties of the two optimal formulations were determined by measuring the angle of repose, critical orifice diameter, flow rate, bulk and tapped densities (see sections 2.2 and 3.6).

Angle of repose was determined by allowing the powder mixture to flow through an orifice onto a surface. Height and radius of the resultant cone were measured (Annexure E). Average height and average radius for the formulation containing Avicel® PH 101 (FA) was 3.27 ± 0.252 cm and 4.83 ± 0.289 cm, respectively, and the average height and average radius for the formulation containing Granulac® 200 (FG) was 3.73 ± 0.351 cm and 5.1 ± 0.328 cm, respectively (see Table 4.7). Angle of repose for the FA formulation was 34.10 º whereas angle of repose for the FG formulation was 36.18 º and therefore showed fair flow properties. According to the USP an angle of repose of between 31º - 35º indicates good flow properties, whereas between 36º - 40º indicates fair flow properties.

The COD is the size of the smallest hole through which a powder discharges when a shutter of a cylinder with interchangeable discs is removed. If the orifice that a powder can flow through is smalls it indicates good flow properties. If it is large it indicates poor flow properties. The COD was determined as 4 mm for both the formulations (FA & FG) (Table 4.6) and therefore indicated good flow properties.

The time at which a powder discharged from the cylinder through an orifice gave an indication of flow rate. The average flow rate of the FA and FG powder mixtures was 16.67 ± 0.244 seconds/100g and 16.47 ± 0.260 seconds/100g respectively (see Table 4.6).

The bulk density is the density of the powder as poured into a measuring cylinder. The tapped density is a limiting density attained after tapping the cylinder. The guide used to predict powder flowability is the Carr's index and Hausner ratio, which is the ratio of the difference between the bulk and tapped densities. Carr's index for the FA and FG formulation were 16.79% and 21.31%, respectively, whereas the Hausner ratio for the FA and FG formulation was 1.2 and 1.27, respectively (see Table 4.6). The FA powder mixture, therefore, exhibited fair flow properties and the FG powder mixture exhibited passable flow properties. In conclusion, it can be said that the FA powder mixture exhibited the best flow properties.

Table 4.6: Comparison of the flow of the two optimal formulations FA and FG.

Properties	FA	FG
Angle of repose	34.10º	36.18º
COD	4 mm	4 mm
Flow rate	16.67 ± 0.244 seconds/100g	16.47 ± 0.260 seconds/100g
Bulk density	0.466 ± 0.002 g/ml	0.554 ± 0.004 g/ml
Tapped density	0.560 ± 0.007 g/ml	0.704 ± 0.000 g/ml
Carr's index	16.79%	21.31%
Hausner ratio	1.2	1.27

4.6 Evaluation of tablet properties

4.6.1 Weight variation

Both the formulations proved to be within the BP standards (see Annexure F). Standard deviation of the FA formulation (0.005) was smaller than the standard deviation of the FG formulation (0.007). Both the formulations had a small standard deviation which indicated a small variation in tablet weight and this observation confirmed good powder flow properties of the two powder mixtures. The difference between the 25^{th} and 75^{th} percentile (the box) of the FA formulation was smaller than that of the FG formulation and the spread of the individual tablet weights of the FA formulation was narrower than the spread of the individual tablet weights of the FG formulation (see Figure 4.12). Weight variation of the FG formulation was significantly lower than that of the FA formulation ($p \leq 0.05$). The average weight for the FA formulation was 852.7 mg and for the FG formulation was 832.2 mg that confirmed good flow properties.

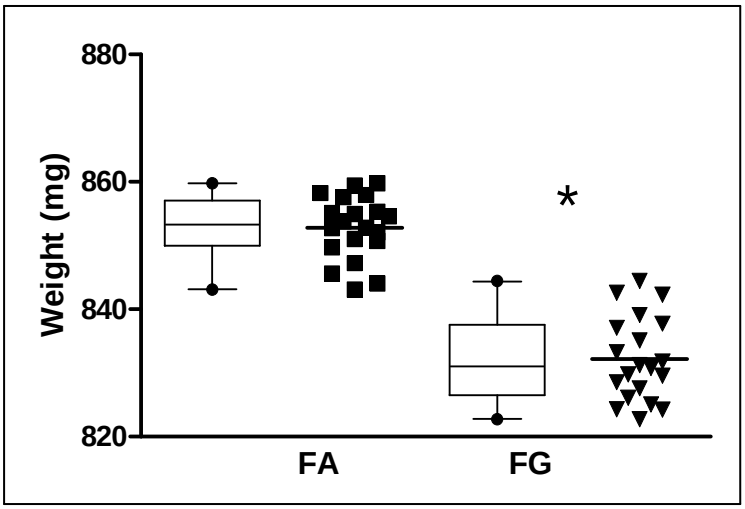


Figure 4.12: The average weight of 20 tablets of the formulations containing Avicel® PH 101 (FA) and Granulac® 200 (FG) as fillers. The first column indicates the box and whiskers of the tablets and the second column is a dot plot indicating the spread of the tablets. The * indicates a statistical significant difference between the two formulations ($p \leq 0.05$).

4.6.2 Friability

Table 4.3 gives a summary of the friability results. Tablets of the FA formulation exhibited a percentage friability of 0.68% and complied with the standard given by the BP. Strictly speaking, tablets from the FG formulation did not comply as it exhibited with a percentage friability of more than 1% (1.01%), but the tablets were not obviously cracked, cleaved or broken and appeared acceptable. Lactose (Granulac® 200) is included under the brittle materials category (Doelker, 1993). That is, lactose generally gives a lower mechanical strength and decrease the resistance of tablets to fragmentation (Roberts & Rowe, 1986). Microcrystalline cellulose (Avicel® PH 101) also contributed to increased friability (Pasqualoto *et* al., 2007

Table 4.7: Friability of the two optimal formulations FA and FG. The results is the sum of ten tablets in g.

	FA (g)	FG (g)
Weight before	8.51	8.35
Weight after	8.46	8.26
% Friability	**0.68%**	**1.01%**

4.6.3 Tablet crushing strength, diameter and thickness

The crushing strength for tablets prepared from the FA and FG formulations were 121.56 Newton and 91.99 Newton, respectively. Both the formulations complied with the BP guidelines. Tablets from the FA formulation exhibited a higher crushing strength (see Figure 4.13) and therefore could contribute to the smaller percentage friability compared to tablets prepared from the FG formulation. Annexure G.1.1 gives the crushing strengths of the individual tablets. The average thickness of tablets form the FA and FG formulations were 5.59 ± 0.022 mm and 5.46 ± 0.025 mm, respectively, and the average diameter for the two formulations (FA & FG) were 14.08 ± 0.009 mm and 14.08 ± 0.015 mm, respectively (see Annexure G). They did not statistically differ significantly. The crushing strength of the FG formulation is significantly lower ($p = \leq 0.05$) therefore the higher % friability. Lactose generally gives a lower mechanical strength and decrease the resistance of tablets to fragmentation (Roberts & Rowe, 1986).

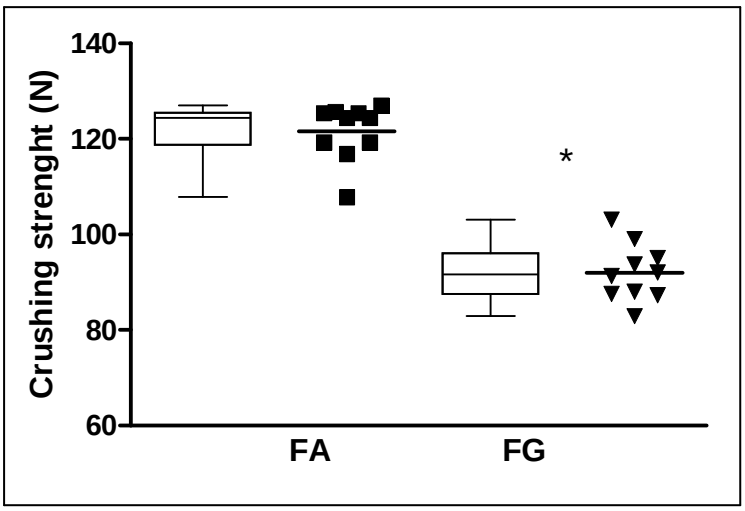


Figure 4.13: The average crushing strength of 10 tablets of the formulations containing Avicel® PH 101 (FA) and Granulac® 200 (FG) as fillers. The first column indicated the box and whiskers of the tablets and the second column is a dot plot indicating the spread of the tablets. The * indicates a statistical significant difference between the two formulations ($p \leq 0.05$).

4.6.4 Disintegration

The average disintegration time for tablets prepared from the FA formulation was faster than the average disintegration time of tablets from the FG formulation, although the tablets prepared from the FA formulation had a much higher crushing strength (121.56 N). The FG-tablets disintegrated between 92 seconds and 140 seconds whereas FA-tablets disintegrated between 44 seconds and 52 seconds. See Annexure H for the individual disintegration times. The average disintegration time for the FA-tablets and FG-tablets were 49.5 seconds and 111.5 seconds, respectively. Results were similar to that of the factorial design. Although the crushing strength of FA was higher the tablets still disintegrate faster. Microcrystalline cellulose also function as a disintegrant and lactose deliver tablets that are brittle (Roberts & Rowe, 1986; Rowe *et al*., 2006).

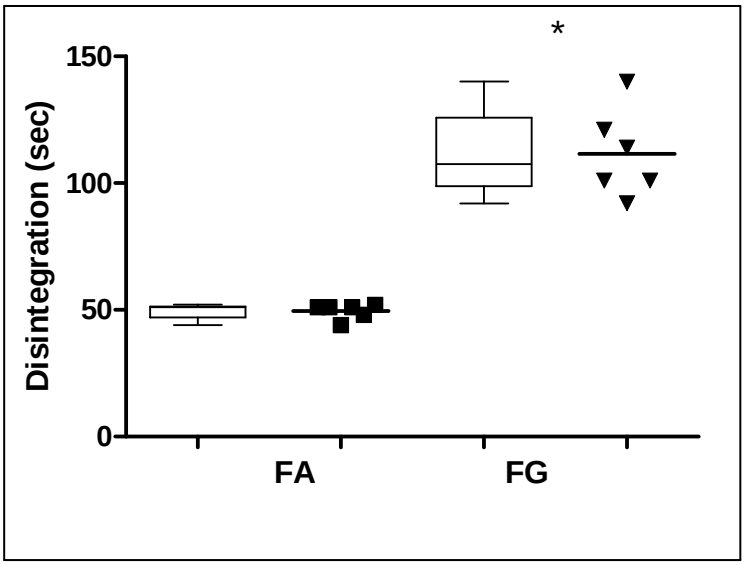


Figure 4.14: The average disintegration of six tablets of the formulations containing Avicel® PH 101 (FA) and Granulac® 200 (FG) as fillers. The first column indicated the box and whiskers of the tablets and the second column is a dot plot indicating the spread of the tablets. The * indicates a statistical significant difference between the two formulations ($p \leq 0.05$).

4.6.5 Assay and dissolution results

A summary of the assay and dissolution results is given in Table 4.11. Assay results depicted the average and %RSD of three individual determinations. Dissolution results depicted the average and %RSD across vessels 1 – 6 (6 individual determinations) per withdrawal time point. The assay was done to determine the amount of API's in the tablets of different formulations before dissolution was done.

Table 4.8: Assay and dissolution results of tablets of the FA formulation (top) and FG formulation (bottom).

FA						
Active	**Assay % (%RSD)**	**Dissolution % (%RSD)**				
		15 minutes	30 minutes	45 minutes	60 minutes	90 minutes
Artesunate	96.9% (0.5%)	98% (1.8%)	98% (1.6%)	97% (1.0%)	NA	NA
Proguanil	95.1% (1.8%)	93% (0.9%)	93% (1.0%)	92% (1.2%)	94% (1.4%)	94% (1.1%)
Dapsone	98.5% (1.1%)	94% (0.9%)	95% (0.9%)	94% (0.7%)	93% (0.9%)	94% (0.2%)
FG						
Artesunate	101.9% (0.3%)	62% (3.6%)	72% (6.0%)	78% (2.9%)	NA	NA
Proguanil	94.1% (0.6%)	85% (4.5%)	89% (2.5%)	89% (1.6%)	91% (1.1%)	91% (1.0%)
Dapsone	98.9% (0.4%)	92% (0.7%)	92% (0.8%)	92% (1.0%)	90% (1.4%)	90% (2.2%)

Artesunate, proguanil and dapsone complied with the dissolution test specifications as artesunate was 98% dissolved, proguanil was 93% dissolved and dapsone is 94% dissolved in 15 minutes for the FA formulation. For the FG formulation artesunate was 62% dissolved, proguanil was 85% dissolved and dapsone was 92% dissolved in 15 minutes. A fast release

was displayed, because more than 60% of the API's was released within 15 minutes for artesunate, proguanil and dapsone.

Artesunate showed immediate release with 98% of the artesunate content release within 15 minutes for the FA formulation (Figure 4.15). For the FG formulation 62% of the artesunate content was released within 15 minutes, increasing to 78% within 45 minutes (Figure 4.15). Proguanil showed immediate release with 93% of the proguanil content released within 15 minutes for the FA formulation (Figure 4.16). For the FG formulation 85% of the proguanil content was released within 15 minutes (Figure 4.16). Dapsone showed immediate release with 94% of the dapsone content released within 15 minutes for the FA formulation (Figure 4.17). For the FG formulation 92% of the dapsone content was released within 15 minutes (Figure 4.17). Therefore both the formulations complied to the BP standards.

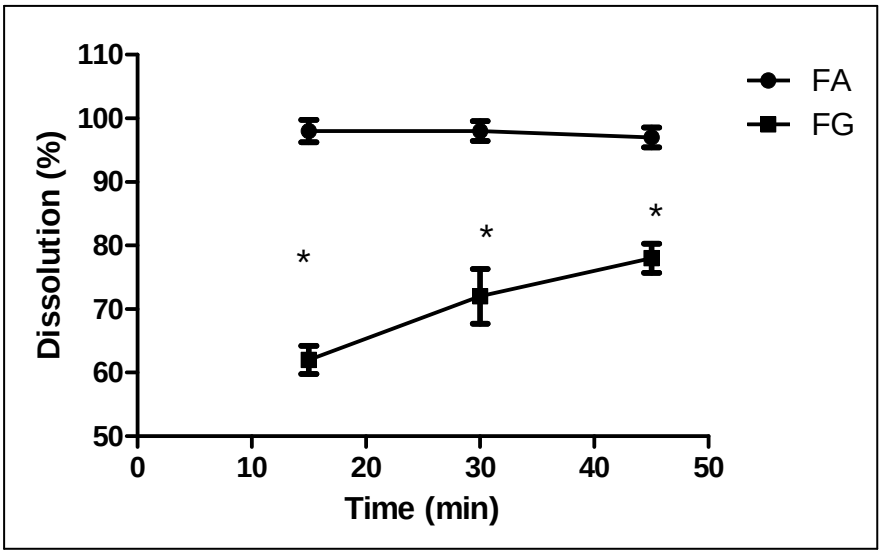


Figure 4.15: Dissolution of artesunate for the FA and FG formulations.

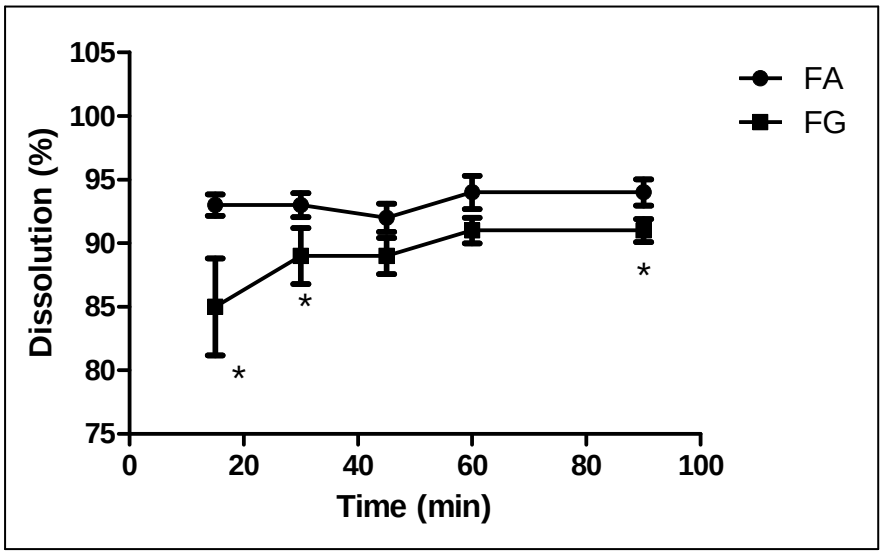


Figure 4.16: Dissolution of proguanil for the FA and FG formulations.

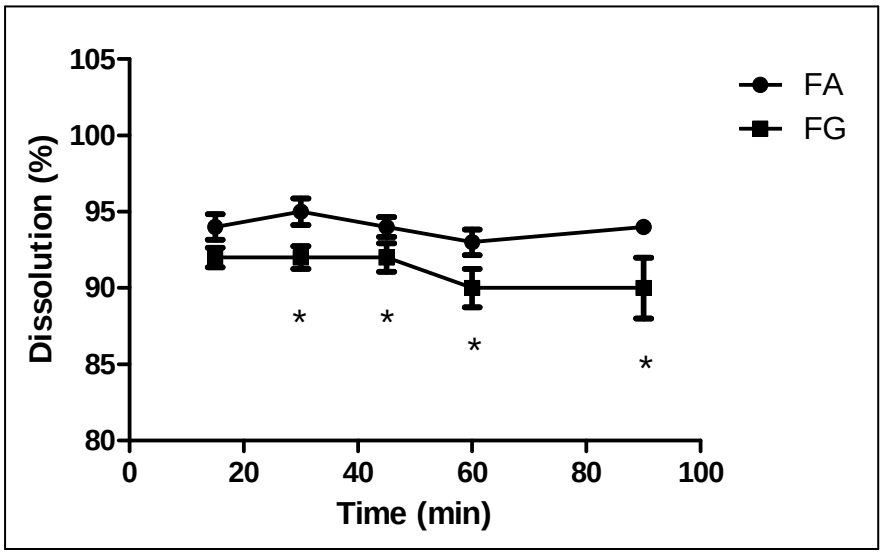


Figure 4.17: Dissolution of dapsone for the FA and FG formulations.

Summary

FA formulation produced tablets with a thickness of 5.59 ± 0.022 mm and a diameter of 14.08 ± 0.009 mm. Average weight was 852.7 mg and the weight variation was 0.005 g. Friability was 0.68% and the average crushing strength was 121.56 ± 5.903 N. Tablets disintegrate fast (49.5 seconds) and there was an immediate release of API's. FG formulation produced tablets with a thickness and diameter of 5.46 ± 0.025 mm and 14.079 ± 0.015 mm, respectively. Average weight was 832.2 mg and the weight variation was 0.00660 g and the friability was 1.01%. Average crushing strength was 91.99 ± 6.008 N and disintegration was slow but there was still dissolution of the API's. Artesunate showed increase in release compared to proguanil and dapsone. There was no difference in dissolution for proguanil and dapsone. The formulation containing Avicel® PH 101 was therefore the better formulation according to all the results.

SUMMARY AND RECOMMENDATIONS

The aim of this study was to develop an oral triple fixed-dose combination tablet containing artesunate, proguanil and dapsone and to evaluate the features of the tablets. An artesunate-proguanil-dapsone fixed-dose combination tablet was able to be manufactured by using wet granulation. Wet granulation was necessary to obtain a powder mixture with flow properties that was acceptable for manufacturing. Flow of both the wet granulation formulations (FA and FG) increased and showed fair and passable flow. Flow properties of a powder mixture influence the weight variation. Artesunate is very unstable and therefore the manufacturing of ACT is very challenging in terms of stability. Manufacturing of tablets is possible with different formulation combinations but wet granulation is necessary. Wet granulation with an aqueous solution can however contribute to the instability of artesunate.

A process for the production of an original compressible powder mixture was evaluated. Different excipients were used to identify two optimal formulations. The excipients included Avicel® PH 101, Granulac® 200, Pruv®, Kollidon® VA 64, Aerosil® and Ac-di-sol®. The parameters (flow properties) to successful granulation were evaluated and the influences of the various factors on the granulation of artesunate-proguanil-dapsone were described and optimum formulations were identified. The only difference between the two optimal formulations was the filler used. The FA formulation contained Avicel® PH 101 as filler and the FG formulation contained Granulac® 200 as filler.

The DSC results of the mixtures showed reduction of melting points of the API's; that could indicate interaction between the API's and excipients. An HPLC method was developed to verify the presence of, and quantify the concentration of artesunate, proguanil and dapsone during dissolution studies. The FA formulation had a smaller weight variation than the FG formulation and that is attributed to the fair flow properties of the FA formulation. Both the formulations had a relative high crushing strength. A maximum weight loss during friability for the FG formulation was more than 1%, but as there was no obviously cracked, cleaved or broken tablets, it still complied to the BP standards. Disintegration times for the FA formulation were shorter than the disintegration time for the FG formulation. Dissolution data showed immediate release for both formulations within 15 minutes. Artesunate is immediately released and with a half-life value with less than one hour there will be immediate action against *P. falciparum*. Proguanil and dapsone are also immediately released, so their action will also take

place immediately, but they have a half-life of approximately 20 hours and 30 hours respectively and therefore, will take action for a while against *P. falciparum*.

The differences found between the formulations containing the two different fillers can possible be attributed to the distinct differences in properties between the two fillers. Microcrystalline cellulose (Avicel® PH 101) is insoluble in water with a bulk density of 0.28 g/ml and an average particle size of 50 microns. Four percent of microcrystalline cellulose is loss when drying (Pformulate, 2011b). Lactose (Granulac® 200) is soluble in water, but had limited flowability. Therefore lactose must be granulated. Lactose has a bulk density of 0.53 g/ml and a particle size of 45 – 75% < 32 microns. Lactose has poor binding properties and therefore a binder is included in the formulation if lactose is used in wet granulation (Pformulate, 2011a). Microcrystalline cellulose produced tablets with a high crushing strength and a fast disintegration time, therefore the FA formulation delivered tablets with a higher crushing strength and a faster disintegration time.

Artemisinin-based products are currently the most effective treatments available to treat malaria infections (Premji *et* al., 2009; Tiono *et al*., 2009). All the studies done on artesunate by Kauss *et al*. (2010), Bradsley *et al*. (2011) and Lacaze *et al*. (2011) showed that artesunate is unstable and there is interactions between artesunate and dapsone (Bradsley *et al*., 2011); and artesunate and amodiaquine (Kauss *et al*., 2010; Lacaze *et al*., 2011). Studies on artesunate-chlorproguanil-dapsone done by Bradsley *et al*. (2011) showed a formation of a degradant after 36 months of stability testing. Kauss *et al*. (2010) and Lacaze *et al*. (2011) both used wet granulation and the same excipients (see Table 5.1) for manufacturing of the fixed-dose tablets containing artesunate and amodiaquine. Excipients used by *Kauss et al*. (2010) and Lacaze *et al*. (2011) included:

- Sodium croscarmellose (Ac-di-sol®) - Disintegrant
- Polyvinylpyrrolidone (PVP) – Binder
- Silica (Aerosil) – Glidant
- Magnesium stearate - Lubricant

Bradsley *et al*. (2011) used mannitol, microcrystalline cellulose, croscarmellose sodium, hypromellose, magnesium stearate and Opadry (distinguishing colour) in the manufacturing of a fixed-dose tablet containing artesunate, chlorproguanil and dapsone. Bradsley *et al*. (2011)

also made used of wet granulation as the method of manufacturing. Okwelogu *et al*. (2010) used mannitol, PVP, aspartame and Ac-di-sol® in the manufacturing of artesunate-amodiaquine fixed-dose paediatric formulation. These results together with the results of this study indicated that it is possible to successfully formulate a triple fixed-dose combination. The aim of this study was achieved and a triple fixed-dose combination was successfully formulated.

As this tablet will be used for malaria treatment in poor countries the formulation has to remain cost effective.

Recommendations

- Use a non-aqueous solution for wet granulation.
- Granulation of artesunate and dapsone individually and coat them before mixing together.
- To develop a bi-layer tablet with artesunate and dapsone in different layers.
- Investigate other excipients such as co-processed excipients with specific properties.
- The DSC analysis of possible interaction was inconclusive and therefore it would be recommended to analysis it with infrared spectroscopy (IR) and X-ray diffraction (XRD). DSC has certain disadvantages as such as exposure of the drug to high temperatures, which may not always be relevant to ambient conditions. DSC results have to be interpreted carefully and the conclusions based on the results alone can then be misleading and in conclusive.
- Do an accelerated or full stability study.

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ANNEXURES

Annexure A: Weight variation (gram) of Formulation 1 – 16:

Formulation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0.8683	0.8545		0.8377	0.8274	0.8314		0.8618	0.8371	**0.8471**	0.8542	**0.8808**	0.8619	0.8275	0.8306	0.8693
2	0.8629	0.8672		0.8578	0.8312	0.8498		0.8655	0.8571	**0.85**	0.8462	**0.875**	0.8581	0.8129	0.8332	0.8531
3	0.8541	0.8462		0.8509	0.83	0.8308		0.857	0.8401	**0.8405**	0.8532	**0.8828**	0.8522	0.8211	0.8261	0.8673
4	0.8561	0.8654		0.8581	0.8162	0.8367		0.8594	0.8364	**0.8456**	0.8496	**0.8886**	0.8541	0.8341	0.8432	0.8675
5	0.8617	0.8497		0.8513	0.8371	0.8046		0.848	0.8487	**0.8466**	0.8594	**0.8694**	0.8558	0.8372	0.8415	0.8557
6	0.8454	0.8986		0.8526	0.8118	0.8358		0.8657	0.8482	**0.857**	0.8477	**0.8866**	0.8651	0.8375	0.8258	0.8661
7	0.8741	0.8714		0.8472	0.8179	0.8301		0.8582	0.8443	**0.8378**	0.849	**0.8753**	0.8607	0.8199	0.8362	0.8757
8	0.8712	0.8738		0.8576	0.8331	0.827		0.8626	0.8458	**0.8564**	0.8557	**0.8788**	0.8685	0.8369	0.8437	0.8559
9	0.8629	0.8686		0.8323	0.8325	0.8281		0.8553	0.8393	**0.8489**	0.8492	**0.888**	0.8623	0.8358	0.8404	0.8548
10	0.8641	0.8701		0.8521	0.8146	0.8346		0.8539	0.9366	**0.8494**	0.8569	**0.8797**	0.8618	0.8296	0.828	0.8575
11	0.8689	0.8578		0.8548	0.809	0.8289		0.8652	0.8574	**0.8544**	0.8564	**0.8836**	0.8693	0.8207	0.8293	0.8527
12	0.8761	0.866		0.8639	0.8635	0.8248		0.8565	0.8539	**0.8526**	0.8312	**0.8814**	0.8582	0.8055	0.8528	0.8619
13	0.8635	0.8648		0.8417	0.8357	0.8205		0.8561	0.8453	**0.8395**	0.8441	**0.8728**	0.8592	0.8288	0.824	0.8672
14	0.864	0.8522		0.8605	0.8185	0.8351		0.8633	0.8517	**0.8457**	0.8296	**0.8815**	0.8595	0.8298	0.8306	0.8756
15	0.8596	0.8627		0.853	0.8227	0.8362		0.8611	0.8436	**0.8567**	0.8574	**0.8732**	0.8593	0.8355	0.822	0.8732
16	0.8525	0.8717		0.853	0.8177	0.8507		0.8649	0.8486	**0.8442**	0.8597	**0.8692**	0.8592	0.8205	0.822	0.8685
17	0.8686	0.8569		0.8462	0.8344	0.8277		0.8659	0.8449	**0.8568**	0.857	**0.8927**	0.8577	0.8322	0.8149	0.8551
18	0.825	0.857		0.8491	0.8141	0.8343		0.8667	0.8496	**0.833**	0.8555	**0.8765**	0.8515	0.8099	0.8295	0.8602
19	0.8699	0.871		0.8662	0.8199	0.83		0.8578	0.8501	**0.8532**	0.8588	**0.8675**	0.8537	0.8244	0.8357	0.8681
20	0.8734	0.865		0.8459	0.8171	0.8262		0.856	0.8428	**0.8614**	0.8533	**0.878**	0.8517	0.8282	0.825	0.8443
Average	0.8621	0.8645		0.8516	0.8252	0.8312		0.86	0.8511	**0.8488**	0.8512	**0.8791**	0.859	0.8264	0.8317	0.8625
Std dev	0.0117	0.0113		0.0084	0.0126	0.0097		0.005	0.021	**0.0074**	0.0084	**0.0069**	0.0051	0.0094	0.0092	0.0086
Rel. std dev	1.3577	1.3047		0.9815	1.5217	1.1628		0.5843	2.4649	**0.8766**	0.9905	**0.7826**	0.5883	1.1376	1.103	0.9978

Annexure B: Friability (percentage) of Formulation 1 – 16:

Formulation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Weight before (g)	8.6198	8.6669		8.5067	8.2534	8.3104		8.5874	8.4419	**8.4812**	8.5209	**8.8041**	8.5998	8.2928	8.3488	8.623
Weight after (g)	8.558	8.6019		8.4243	0.8235	8.2193		8.4819	8.3758	**8.4142**	8.4413	**8.7268**	8.5217	8.1881	7.4416	8.5245
% Friability	0.71695	0.74998		0.96865	90.0223	1.09622		1.22854	0.783	**0.78998**	0.93417	**0.878**	0.90816	1.26254	10.8662	1.14229

Annexure C: Crushing strength (Newton), thickness (mm) and diameter (mm) of Formulation 1 – 16:

C.1.1: Crushing strength (mm)

Formulation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	106.2	98.4		106.8	76.2	81.2		85.6	100.8	**92.3**	104.5	**132.1**	97.1	68.1	83.6	101.4
2	102.8	97.1		113.6	61.7	86.6		99.4	103.5	**96.4**	120	**128.4**	111.5	74.5	73.8	102.8
3	125.7	90		104.8	68.1	78.2		90.7	94.4	**105.5**	111.9	**112.6**	104.1	70.8	80.5	86.9
4	119	102.1		106.2	54.9	75.2		91.7	95.7	**100.4**	102.8	**110.9**	95.7	69.4	96.4	93
5	119	104.1		107.2	55.3	86.6		88.3	81.9	**102.1**	97.4	**109.2**	106.5	75.5	95	91
6	103.1	95.4		113.6	44.8	73.1		96	97.1	**96.7**	108.9	**121.7**	95.7	71.1	97.7	90.7
7	115.3	110.2		104.5	47.2	67.4		81.6	100.1	**97.1**	103.8	**62**	94.7	69.8	111.2	107.5
8	113.2	112.9		123.7	66.7	81.6		86.6	89.3	**100.8**	88.3	**124**	107.2	74.1	93.3	97.1
9	121.3	104.8		103.5	63.7	81.6		67.1	105.1	**90.7**	126	**116.9**	106.8	71.1	97.7	97.7
10	76.8	107.2		107.5	54.3	80.9		84.9	88.3	**92.7**	118.3	**116.9**	106.8	72.1	71.1	99.4
Average	110.2	102.22		109.1	59.29	79.24		87.19	95.62	**97.47**	108.2	**113.5**	102.6	71.65	90.03	96.75
Min	76.8	90		103.5	44.8	67.4		67.1	81.9	**90.7**	88.3	**62**	94.7	68.1	71.1	86.9
Max	125.7	112.9		123.7	76.2	86.6		99.4	105.1	**105.5**	126	**132.1**	111.5	75.5	111.2	107.5

C.1.2: Thickness (mm)

Formulation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	5.96	5.75		5.53	5.76	5.70		5.68	5.77	**5.65**	5.55	**5.74**	5.69	5.66	5.55	5.63
2	5.96	5.75		5.59	5.73	5.68		5.64	5.75	**5.67**	5.60	**5.72**	5.73	5.63	5.52	5.64
3	6.04	5.71		5.54	5.75	5.63		5.67	5.75	**5.72**	5.58	**5.69**	5.69	5.60	5.55	5.59
4	5.98	5.73		5.54	5.75	5.64		5.67	5.77	**5.74**	5.57	**5.69**	5.68	5.62	5.57	5.66
5	6.01	5.74		5.64	5.81	5.66		5.65	5.72	**5.75**	5.53	**5.65**	5.71	5.67	5.53	5.59
6	5.98	5.71		5.56	5.71	5.68		5.73	5.77	**5.69**	5.54	**5.71**	5.68	5.66	5.57	5.62
7	5.97	5.76		5.53	5.86	5.65		5.65	5.77	**5.67**	5.55	**5.52**	5.67	5.63	5.52	5.60
8	5.98	5.76		5.57	5.83	5.69		5.70	5.72	**5.75**	5.51	**5.69**	5.72	5.65	5.55	5.65
9	6.00	5.77		5.54	5.72	5.66		5.59	5.78	**5.66**	5.63	**5.69**	5.71	5.64	5.53	5.58
10	5.94	5.76		5.57	6.01	5.68		5.67	5.75	**5.68**	5.61	**5.65**	5.73	5.68	5.48	5.63
Average	**5.982**	**5.744**		**5.561**	**5.793**	**5.667**		**5.663**	**5.755**	**5.698**	**5.567**	**5.675**	**5.701**	**5.644**	**5.537**	**5.619**

C.1.3: Diameter (mm):

Formulation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	14.06	14.09		14.09	14.08	14.14		14.09	14.10	**14.09**	14.06	**14.06**	14.07	14.10	14.05	14.07
2	14.07	14.11		14.11	14.10	14.11		14.07	14.09	**14.09**	14.09	**14.09**	14.13	14.09	14.06	14.06
3	14.10	14.10		14.09	14.08	14.11		14.09	14.09	**14.10**	14.08	**14.07**	14.06	14.11	14.07	14.06
4	14.24	14.10		14.07	14.15	14.12		14.08	14.07	**14.10**	14.06	**14.06**	14.06	14.08	14.06	14.09
5	14.08	14.18		14.12	14.10	14.10		14.08	14.08	**14.11**	14.05	**14.07**	14.07	14.10	14.07	14.07
6	14.08	14.11		14.10	14.12	14.31		14.09	14.08	**14.10**	14.06	**14.07**	14.05	14.10	14.08	14.09
7	14.08	14.11		14.09	14.09	14.12		14.08	14.08	**14.08**	14.05	**14.06**	14.07	14.09	14.06	14.08
8	14.21	14.09		14.10	14.09	14.10		14.09	14.07	**14.11**	14.06	**14.07**	14.07	14.09	14.06	14.13
9	14.07	14.12		14.09	14.08	14.11		14.08	14.11	**14.12**	14.06	**14.09**	14.07	14.08	14.06	14.08
10	14.09	14.10		14.09	14.09	14.11		14.09	14.08	**14.12**	14.06	**14.07**	14.08	14.09	14.05	14.07
Average	**14.108**	**14.111**		**14.095**	**14.098**	**14.133**		**14.084**	**14.085**	**14.102**	**14.063**	**14.071**	**14.073**	**14.093**	**14.062**	**14.08**

Annexure D: Disintegration (seconds) of Formulation 1 – 16:

Formulation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	35	40		98	23	31		37	31	**39**	57	**100**	25	30	40	41
2	36	40		98	23	31		38	31	**39**	59	**107**	25	30	42	49
3	38	41		109	23	31		39	31	**40**	59	**107**	25	30	42	50
4	39	42		109	23	32		42	32	**42**	65	**122**	27	32	44	55
5	39	43		123	24	33		43	33	**45**	65	**125**	27	32	44	55
6	40	44		152	24	33		45	34	**45**	67	**131**	29	32	46	59
Average	37.8	41.7		115	23.3	31.8		40.7	32	**41.7**	62	**115**	26.3	31	43	51.5

Annexure E: Flow properties

Annexure E.1.1: Height and radius of the FA and FG formulations used to determine angle of repose:

FA		**FG**	
Height	**Radius**	**Height**	**Radius**
3.3 cm	5.0 cm	3.7 cm	5.15 cm
3.5 cm	5.0 cm	3.4 cm	4.75 cm
3.0 cm	4.5 cm	4.1 cm	5.4 cm
Average: 3.27 cm	**Average: 4.83 cm**	**Average: 3.73 cm**	**Average: 5.1 cm**

Annexure E.1.2: Flow rates of the FA and FG formulations:

FA	**FG**
16.62 seconds/100 g	16.75 seconds/100 g
16.94 seconds/100 g	16.41 seconds/100 g
16.46 seconds/100 g	16.24 seconds/100 g

Annexure E.1.3: Bulk density and tapped density (g/ml) used in determining Carr's index and Hausner ration

	FA			Average	SD
Bulk density	0.467	0.463	0.467	**0.466**	**0.002**
Tapped density	0.556	0.568	0.556	**0.560**	**0.007**

	FG			Average	SD
Bulk density	0.550	0.556	0.556	**0.554**	**0.004**
Tapped density	0.704	0.704	0.704	**0.704**	**0.000**

Annexure F: Weight variation of the FA and FG formulations:

	FA	FG
1	0.8510	0.8312
2	0.8546	0.8426
3	0.8472	0.8391
4	0.8456	0.8227
5	0.8521	0.8371
6	0.8528	0.8444
7	0.8440	0.8351
8	0.8553	0.8298
9	0.8527	0.8332
10	0.8594	0.8318
11	0.8507	0.8423
12	0.8431	0.8296
13	0.8598	0.8308
14	0.8551	0.8242
15	0.8538	0.8285
16	0.8549	0.8250
17	0.8497	0.8377
18	0.8582	0.8276
19	0.8579	0.8243
20	0.8576	0.8261
Average	**0.8527**	**0.832155**
Standard deviation	**0.004939**	**0.006595**
Relative standard deviation	**0.579141%**	**0.792551%**

Annexure G: Crushing strength (Newton), Thickness (mm) and diameter (mm) of the FA and FG formulations:

G.1.1: Crushing strength (Newton) of the FA and FG formulations:

	FA	FG
1	124.4	92
2	116.9	99
3	124.4	88
4	127	82.9
5	125.4	103.1
6	125.4	91.3
7	125.7	93.7
8	119.3	95
9	119.3	87.3
10	107.8	87.6
Average	**121.56**	**91.99**
SD	**0.022**	**6.008**

G.1.2: Thickness and diameter (mm) of the FA & FG formulations:

Formulation	FA		FG	
	Thickness	Diameter	Thickness	Diameter
1	5.59	14.08	5.46	14.07
2	5.59	14.09	5.49	14.07
3	5.56	14.07	5.47	14.10
4	5.59	14.07	5.44	14.11
5	5.58	14.07	5.51	14.07
6	5.58	14.07	5.47	14.07
7	5.57	14.07	5.45	14.07
8	5.64	14.09	5.46	14.08
9	5.61	14.07	5.47	14.07
10	5.59	14.07	5.42	14.08
Average	**5.59**	**14.075**	**5.464**	**14.079**
SD	**0.022**	**0.009**	**0.025**	**0.015**

Annexure H: Disintegration (seconds) of the FA and FG formulations:

	FA	FG
1	44 sec	92 sec
2	48 sec	101 sec
3	51 sec	101 sec
4	51 sec	114 sec
5	51 sec	121 sec
6	52 sec	140 sec
Average	**49.5 sec**	**115.5 sec**

Annexure I: Data by Graphpad Prism:

Effect	Univariate Test of Significance, Effect Sizes, and Powers for Hardness (Results) Sigma-restricted parameterization Effective hypothesis decomposition						
	SS	Degr. of Freedom	MS	F	p	Partial eta-squared	Non-centrality
Intercept	1156097	1	1156097	6674.104	0.000000	0.980174	6674.104
FILLER	3520	1	3520	20.323	0.000014	0.130846	20.323
LUBRICANT	20	1	20	0.116	0.734321	0.000856	0.116
GLIDANT	480	1	480	2.772	0.098238	0.020121	2.772
BINDER	15984	1	15984	92.273	0.000000	.406001	92.273
Error	23385	135	173				

Cell No.	FILLER; LS Means (Results Hardness) Current effect: F(1, 135)=20.323, p=.00001 Effective hypothesis decomposition					
	FILLER	Hardness Mean	Hardness Std.Err.	Hardness -95.00%	Hardness +95.00%	N
1	1	89.7925	1.471484	86.88236	92.7026	80
2	2	100.2812	1.802193	96.71707	103.8454	60

Cell No.	LUBRICANT; LS Means (Results Hardness) Current effect: F(1, 135)=.11566, p=.73432 Effective hypothesis decomposition					
	LUBRICANT	Hardness Mean	Hardness Std.Err.	Hardness -95.00%	Hardness +95.00%	N
1	1	95.43250	1.802193	91.86832	98.99668	60
2	2	94.64125	1.471484	91.73111	97.55139	80

Cell No.	GLIDANT; LS Means (Results Hardness) Current effect: F(1, 135)=2.7721, p=.09824 Effective hypothesis decomposition					
	GLIDANT	Hardness Mean	Hardness Std.Err.	Hardness -95.00%	Hardness +95.00%	N
1	1	96.97375	1.471484	94.06361	99.88389	80
2	2	93.10000	1.802193	89.53582	96.66418	60

Cell No.	BINDER; LS Means (Results Hardness) Current effect: F(1, 135)=92.273, p=.00000 Effective hypothesis decomposition					
	BINDER	Hardness Mean	Hardness Std.Err.	Hardness - 95.00%	Hardness +95.00%	N
1	1	84.3519	1.609530	81.1687	87.5350	70
2	2	105.7219	1.609530	102.5387	108.9050	70

Effect	Univariate Test of Significance, Effect Sizes, and Powers for Disintegration (Results) Sigma-restricted parameterization Effective hypothesis decomposition						
	SS	Degr. of Freedom	MS	F	p	Partial eta-squared	Non-centrality
Intercept	200491.9	1	200491.9	788.4010	0.000000	0.908923	788.4010
FILLER	24567.4	1	24567.4	96.6072	0.000000	0.550132	96.6072
LUBRICANT	4284.1	1	4284.1	16.8464	0.000098	0.175765	16.8464
GLIDANT	42.0	1	42.0	0.1652	0.685521	0.002087	0.1652
BINDER	16745.2	1	16745.2	65.8477	0.000000	0.454599	65.8477
Error	20089.9	79	254.3				

Cell No.	FILLER; LS Means (Results Disintegration) Current effect: F(1, 79)=96.607, p=.00000 Effective hypothesis decomposition					
	FILLER	Disintegration Mean	Disintegration Std.Err.	Disintegration -95.00%	Disintegration +95.00%	N
1	1	33.20833	2.301729	28.62686	37.78981	48
2	2	68.97917	2.819031	63.36803	74.5031	36

Cell No.	LUBRICANT; LS Means (Results Disintegration) Current effect: F(1, 79)=16.846, p=.00010 Effective hypothesis decomposition					
	LUBRICANT	Disintegration Mean	Disintegration Std.Err.	Disintegration - 95.00%	Disintegration +95.00%	N
1	1	43.62500	2.819031	38.01386	49.23614	36
2	2	58.56250	2.301729	53.98102	63.14398	48

Cell No.	GLIDANT; LS Means (Results Disintegration) Current effect: F(1, 79)=.16519, p=.68552 Effective hypothesis decomposition					
	GLIDANT	Disintegration Mean	Disintegration Std.Err.	Disintegration - 95.00%	Disintegration +95.00%	N
1	1	50.35417	2.301729	45.77269	54.93564	48
2	2	51.83333	2.819031	46.22219	57.44447	36

Cell No.	BINDER; LS Means (Results Disintegration) Current effect: F(1, 79)=65.848, p=.00000 Effective hypothesis decomposition					
	BINDER	Disintegration Mean	Disintegration Std.Err.	Disintegration -95.00%	Disintegration +95.00%	N
1	1	36.97470	2.517663	31.96342	41.98599	42
2	2	65.21280	2.517663	60.20151	70.22408	42

Effect	Univariate Test of Significance, Effect Sizes, and Powers for Weight variation (Results) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p	Partial eta-squared	Non-centrality
Intercept	185.1720	1	185.1720	727345.5	0.000000	0.999622	727345.5
FILLER	0.0051	1	0.0051	19.9	0.000012	0.067383	19.9
LUBRICANT	0.0016	1	0.0016	6.4	0.011759	0.022856	6.4
GLIDANT	0.0001	1	0.0001	0.5	0.460682	0.001981	0.5
BINDER	0.0180	1	0.0180	70.9	0.000000	0.204955	70.9
Error	0.0700	275	0.0003				

Cell No.	FILLER; LS Means (Results Weight variation) Current effect: F(1, 275)=19.869, p=.00001 Effective hypothesis decomposition FILLER	Weight variation Mean	Weight variation Std.Err.	Weight variation -95.00%	Weight variation +95.00%	N
1	1	0.846042	0.001261	0.843559	0.848525	160
2	2	0.854932	0.001545	0.851891	0.857974	120

Cell No.	LUBRICANT; LS Means (Results Weight variation) Current effect: F(1, 275)=6.4325, p=.01176 Effective hypothesis decomposition LUBRICANT	Weight variation Mean	Weight variation Std.Err.	Weight variation -95.00%	Weight variation +95.00%	N
1	1	0.847958	0.001545	0.844916	0.850999	120
2	2	0.853016	0.001261	0.850533	0.855500	160

Cell No.	GLIDANT; LS Means (Results Weight variation) Current effect: F(1, 275)=5.4577, p=.46068 Effective hypothesis decomposition GLIDANT	Weight variation Mean	Weight variation Std.Err.	Weight variation -95.00%	Weight variation +95.00%	N
1	1	0.851224	0.001261	0.848740	0.853707	160
2	2	0.849750	0.001545	0.846709	0.85792	120

Cell No.	BINDER; LS Means (Results Weight variation) Current effect: F(1, 275)=70.892, p=.00000 Effective hypothesis decomposition BINDER	Weight variation Mean	Weight variation Std.Err.	Weight variation -95.00%	Weight variation +95.00%	N
1	1	0.842458	0.001380	0.839742	0.845175	140
2	2	0.858516	0.001380	0.855799	0.861232	140

Effect	Univariate Test of Significance, Effect Sizes, and Powers for Friability (Results) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p	Partial eta-squared	Non-centrality
Intercept	1339.707	1	1339.707	2.561045	0.143990	0.221524	2.561045
FILLER	41.960	1	41.960	0.080213	0.783415	0.008834	0.080213
LUBRICANT	1087.175	1	1087.175	2.078292	0.183283	0.187600	2.078292
GLIDANT	826.389	1	826.389	1.579762	0.240434	0.149319	1.579762
BINDER	724.400	1	724.400	1.384797	0.269470	0.133348	1.384797
Error	4707.987	9	523.110				

Cell No.	FILLER; LS Means (Results Friability) Current effect: F(1, 9)=.08021, p=.78342 Effective hypothesis decomposition FILLER	Friability Mean	Friability Std.Err.	Friability -95.00%	Friability +95.00%	N
1	1	12.04114	8.086328	-6.2514	30.33369	8
2	2	8.42001	9.903689	-13.9837	30.82371	6

Cell No.	LUBRICANT; LS Means (Results Friability) Current effect: F(1, 9)=2.0783. p=.18328 Effective hypothesis decomposition LUBRICANT	Friability Mean	Friability Std.Err.	Friability -95.00%	Friability +95.00%	N
1	1	19.44662	9.903689	-2.9571	41.85032	6
2	2	1.01453	8.086328	-17.2780	19.30707	8

Cell No.	GLIDANT; LS Means (Results Friability) Current effect: F(1, 9)=1.5798, p=.24043 Effective hypothesis decomposition GLIDANT	Friability Mean	Friability Std.Err.	Friability -95.00%	Friability +95.00%	N
1	1	2.19555	8.086328	-16.0970	20.48809	8
2	2	18.26560	9.903689	-4.1381	40.66930	6

Cell No.	BINDER; LS Means (Results Friability) Current effect: F(1, 9)=1.3848, p=.26947 Effective hypothesis decomposition BINDER	Friability Mean	Friability Std.Err.	Friability -95.00%	Friability +95.00%	N
1	1	17.42383	8.844937	-2.5848	37.43246	7
2	2	3.03732	8.844937	-16.9713	23.04596	7