



FACULTEIT FARMACEUTISCHE WETENSCHAPPEN

Laboratory of Pharmaceutical Technology

TASTE - MASKED QUININE FORMULATIONS FOR FLEXIBLE PEDIATRIC DRUG DOSING IN ORAL TREATMENT OF MALARIA

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General introduction and objectives

1. Lack of pediatric formulations

Most authorized or licensed oral medicines are intended for adults and are presented as tablets or capsules, often in a unit intended as a single adult dose. Nahata (1999) stated that only 20% of drugs marketed in the United States have labelling for pediatric use and only five of the 80 drugs most commonly used in newborns and infants are approved for pediatric use. Between January 1995 and April 1998, a total of 45 drugs were licensed through the European Medicines Evaluation Agency (EMEA), and 29 of them were of potential use in children, but only 10 of these (34%) were licensed for pediatric use (Impicciatore and Choonara, 1999). According to Ceci and collaborators (2002), the situation had not changed in September 2001. Only 47 of 141 (33%) potentially useful medicines had been licensed by the EMEA for use in children. The pediatric patient group with the highest incidence of unlicensed drug prescriptions in Europe is neonates, with 90% of babies in neonatal intensive care receiving at least one unlicensed or off-label drug prescription (Choonara and Conroy, 2002). The problem is not only the lack of pediatric formulations, but also the lack of product information for pediatric use. Tan and collaborators (2002) reviewed the product information (PI) of 1497 commercially available drugs from 21 therapeutic classes. The objectives were to determine the extent and nature of information available on pediatric dosing and the availability of pediatric dosage formulations. These authors reported that up to 81% of PIs of medicines that were not contraindicated for use in children gave inadequate pediatric dosing information. The proportion of PIs with inadequate dosing information decreased with increasing age group, from 79.1% for the 1-3 months to 71.6% for the 6-12 years age group. The

proportion of PIs, for each age group, that gave specific pediatric dosing information, but did not provide a pediatric dosage form were: 26.5% for age < 1 month, 25% for 1-3 months, 23.3% for 3 months-2 years, 21.9% for 2-6 years and 24% for 6-12 years.

Challenges leading to the lack of pediatric drug formulations on the market

The development of pediatric formulations, particularly those suitable for very young children, can be challenging to the pharmaceutical scientist. As reported by the EMEA, there is only limited knowledge available on the acceptability of different dosage forms, administration volumes, dosage form size, taste, and importantly, the acceptability and safety of formulation excipients in relation to the age and development status of the child (EMA/CHMP/PEG/194810/2005).

Liquid formulations

In general, for oral administration, liquid formulations should be administered whenever appropriate as they are easy to administer and swallow, but there are some limitations and disadvantages. The major challenge for liquid formulations is drug hydrolysis which compromises the chemical stability. The dose volume is a major consideration for the acceptability of a liquid formulation. Typical target dose volumes for pediatric liquid formulations are < 5 ml for children under 5 years and < 10 ml for children of 5 years and older (EMA/CHMP/PEG/194810/2005). Poorly soluble drugs require the addition of cosolvents and surfactants. Unfortunately some solubilisers such as ethanol and propylene glycol are not desirable for administration to children. In addition,

preservatives, detergents, antioxidants, sweeteners, flavours or coloring agents are added to avoid drug or dosage form instability and to improve the organoleptic properties of the formulation. Although those excipients are called “inactive ingredients”, many have some effects and can produce adverse reactions in patients (Pawar and Kumar, 2002). Of crucial importance is the ability to mask the unpleasant taste with sweeteners and flavours. In case of very bitter drugs (e.g. ranitidine HCl, prednisolone Na, quinine), this approach is not achievable and more sophisticated formulation approaches are required, bringing higher technical challenges and consequently, research and development will be more lengthy and costly. Another disadvantage of liquid drug formulation is the low feasibility of controlling the release of the drug. Furthermore, liquids are not the dosage forms of choice in resource-limited settings because of their higher weight resulting in higher expenses during transport.

Rectal dosage forms

Suppositories are often used as drug delivery system in case of nausea or vomiting, in case of oral administration rejection due to the bad taste or in case a medication is readily decomposed in gastric fluid. However, compliance may be lower than for oral dosage forms, as the rectal route of administration is poorly accepted by patients and caregivers in certain countries and cultures (EMEA/CHMP/PEG/194810/2005). Moreover, drug absorption can be decreased secondary to defecation of the drug or to incomplete dissolution depending on the solubility of the drug and the lower fluid volume in the rectum (De Boer et al., 1982). Additionally, depending on the nature of the suppository base, stability problems can occur at elevated temperatures in the tropical countries.

Solid dosage forms

Compared with liquid formulations, solid preparations exhibit higher drug stability as well as higher drug content per single dosage form. Most commonly used excipients such as cellulose derivatives, starches, lactose are non-toxic and safe for use in children. Another advantage of solid dosage forms is the possibility of sustained drug release and gastro-enteric protection. However, the problem with tablets (the most popular solid dosage form) is a dosing issue as most commercially available tablets are in doses that are significantly too high for the pediatric population. Multiple dosage forms based on small solid particles like pellets, granulates, powders and sprinkles could offer a solution for this dosing problem. They are suitable for pediatric use since they are usually mixed with food (solid or liquid) for easy swallowing. They can be filled either into capsules (which are emptied before dose administration), bottles (the required dose is withdrawn by dosage spoons), or sachets (packaged as single doses) (Breitkreutz et al., 1999). Such particles can be compressed into tablets which disperse in the mouth or in a small amount of liquid on a spoon. A variety of such tablet preparations is available as fast dispersing dosage forms (FDDFs), for example Calpol Fast Melts[®] (Pfizer Consumer Health), Nurofen[®] Meltlets (Crookes Healthcare), Benadryl[®] orodispersible tablets. These products are placed in the mouth where they 'melt' on the tongue in the saliva or can be dispersed in a small amount of liquid on a spoon. They are easy to administer providing that taste is acceptable and they provide accuracy of dose. However, none of the products currently has a license for children less than 6 years of age due to the dose strength available, many of these technologies are proprietary and consequently their use will

require licensing agreements, and development costs are higher than for conventional oral dosage forms (Nunn and Williams, 2005).

The lack of pediatric formulations is dictated by two major limitations, being technology and market conditions. The pediatric population is categorised into various groups because neonates, infants, children and adolescents have different body composition (for example percentage of body water and fat) and have body organs in different stage of development. The pediatric age classification according to EMEA (CPMP/ICH/2711/99) is presented in Table 1.

Table 1: Pediatric age classification

Definition	Age
Preterm newborn infants	
Term newborn infants	0 – 27 days
Infants and toddlers	28 days – 23 months
Children	2 – 11 years
Adolescents	12 to 17 years

As pediatric formulations must allow accurate administration of the dose to patients of widely varying age and weight, the development of age-adapted dosage forms is a formidable challenge for formulation scientists.

Moreover, a bitter or metallic taste of the drug can lead to its rejection by children, but they also may not like the taste of “masked” formulations, as flavor and palatability

preferences are influenced by culture. This requires additional studies taking into account cross-cultural preferences for flavors (Giaccoia et al., 2007).

The second challenge is related to the economic reality of the pediatric market, which is relatively small at about 20 to 25 % of the total adult market. Owing to the high cost of developing drugs, the manufacturers are compelled to address the large markets first. This often means postponing or omitting drug development of pediatric formulations (Gupta et al., 2006). Developing pediatric drugs requires additional clinical studies and many reasons are given by the pharmaceutical industry for not testing drugs in children e.g. ethical issues, technical and methodological concerns. However, according to Conroy (2006), the real reason is that it is time consuming and expensive to perform trials in children, and without a legal obligation and/or financial incentives to do so they are unlikely to happen. This statement is based on the fact that most of the drugs submitted in 2001 to the EMEA for licensing for pediatric use were vaccines (with a large pediatric market) and anti-HIV drugs (for which financial incentives for development had been made available).

In-practice alternative: unlicensed and off-label drug use in children

As a consequence of the lack of pediatric drug formulations, health professionals working with children are forced into a situation whereby they often need to use unlicensed (UL) drugs or licensed drugs in ways not covered by the license (off label, OL), to ensure that children receive an appropriate treatment.

Unlicensed drug use is defined as modifications to a licensed medicine. In practice the licensed drug is modified into extemporaneous prepared medicines. Extemporaneous preparation describes the manipulation by pharmacists of various drug and chemical ingredients using traditional compounding techniques to produce suitable medicines. The use of these techniques is widespread in pediatric pharmacy practice. This involves crushing tablets, opening capsules, suspending the drug with various excipients in a liquid, diluting the drug with a bulking agent (e.g. lactose) to a specific strength and supplying the powder mixture in a sachet or as a capsule to the patient. Such liquids, capsules and powders are produced in large volumes in hospital pharmacy departments throughout Europe (Brion et al., 2003), the importance of each dosage form differing from country to country: hospitals in Denmark, England, Ireland, Norway and Sweden mainly prepared oral liquids (> 60% of doses); in Finland, Italy and Scotland mainly powder; and in Belgium, Croatia, France and Switzerland mainly capsules.

The *off-label* use of drugs refers to use outside the conditions of the license or marketing authorisation. This may be due to (Turner et al., 1997):

- dose: a lower (or higher) dose than recommended in the license is administered, depending on the age and weight of the child.
- age: drugs are often not licensed in children under a certain age, or not at all for children, even if they are commonly used for pediatric applications.
- indication: drugs may be used to treat childhood illness not covered by the license.
- route of administration

Unlicensed/off-label drug use in developing countries

The problems affecting pediatric drug use in developed countries are different from those in developing countries, where limited availability, distribution problems and low quality formulations are reported. The WHO Essential Medicines List model has been made to assist low income countries to develop their own national lists with the aim of improving drug use through rational choice; however, the list lacks pediatric focus. As reported by Beggs and collaborators (2005), this is illustrated by the fact that on its 13th edition (2003), of the 160 medication listings that could include a pediatric formulation, only 47 do so. Fortunately, following recommendations from the Expert Committee that updated the current 15th edition of Essential Medicines List, the World Health Organization (WHO) announced the initiation of the work to create a medicines list specifically tailored to children's needs to tackle diseases with high pediatric mortality and morbidity (WHO website). The development of a pediatric-specific essential medicine list would increase the awareness of the need for pediatric-specific formulations and highlight areas of priority where medications are lacking. The lack of pediatric formulations leads to the extended unlicensed and off-label drug use as illustrated by the following example for quinine in Africa. In Kenyan hospitals, vials of quinine dihydrochloride (licensed for intravenous injection) are mixed with syrup base and the resulting extemporaneous quinine dihydrochloride syrup is administered orally for malaria treatment in the pediatric population (Essential drugs website).

In addition, inadequate drug prescription in children and poor advice given to mothers/guardians of children in health care facilities on how to use/administer drugs at home (Nsimba, 2006), can increase unlicensed and off-label drug use.

Risks associated with unlicensed and off-label drug use in children

When a pharmaceutical company submits an application to the licensing authority, it includes data from extensive clinical trials looking at different dosage regimes, pharmacokinetics and drug toxicity. However, many drugs have not been formally tested in children during clinical trials; consequently, the general lack of information on drug formulations to support their administration to children may expose them to toxic or under dosing effects. Some case examples are reported below.

A study undertaken by Turner and collaborators (1999) evaluated the relationship between unlicensed and off-label prescribing and adverse drug reactions (ADRs). On a total of 1046 admissions, 507 (48%) of the patients received one or more unlicensed or off-label drugs and ADRs occurred in 116 (11%) patients. Since these ADRs were linked to licensed drug prescriptions in 3.9% of the cases and to unlicensed and off-label drug prescriptions in 6%, the authors concluded that unlicensed and off-label drug use was significantly associated with ADR risk.

In the Trent region (UK), 95 reports of ADR were addressed by the pediatric regional monitoring center in 1998. These reports involved 105 drugs suspected of being responsible for 171 ADRs. About 25% of the reports had at least one drug that was used off-label (Clarkson et al., 2001).

A prospective pharmacovigilance study involving 1419 children less than 16 years was conducted in the south west of France. The authors found that 18.9% of the prescriptions were off-label (11.5% for a different indication, 4.7% for a different dosage, 1.1% for age, 0.6% for inadvisable co-prescription, 0.6% for contra-indication and 0.4% for route of administration). Twenty non-serious (8 gastrointestinal, 6 cutaneous, 3 neurological, 2 fever and rhinitis) ADRs were reported. ADR incidence increased from 1.4% overall to 2.0% with off-label use (Horen et al., 2002).

For extemporaneous prepared formulations, the lack of reliable information and lack of standards are two of the biggest obstacles in compounding medications for children. Very little is known about the effect on children's health of excipients used in compounding. Adverse effects caused by excipients could be a cause of noncompliance, e.g. patient who experiences osmotic diarrhoea after ingesting a compound containing glycerol or various sugars (sucrose, manitol, glucose) (Pawar et al., 2002). Hurtado and Moffett (2007) evaluated compounded pediatric formulations for consistency in concentration and formulation ingredients to ensure that the same medication was available to children who had undergone cardiac transplant once they were discharged from the hospital. These authors reported that only 50% of the pharmacies used formulations consistent with the formulation used by the discharging hospital, 25% used formulations that could not be shared because of the pharmacy policy, and 20% of the pharmacies used unpublished formulations. These authors suggested to use published information for compounding when available, otherwise the use of pharmacopoeia or other scientific references for chemical solubility and stability information.

Which initiatives have already been taken to improve the access of the pediatric population to appropriate drug formulations?

Already in 1997, the Food and Drug Administration (FDA) provided incentives for pharmaceutical companies to perform specific research for pediatric medications. This was extended in 2002 via the Best Pharmaceuticals for Children Act (BCPA) which provided a significant financial incentive (i.e. 6 month patent extension) if the manufacturer conducts clinical trials to determine the safety, efficacy and dosing of a product for the pediatric population and updates the drug's label accordingly. As a result of these initiatives the labelling of more than 100 drugs has been changed to include information for pediatric applications. To further stimulate the development of pediatric formulations, the Pediatric Research Equity Act (PREA) came into effect in the US (2003) which requires pharmaceutical companies testing a new drug to conduct pediatric clinical trials if the drug is likely to be used in children (FDA website, accessed on 26 Nov. 2007).

More recently a similar regulation on medicinal products for pediatric use was published by the European authorities (EC No 1901/2006) to offer incentives for pharmaceutical companies. Rewards for testing a product in children can be (a) a 6-month extension of the protection for patented medicines (regardless of the outcome of the study), (b) an additional 2 year market exclusivity (i.e. 10+2 years in total) for orphan medicines, or (c) a specific type of marketing authorization (i.e. Pediatric Use Marketing Authorization, providing 10 years data protection) for off-patent medicines specifically developed for children (Ramet, 2005). In addition, the European Medicines Agency (EMA) has issued

papers to assist pharmaceutical companies and research group developing pediatric drug formulations:

- Reflection paper on formulations of choice for the pediatric population (EMA 194810/2005) which specifically provides points to consider for those formulating drug dosage forms for children (e.g. info about routes of administration, excipients, dose delivery devices, ...)
- Updated priority for studies into off-patent pediatric medicinal products (EMA/197972/2007). The development of formulation containing the drugs listed in this paper is specifically encouraged in the call of the Seventh Research Framework Programme (FP7) of the European Union.

Conclusion

There is an urgent need to address the lack of adequate formulations for the pediatric population. Specific concerns that need to be addressed include the identification of economic, scientific and technical barriers, the definition of the role of unlicensed and off-label drug formulations (especially extemporaneous formulations) and how the effectiveness and safety of these preparations can be realistically monitored as these formulations may remain an important alternative if licensed pediatric drug formulations are not available.

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2. Malaria

Malaria is a disease caused by protozoan parasites of the genus *Plasmodium*. Four plasmodia species commonly infect humans: *P. falciparum*, *P. malariae*, *P. vivax* and *P. ovale*. All four species are found in the tropics and sub-tropics around the world. The vast majority of clinical disease and practically all malaria-related deaths in Africa are due to *P. falciparum* (Marsh and Makani, 2004).

Human infection results from the bite of the female Anopheline mosquito. Disease symptoms appear following a complicated life cycle of the malaria parasites as shown in Figure 1. Mosquitoes inject parasites (sporozoites) into the blood circulation. These reach the liver in a matter of minutes and start to reproduce, becoming hepatic schizonts which subsequently rupture and release parasites (merozoites) into the bloodstream. These merozoites rapidly infect red blood cells. The time from mosquito bite to hepatic schizont rupture is generally between 1 and 2 weeks.

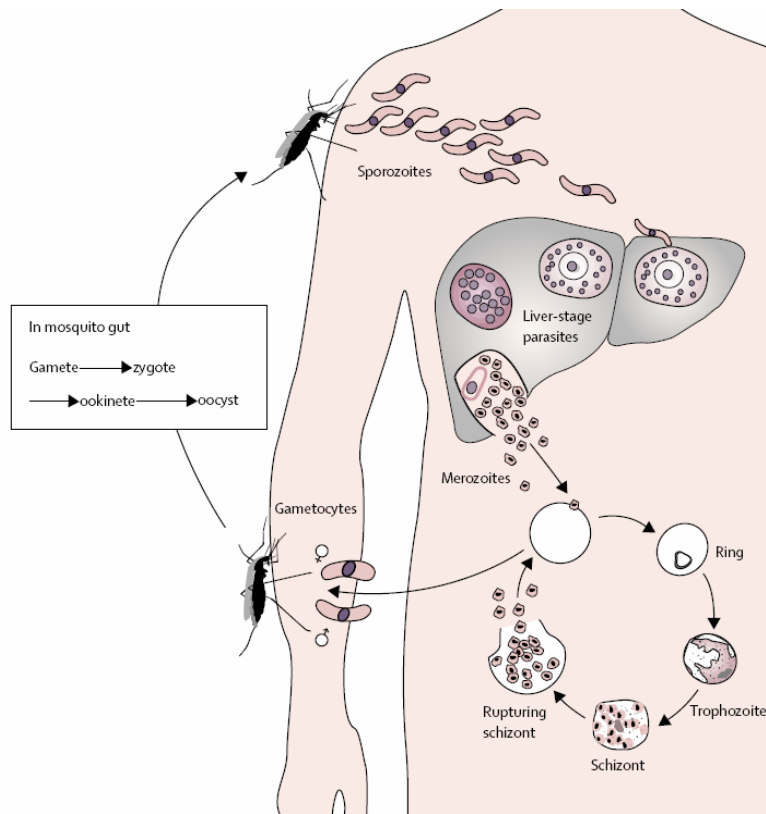


Figure 1: Life cycle of the malaria parasite. (From Greenwood et al., 2005)

In the red blood cells parasites consume haemoglobin, multiply and develop into schizonts. Rupture of the schizonts releases more merozoites into the blood stream to invade yet more red blood cells, causing malaria symptoms (fever) and the infective biomass to expand. This asexual life-cycle from the invasion of red blood cells by merozoites until schizont rupture takes 48 h for *P. falciparum*. The female Anopheline mosquitoes become infected by biting an infected human and ingesting blood containing male and female gametocytes. In the mosquito gut the gametocytes fuse to form a zygote, which develops into new sporozoites to complete the life-cycle (Ashley, 2006).

Malaria clinical manifestations range from a self-limiting fever to a severe illness. Since the malaria symptoms may mimic many other infectious diseases, investigations of the blood film is necessary to confirm the diagnosis and assess parasite density.

Children with malaria typically develop fever, vomiting and headache, while symptoms of severe malaria include prostration, impaired consciousness, severe anemia, hypoglycemia and multiple convulsions (Greenwood et al., 2005).

Each year an estimated 300 to 500 million clinical cases of malaria including 2-3 million severe attacks occur, making it one of the most common infectious disease worldwide. In many malaria areas, especially sub-Saharan Africa, malaria is ranked among the most frequent causes of morbidity and mortality among children. Rowe and collaborators (2006) reported that between 700.000 and 900.000 children in sub-Saharan Africa aged under 5 years died of the disease in 2000, accounting for 16 to 20% of deaths in that age group. Nearly all of deaths (94%) were in areas with high intensity transmission in the central regions of Africa, i.e. two thirds (68%) of these deaths occurred in rural areas and a quarter (26%) in urban areas. For all of sub-Saharan Africa, including populations not exposed to malaria, malaria caused permanent neurological damage in approximately 7% of the patients (WHO, Roll Back Malaria).

2.1 World Health Organization (WHO) guidelines for malaria treatment

In November 2000, an Informal Consultation on the Use of Antimalarial Drugs was convened by the WHO in Geneva. The meeting acknowledged the limited number of treatment options that are available to countries to improve their treatment policies. This

is of particular concern in areas of highest resource constraints such as sub-Saharan Africa where a lack of resources has contributed to the continued use of drugs whose effectiveness have been compromised by drug resistance.

Malaria control efforts in the region have been greatly affected by the emergence and spread of resistance to chloroquine, the most used antimalarial drug. Sulfadoxine-pyrimethamine (SP) was seen as the obvious successor to chloroquine. However, resistance to SP developed quickly (Ogutu et al., 2000; Trigg et al., 1997), thus reducing the useful therapeutic life of this drug.

2.1.1 Uncomplicated malaria

Uncomplicated malaria is defined as symptomatic malaria without signs of severity or evidence of vital organ dysfunction. In acute falciparum malaria there is a continuum from mild to severe malaria. Young children and non-immune adults with malaria may deteriorate rapidly.

Antimalarial combination therapy

The existing antimalarials were used as monotherapies. However, following to the resistance to the two most used drugs (chloroquine and Sulfadoxine-pyrimethamine) the protection offered by antimalarials was questioned and the potential value of malaria therapy using combinations of drugs (White, 1998a; 1998b) was identified as a strategic and viable option in improving efficacy, and delaying development and selection of resistant parasites.

The concept of combination therapy is based on the synergistic or additive potential of two or more drugs, to improve therapeutic efficacy and also delay the development of resistance to the individual components of the combination. In practice it is the simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite. The concept of combinations of antimalarials is now recommended by the WHO for the treatment of *P. falciparum* malaria (WHO, 2006).

Non-artemisinin based combinations

Chloroquine plus sulfadoxine-pyrimethamine

Chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) are antimalarial drugs that were used frequently in Africa either as first-line or second-line drug for the treatment of *P. falciparum* malaria. In areas with high levels of *P. falciparum* resistance to CQ and moderate resistance to SP, the combination of CQ+SP would not be expected to achieve significantly better cure rates than SP alone. However, studies in Gambia (Bojang et al., 1998) and Papua New Guinea (Jayatilaka, 2003) which compared the efficacy and safety of CQ+SP to that of SP alone showed that the efficacy of the combination was dependent on the levels of resistance to the individual components. Overall, the available evidence has shown that the CQ+SP combination is unlikely to have a significant advantage over SP alone in areas of predominant *P. falciparum* transmission with high levels of resistance to CQ. Since this reflects the current situation in most of sub-Saharan Africa, a

change to this combination as a first-line treatment policy did not give any significant useful long-term advantage.

Amodiaquine plus sulfadoxine-pyrimethamine

Amodiaquine (AQ) is a 4-aminoquinoline similar in structure and activity to chloroquine. A recent review of studies on the treatment of uncomplicated falciparum malaria conducted over the past ten years in Africa showed a higher therapeutic efficacy of amodiaquine over chloroquine, with a tendency towards faster clinical recovery. This difference was also observed in areas with mild to moderate parasite resistance to chloroquine (Olliaro et al., 1996; Hatton et al., 1996; Brasseur et al., 1999). The global use of amodiaquine has declined owing to reports of severe adverse reactions including neutropenia following its use for chemoprophylaxis of malaria (Hatton et al., 1986).

The WHO concluded that SP+AQ can be more effective than either drug alone, but the combination needs to be considered in the light of comparison with artemisinin-based combinations (ACTs). If ACTs are not available and AQ and SP are effective, AQ+SP may be used as an interim measure (WHO, 2006).

Mefloquine-sulfadoxine-pyrimethamine (Fansimef[®], Roche)

The combination of mefloquine-sulfadoxine-pyrimethamine (MSP) was developed for therapeutic use on the basis of the observation that its components display at least additive activity and that their combination might delay the emergence of parasite resistance (WHO, 1984). The long elimination half-life of mefloquine (20 days in adults) is an advantage for single dose treatment, but a disadvantage in areas with intensive

malaria transmission where the residual drug level for a long duration is likely to exert high selection pressure on the parasite population (Watkins and Mosobo, 1993). However, the use of MSP as a first-line treatment for uncomplicated *P. falciparum* infections in Thailand was associated with a rapid development of resistance to mefloquine on the Thai-Cambodian border (White, 1992; Nosten et al., 1991). It is thought that this was caused by residual, post-therapeutic drug blood levels in individuals who returned to areas with intensive malaria transmission and contracted new infections (Wernsdorfer et al., 1994). As a result, MSP has not been recommended for general use by malaria control programs for either prophylaxis or treatment.

Artemisinin-based combinations

Artemisinin (qinghaosu) and its derivatives (artesunate, artemether, artemotil and dihydroartemisinin) produce rapid clearance of parasitaemia and rapid resolution of symptoms. They are active against all four species of malaria parasites that infect humans and are generally well tolerated. Artemisinin and its derivatives are eliminated rapidly. When given in combination with rapidly eliminated compounds (tetracyclines, clindamycin), a 7-day course of treatment with an artemisinin compound is required; but when given in combination with a longer half-life partner antimalarial drug shorter courses of treatment (3 days) are effective.

Pre-clinical studies have shown that artemisinin and its derivatives do not exhibit mutagenic or teratogenic activity. However, the drugs have caused fetal resorption in rodents at relatively low doses of 10 mg/kg, when given after the sixth day of gestation

(Qinghaosu, 1979). Reports on the use of these drugs in humans during pregnancy are limited (Li et al., 1994; Wang, 1989). Thus, because of the effects in rodents and the very limited data in humans, the artemisinin derivatives are currently not recommended for use in the first trimester of pregnancy (McGready, 1998).

Artesunate plus chloroquine (AS+CQ)

Efficacy and safety of the combination of artesunate plus chloroquine have been evaluated in two randomized, double-blind, placebo-controlled clinical trials conducted in children from Burkina Faso (Sirima et al., 2003) and Sao Tome and Principe (Gil et al., 2003). The combination was well tolerated with rapid parasitemia clearance by day 14. However at day 28, a high failure rate (mostly due to CQ resistance level) of 19.0% and 32.4%, respectively, were observed. Based on these findings, artesunate + CQ does not appear to be a viable option in areas with moderate to high levels of *P. falciparum* resistance to CQ.

Artesunate plus amodiaquine

This combination is currently available as two separate tablets containing 50 mg artesunate and 153 mg amodiaquine base, respectively. Co-formulated tablets are under development. The recommended dose is 4 mg/kg body weight of artesunate and 10 mg/kg body weight of amodiaquine base given once a day for 3 days.

The efficacy and safety of artesunate plus amodiaquine have been evaluated in three randomized, double-blind, placebo-controlled clinical trials conducted in Senegal (Trape et al., 2003) Burundi (Ndayiragije et al, 2004) and Gabon (Oyakhrome et al., 2007). The

combination was efficacious and well tolerated. The level of efficacy coincides with low levels of AQ resistance in the study sites. The 14 day parasitological cure rate of the combination was > 90% at all sites. Artesunate plus amodiaquine appears to be a viable option, particularly in areas where CQ efficacy is already compromised. However, continued monitoring of resistance to AQ and the impact of AQ resistance on the effectiveness of the combination would need to be carefully monitored.

Artesunate plus sulfadoxine-pyrimethamine

The efficacy and safety of SP plus artesunate have been evaluated in three randomized, double-blind, placebo-controlled clinical trials in Gambia (Doherty et al., 1999), Kenya (Ochola et al., 2003) and Uganda (Priotto et al., 2003). This combination was very well tolerated and, as with CQ and AQ combinations with artesunate, the therapeutic efficacy was dependent on the level of pre-existing resistance to the partner drug. The increasing levels of resistance to SP will limit the use of artesunate + SP, particularly in the eastern parts of Africa. However, it may still be a viable option for some countries of West Africa and other areas where SP efficacy is not yet compromised by resistance (WHO, 2001). It is currently available as 2 individual tablets, one containing 50 mg artesunate tablets, and one containing 500 mg sulfadoxine and 25 mg pyrimethamine tablets.

The total recommended treatment is 4 mg/kg body weight of artesunate given once a day for 3 days and a single administration of sulfadoxine-pyrimethamine (25 and 1.25 mg/kg body weight for sulfadoxine and pyrimethamine, respectively) on day 1.

Artesunate plus mefloquine

The co-administration of artesunate plus mefloquine has been in use for many years in parts of Thailand and has become the first-line treatment in several parts of South-East Asia (WHO, 1998a; 1998b). Adverse reactions reported with the use of mefloquine included severe adverse neuropsychiatric effects, a few cases of cardiotoxic effects (Havaldar, 2000; Potasman et al., 2000). There is concern that the long half-life of mefloquine may lead to the selection of resistant parasites in areas of intense transmission. Furthermore, there are also concerns of a possible increase of mefloquine-related adverse reactions when used unsupervised on a large scale for treatment of malaria (WHO, 2001).

Artemether-lumefantrine (Coartem[®], Riamet[®], Novartis)

This is a co-formulation of artemether and lumefantrine (an aryl alcohol related to quinine and mefloquine) that has proved as effective and better tolerated as artesunate plus mefloquine in the treatment of multi-drug resistant *P. falciparum*. This is currently available as co-formulated tablets containing 20 mg artemether and 120 mg lumefantrine. The total recommended treatment is a 6-dose regimen of artemether-lumefantrine twice a day for 3 days. An advantage of this combination is that lumefantrine is not available as a monotherapy and has never been used by itself for the treatment of malaria. However, lumefantrine absorption is enhanced by co-administration with fat. Low blood levels, with resultant treatment failure, could potentially result from inadequate fat intake, and so it is essential that patients or care givers are informed of the need to take this ACT with milk or fat-containing food – particularly on the second and third days of treatment.

There are as yet no serious adverse reactions documented, and studies show no indication of cardiotoxicity. However, the drug is not recommended for use in pregnant and lactating women, as safety for use in these groups has not yet been established. Artemether-lumefantrine is the most viable artemisinin combination treatment available at the moment, because in addition to its efficacy, safety and tolerance profile, it is available as a fixed-dose formulation, increasing the likelihood of patient compliance with the drug regimen (WHO, 2006).

Deployment considerations affecting choice

Although for many countries, artemether-lumefantrine and artesunate + mefloquine may give the highest cure rates, there may be problems of affordability and availability of these products. Also, there is currently insufficient safety and tolerability data on artesunate + mefloquine at the recommended dose of 25mg/kg in African children to support its recommendation. Trials with mefloquine monotherapy (25mg/kg) have raised concerns of tolerability in African children. Countries may therefore opt instead to use artesunate + amodiaquine and artesunate + sulfadoxine–pyrimethamine, which may have lower cure rates because of resistance.

Based on available safety and efficacy data, the WHO recommended the following therapeutic options in prioritized order if costs were not an issue (WHO, 2001):

1. artemether-lumefantrine (Coartem[®])
2. artesunate plus amodiaquine

3. artesunate plus sulfadoxine-pyrimethamine (SP) in areas where SP efficacy remains high
4. SP plus amodiaquine in areas where efficacy of both amodiaquine and SP remain high. This is mainly limited to countries in West Africa.

Furthermore, continued documentation of safety and efficacy especially among very young children, pregnant women, and breastfeeding mothers and their babies was recommended for these combination options. The current assessment of benefits compared with potential risks suggests that the artemisinin derivatives should be used to treat uncomplicated falciparum malaria in the second and third trimesters of pregnancy, but should not be used in the first trimester until more information becomes available. The antimalarials considered safe in the first trimester of pregnancy are quinine, chloroquine, proguanil and sulfadoxine–pyrimethamine. Of these, quinine remains the most effective and can be used in all trimesters of pregnancy including the first trimester (WHO, 2006).

2.1.2 Severe malaria

In a patient with asexual *P. falciparum* parasitaemia and no other obvious cause of their symptoms, the presence of one or more of the following clinical or laboratory features classifies the patient as suffering from severe malaria.

Clinical manifestations: prostration, impaired consciousness, respiratory distress, multiple convulsions, circulatory collapse, pulmonary oedema, abnormal bleeding, jaundice, haemoglobinuria.

Laboratory test: severe anemia, hypoglycemia, acidosis, renal impairment, hyperlactatemia, hyperparasitemia.

Two classes of drugs are currently available for the parenteral treatment of severe malaria: the cinchona alkaloids (quinine and quinidine) and the artemisinin derivatives (artesunate, artemether and artemotil).

Quinine

Quinine (Figure 2) exists under different salts forms and 100 mg of anhydrous quinine base is approximately equivalent to 169 mg of quinine bisulphate, 122 mg of quinine dihydrochloride, 122 mg of quinine etabonate, 130 mg of quinine hydrobromide, 122 mg of quinine hydrochloride and 121 mg of quinine sulphate.

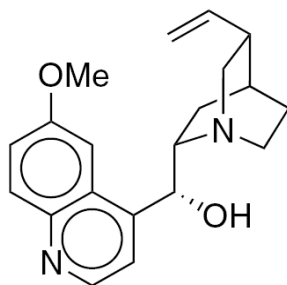


Figure 2: Chemical structure of quinine

For the treatment of malaria quinine is given by mouth, usually as sulphate or hydrochloride salt, or in case of severe malaria when the patient is unable to take oral medication, it is administered parenterally by slow intravenous infusion as dihydrochloride salt (Martindale, 2005).

For oral administration, quinine is commercially available as tablets in most of cases containing sulfate or hydrochloride salts in a dose of 200 or 300 mg per tablet. Although this molecule has been commercially used for decades, no liquid formulation for pediatric applications is available. Tablet breaking is the only way to adapt the dose to body weight of children. The accuracy of quinine dose following tablet breaking will be discussed in Chapter I of the present work.

Quinine is a reasonable option for treatment in travellers returning to non-endemic areas who develop malaria, since the drug-resistance pattern of the parasite may not be known and a fully efficacious drug is needed in non-immunes to prevent progression of uncomplicated malaria to severe disease.

Quinine can be used as a second-line treatment for patients who fail to respond to the standard first-line therapy and/or in case they are contra-indicated. To improve compliance and maintain its efficacy (WHO, 2001), quinine is usually combined with

tetracycline or doxycycline. However, these drugs are contra-indicated in children and pregnant women, clindamycin can be used for these groups.

For parenteral use, a loading dose of quinine twice the maintenance dose (i.e. 20 mg salt/kg) reduces the time required to reach therapeutic plasma concentrations (4 h by infusion, while therapeutic concentrations may not be reached in the first 12 h of treatment if no loading dose is administered (Tombe et al., 1992; Assimadi et al., 2002)). After the first day of treatment, the daily maintenance dose of quinine is 30 mg salt/kg usually divided into three equal administrations at 8 h intervals. Rate-controlled intravenous (IV) infusion is the preferred route of quinine administration, but if this cannot be given safely, then intramuscular (IM) injection is an alternative, but side effects have been reported. In some countries, the IM injection is the most common cause of lower limb paralysis when administered mistakenly into the sciatic nerve (Barennes et al., 1999). Thus, the rectal use was used to overcome problems associated with IM quinine administration. A diluted injectable formulation has been evaluated (20 mg/kg) but the drug was sometimes expelled from the rectum; in addition the calculated intrarectal bioavailability was only about 40% (Barennes et al., 1996).

Whenever parenteral quinine is used, oral treatment should be resumed as soon as the patient is able to take it, and continued for the completion of the course.

Recommended oral treatment

For oral administration quinine should be given by one of the following regimens:

- *Areas where parasites are sensitive to quinine:*

Quinine (8 mg of base per kg, three times daily for 7 days)

- *Areas with marked decrease in susceptibility of P. falciparum to quinine*

Quinine (8 mg of base per kg, three times daily for 7 days)

In combination with (a) doxycycline (100 mg of salt, daily for 7 days, not in children under 8 years of age and not during pregnancy), (b) tetracycline (250 mg, four times daily for 7 days, not in children under 8 years of age and not during pregnancy) or (c) clindamycin (300 mg, four times daily for 5 days, not contra-indicated in children and during pregnancy).

Drug disposition

Quinine is rapidly absorbed when taken orally, and peak plasma concentrations are reached within 1–3 h. The drug is distributed throughout body fluids and is highly protein bound. It readily crosses the placental barrier and is found in cerebrospinal fluid. Quinine is extensively metabolized in the liver, has an elimination half-life of 10–12 h in healthy individuals and is subsequently excreted in the urine, mainly as hydroxylated metabolites (WHO, 1990). Several pharmacokinetic characteristics differ according to the age of the subject, and are also affected by malaria. The volume of distribution is less in young children than in adults, and the rate of elimination is slower in the elderly than in young adults (Wanwimolruk, 1991). In patients with acute malaria, the volume of distribution is reduced and systemic clearance is slower than in healthy subjects, these changes being proportional to the severity of the disease. Protein binding of quinine is, however, increased in patients with malaria, as a result of the increased circulating concentration of the binding-protein alpha-1 acid glycoprotein (Winstanley, 1993).

Adverse effects

Cinchonism, a symptom complex characterized by tinnitus, hearing impairment, and sometimes vertigo or dizziness, occurs in a high proportion of treated patients. These symptoms, which are usually reversible, generally develop on the second or third day of treatment and rarely constitute a reason for withdrawing the drug.

Hypoglycemia may be caused by quinine since the drug stimulates secretion of insulin from pancreatic beta-cells. Hypoglycemia is particularly likely to develop after intravenous infusion of quinine in pregnancy, since beta-cells are more susceptible to a variety of stimuli at that time (WHO, 1990).

Resistance to quinine

The first reports of possible quinine resistance occurred in Brazil almost 100 years ago. Even today, however, clinical resistance to quinine monotherapy is only sporadically reported in South-East Asia and western Oceania. Strains of *P. falciparum* from Africa are generally highly sensitive to quinine.

Widespread use of quinine in Thailand in the 1980s led to a significant reduction of its sensitivity (Wernsdorfer, 1994). There is some cross-resistance between quinine and mefloquine, suggesting that the wide use of quinine in Thailand might have influenced the development of resistance to mefloquine in that country (Suebsaeng et al., 1986).

Artemisinin derivatives

Various artemisinin derivatives have been evaluated in the treatment of severe malaria including artemether, artemisinin, artemotil and artesunate. Concerns have been raised,

regarding the possibility that the intrinsic benefits of artemether as an antimalarial may have been negated by its erratic absorption following intramuscular injection. Artemotil is very similar to artemether, but very few trials have been conducted.

Artesunate is the most documented. A trial (Newton et al., 2003) comparing IV artesunate to IV quinine in 113 adults from west Thailand with severe malaria found no significant difference in mortality between the treatments. It found that artesunate significantly improved parasite clearance time, but that there was no significant difference in fever clearance time or coma recovery time. However, treatment with artesunate was well tolerated, whereas quinine was associated with hypoglycemia.

A large multi-centre trial involving 1461 patients was conducted in Bangladesh, India, Indonesia and Myanmar by the South-East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) group. It found that the mortality (15%) in the artesunate group was significantly lower compared to the quinine group (22%) (SEAQUAMAT group). Artesunate should be used in hyperparasitemic adult patients. However, it is not clear if artesunate is superior to quinine in children in sub-Saharan Africa as the existing data are insufficient to answer this question and the issue is currently being addressed in trials (Checkley and Whitty, 2007).

2.1.3 Choice of antimalarial drug for children

In endemic countries, malaria is common in children under 5 years of age. Although case-fatality is higher in this age group than in adults, drug assessment in this group is lower which complicate the choice of appropriate treatment. As an example, the WHO

recommends the following antimalarial medicines for severe malaria treatment in children, as there is insufficient evidence to recommend any of these antimalarial medicines over another (WHO, 2006):

- artesunate (2.4 mg/kg body weight, IV or IM administration on admission, after 12 and 24 h, then once a day),
- artemether (3.2 mg/kg body weight, IM administration on admission, then 1.6 mg/kg body weight per day),
- quinine (20 mg salt/kg body weight on admission via IV infusion or divided IM injection, then 10 mg/kg body weight every 8 h; infusion rate should not exceed 5 mg salt/kg body weight per hour).

As IM injections may lead to paralysis and IV perfusions are not available in the rural facilities, many of children die during transfer to appropriate health facilities because of the treatment delay (White and Krishna, 1989). Moreover, among children admitted to a hospital, 50–70% die during the first hours of their admission whatever the administered treatment (White and Krishna, 1989). These observations indicate that there is a need to establish an early and easy treatment in children, which can be administered in house by educated mothers or in a rural dispensary by a health technician. Some alternatives have been developed for children care, mostly based on rectal route:

- Quinimax[®] (a water-soluble combination of cinchona alkaloids) appeared to be efficacious and well tolerated by rectal route in the treatment of acute uncomplicated malaria of the child. However, bioavailability was hampered by loss of part of the liquid solution during or shortly after rectal introduction.

- A cream (Sanofi, France) containing 5.33 g quinine gluconate per 100 g cream has been specifically designed for the intrarectal route. It was presented as a graduated syringe-nozzle containing 3.75 g cream, corresponding to 200 mg quinine gluconate and 125 mg quinine base. This formulation was tested in French speaking parts of Africa (Barennes et al., 1996). In contrast to the injectable Quinimax[®] solution, the authors have noted the good tolerability of this cream since no expulsion of the product was observed. However, C_{\max} (3.2 ± 0.7 mg/l) was lower than with the intravenous route (5.1 ± 1.4 mg/l). Areas under the curve (AUC_{0-8h}) were smaller with the intrarectal (17.0 ± 7 mg l/h) than with intramuscular (19.4 ± 4.8 mg/l) and intravenous (27.8 ± 8.2 mg l/h) routes. The approximate bioavailability of intrarectal quinine from 0 to 8 h was 36% vs. intravenous quinine. The low bioavailability may be caused by the difficulty in controlling the administered dose. Therefore, none of the reported quinine rectal formulations had both a good tolerability and a high bioavailability.
- Two quinine rectal gels, namely a mucoadhesive hydroxypropyl methylcellulose 4000 (HPMC) gel and a thermosensitive Poloxamer 407 gel, containing 20 mg quinine base/g were developed and evaluated in vitro and in vivo in rabbits (Fawaz et al., 2004). Following administration to rabbits, the absolute bioavailability of quinine hydrochloride was 86.3% and 66.3% for the HPMC and poloxamer 407 gels, respectively. However, no further investigations have been conducted and the formulation has not been commercialized (Personal communication).

- Recently, artesunate suppositories have been developed by the UNDP/UNICEF/World Bank/WHO Special Program for Research and Training in Tropical Diseases in order to provide therapeutic cover for the initial 24 h of treatment (WHO, 2006). The individual suppositories contain 50, 100 or 400 mg artesunate. Such artesunate suppositories (Plasmotrim Rectocaps, Mepha, Switzerland) have been evaluated in 109 children and 35 adults from Malawi suffering from moderately severe malaria (Barnes et al., 2004). All artesunate-treated patients had pharmacodynamic or pharmacokinetic evidence of adequate drug absorption. After 12h, 92% of artesunate-treated children had a parasite density lower than 60% of baseline, compared with 14% receiving parenteral quinine. In adults, parasitaemia at 12 h was lower than 60% of baseline in 96% of the patients receiving artesunate, compared with 38% for those receiving quinine. Clinical response was equivalent with rectal artesunate and parenteral quinine. They concluded that a single rectal dose of artesunate is associated with rapid reduction in parasite density within the initial 24 h of treatment. This option is useful for initiation of treatment in patients unable to take oral medication, particularly where parenteral treatment is unavailable.

As there are not sufficient data proving that rectal artesunate is as good as intravenous or intramuscular option, the WHO recommended that artesunate suppositories should be used only as a single pre-referral dose (Table 2) before patient transfer to a facility where complete parenteral treatment should be administered.

Table 2: Doses for artesunate suppositories for pre-referral treatment of severe malaria

Weight (Kg)	Dose (mg)	Regimen (single dose)
5-8.9	50	One 50-mg suppository
9-19	100	One 100-mg suppository
20-29	200	Two 100-mg suppositories
30-39	300	Three 100-mg suppositories
>40	400	One 400-mg suppository

For oral treatment, the weight-adjusted doses of antimalarials in infants are similar to those used in adults. However, the lack of an infant formulation for the majority of antimalarials necessitates the division of adult formulations, which leads to inaccurate dosing. Furthermore, taste, volume and gastrointestinal tolerability are important determinants of treatment acceptability by children.

Dry suspensions with artemisinin derivatives (artesunate, artemether and dihydroartemisinin) for pediatric use have been developed by Gabriëls and Plaizier-Vercammen (2004). Xanthan gum and Avicel[®] CL 611 have been used as viscosity-enhancing agents. A 3 mg/ml dose was obtained after reconstitution with water. Dry suspensions with 0.20% w/v xanthan gum in combination with 2.0, 2.4 and 2.3% w/v Avicel[®] CL 611 for formulations containing artesunate, artemether and dihydroartemisinin, respectively, showed suitable viscosity over a one week sedimentation test. However at 25°C, a reconstituted artesunate suspension degraded up to 50% within 12 days, dihydroartemisinin showed degradation up to 65% after 21 days, while artemether remained stable over this period. Despite the molecular stability of artemether, significant crystal growth was observed in the reconstituted suspension at 25

and 45°C after 4 weeks. The authors concluded that the temperature and conservation period have to be clearly defined in the storage conditions of the reconstituted suspension.

Based on these findings, there is an urgent need to develop oral formulations for antimalarials in order to improve the accuracy and reliability of dosing in children.

2.2 Malaria in Rwanda

Rwanda is situated in Central Africa where malaria is endemic. Malaria transmission in Rwanda has increased over the last ten years for a number of reasons: greater population density and population movements, increased human and economic activities such as rice farming, brick making and mining, which increase breeding areas for mosquitoes. Malaria is now evident in high altitude areas and other areas where the disease was previously not a public health problem. Often, inhabitants of these areas have little or no immunity to the disease and are therefore prone to severe forms of malaria. Health facility data show that malaria is the overall leading cause of morbidity and mortality in Rwanda. More than 1.2 million episodes of uncomplicated malaria were treated in public health centers during 2004. In 2005, this number increased to over 1.5 million.

Malaria was responsible for 49.4% of all in-hospital patient deaths and 43.4% of all outpatients' attendance in 2004. With 40 % of deaths in children under five, malaria is the most killing disease for this age group. In 2000 malaria-related mortality was 200 per 10.000 people and for children under five it was 1.049 per 10.000. In 2001, throughout the country there were about 1 million new cases of malaria and of these 33% were

children under five. However, this number significantly underestimates the total number of annual episodes in the population since only 32% of the population utilized health services during the same period. Most of 8 million Rwanda's inhabitants suffer an estimated two or three episodes per year and in many areas the transmission period lasts more than a half year (Figure 3).

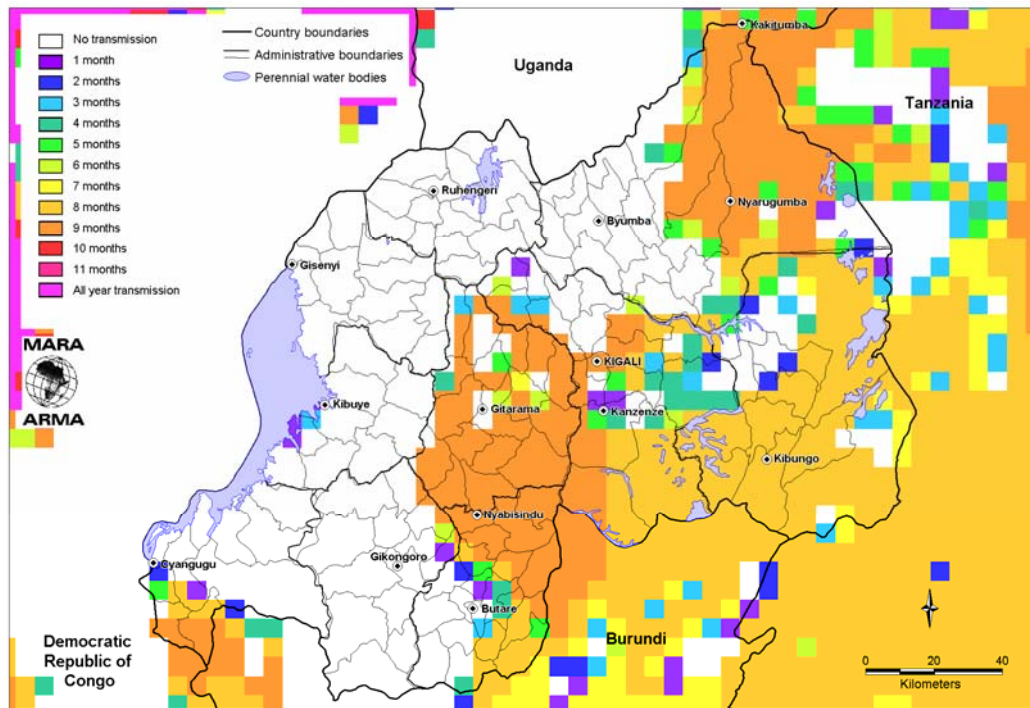


Figure 3: Map of the duration of malaria transmission seasons in Rwanda (Mapping Malaria Risk in Africa (MARA), 2001)

2.2.1 Rwandan National Policy for malaria treatment

Uncomplicated malaria

In 2001, following high levels of chloroquine (CQ) resistance in Rwanda, the combination amodiaquine + sulfadoxine/pyrimethamine (AQ + SP) was selected as the first-line antimalarial treatment. Although the clinical response to this combination was relatively good in 2001, its efficacy has steadily declined: in 2002 the rate of successful treatment was 83% (Rwagacondo et al., 2003) and in 2003 it was only 74% (Karema et al., 2006). In order to address these treatment failures and to preserve the efficacy of SP for preventive treatment during pregnancy, the Rwandan ministry of health decided in July 2005 to introduce artemisinin-based combination treatments (ACTs) as first-line treatment for uncomplicated malaria as recommended by the World Health Organization (WHO). ACTs were well tolerated and highly effective against *Plasmodium falciparum* malaria in Southeast Asia, a region where a high resistance to single-therapy antimalarials has been reported (Bloland, 2001). Different ACTs like amodiaquine + artesunate (AQ+AS) and dihydroartemisinin + piperaquine (DHA+PPQ) have been tested as possible alternatives to AQ+SP treatment (Karema et al., 2006). DHA+PPQ and AQ+AS were efficacious for the treatment of uncomplicated *P. falciparum* malaria, with a significantly higher prevalence of successful treatment in children compared to the AQ+SP combination (95.2, 92.0 and 84.7%, respectively). An artemisinin-based combination consisting of artemether (20 mg) and lumefantrine (120 mg) (Coartem[®], Novartis), given in six doses, provided the highest efficiency: 96.7% of good clinical and parasitological responses in children under five from the eastern region of Rwanda, an

area where the commonly-used antimalarials are less effective (Fanello et al., 2007). This last combination was selected for treatment of uncomplicated malaria.

Severe malaria

Unfortunately due to insufficient information, Coartem[®] is contra-indicated in children of less than 5 kg and during pregnancy. In these cases orally administered quinine is indicated as alternative. This treatment is also recommended as the second-line drug in case of treatment failure (Rwandan Ministry of Health, 2006).

In most rural area health facilities where intravenous infusion is not possible, quinine was given by *intramuscular* injection. However, pain and inflammation at the injection site were reported, and fever due to inflammation at the injection site can be mistaken for treatment failure. Limb paralysis associated with intramuscular quinine is rare, but has devastating consequences as reported previously. Therefore, in Rwanda the intramuscular way was replaced by *intrarectal administration* (off-label use of quinine dihydrochloride) in children to avoid the above mentioned adverse effects. For the intrarectal administration, a dose of 15 mg/kg quinine dihydrochloride is dissolved in 4 ml of water or normal saline solution and administered with a syringe. The care giver is required to hold the buttocks together for 5 minutes to avoid reflux expulsion. If the drug is expelled within 10 min, half of the dose is re-administered (Rwandan Ministry of Health, 2006). However, in addition to the reported low quinine bioavailability, diarrhoea accompanying malaria fever considerably limits the use of this alternative route of drug administration, reducing thereby the options for children to be correctly treated.

2.3 Conclusion

Malaria is a major worldwide concern. Malaria control efforts are greatly affected by problems of resistance to the commonly used drugs. The concept of combinations of antimalarials is now recommended by the WHO as first line treatment of *P. falciparum* malaria. However, in most of Sub-Saharan African countries a change to this combination policy can be compromised by the availability and affordability of antimalarial combination therapies. The attention must be focused on children as they represent the most vulnerable group. Although quinine is very important in the treatment of malaria and commercialized a long time ago, there is still a need for the development of its pediatric formulation for oral administration.

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3. Objectives

Malaria is a public health problem worldwide, especially in tropical Africa where it kills around a million of people a year, of which 75% are children under 5 years of age. Although quinine is re-emerging as an important drug in the treatment of multiple-drug resistant or severe *Plasmodium falciparum* malaria, no pediatric formulation is commercially available. In practice, tablets are broken to obtain the pediatric dose.

The objective of the first chapter of the present work was to evaluate the dose accuracy when children are treated by means of quinine sulphate tablet broken into fragments to adjust for body weight.

The second part explores the concept of multiparticulate dosage forms as a flexible system that allows dose adaptation to the child body weight, the aim being the development of quinine sulphate pellets via extrusion-spheronisation. An additional challenge for an oral quinine sulphate formulation is its extremely bitter taste. Since the spherical shape of the pellets promotes the efficiency of the coating process, quinine sulphate pellets will be coated using a polymer for taste-masking purpose. In vitro tests will be conducted in order to optimise the coating process and consequently taste-masking efficiency.

The third part will focus on the in vivo evaluation of quinine taste-masked pellets: pharmacokinetics study in healthy volunteers comparing taste-masked pellets and tablets commercially available in Rwanda, and efficacy of taste-masked quinine sulphate pellets in children with uncomplicated *Plasmodium falciparum* malaria.

The last part of this work focuses on the development of a taste-masked quinine suspension by precipitating quinine hydrochloride with disodium pamoate salt. The poor solubility of the resulting quinine pamoate salt is correlated with the bitterness masking. The suspension development and stability studies are reported and pharmacokinetics studies are performed in dogs.

Chapter I

Lack of quinine sulphate pediatric formulations: tablet breaking as alternative in the treatment of children

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Impact of breaking of quinine sulphate tablets on the accuracy of the dose given to children suffering from malaria

I. Introduction

Per oral administration is a common drug administration route in the treatment of children, next to the parenteral and rectal route. However, most drugs that are administered to children are not specifically designed for pediatric use. As pediatricians prescribe doses depending on the children's weight and drugs are not always available in the prescribed dose, tablet breaking is a frequent method to obtain the desired dose. Several studies on the assessment of the accuracy of tablet breaking reported that this resulted in significant weight variations of the tablet fragments which may compromise the clinical outcomes or increase the risk of adverse effects, depending on the dose-response curve and therapeutic window of the particular drug. Such weight variability may be caused by difficulties during tablet breaking due to the tablet size and shape, the presence of a score line, the breaking methodology and the patient's or caregiver's age.

As reported by Gupta and Gupta (1988) following manual breaking of tablets into halves, round tablets scored on one side tablets broke unevenly with large weight deviations, whereas elongated tablets with a deep score on both sides broke cleanly (weight deviation less than 10% in 97% of the evaluated tablets). Small tablets were most difficult to break accurately and 40% of the evaluated tablets deviated by more than 20% of the theoretical weight. Weight loss from fragmentation and powdering was appreciable for the round tablets (up to 2.6%), but negligible for the elongated tablets.

Michael et al. (1985) determined the weight deviation following manual breaking into halves of 100 scored tablets from 34 brands of commercially available antihypertensive drugs. They classified the brands into excellent, good, moderate and poor divisibility categories if $\geq 95\%$, $\geq 85\%$, $\geq 50\%$ and $\leq 50\%$ of halves deviated less than 5% of the expected weight, respectively. They found that weight deviations of 10% or more were frequent. Finally 18% of the brands (including commonly used brands such as Inderal[®] 80, Sotalex[®] and Tenormin) were categorised as unsuitable for breaking by hand. The authors suggested to the companies either to optimize the divisibility of these tablets or to commercialize, despite higher production costs, wide range of unscored tablets.

Myriam et al. (1994) evaluated a tablet-splitting device for accuracy by dividing tablets of various shapes and sizes (round, oblong, flat, oval, plain, coated, scored) into halves. Some tablets were friable and broke into more than two parts on several occasions. Most of the half tablets from coated tablets deviated more than 15% of the target weight. The best results were found with larger tablets, oblong tablets and tablets having a flat shape.

Mc Devitt et al. (1998) determined the accuracy of manually breaking of 25 mg hydrochlorothiazide tablets. They did not find a gender effect in tablet splitting, but an effect of age was detected as younger and older volunteers caused more loss by powdering than middle-age volunteers. More than 87% of the portions of oval 10-mm tablets with deep scores on both sides were within 10% of the ideal weight. Smaller round tablets were more likely to yield variable segment weight as 55% of round 8- or 9-mm and 44% of round 7-mm tablet deviated by more than 10 and 20%, respectively.

Lieven et al. (2002) investigated the influence of breaking methodology on mass uniformity of half- and quarter-tablets by performing several breakability test methods

using a model tablet (Figure 1). It has been demonstrated that - beside the possible interaction between the methodology and the person breaking the tablets - the major factor significantly influencing the mass uniformity of broken tablets is the breaking methodology. The best breakability (i.e. lowest loss and variability) was obtained when the breaking force applied by the thumbs was directed towards the score side of the tablet, i.e. by “opening” the score, independent of score line orientation (upwards (1) versus downwards (3)). The use of knife resulted in a higher variability and greater loss of particles.

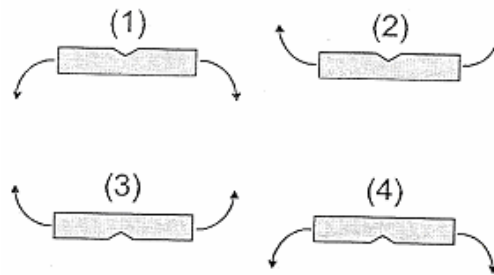


Figure 1: Visualisation of the breakability methods (Lieven et al., 2002)

We did not find a study that evaluated the impact of tablet breaking on the therapeutic outcome. So it is not yet clearly defined at which level the mass unconformity can compromise the therapeutic efficacy of the drug.

Since no specific quinine formulations are available for pediatric application, breaking of commercially available tablets intended for adults (200 or 300 mg quinine sulphate dose

per tablet) is required to adjust the dose in function of body weight. Therefore, specific dosage schemes (Table 1) are recommended by the WHO (WHO, 2000).

Table 1: Dosage scheme of quinine sulphate tablets for treatment of children based on body weight.

Quinine sulphate dose per tablet	Body weight				
	3- <6 kg	6- <10 kg	10- <15 kg	15- <20 kg	20- <29 kg
200 mg	1/4	1/2	3/4	1	1 1/2
300 mg	-	-	1/2	1/2	1

The aim of this study was to evaluate the dose accuracy when the WHO dosage scheme is applied to treat children using quinine sulphate tablets commercially available on the Rwandan market.

II. Materials and methods

Materials

The study was conducted with three different types of quinine sulphate (QS) tablets commercially available on the Rwandan market. They were selected based on the presence of a score line. Figure 2 shows the studied tablets and Table 2 summarises their characteristics.

For simplicity the different types will be referred to as S (score line on one side of the tablet), NS (without score line) and DS (score line on both sides of the tablet).

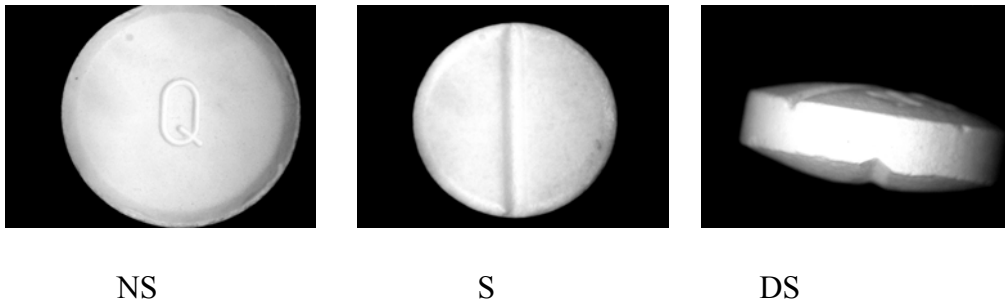


Figure 2: Images of different QS tablets used during the tablet breaking study

Table 2: Characteristics of QS tablets evaluated

Type	Manufacturer	Diameter (mm)	Thickness (mm)	Drug content (mg QS/tablet)	Shape
S	Changcheng Pharm Factory, China	10	3	200	Flat and round
NS	Pharmakina, Bukavu, Dem Rep Congo	11.6	3.5	300	Flat and round
DS	Laboratory&Allied, Nairobi, Kenya	12.8	3.6	300	Biconvex and round

Methods

Of each type, 30 tablets were taken at random, weighed and the mean weight per tablet calculated. They were distributed among 3 persons (10 tablets per person). Each person was asked to manually break the tablets into halves. Of each tablet, one half was weighed. The average weight of the half tablets was determined. The deviation of each individual fragment from the average weight was calculated. In the same way, the person was asked to break tablets into quarters, and of each tablet, one quarter was evaluated. For the entire, half and quarter tablets, the interval of 85 - 115% and 75 - 125% based on the respective mean weight was calculated.

III. Results and discussion

Table 3 represents the mean weight and weight range of the entire, half and quarter tablet. All tablet types (NS, S and DS) were conform to the European Pharmacopoeia mass uniformity requirements as the weight of all intact tablets was between 85% and 115% of the respective average weight. However, the quality of the tablets could be improved as the weight range is quite large compared to other industrially prepared tablets.

Table 3: Mean weight and weight ranges for the evaluated tablets

Tablet type	Mean (mg \pm SD)	Weight range	
		mg	%
<u>Entire tablets</u>			
NS	417.7 \pm 11.3	355.0 - 480.3	85.0 - 115.0
S	300.2 \pm 8.6	255.1 - 345.0	85.0 - 114.9
DS	655.5 \pm 18.3	557.1 - 753.2	85.0 - 114.9
<u>Half tablets</u>			
NS	205.2 \pm 31.8	153.3 - 297.2	74.7 - 144.8
S	149.8 \pm 12.8	124.3 - 174.1	83.0 - 116.2
DS	326.3 \pm 11.6	303.2 - 349.7	92.9 - 107.2
<u>Quarter tablets</u>			
NS	103.2 \pm 24.6	64.5 - 145.1	62.5 - 140.6
S	74.1 \pm 11.7	53.5 - 97.3	72.2 - 131.3
DS	160.9 \pm 32.6	114.8 - 214.8	71.3 - 133.5

An indication of the deviation of the halves and quarters from the mean weight is presented in Table 4.

Table 4: Percentage of fragments (halves and quarters) whose weight fall within the indicated interval.

Deviation interval from the mean weight	Percentage of fragments (\pm SD) that fall within the interval		
	NS	S	DS
	<u>Half tablets</u>		
< 15%	63.3 \pm 2.1	86.6 \pm 2.3	100
> 15 - < 25%	30.0 \pm 1.0	13.3 \pm 2.3	0
> 25%	6.7 \pm 0.6	0.1 \pm 2.1	0
	<u>Quarter tablets</u>		
< 15%	27.0 \pm 0.6	60.0 \pm 2.3	40 \pm 1.1
> 15 - < 25%	37.0 \pm 0.6	27.0 \pm 2.3	30 \pm 1.0
> 25%	36.0 \pm 1.2	13.0 \pm 2.1	30 \pm 0.6

Only halves from DS tablets complied with the Eur. Pharm. (Pharmeuropa, 2004): the weight of each half tablet deviated less than 15% from the mean weight. About 37 and 13 % of the halves from NS and S tablets, respectively, deviated more than 15% from the mean. For NS about 7% of the halves deviated even more than 25% from the mean

weight. The situation worsened for quarter tablets, none of the evaluated formulations complied with the Eur. Pharm requirements. Only 27, 60 and 40 % of NS, S and DS quarters, respectively, were within a 15% weight interval of the mean. Deviations of more than 35% were observed in 13 and 3% for NS and DS, respectively. Since in the present study the tablets were round and of a similar size (10 - 12 mm diameter), the observed mass variability was mainly related to the score line. In case of NS tablets, the large weight deviations can be explained by the absence of a score line, whereas the scored tablets performed better. However, only halves from the tablet scored at both sides (DS) complied with the European Pharmacopoeia, despite the poor alignment of the score lines (Figure 2). Similar to NS and S, breaking DS into quarters resulted in a poor weight distribution as the advantage of breaking along a score line was lost. According the WHO dosing regimen, the patient should receive a dose of 10 mg per kg body weight. However, even if a tablet would be broken evenly the dose administered to children of different body weight will vary if the caretaker strictly adheres to the dosing scheme proposed by the WHO (Table 1). The dose variation is illustrated in Table 5 for some selected body weights. In case of exact tablets breaking, a child of 3 kg will receive (every 8 h) 16.7 mg/kg, while a child of 5 kg treated with the same tablets (quarters of 200 mg QS tablet) will receive 10 mg/kg. A child weighing 15 kilos would even receive a different dose depending on which tablets are available at the time of treatment (200 or 300 QS mg/tablet).

Table 5: Dose (mg/kg) to be administered based on the WHO scheme for some selected bodyweights

Quinine sulphate per tablet	Administered dose (mg/kg)			
	3 kg	5 kg	10 kg	15 kg
200 mg	16.7	10	15	13.3
300 mg	-	-	15	10

However, due to the weight variability during tablet breaking the actual dose administered to the child could vary even more. To simulate this variation based on actual data, the fragments obtained in the present study were used to calculate the dose (expressed as mg QS per kg body weight) administered per dosing interval (n=20) to the children with differing body weight (Figure 3). The 20 fragments used to simulate the dose administered were chosen randomly from the batches that resulted in large deviations from the average weight (halves of S and quarters of NS).

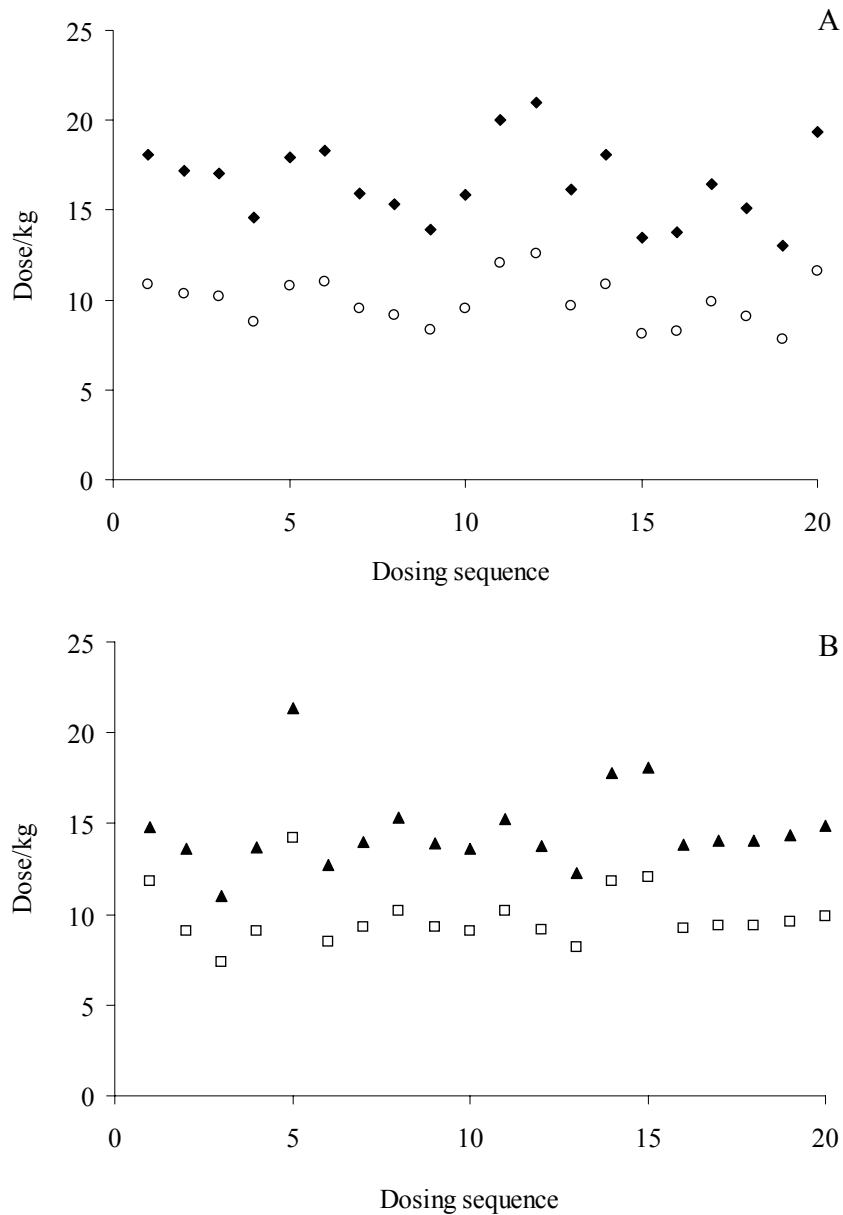


Figure 3: Dose variability during a dosing sequence (n=20), following the dose scheme proposed by WHO and using tablet fragments from the present study.

A: children of 3 (◆) 5 kg (○) are treated with quarter tablets from S (200 mg QS/tablet)

B: children of 10 (▲) and 15 kg (□) are treated with halves from NS (300 QS mg/tablet)

From these figures it is clear that a child of 3 kg will always receive a dose that exceeds the recommended dose (10 mg/kg). Even in 45% (9/20) of the cases, a dose exceeding 16.7 mg/kg will be administered, the minimal and maximal doses being 13 and 21 mg/kg, respectively. Despite the observed deviations, from practical point of view it is not possible to get closer to 10 mg/kg for children weighing lower than 5 kg, as one can not break the existing tablets beyond a quarter of tablet (i.e. lower than 50 mg per dose). Commercializing tablets with a dose less than 200 mg could solve this dosing problem, but companies are probably not interested to do this as the market potential for such a tablet would be very low. A child of 5 kg treated with the quarter tablets would receive in 35% of the cases a dose < 10 mg/kg, the minimum and maximum dose being 8 and 13 mg/kg, respectively. Children of 10 and 15 kg treated with halves of NS will receive maximum QS doses of 21 and 14 mg/kg, respectively. A child of 10 kg will receive in 100% of the cases a doses > 10 mg/kg with a minimum of 11 mg/kg.

Considering the variability in doses administered, the question rises if the same therapeutic effect will be obtained during the entire treatment period. Although the therapeutic outcome can not be assessed based on our results, and no literature reports about the therapeutic outcomes after tablet breaking are available, the recommended dose of 10 mg/kg is not respected due to the poor reproducibility during tablet breaking. In addition, as the dose range administered to the children is quite large the toxicity in those children continuously treated with high doses or the risk of suboptimal treatment in children receiving lower doses could be an issue. It has been reported that even at a dose of 10 mg/kg a significant proportion of children (Krishna et al., 2001) and adults

(Pukrittayakamee et al., 2003) is likely to be insufficiently treated in areas where parasites are not fully quinine sensitive.

Even if the current practice of tablet breaking would not result in toxicity and/or suboptimal treatment, according to Good Clinical Practice it is recommended to administer a constant amount of drug every dosing time. Hence, there is a pressing need for new quinine sulphate dosage forms allowing easy dose adaptation in function of body weight.

IV. Conclusion

Large weight variations have been observed when QS tablets were broken into halves and quarters. Those deviations were related to the presence and position of the score line. In developing countries where the drug accessibility is low and where it is common to find only one type of tablet in a health facility, the breaking of tablets without score line would exacerbate the weight variability of the fragments and complicate patient assessment. This could lead to confusion about whether failure of treatment is due to *Plasmodium falciparum* resistance or to the poor quality of the products. Further studies focusing on plasma quinine concentrations during per oral treatment using tablet fragments would provide precious information about the impact of such practice on therapeutic outcome. Next to that, there is a need for the development of dosage forms that are more flexible to the dose adaptation based to the child's body weight.

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Chapter II

**Taste-masked quinine sulphate pellets as alternative to tablets breaking in oral
treatment of malaria**

Published in *Eur. J. Pharm. Biopharm.* 66 (3): 460 – 465.

II.1 Quinine sulphate pellets produced by extrusion-spheronisation

II.1.1 Introduction

The most suitable dosage forms for per oral administration to children are often not available, hence tablets have to be split (or even crushed) to adjust the dose to the body weight of the patient. However, as illustrated in the previous chapter limited dosing accuracy due to the poor reproducibility of tablet breaking could compromise the efficiency of the treatment.

The concept of multiple unit dosage forms was introduced in the early 1950s. These forms can be defined as oral dosage forms consisting of a multiplicity of small units. These multiple units are produced by agglomerating fine powders or granules of bulk drugs and excipients into small, free-flowing, spherical or semi-spherical units, referred to as pellets. These pellets usually range in size from 0.5–1.5 mm, though other sizes could be prepared depending on the processing technologies, the application and the wish of the producer (Ghebre Sellasie, 1989).

Pellets can be produced in different ways: spraying a drug/binder-solution (or suspension) onto an inert core building the pellet layer after layer, spraying a melt of fats and waxes from the top into a cold tower (spray-congealing) forming pellets due to the hardening of the molten droplets, spray-drying a drug solution or suspension forming pellets due to the evaporation of the liquid phase, spraying a binder solution into the whirling powder using

a fluidized bed and via extrusion-spheronisation. The latter technique has been used in this study.

Extrusion-spheronisation

The technique involves four steps:

- Granulation – preparation of the wet mass;
- Extrusion – shaping the wet mass into cylinders;
- Spheronisation – breaking up the extrudate and rounding the particles into spheres;
- Drying – drying of the pellets.

The first step of the extrusion and spheronisation process is the preparation of the wet mass. Although different granulators are used, planetary mixers are commonly used (Vervaet et al., 1995). Extrusion is the second step of the process and the wet mass is forced through the screen with uniform perforations, shaping it into long rods. The third step involves the dumping of the cylinders onto the spinning plate (friction plate) of the spheroniser, upon which the extrudate is broken up into smaller cylinders with a length equal to their diameter. The process of spheronisation has been divided into various stages in terms of the changes in the shape of the extrudate. According to Rowe (1985), cylinders transform into cylinders with rounded edges then to dumb-bells and elliptical particles and eventually form perfect spheres. Baert and Remon, (1993) suggested another mechanism: twisting of the cylinder occurs after the formation of cylinders with rounded edges, finally resulting in the breaking of the cylinder into two distinct parts both

having a round and a flat side. Due to the rotational and frictional forces involved in the spheronisation process the edges of the flat side fold together like a flower forming the cavity observed in certain pellets (Figure 1).

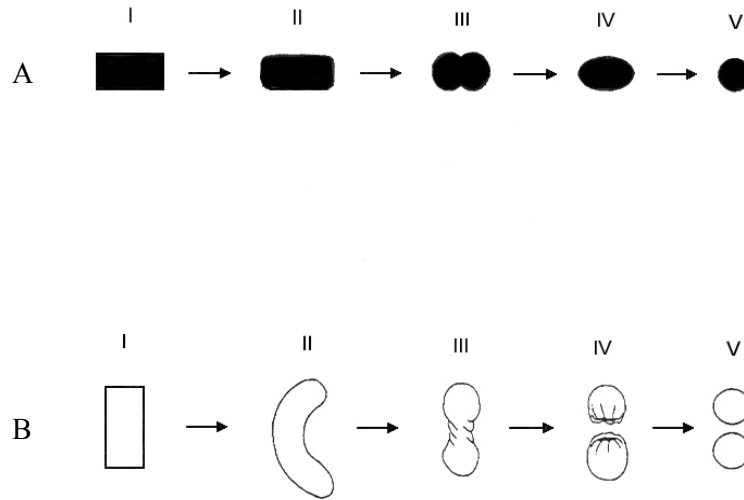


Figure 1: Pellet-forming mechanism according to Rowe (A): I, cylinder; II, cylinder with rounded edges; III, dumb-bell; IV, ellipse; V, sphere; Baert and Remon (B): I, cylinder; II, rope; III, dumb-bell; IV, sphere with a cavity outside; V, spheres.

Applications are found not only in the pharmaceutical industry, but also in the agribusiness (fertilizer and fish food) and polymer industry (Vervaet et al., 1995). The use of pellets as a vehicle for drug delivery has received significant attention because of the numerous advantages they offer (Rajesh et al., 1999).

- Pellets disperse freely in the gastrointestinal tract, and so they invariably maximize drug absorption, reduce peak plasma fluctuation, and minimize potential side effects without lowering drug bioavailability.

- Pellets also reduce variations in gastric emptying rate and overall transit time. Thus inter- and intra-subject variability of plasma profiles, which is common with single-unit regimens, is minimized.
- High local concentration of bioactive agents, which may inherently be irritative, can be avoided.
- When formulated as modified-release dosage forms, pellets are less susceptible to dose dumping than the reservoir-type, single-unit formulations.
- Better flow properties, narrow particle size distribution, less friable dosage form and uniform packing.
- The spherical shape makes pellets ideally suited for film coating for controlled release or taste-masking. They can also be made attractive because of the various shades of colour that can be easily imparted to them during the manufacturing process, thus enhancing the product elegance and organoleptic properties.
- In pediatrics they offer a flexible dosing system. Since each individual particle contains a known amount of drug, the drug dose can be easily adjusted to the patient's body weight by measuring a specific volume of these multiparticulates. Next to the dosing flexibility, these small multiparticulates offer the advantage that they can be sprinkled on food, mixed with fluids (water, milk or jelly) or directly swallowed, improving patient compliance (Breitkreutz et al., 1999).

The aim of this work was to develop a multiparticulate solid dosage form of quinine sulphate (QS) which allows flexible drug dosing. To this end, quinine sulphate pellets

ranging between 300 and 700 μm of diameter were developed via extrusion-spheronisation.

II.1.2 Production of quinine sulphate pellets

Materials and methods

Materials

Quinine sulphate (QS) was purchased from BUFA (Uitgeest, Holland)

The microcrystalline cellulose grades (Avicel[®] PH 101 and Avicel[®] CL 611) used as spheronisation aid were obtained from FMC (Cork, Ireland).

Methods

Granulation

QS was blended with a mixture of Avicel[®] PH 101 and Avicel[®] CL 611 (ratio PH101/CL 611: 1/3). The batch size was 300 g of dry material and the quinine sulphate load varied from 50 to 75% (w/w). The powders were dry mixed for 5 min at 60 rpm in a planetary mixer equipped with a “K-shaped” mixing arm (Kenwood Major Classic, Hampshire, UK). The mixture was wetted with demineralised water (40 - 43% of the total mass) and granulated for 5 min using the same equipment and mixing speed.

Extrusion

The wet mass was extruded at an extrusion speed of 60 rpm by means of a single-screw extruder (Model DG-L1, Fuji Paudal, Osaka, Japan) equipped with a domed screen having perforations of 400, 600 or 1000 μm diameter.

Spheronisation

The extrudates were spheronized (at 750 rpm during 8 min) in a spheronizer (Caleva Model 15, Sturminster Newton, UK) using a friction plate with cross-hatched geometry.

Drying

The pellets were dried overnight in a forced-air oven (Mettert, Belgium) at 40°C.

II.1.3 Quinine sulphate pellets evaluation

Size distribution

The particle size distribution of the pellets was determined by sieve analysis, using a sieve shaker (VE, Retsch, Haan, Germany) equipped with 800, 700, 500, 300 and 250 μm sieves for 5 min at an amplitude of 2 mm. The pellet yield was calculated based on the pellet fraction between 300-700 μm and presented as the percent of the total pellet weight. This size fraction was used for all further measurements.

Friability

The mechanical stability of the pellets was checked by tumbling a 10g sample of pellets (300-700 µm) together with 200 glass beads (mean diameter 4 mm) in a friabilator (PTE Pharma test, Hainburg, Germany) (Shah et al., 1995). After 10 min tumbling (25 rpm), the beads were removed and the pellets sieved through a 300 µm sieve diameter. The pellets retained on the sieve were weighed and the % friability calculated via the following equation (Eq. 1):

$$\frac{W_1 - W_2}{W_1} * 100 \quad (1)$$

Where W_1 and W_2 are the weight of pellets before and after friability test, respectively.

Sphericity and shape

The aspect ratio and shape of the pellets were determined using an image analysis system. Photomicrographs of pellets were taken with a digital camera (Camedia C-3030 Zoom, Olympus, Tokyo, Japan), linked with a stereomicroscope system (SZX9 DF PL 1.5, Olympus, Tokyo, Japan). A cold light source (Highlight 2100, Olympus, Germany) and a ring light guide (LGR66, Olympus, Germany) were used to obtain top light illumination of the pellets against a dark surface. The images were analysed by image analysis software (AnalySIS, Soft Imaging System, Münster, Germany). The magnification was set in a way that one pixel corresponded to 5.7 µm and around 300 pellets were analysed

for every batch. Each individual pellet was characterised by aspect ratio (AR) (ratio of longest Feret diameter and its longest perpendicular diameter) and two-dimensional shape factor (eR) (as described by Podczeck and Newton (1994) (Eq. 2) :

$$e_r = \frac{2\pi r}{P_m} - \sqrt{1 - \left(\frac{b}{l}\right)^2} \quad (2)$$

Where r is the pellet radius, P_m is perimeter, l is the length of the pellet (longest Feret diameter) and b its width (longest diameter perpendicular to the longest Feret diameter).

Drug content assay

A sample of pellets was ground in a mortar. An accurately weighed portion of powder, equivalent to 100 mg of QS, was dissolved in 100 ml methanol and stirred for 30 minutes. The mixture was filtered through a 0.2 µm cellulose acetate filter (Sartorius, Goettingen, Germany). The QS content was assessed using a HPLC equipment consisting of a solvent pump (L-7110, Hitachi, Tokyo, Japan) set at a constant flow rate of 0.8 ml/min, a variable wavelength detector (L-7480 fluorescence detector, Hitachi, Tokyo, Japan) set at 325 and 375 nm as excitation and emission wavelength, respectively, a C18 reversed phase column (Lichrospher 100 RP 18 (5µm), Merck, Darmstadt, Germany) and an automatic integration system (L-7000, Hitachi, Tokyo, Japan). The mobile phase was based on the composition described by Samanidou et al. (2005). It consisted of a filtered and degassed mixture of 0.1 M ammonium acetate, acetonitrile and methanol (40: 15: 45). The pH was adjusted to 3 (McCalley, 2002) using perchloric acid (~0.4 ml/100 ml).

QS content was calculated by means of a standard calibration curve of concentration (0.25 – 4 mg/l) versus peak areas.

Dissolution testing

Dissolution tests (USP XXVII) of QS pellets were carried out using the basket method (USP Method 1) and an automated dissolution tester (VanKel, Edison, NJ, USA) at a rotational speed of 100 rpm. 900 ml 0.1N hydrochloric acid maintained at $37 \pm 0.5^\circ\text{C}$ was used as dissolution medium. 5 ml samples were withdrawn from the dissolution medium over a period of 45 minutes, diluted (1:40) and the QS was spectrophotometrically measured using UV detection (Lambda 12, Perkin Elmer, Norwalk CT, USA) at 248 nm. The amount of drug dissolved was calculated by means of a calibration curve (absorbance vs. concentration) constructed using standard solutions with QS concentrations from 3 to 16 mg/l. The dissolution tests were performed in triplicate.

II.1.4 Results and discussion

Pellets were selected as dosage forms for flexible pediatric dosing as their multi-particulate nature allows precise adjustment of the dose depending on the body weight of the child.

The initial experiments using an extrusion screen with 1 mm cylindrical perforations showed that pellets could be produced at a QS concentration from 50 to 70%, the majority of the particles (> 80%) having a diameter above 800 μm and a friability less

than 1% (Figure 2 A). However, at quinine sulphate concentration of 75%, pellets were less spherical (Figure 2 B) and more friable (friability of 2.5 %).

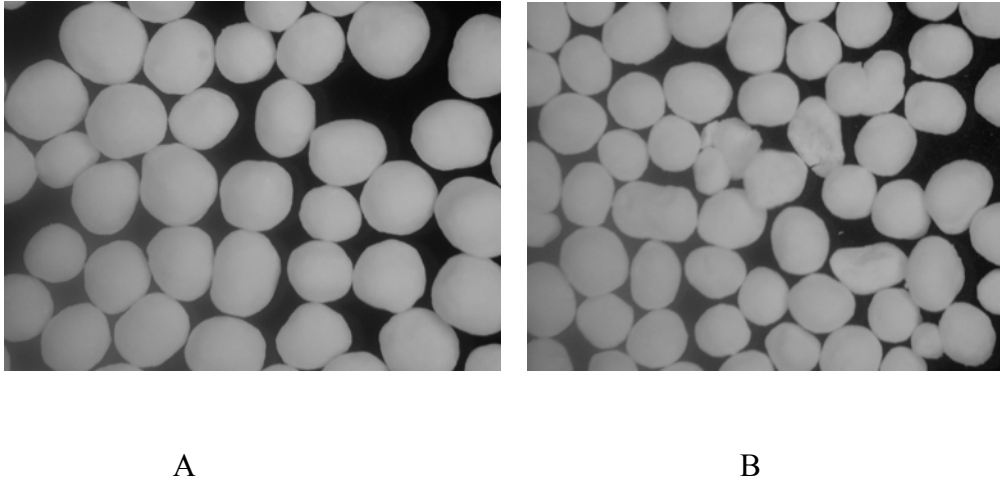


Figure 2: QS pellets produced via extrusion/spheronisation of a QS/Avicel[®] mixture. Ratio: 70/30 (A), 75/25 (B).

Preliminary tests showed that these larger pellets ($> 800 \mu\text{m}$) have a less acceptable mouth feel when mixed with fluids or semisolids. In contrast, when smaller pellets ($< 300 \mu\text{m}$ diameter) were used their mouth feel was acceptable, but a fraction of these smaller particles tended to remain in the mouth which would induce an unacceptable bitter taste some time after drug administration. Therefore, the size range of 300 to 700 μm was selected for the development of a multiparticulate quinine sulphate dosage form. Since the yield of this size fraction is very low when the pellets were manufactured using an extrusion screen with 1 mm perforations, screens with 400 and 600 μm perforations were tested to maximize the yield of the targeted interval.

Although pellets having a high drug fraction could be formulated using extrusion/spheronisation, a dosing simulation test showed that at high QS concentrations the total amount of pellets required per dosing would be too small to ensure accurate dosing, especially in children with lower body weight. As an example, the amount of pellets dosed at 70% QS that can be packed in a n° 4 capsule is about 175 mg (n = 30). Since this corresponds to 122.5 mg QS, a dose for a child of more than 12 kg, it would not be possible to provide accurate doses for children below this weight. Therefore, the drug load for all subsequent formulations was fixed at 20% (w/w), as in that case we observed that a capsule n° 4 contained a pellet amount equivalent to about 20 mg QS, a dose for a child of 2 kg body weight.

Figure 3 summarizes the size distribution of pellets containing 20% quinine sulphate and produced via extrusion/spheronisation using extrusion screens having different perforation diameters (400 and 600 μm). For both screens the 500-700 μm size fraction contained most of the pellets. However, the 600 μm screen yielded only 66.4% pellets within the required range (300-700 μm) as in excess of 30% of the particles was larger than 700 μm . The 400 μm screen was more suitable to produce 300-700 μm pellets as 95.9% of the pellets were within this interval.

The aspect ratio and two-dimensional shape factor of the pellets produced with the 400 μm screen were 1.15 ± 0.08 and 0.52 ± 0.1 , respectively.

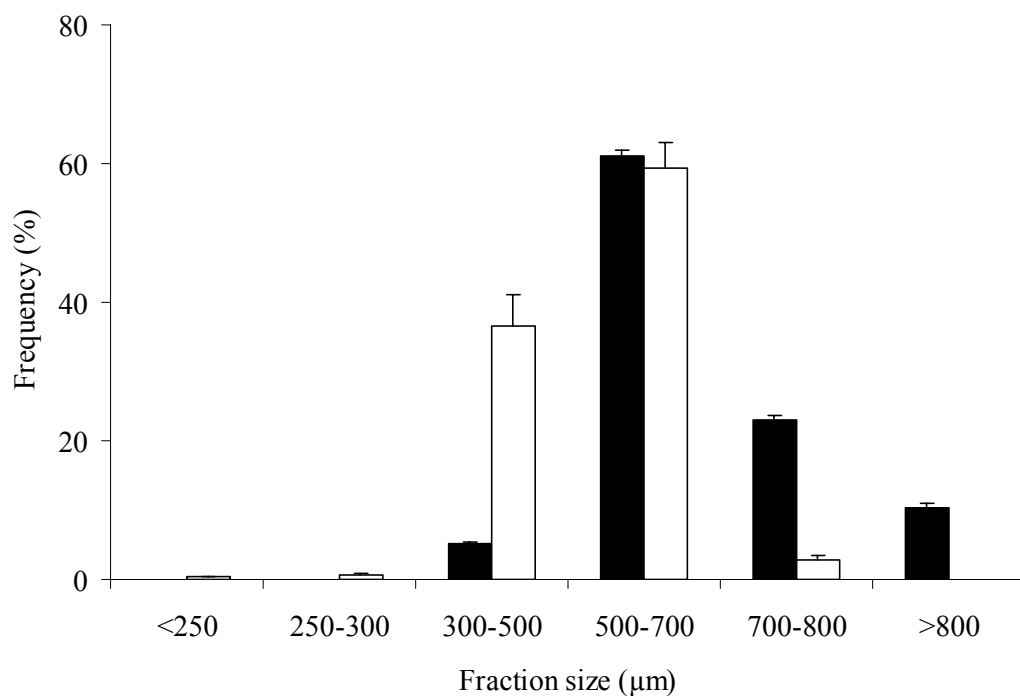


Figure 3: Size distribution of the pellets containing 20% QS and produced via extrusion/spheronisation using extrusion screens having 400 µm (□) and 600 µm (■) perforations diameter.

Using an extrusion screen having 400 µm perforations diameter, no impact of pellet size on the quinine sulphate release rate was observed in 0.1N hydrochloric acid: drug release from all pellet fractions (300 - 500µm, 500 - 700µm and 700 - 800µm) was complete within 10 min (Figure 4), meeting the USP XXVII requirements (more than 75 % should dissolve within 45 min).

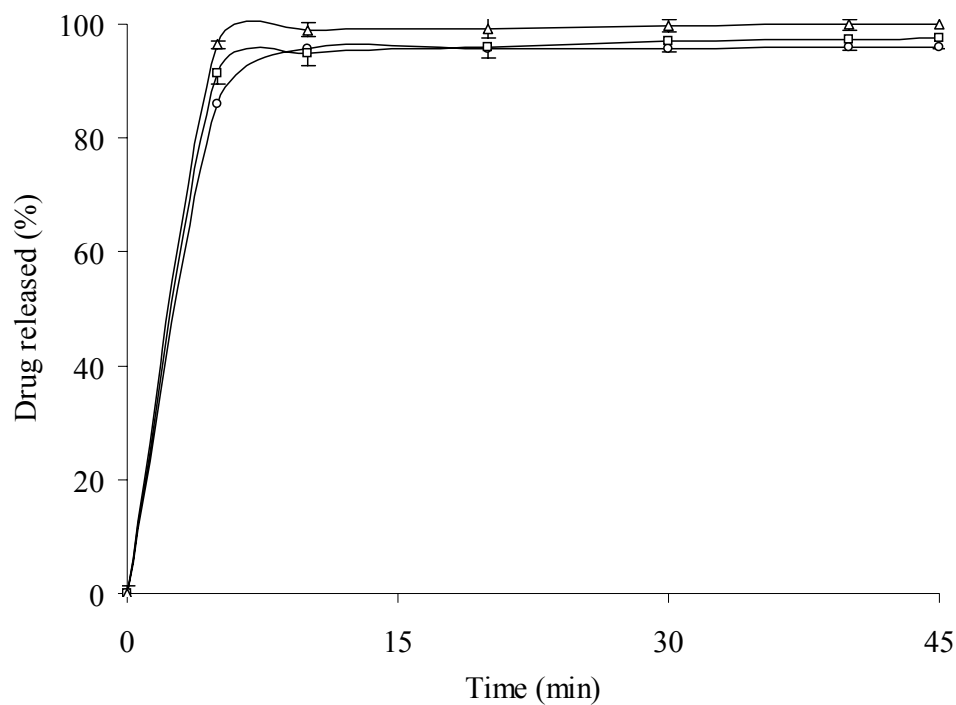


Figure 4: Release (%) of QS in 0.1N hydrochloric acid (n=3), from pellets produced using an extrusion screen having 400 μm perforations. Size fraction of the pellets: (Δ) 300 - 500 μm, (□) 500 - 700μm and (○) 700 - 800μm.

The mean quinine content was 96.8 ± 1.8 % (n=6), complying with the USP 27 interval of 90 - 110% required for QS content.

II.1.5 Conclusion

QS pellets ranging between 300 and 700 μm have been successfully produced via extrusion/spheronisation, using an extrusion screen having perforations of 400 μm diameter. Although pellets having a high drug fraction could be formulated using

extrusion/spheronisation, the drug dose was fixed at 20% (w/w) to obtain sufficient bulk volume of the formulation in order to allow accurate dosing.

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II.2 Quinine sulphate taste-masking by Eudragit[®] E PO polymer film coating

II.2.1 Introduction

Quinine sulphate (QS) pellets (300-700 μ m diameter) have been produced for pediatric application. However, the drug is extremely bitter: a 0.025% solution (w/v) was classified at the highest score on a bitter taste scale; only solutions below 0.001% were considered as having an acceptable bitter taste (Katsuragi et al., 1997; Suzuki et al., 2003). Therefore, efficient taste-masking is required to ensure patient compliance and effective pharmacotherapy, especially in pediatric applications.

The addition of flavours or sweeteners may not be efficient to sufficiently mask the taste of drugs and it may be necessary to use additional technological processes. Taste masking by coating QS pellets with a polymer was selected since the spherical shape of the pellets promotes the efficiency of the coating process and this option is the simplest and most feasible to achieve taste masking (Sohi et al., 2004).

Eudragit[®] E PO was selected as taste-masking polymer. This polymer is a butylmethacrylat-(2-dimethylaminoethyl) methacrylate-methylmethacrylate cationic copolymer (1:2:1), swellable and permeable above pH 5 and soluble below pH 5 (Pharma-polymers website). By that dissolution behaviour, Eudragit[®] E PO can sufficiently delay the release of quinine sulphate in saliva whose pH is between 6.5 and 7.4 (Pedersen et al., 2002) and allow fast release into the gastric fluids (pH 1.0-1.5) (Ishikawa et al., 1999). Eudragit[®] E PO, the micronized powder of Eudragit[®] E 100, allows aqueous coating processes. In combination with a plasticizer (stearic acid, dibutyl

or diethyl sebacate or acetyl tributyl citrate) and an emulsifier (sodium lauryl sulphate), the powder can be dispersed in water by simple stirring at room temperature to form a latex of approximately 25 nm. This coating dispersion can be combined with glidants and/or pigments, in order to get a coating suspension which can be applied using conventional coating technology in pan or fluidized bed systems.

The objective of the present study was to mask the bitter taste of QS by coating QS pellets in a fluidized bed using a Eudragit[®] E PO-based aqueous suspension.

II.2.2 Pellets coating process

Materials and methods

Materials

Eudragit[®] E PO (11.4% w/w suspended in water) (Rohm Degussa, Darmstadt, Germany) was used in an aqueous dispersion for QS pellets coating. Sodium lauryl sulphate (SLS, 10% w/w based on dry polymer weight) used as emulsifier was purchased from Federa (Brussels, Belgium). Two plasticizers (10-15% w/w based on dry polymer weight) were evaluated: stearic acid (StA) (Federa, Brussels, Belgium) and dibutyl sebacate (DBS) (Sigma-Aldrich, Bornem, Belgium). Magnesium stearate (35% w/w based on dry polymer weight) (Alpha pharma, Nazareth, Belgium) was added as antisticking agent.

Methods

Sodium lauryl sulphate and the plasticizer were dispersed in part of the water and homogenized by means of a magnetic stirrer. Next Eudragit[®] E PO was added progressively. The mixture was homogenized for 30 min by means of a magnetic stirrer. Magnesium stearate was homogeneously suspended in the remaining part of water using a high-shear mixer (Silverson, Bucks, UK) for 10 min. Afterwards, the magnesium stearate suspension was added to the polymer dispersion and homogenized for an additional 30 min using a high-shear mixer. The coating suspension was passed through a 250 μm sieve before use. Gentle stirring was continued during the entire coating process using the magnetic stirrer. 300g pellets (300-700 μm) were preheated for 30 min to 30°C and coated in a fluid bed used in the bottom-spray mode with the Wurster setup (GPCG1, Glatt, Binzen, Germany) (Figure 1). The coating conditions are presented in Table 1. After coating, the pellets were cured for 30 min at the same conditions as during the coating process.



Figure 1: Fluid bed (Glatt, Binzen, Germany)

Table 1: Parameters used during the coating process of QS pellets in GPCG1-fluid bed (Glatt).

Coating process parameters	Set values
Product load (g)	300
Nozzle diameter (mm)	0.8
Spray rate (g/min)	3.5 - 4.6
Atomizing air pressure (bar)	1.5
Inlet air temperature (°C)	30 - 35
Bed temperature (°C)	27 - 30

II.2.3 Evaluation of the coating effectiveness and taste-masking efficiency

Dissolution testing

Dissolution tests (USP XXVII) of uncoated and coated pellets (equivalent to 100 mg QS) were carried out using the basket method (USP Method 1) and an automated dissolution tester (VanKel, Edison, NJ, USA) at a rotational speed of 100 rpm. 900 ml 0.1N hydrochloric acid and demineralised water ($37 \pm 0.5^\circ\text{C}$) were used as dissolution media to evaluate the influence of polymer coating on quinine sulphate release and to determine the taste-masking efficiency, respectively. 5 ml samples were withdrawn from the dissolution medium over a period of 2 hours. The concentration of QS was spectrophotometrically measured using UV detection (Lambda 12, Perkin Elmer,

Norwalk CT, USA) at 248 and 235 nm for acidic and water samples, respectively. The dissolution tests were performed in triplicate.

Evaluation of the bitter taste using an electronic tongue

As an alternative to human sensory evaluation, the taste-masking properties of the formulations were evaluated via a sensor-based system, the Astree Electronic Tongue (Alpha M.O.S., Toulouse, France). Therefore, an amount of pellets (uncoated and coated with 10, 20 and 30% (w/w) Eudragit[®] E PO) corresponding to 100 mg QS was added to a beaker containing 100 ml water. After a specific time interval (1 to 5 min) the liquid was filtered (0.45 µm) to remove the pellets and any undissolved material, and the solutions were analyzed by the Astree Electronic Tongue equipped with the Bitterness Prediction Module (BPM). The BPM uses a 7-sensor array (specifically developed to evaluate bitterness) and a statistical model between instrumental and human sensory scores (elaborated using a set of bitter reference compounds). Based on the model (Partial Least Square analysis) the bitterness score of the samples (used as a marker for quinine sulphate release and coating integrity) is determined on a scale ranging from 1 to 20 (corresponding to a bitterness qualified as "non detectable" and "unacceptable", respectively)(Figure 2). A detailed review of the Electronic Tongue system has been published by Vlasov et al. (2002).

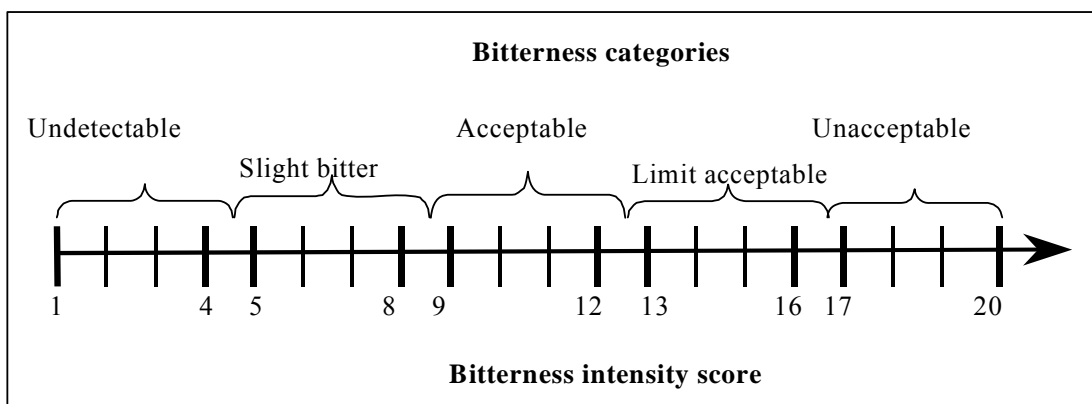


Figure 2: Bitterness intensity score and corresponding bitterness categories

Scanning electron microscopy

The morphology of the coating surface and the coating thickness were examined by scanning electron microscopy (SEM) (Joel JSM 5600 LV, Jeol, Tokyo, Japan). Pellets were cut into two halves which were platina coated using a sputter coater (Auto Fine Coater, JFC-1300, Jeol, Tokyo, Japan). The coating thickness is expressed as the mean of five pellets, with measurements at three sites per pellet.

II.2.4 Results and discussion

Coating of the 300-700 μ m pellet fraction using a Eudragit[®] E PO-based dispersion was possible. However, using 15% DBS (based on polymer weight) as plasticizer in the formulation caused pellet agglomeration after one week storage at 40°C and 75% relative humidity. A high concentration of plasticizer decreased the minimum film formation temperature of the polymer, correlated with an increase in tackiness of the film

(Wesseling et al., 1999). In addition to the low T_g when Eudragit[®] E PO was plasticized with DBS (~10°C) (Petereit, 2001), this agglomeration process was accelerated by the plasticizing effect of water when it was absorbed by the polymer film during storage at high relative humidity (Petereit, 1999). Similar agglomeration phenomena have been observed following storage at high temperature and relative humidity of pellets coated with acrylic and cellulosic polymer films (Thoma et al., 1999). Reducing the plasticizer concentration to 10% (w/w based on polymer weight) did not prevent pellet agglomeration during storage and in addition this plasticizer concentration resulted in a higher drug release rate during the initial stages of the dissolution test (Figure 3). This fast drug release was due to imperfections in the coating film (Figure 4) which allowed the dissolution medium to penetrate into the pellet and dissolve the drug. These imperfections were a consequence of the suboptimal plasticizer concentration, resulting in poor coalescence of the suspension drops on the surface of the pellets. The faster dissolution observed for this formulation is correlated with the loss of the taste-masking properties of the formulation.

Substituting DBS as a plasticizer by StA (15% based on polymer weight) yielded pellets which were less sensitive to sticking. This might be due to the higher glass transition temperature (T_g) when Eudragit[®] EPO is plasticized by StA (26°C) (Petereit, 2001). Furthermore, similar dissolution profiles (Figure 3) were obtained, indicating that the substitution of DBS by StA did not affect the taste-masking potential.

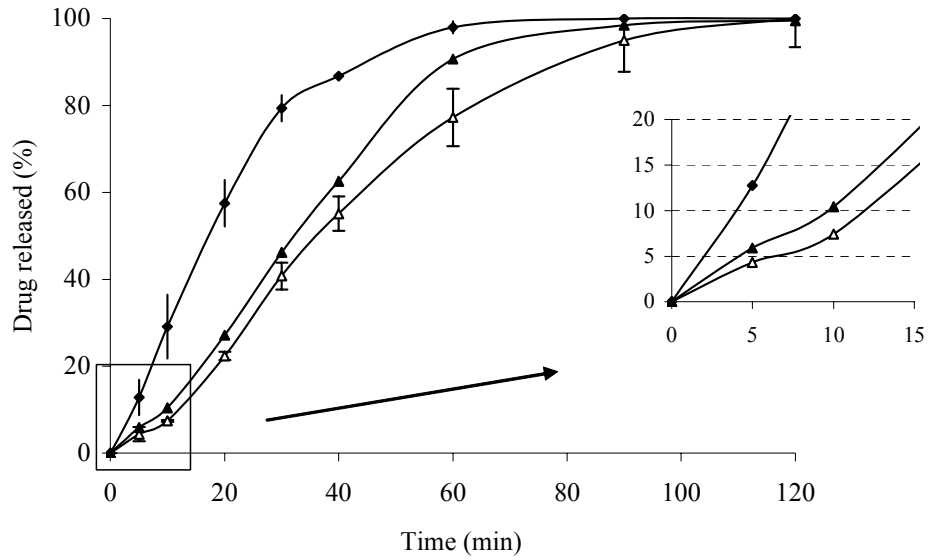


Figure 3: Release (%) of QS in water (n=3), from pellets coated with 20% (w/w) Eudragit® E PO using 15% (Δ) DBS, 10% (\blacklozenge) DBS and 15% StA (\blacktriangle) as plasticizer.

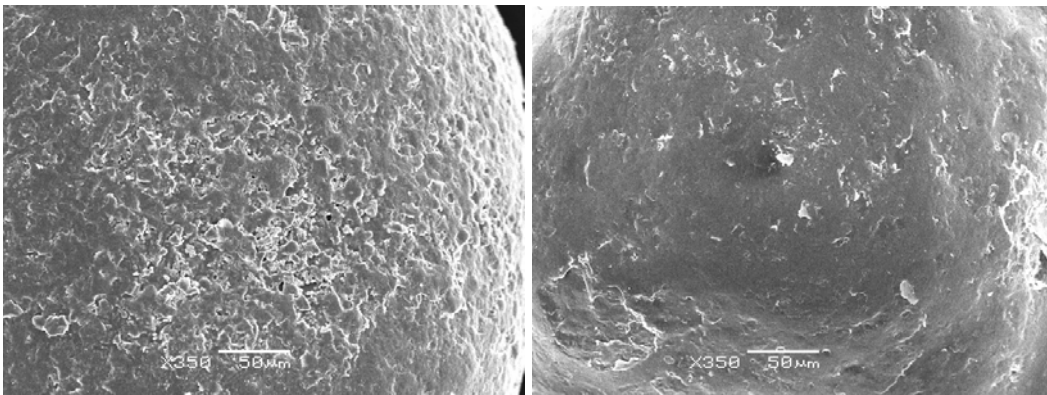


Figure 4: SEM picture of the surface of pellets coated with 20% (w/w) Eudragit® E PO using 10% (*left*) and 15% (*right*) DBS as plasticizer.

As a delay in the onset of drug release was essential to obtain a taste-masking effect, the dissolution profiles of the coated pellets in water (Figure 5) confirmed the ability of a Eudragit® E PO-based coating to delay quinine sulphate release from pellets.

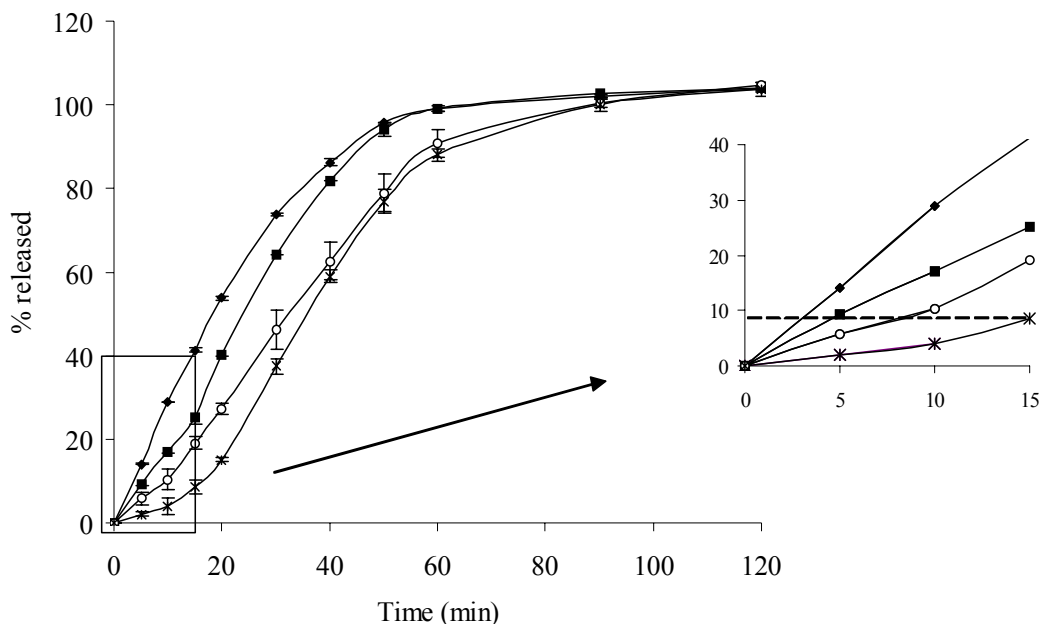


Figure 5: Release of QS in water (n=3), from uncoated pellets (◆) and coated pellets with 10 (■), 20 (○) and 30% (x) (w/w) Eudragit® E PO.

Dashed line (~9.4 mg/l quinine sulphate) is indicative of the maximum concentrations without bitter taste perception according Katsuragi et al. (1997) and Suzuki et al. (2003).

These release data in water (pH ~ 7) suggested that Eudragit® E PO can sufficiently delay the release of QS in saliva whose pH is between 6.5 and 7.4. Whereas about 14% of quinine sulphate was released from uncoated pellets within the first 5 min, the initial release was reduced depending on the amount of coating: 9.2, 5.9 and 2.1% drug released from pellets coated with 10, 20 and 30% (w/w) Eudragit® E PO, respectively. Based on papers of Katsuragi et al. (1997) and Suzuki et al. (2003), a drug release below 9%

resulted in a solution with an acceptable bitter taste (i.e. QS solutions having a concentration below 10mg/l).

However, based on the dissolution profiles it was impossible to assess which pellet formulations will have an acceptable taste perception for the patient during in-vivo application (due to the different experimental conditions, e.g. volume and mixing hydrodynamics). Therefore, pellets were evaluated under conditions which are a better representation of the conditions during administration of these formulations: immersion in 100ml water during 1 to 5 min (pellets are mixed with food or fluids before administration). The bitterness score of the resulting QS solutions was evaluated in function of time using the bitterness prediction module of the electronic tongue (providing a direct correlation with the in-vivo bitterness perception via a model established with a taste panel) (Figure 6).

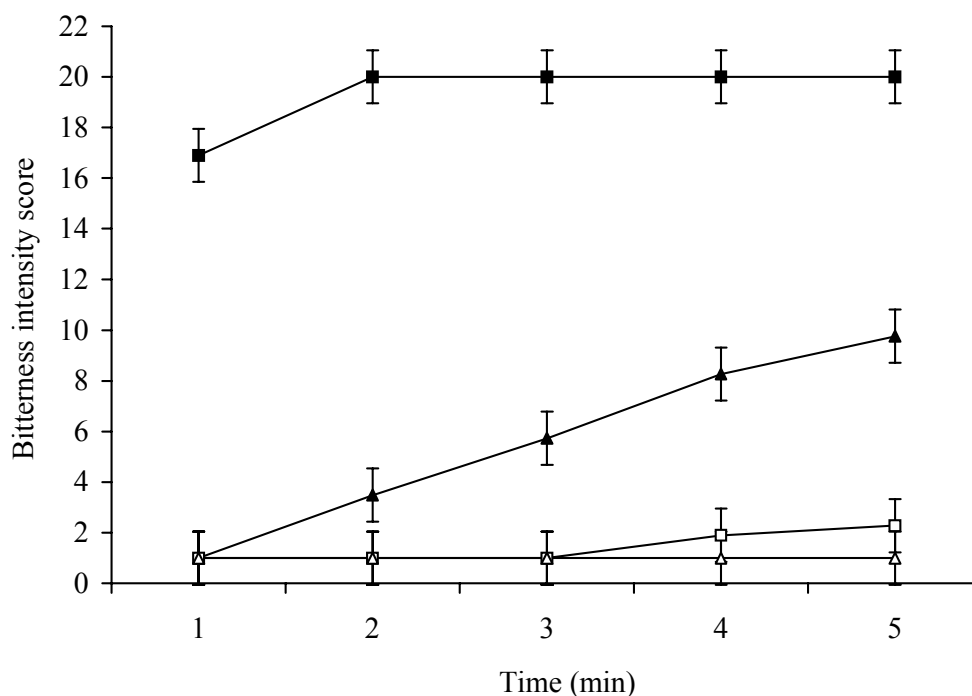


Figure 6: Bitterness score (\pm stand. dev., $n=6$) of QS pellets in function of time. Bitterness of QS pellets (uncoated (■) and coated with 10 (▲), 20 (□) and 30% (Δ) (w/w) Eudragit[®] E PO) was measured using the Astree electronic tongue and its Bitterness Prediction Module.

Without coating the bitterness reached an unacceptable level (intensity score ≥ 16.5) within the first minute. Using a 10% (w/w) Eudragit[®] E PO coat, QS release was delayed, yielding a bitterness score of 3.5 (undetectable) and 9.8 (acceptable) after 3 and 5 min, respectively. When applying $\geq 20\%$ (w/w) Eudragit[®] E PO to the pellets no bitterness was detected (score < 4.5) even after 5 min. The standard deviation of these measurements (taking into account all 7 sensors) was lower than 3%, indicating a very good repeatability of the results. As a delay in drug release of even a few minutes has been reported to prevent the sensation of an unpleasant taste (Klanche, 2003), the drug

release of QS pellets coated with 20% (w/w) Eudragit[®] E PO is considered sufficiently delayed for the patient to swallow the pellets without experiencing any discomfort due to quinine bitterness.

SEM pictures confirmed the potential of the 20% (w/w) Eudragit[®] E PO-formulation for taste-masking purposes since the film (coating thickness: $26.1 \pm 1.5 \mu\text{m}$) appeared smooth and continuous (Figure 7 A), constituting an efficient barrier between the pellet core and dissolution medium. In contrast, at a coating level of 10% (w/w) Eudragit[®] E PO (Figure 7 B) the film appeared thin and discontinuous, suggesting that taste-masking might not be sufficient.

The Eudragit[®] E PO coating had no impact on the QS release profile in acid medium as the dissolution profiles of uncoated and coated pellets were similar, more than 80% quinine sulphate was released within the first 10 min independent of the coating thickness (Figure 8).

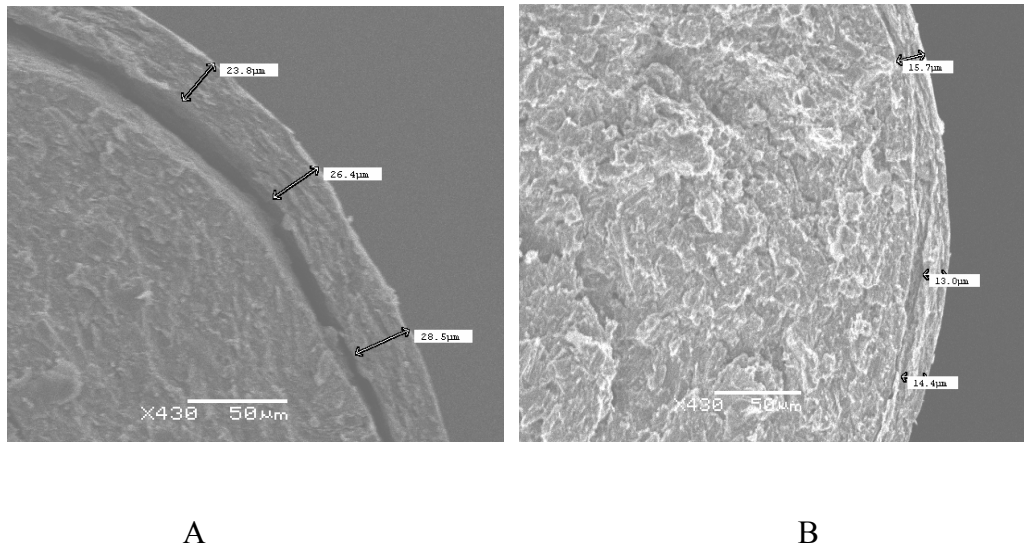


Figure 7: SEM picture of a cross-section of a pellet coated with (A) 20 and (B) 10% (w/w) Eudragit[®] E PO.

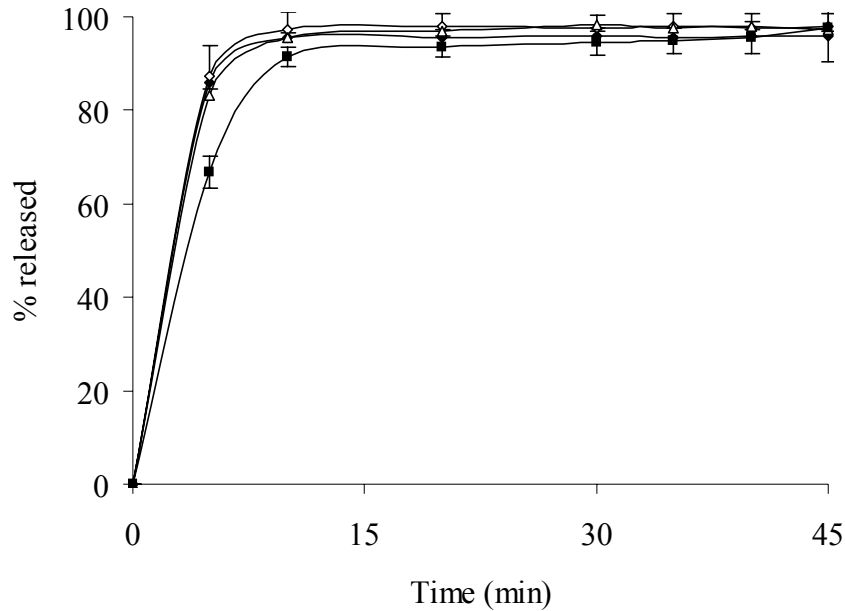


Figure 8: Release of QS in 0.1N hydrochloric acid (n=3), from uncoated pellets (◇) and coated pellets with 10 (◆), 20 (Δ) and 30% (■) (w/w) Eudragit[®] E PO.

II.2.5 Conclusion

QS pellets were successfully coated with Eudragit[®] E PO polymer for taste-masking purposes. Based on dissolution tests and in-vitro evaluation of bitterness evolution via the Astree electronic tongue, the taste masking efficiency of pellets was achieved with a 20% (w/w) Eudragit[®] E PO coat. The drug release was sufficiently lowered to delay the release of a bitter taste during administration. The electronic tongue provided valuable information about the evolution of bitterness intensity in function of time, which was essential for selecting the optimal formulation among pellets having different coating thickness.

Based on these data QS taste-masked pellets are proposed in pediatrics as alternative to tablet breaking and can be used as flexible dosage form for dose adaptation to a child's body weight.

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Chapter III

Human bioavailability of quinine sulphate from taste-masked pellets.

Ann. Trop. Paed. (Submitted)

I. Introduction

Since no quinine sulphate formulation suitable for pediatric application is available, taste-masked quinine sulphate pellets have been developed (Chapter II) as they offer flexibility for dose adaptation to the body weight; additionally they increase compliance in children since the bitter taste is masked. The aim of the present study was to evaluate the oral bioavailability of quinine sulphate formulated as pellets, using a commercially available quinine sulphate tablet as reference. According to the International Committee on Harmonization (ICH), comparison of the relative bioavailability of pediatric formulations with a formulation intended for adults should be done in adults, prior definitive pharmacokinetic studies in pediatric population (ICH Topic E 11, 2001). Therefore, in a randomized cross-over study a single dose of 600 mg quinine sulphate was administered to adults either as taste-masked pellets or as commercially available tablets.

II. Materials and methods

Materials

Quinine sulphate 300 mg tablets (Batch SB 02-2008, Pharmakina, Bukavu, Democratic Republic of Congo) were kindly obtained from the Kigali University Hospital (Centre Hospitalier Universitaire de Kigali). Quinine sulphate taste-masked pellets were prepared as previously described in Chapter II, packed in aluminum bags (Vapor Flex[®], USA),

sealed and labeled with the name and address of the investigator, full composition, batch number, dosing instructions.

Propranolol hydrochloride was supplied by Pharminnova (Waregem, Belgium). Diethylether, ammonium acetate and sodium hydroxide were obtained from VWR International (Leuven, Belgium). Acetonitrile and methanol were purchased from Biosolve (Valkenswaard, The Netherlands). All reagents were of analytical or HPLC grade.

Methods

1. Clinical protocol

The study was performed at the Kigali University Hospital, Rwanda. The study protocol was approved by the Medical Ethics Committee of the Rwandan Ministry of Health. The study was conducted in accordance with the Declaration of Helsinki ethical principles (WMA, Edinburgh, October 2000). Twelve volunteers gave written informed consent after receiving a detailed explanation of the investigational nature of the study. They were non-smokers, and were judged healthy on the basis of medical history, physical examination, electrocardiogram and investigation of biochemical, immunological, parasitological and haematological parameters in blood and urine. Pregnant, lactating females were excluded from the study; a pregnancy test was done for all females included in the study.

Prior and concomitant therapy

The subjects abstained from intake of medication from two weeks prior and during the study. The exceptions to this rule were paracetamol and oral contraceptives, and the dose and dosage regimen had to be recorded on the Concomitant Therapy Form which is part of the Case Report Form (CRF). They were asked to abstain from beverages containing alcohol or quinine between 24 hours before and 48 hours after drug dosing per experimental period. Drinking of water was allowed up to 2 hours before drug administration. The subjects fasted for at least 8 hours before entering the test facility.

Procedure

Before drug administration, an intravenous canula was placed in an antecubital vein and kept patent by use of a saline solution. The drug was administered with a glass of water (about 200 ml). The subjects remained in the test facility for 12 h after receiving the dose. From two hours after dosing, intake of water was allowed. A standard lunch and dinner were served at 4 and 10 h post-dosing, respectively.

Blood sampling

Venous blood samples (5 ml) were taken before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 h after drug intake. Exact times of blood sampling were noted in the Case Report Form. Blood samples were collected in heparinized tubes and centrifuged for 10 min at 1500g within 2 h after collection. Separated plasma was aspirated with a disposable pipette and transferred to plastic tubes. The tubes were sealed by means of polyethylene stoppers and labeled with the investigator's name, trial number, CRF ID, subject initials, date and time of sampling and stored at -20°C until assay of quinine.

Randomisation

The study was conducted in a randomized crossover design. Subjects were randomly assigned to receive a single dose of 600 mg quinine sulphate as taste-masked pellets or as commercially available tablets (2 tablets from Pharmakina containing 300 mg quinine sulphate per tablet). A washout period of 1 week separated both drug intakes. Subjects entering the study were allocated a number from 1 to 12 and a randomization scheme shown in Table 1 was used to assign them to either of the two treatments:

- F-1: 600 mg quinine sulphate as taste-masked pellets
- F-2: 2 x 300 mg quinine sulphate tablets

Table 1: Randomization scheme

Volunteer No.	Session 1	Session 2
1	F-1	F-2
2	F-1	F-2
3	F-2	F-1
4	F-1	F-2
5	F-2	F-1
6	F-1	F-2
7	F-1	F-2
8	F-1	F-2
9	F-2	F-1
10	F-2	F-1
11	F-1	F-2
12	F-2	F-1

2. Quinine sulphate assay

Quinine sulphate plasma concentrations were measured by a reversed-phase HPLC method with fluorescence detection set at excitation and emission wavelengths of 325 and 375 nm, respectively, using propranolol hydrochloride as internal standard.

Extraction procedure

Quinine was extracted using a liquid-liquid extraction method based on the procedure described by Salako et al. (1992). 500 µl of the internal standard solution (5 µg/ml propranolol hydrochloride) and 500 µl of 2 N sodium hydroxide were added to 500 µl of plasma sample (or blank plasma) and vortexed for 1 min. Extraction was achieved by adding 3 ml of diethylether, mixing and centrifuging for 5 min at 2500g. The upper ether layer was transferred to a disposable tube and evaporated to dryness under a nitrogen stream. The residue was reconstituted in 500 µl mobile phase and 100µl was injected into the HPLC system.

Chromatographic conditions

The HPLC equipment consisted of a solvent pump (L-7110, Hitachi, Tokyo, Japan) set at a constant flow rate of 0.8 ml/min, a variable wavelength detector (L-7480 fluorescence detector, Hitachi, Tokyo, Japan) set at 325 and 375 nm as excitation and emission wavelength, respectively, a C₁₈ reversed phase precolumn and column (Lichrospher 100 RP 18 (5µm), Merck, Darmstadt, Germany) and an automatic integration system (L-7000, Hitachi, Tokyo, Japan). The mobile phase was based on the composition described

by Samanidou et al. (2005). It consisted of a filtered and degassed mixture of 0.1 M ammonium acetate, acetonitrile and methanol (40/15/45; v/v/v). The pH was adjusted to 3.0 (McCalley, 2002) using perchloric acid (~ 0.4 ml / 100ml).

3. Validation of HPLC method

The HPLC analysis method was validated based on the guidelines of the Food and Drug Administration (FDA) for bioanalytical method validation (FDA, May 2001). The method specificity, accuracy, precision, linearity, recovery, detection and quantification limit were checked.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of interfering components. It was assessed by comparing the chromatogram of blank plasma with the one obtained from blank plasma spiked with quinine sulphate and internal standard. Figure 1 shows the chromatograms after extraction of (a) blank plasma and (b) plasma spiked with quinine sulphate and propranolol hydrochloride.

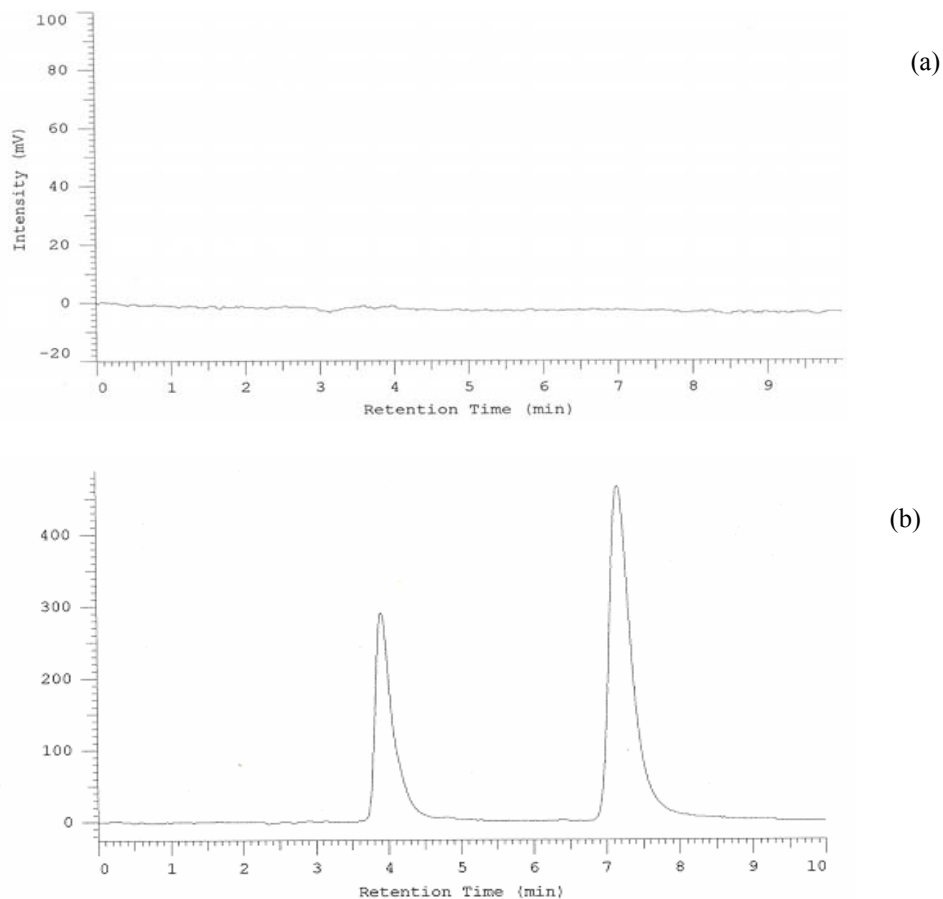


Figure 1: Specificity of the HPLC method. Chromatogram after extraction of (a) blank plasma and (b) plasma spiked with quinine sulphate (retention time at 3.92 min) and propranolol hydrochloride (retention time at 7.18 min).

As no interfering peaks were observed, it was concluded that the method is selective to determine quinine sulphate in human plasma.

Accuracy

Accuracy of an analytical procedure expresses the closeness of agreement between the true value and the determined value and is expressed as the percent agreement between the mean determined value and the true concentration. The accuracy was investigated at

four concentrations on plasma spiked with known amounts of the analyte, then treating the resulting solution as indicated by the method. The results of the measured quinine sulphate concentrations from spiked plasma samples and their closeness to the nominal concentrations are listed in the Table 2.

Table 2: Accuracy (n=5) for determination of quinine in human plasma

Quinine concentrations ($\mu\text{g/ml}$)	Average accuracy (%)	SDV
0.1	97.4	8.6
0.3	93.7	7.5
0.5	97.1	4.3
1.0	87.3	5.2

At all concentrations tested the mean values are within 15% of the nominal concentration which is the acceptance criterion.

Precision

The precision refers to the closeness of agreement between repeated determinations. It may be considered at three levels: repeatability (intra-assay precision), intermediate precision (within-laboratory precision) and reproducibility (between-laboratories precision). As all the experiments have been conducted by the same person using the same equipment in the same laboratory, only repeatability was evaluated. The within-day and between-days precision was calculated as the relative standard deviation (RSD) of

the mean peak area obtained after repeated injection (three times a day and within three days). The results are presented in Table 3.

Table 3: Precision for determination of quinine in plasma (n=3)

Concentrations ($\mu\text{g/ml}$)	Precision (RSD%)	
	Intra-day	Between-days
0.1	3.9	12.2
0.3	7.0	11.9
0.5	5.0	6.7
1.0	2.6	3.6

A relative standard deviation (RSD) between 2.6 and 7.0% and between 3.6 and 12.2% for intra-day and between-days, respectively, was obtained. All values are within the required acceptance criterion (RSD less than 15%).

Linearity

The linearity of an analytical procedure is the ability - with a given range - to obtain test results which are directly proportional to the concentration of analyte in the sample. During validation of the HPLC method and analysis of the samples, several calibration curves were analyzed. The linearity of the calibration curves was evaluated by the determination coefficient R^2 . The mean calibration curve of quinine sulphate concentration versus the ratio of peak areas of quinine sulphate and propranolol hydrochloride was calculated: $Y = 1.269 (\pm 0.058) x - 0.015 (\pm 0.004)$ with a coefficient

of determination (R^2) of 0.999 (± 0.007) ($n=5$) indicating that the relationship between response and concentration was linear within the tested quinine sulphate concentration range (0.1 - 3 $\mu\text{g/ml}$).

Recovery

The recovery was determined for the different quinine sulphate concentrations and for the internal standard. It can be defined as the closeness of agreement (in %) between the surface area of an extracted sample versus a non-extracted sample. Appropriate concentrations of quinine sulphate (0.1, 0.3, 0.5 and 1.0 $\mu\text{g/ml}$) and 5 $\mu\text{g/ml}$ propranolol hydrochloride were added to blank plasma. After extraction and reconstitution, each peak was compared with the one obtained by injecting the same non-extracted sample. The mean recoveries are presented in Table 4.

Table 4: Mean recoveries ($n=5$) (\pm SD) of quinine and propranolol in human plasma.

	Concentrations ($\mu\text{g/ml}$)	Recovery (%)
Quinine sulphate	0.1	94.1 \pm 4.7
	0.3	89.3 \pm 2.3
	0.5	90.9 \pm 11.5
	1.0	96.8 \pm 5.7
Propranolol hydrochloride	5.0	90.7 \pm 7.3

Detection and quantification limit

The detection limit of an analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of detection (LOD) was defined as the concentration of the analyte that produced a response equivalent to the blank signal plus three times the standard deviation of the blank signal (mean of the Y-intercept of the calibration curves). The quantification limit is the lowest amount of analyte in a sample which can be adequately determined with suitable precision and accuracy.

$$DL = \frac{3.3 \times \sigma}{S}$$

$$QL = \frac{10 \times \sigma}{S}$$

With

DL = detection limit

QL = quantification limit

σ = standard deviation of the Y-intercept of the mean calibration curve

S = slope of the mean calibration curve

The detection and quantification limits of quinine in human plasma were 0.028 and 0.086 $\mu\text{g/ml}$, respectively.

4. Data analysis

The peak quinine plasma concentration (C_{max}) and the time at which this was reached (t_{max}) were derived from the plasma concentration versus time profiles. The area under

the plasma concentration-time curve to 24 h (AUC_{0-24}) and the terminal half-life ($t_{1/2}$) were calculated using the MW-PHARM program version 3.0 (Mediware 1987-1991, Utrecht, the Netherlands). Quinine pharmacokinetic parameters were statistically evaluated by a two-way ANOVA test. A significance level of 0.05 was used. All statistical analyses were performed using SPSS 12.0.

III. Results and discussion

The study involved 7 male and 5 female volunteers aged between 19 and 37 years, weighing from 51 to 93 kg (mean 64.3 ± 12.3 kg). The pharmacokinetic parameters and the mean ($n=12$) plasma concentrations/time profiles after oral administration of 600 mg QS as commercially available tablets and the taste-masked pellets to healthy human volunteers are presented in Table 5 and Figure 2, respectively.

Table 5: Mean pharmacokinetic parameters (\pm SD) ($n=12$) after oral administration of 600 mg QS to healthy volunteers as taste-masked pellets and as commercially available tablets.

	Parameter			
	C_{\max} ($\mu\text{g}\cdot\text{ml}^{-1}$)	t_{\max} (h)	$t_{1/2}$ (h)	AUC_{0-24} ($\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$)
Pellets	$4.7 \pm 0.8^*$	2.4 ± 0.5	9.8 ± 1.1	$63.5 \pm 13.9^*$
Tablets	3.7 ± 0.8	3.3 ± 0.5	9.1 ± 1.2	54.2 ± 14.4

* Significantly different from tablets ($p < 0.05$)

The QS plasma profile of both dosage forms was similar. However, C_{\max} and AUC_{0-24h} of the tablets were significantly lower and t_{\max} occurred later compared to the pellets. The relative bioavailability of QS taste-masked pellets vs. tablets was 111%.

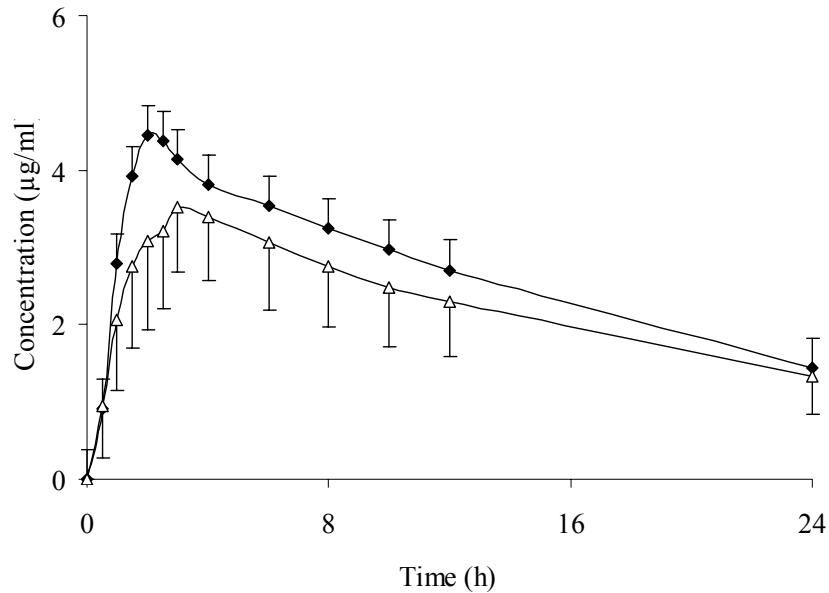


Figure 2: Mean (n=12) plasma concentration-time profiles after oral administration of 600 mg QS as taste-masked pellets (◆) and commercially available tablet (Δ) to healthy human volunteers.

Values for both formulations were within the concentration ranges reported in literature. As reviewed by Krishna and White (1996) and Lebrun-Vignes (1999), quinine was rapidly and reliably absorbed via the oral route in uninfected study participants. According to different studies these authors are referring to, peak plasma concentrations ranged between 3.4 and 6.2 µg/ml after a single oral administration of 600 mg quinine salts, and t_{\max} values between 1.5 and 3.4 h were reported. Quinine elimination half-life

values found in this study are similar to those reported in the literature for healthy volunteers: 9.7 h (Auprayoon et al., 1995) and 10.2 h (Wanwimolruk and Shalcroft, 1991) following an oral administration of 600 mg quinine sulphate.

IV. Conclusion

As evidenced by the respective t_{max} values, taste-masked pellets offer an immediate release and rapid absorption of quinine sulphate. Based on pharmacokinetic values it was concluded that formulating quinine sulphate as taste-masked pellets did not affect quinine bioavailability and that this new quinine sulphate dosage form was considered safe for use in children with uncomplicated *Plasmodium falciparum* malaria.

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Chapter IV

Efficacy of taste-masked quinine sulphate pellets against uncomplicated *Plasmodium falciparum* malaria

Efficacy and steady-state pharmacokinetics of taste-masked quinine sulphate pellets in children with uncomplicated *Plasmodium falciparum* malaria.

I. Introduction

In the previous chapter the quinine bioavailability in adult volunteers was evaluated following single administration of 600 mg quinine sulphate (QS) formulated as taste-masked pellets. According to the results, these pellets had a good bioavailability compared to the available literature data. Since these pellets have been formulated for pediatric use allowing accurate dosing based on body weight, the goal of the present study was to evaluate the formulation's efficacy in this target group. 56 children with uncomplicated malaria were treated for 7 days with QS administered as taste-masked pellets. As signs of therapeutic response, parasitaemia clearance and axillary temperature were recorded and the treatment outcome classification was inspired by the WHO guidelines for drug efficacy assessment of antimalarials (WHO/HTM/RBM/2003.50). In addition, steady-state pharmacokinetics of QS were evaluated.

II. Materials and methods

Patients

A total of 56 children between 6 and 59 months with *Plasmodium falciparum* mono-infection (confirmed by blood film examination) and a parasite density between 2000 and 200 000 parasites/ μ l were recruited among patients consulting the University Hospital of Butare and nearby health centers after a parent or guardian gave full informed consent.

Inclusion and exclusion criteria

During the screening phase children were excluded if they had (i) danger signs (unable to drink or breastfeed; vomiting more than twice in 24 h; recent history of convulsions; unconscious state or unable to sit or stand), (ii) signs of severe malaria, (iii) a packed cell volume (PCV) below 15%, (iv) severe malnutrition (v) respiratory distress and/or acidosis, (vi) any evidence of chronic disease, (vii) history of allergy to quinine, (viii) use of halofantrine or quinine within the two preceding weeks. As final enrollment step, a randomization number was assigned; the bodyweight, parasitaemia charge at entrance and temperature were recorded in the Case Report Form (CRF).

Patient follow-up

As proper patient management takes priority over continuation of the test, patients were hospitalized for continuous monitoring during the initial 4 days of the treatment. Upon discharge from hospital, parents/guardians were asked to return the children to the clinic

for examination on 7th and 14th day of the study. They were encouraged to return to the hospital at any time the child was unwell.

Clinical examination and two parameters related to the clinical response (parasitaemia clearance and axillary temperature) were recorded before the first dose of the day was administered (Table 1).

Table 1: Follow-up schedule

Day	1	2	3	4	7	14
Clinical assesment	X	X	X	X	X	X
Axillary temperature (°C)	X	X	X	X	X	X
Blood slide for parasite count	X	X	X	X	X	X
Blood sample for QS pharmacokinetics				X		

Laboratory methods

May-Gründwald-Giemsa-stained thick and thin films were prepared following finger prick. Parasite density, expressed as the number of asexual parasites per microliter (μl), was calculated by counting the number of asexual parasites against a set number of white blood cells (WBCs) using a hand tally counter, assuming a total WBC count of 8000/ μl . Therefore, the number of asexual parasites was divided by the number of WBCs counted and then multiplied by 8000.

Medication

After manufacturing of the taste-masked QS pellets (Kayumba et al., 2007), they were filled into n° 0 and n° 4 HPMC capsules (corresponding to 100 and 20 mg QS per capsule, respectively) and packed in aluminum bags (Vapor Flex[®], USA). Children were treated with taste-masked pellets for seven days (drug administration every 8 h) according to the dosing scheme in function of body weight shown in Table 2, corresponding to a dose between 10 and 12.5 mg/kg. The required number of capsules was dispensed by the pharmacist to the trial nurse. Prior to drug administration the capsules were opened and the pellets mixed with a small amount of soft food (sorghum pudding). All doses were given under direct observation of the trial nurse for at least 30 min to ascertain retention of the drug. If the patient vomited within 30 min post-administration, a new dose was administered. Children with persistent vomiting were excluded from the study and referred to the appropriate department within the hospital for parenteral treatment. Only the administration of an antipyretic drug was allowed if the patient's conditions warranted such medication.

Table 2: Dosing scheme of QS taste-masked pellets based on child's body weight

Body weight (kg)	QS dose (mg)	Dose range (mg/kg)	Number of capsules	
			n° 0	n°4
$7 < X \leq 8$	80	10 - 11.4	-	4
$8 < X \leq 10$	100	10 - 12.5	1	-
$10 < X \leq 12$	120	10 - 12	1	1
$12 < X \leq 14$	140	10 - 11.6	1	2
$14 < X \leq 16$	160	10 - 11.4	1	3
$16 < X \leq 18$	180	10 - 11.2	1	4
$18 < X \leq 20$	200	10 - 11.1	2	-
$20 < X \leq 22$	220	10 - 11	2	1

Steady-state pharmacokinetics

Before discharge of the patient from the hospital, quinine pharmacokinetic parameters were evaluated. Therefore, the patients were randomized into seven groups (8 children/group) and one venous blood sample (3 ml) per child was collected before administration of the first dose of the day for group I and 1, 2, 3, 4, 6 and 8 h post-dose for group II to VII, respectively. This allowed determining the plasma concentration/time profile of QS under steady-state conditions via population kinetics. The samples were treated and analyzed by the same validated HPLC method as described in the previous study in healthy volunteers.

Upon discharge from hospital (4th day after blood sampling) parents/guardians were given the rest of medication to complete the seven days treatment. Each dose was contained in an individual plastic bag to ensure accurate dosing and the medication was provided in a MEMS (Medication Events Monitoring System) bottle which registered when the bottle was opened via a chip in the cap. This allowed monitoring of the dosing time during ambulatory care setting.

Treatment outcomes classification

The treatment outcomes were inspired by the WHO guidelines on antimalarial drug efficacy assessment (WHO/HTM/RBM/2003.50) and were defined as Early Treatment Failure (ETF) (patient developing severe malaria on or before day 3, or in case parasitaemia at day 2 exceeded the parasitaemia values of day 1 irrespective of axillary temperature, or in case parasitaemia at day 3 was above 25% of that on day 1) and Late Treatment Failure (LTF) (development of symptoms or signs of severe malaria without previously meeting any of the criteria of early treatment failure, or any parasitaemia after day 4 without previously meeting any of the criteria of early treatment failure). All other responses were regarded as Adequate Clinical Responses (ACR).

III. Results and discussion

Since the taste-masked QS pellets were formulated for pediatric use as an accurate and flexible way of adjusting the drug dose to the child's body weight, the efficacy of taste-masked QS pellets was evaluated in children with uncomplicated malaria during a 7-day

treatment. However, as no pediatric data are available about the efficacy of QS after oral administration to children (specifically children under 5 years), results will be discussed based on data reported for adults after oral administration and for children following other routes of administration. Table 3 represents the clinical and parasitological profiles at study enrollment.

Table 3: Baseline characteristics in 56 children under five years at the study enrollment.

Parameter	Values
Mean age (months)(SD, range)	29.7 (13.7, 8 - 54)
Mean weight (kg)(SD, range)	12.1 (3.2, 7 - 21)
Mean axillary temperature (°C)(SD, range)	37.7 (0.7, 36.5 - 39.2)
Median parasitaemia (μl^{-1})(range)	41598 (2600 - 198720)

Pellets were well accepted by the children and all of them completed the full 14-day follow-up. After 72 h of treatment (9 doses) the axillary temperature returned to normal ($36.5 \pm 0.5^\circ\text{C}$) in 91% (51/56) of the children and no temperature increase was observed until discharge from the hospital at the 4th day. Furthermore, no temperature increase was observed on the 7th and 14th day. The mean Fever Clearance Time (time for temperature to fall to the normal level) was 60.7 ± 17.8 h.

The parasitaemia evolution is shown on Figure 1. Within 24 h of treatment (3 doses), the parasitaemia fell to about 24% of the initial parasitic charge. The mean estimated time for

a 50% decrease of initial parasitaemia (PCT₅₀) was 16.7 ± 5.5 h, similar to the values found in non-smoking Thai adults with uncomplicated malaria (Pukrittayakamee et al., 2002).

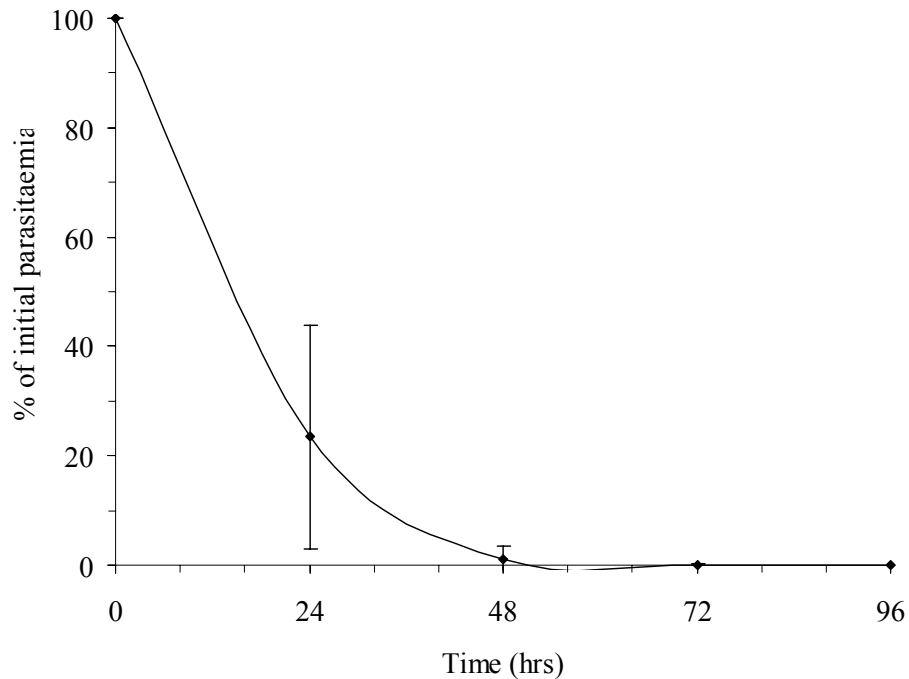


Figure 1: Parasitaemia (expressed as the mean percentage of the initial parasitic charge, \pm stand. dev.) in function of treatment time (n=56).

After 72 h (9 doses) of treatment, 75% of the children (42/56) were aparasitic and 25% (14/56) had a parasitaemia between 0.1 and 1.1% of the initial baseline. At discharge from the hospital on the 4th day, all patients were free of parasites and no parasite recrudescence was found at the 7th and 14th day check-up. The mean Parasitaemia Clearance Time (PCT) (\pm SD) was estimated at 72.8 ± 16.5 h, similar to those observed in adults (73 h) from Thailand following oral administration of quinine sulphate 10 mg/kg

every 8 h for 7 days (Pukrittayakamee et al., 2003). Figure 2 represents quinine plasma concentrations on the 4th day before discharge from the hospital.

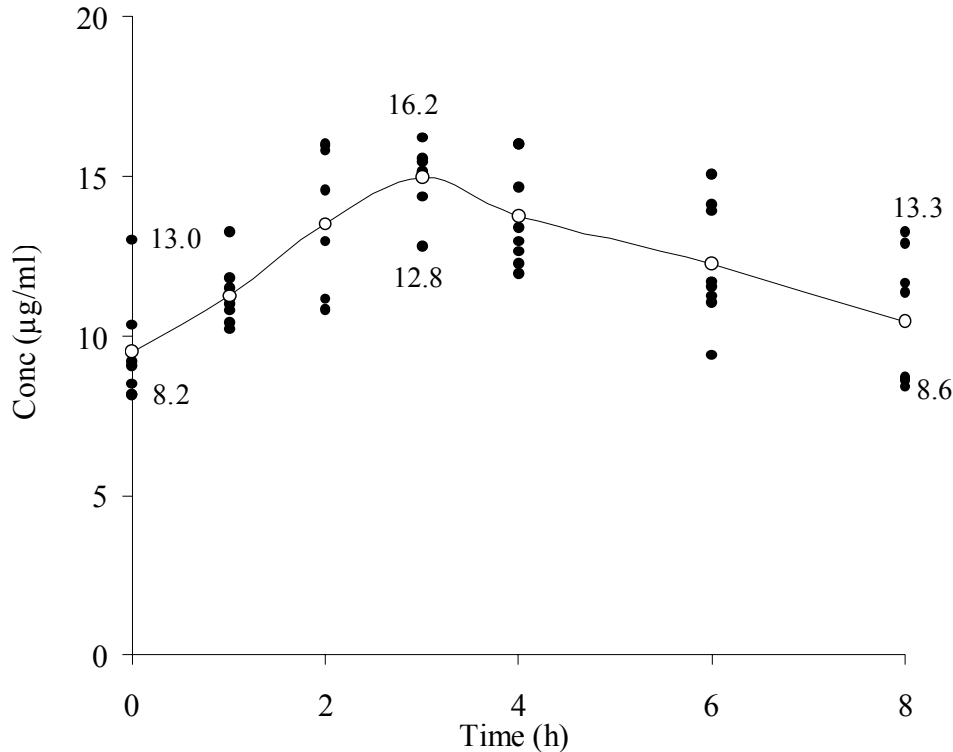


Figure 2: Individual (●) and mean (○) population (n=56) quinine plasma concentrations on the 4th day of treatment (interval between 9th and 10th dosing).

The mean peak concentration during steady state ($14.9 \pm 1.0 \mu\text{g/ml}$) was detected 3 h after dose administration. This concentration range is similar to the 14.7 - 20.7 mg/l peak plasma concentration obtained in Gambian children following an intravenous infusion of 10 mg/kg quinine dihydrochloride (Van Hensbroek et al., 1996). Eight hours after dose administration, the plasma concentration decreased to $10.4 \pm 2.1 \mu\text{g/ml}$ (range: 8.6 - 13.3 $\mu\text{g/ml}$). A concentration difference of 3 – 5 $\mu\text{g/ml}$ QS was observed between minimum

and maximum plasma concentrations. This narrow and regular plasma concentration range is associated with the pellet properties (i.e. as pellets disperse freely in the gastrointestinal tract, variations in gastric emptying rate are reduced and result in a lower inter-subject variability of plasma profiles) and mainly with the narrow dose interval (pellets provided accurate dosing according to body weight, 10 - 11.4 mg/kg). Figure 3 compares the QS doses (mg/kg) given to the recruited children via taste-masked pellets and the dose that would be given if QS tablets were broken according the WHO guidelines.

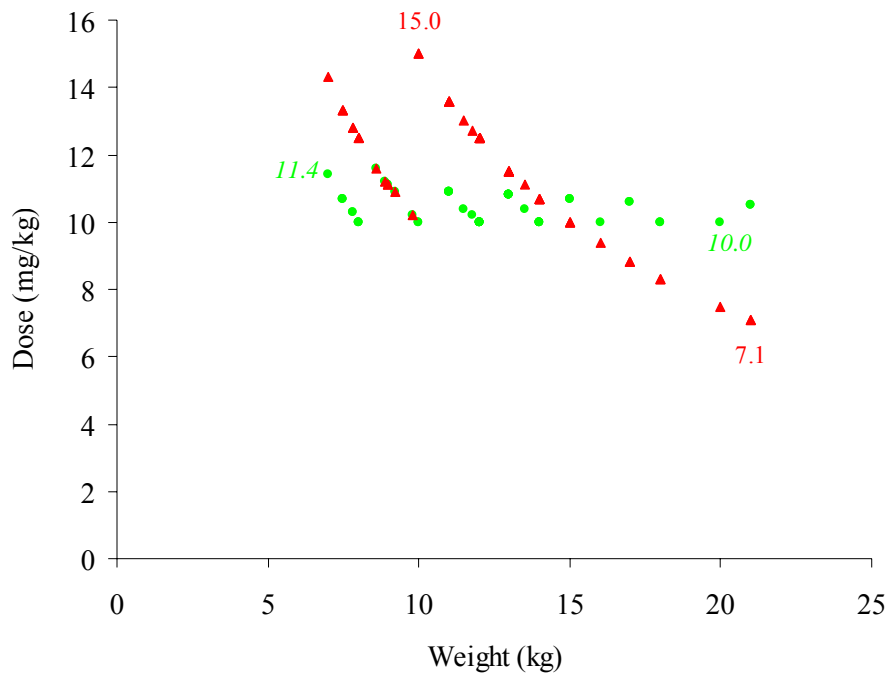


Figure 3: Quinine sulphate doses (mg/kg) administered as taste-masked pellets (●) to the enrolled children compared to the doses that would be given according the WHO dosing scheme via tablet breaking (▲).

It is clear that QS dose administered via pellets was more accurate and this dosage form was more flexible towards dose adaptation in function of body weight. Furthermore, by means of pellets, accurate doses could be delivered over the entire weight range. For tablets, the actual situation can be even worse than the one presented in Figure 3 due to poor breakability of tablets as described in Chapter I of the present research work.

In the present study, the lowest quinine plasma concentration in the 56 recruited children was 8.2 µg/ml, still within the therapeutic range of 8-20 µg/ml reported by White (White, 1996); indicating that there is no risk of sub-optimal concentrations during the 7-day treatment. According to our findings, QS formulated as taste-masked pellets was fully efficient, no treatment failure was found. From the data recorded by the MEMS bottles, it was evidenced that adherence to the dosing scheme was less during drug administration at home by the parents or guardians of the children compared to the hospital setting (drug administration under direct supervision of trial staff): dosing time was not respected in 12.6% vs. 0.9 % in the hospital. Nevertheless, these observations did not affect the therapeutic outcome, since no parasites were detectable at the time of discharge from the hospital.

IV. Conclusion

Based on our results, taste-masked quinine sulphate pellets were shown to be efficacious against uncomplicated *Plasmodium falciparum* in children (< 5 years) as evidenced by parasitaemia clearance and axillary temperature. As a multiparticulate dosage form these QS pellets offer the possibility to easily adjust the QS dose to the body weight of the

child, reducing the risk of under- or over-dosing as evidenced by plasma concentration during steady-state.

V. References

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Chapter V

Development of a taste-masked quinine based suspension

I. Introduction

Taste is one of most important parameters governing patient compliance. Bitterness of the active ingredient is one of several challenges for development of oral pharmaceuticals. Formulation of a bitter drug as liquid dosage form with an acceptable degree of palatability is a key issue for formulation scientists, especially in case of oral administration for the pediatric population. Sohi and collaborators (2004) reviewed different recent developments and approaches for taste masking in oral pharmaceuticals. In case of liquid formulations, the bitter taste can be masked with sweeteners like aspartame, sodium saccharin and refined sugar. However, this approach is not successful for highly bitter and highly water soluble drugs. Artificial sweeteners and flavours are then used along with other taste-masking techniques. The authors also reported the taste masking by inclusion complexation, where the drug molecule fits into the cavity of a complexing agent. β -cyclodextrin is the most widely used for this approach. The complexing agent masks the bitter taste of drug by either decreasing its oral solubility on ingestion or decreasing the amount of drug molecules exposed to taste sensors. However, this method is suitable only for low dosed drugs.

Since the bitterness sensation is a function of the drug concentration in contact with the taste sensors, the approach of decreasing the oral drug solubility is of high interest to reduce the bitter taste of a molecule.

As the present chapter is based on drug poor solubility as a possible means for taste masking, an introduction on the formation of poorly soluble drug salts is presented.

Reduced solubility may be conferred on a drug by formation of poorly water-soluble drug salts if the drug contains ionisable groups (Madan, 1985). Since the majority of marketed drugs are weak electrolytes the potential of achieving a low solubility by salt formation is huge. Therefore, proper selection of a counterion that would confer the desired solubility is warranted. Preparation of poorly soluble salts has been extensively utilized in the literature with the intention of obtaining a prolonged duration of action, the most well known being procaine penicillin and benzathine penicillin, which are marketed as aqueous suspensions for achieving the depot effect after parenteral administration (Brunner & Giovannini, 1956; Giovannini, 1956). A similar principle has been applied on various classes of therapeutic agents (Table 1).

As can be noticed from Table 1, only a limited number of counterions have been used to prepare poorly soluble salts. For preparation of poorly soluble salts, aromatic ortho-hydroxy carboxylic acids have been used as salt-forming agent, the planar and symmetrical pamoic acid (Figure 1) being the most widely used as salt former.

Table 1: Examples of poorly soluble drug salts prepared for sustained release.

Therapeutic class	Drug salt	Reference
Anoretics	Benzphetamine pamoate	Morozowich et al., 1962
	Phendimetrazine pamoate	Saias et al., 1969
Anthelmintics	Pyrantel pamoate	Desowitz et al., 1970
	Pyrvinium pamoate	Martindale, 2002
Antiasmatics	Clorprenaline pamoate	Xue-qiu et al., 1982
Antibiotics	Procaine penicillin	Bruner&Giovannini, 1956
	Benzathine penicillin	Giovannini, 1956
Antidepressants	Imipramine pamoate	Miller et al., 1973
Antihistamines	Phenylephrine tannate	Kile, 1958
Antimalarials	Cycloguanil pamoate	Thompson et al., 1963
	Pyrimethamine pamoate	Colemann et al., 1986
Beta-adrenergic blockers	Propranolol laurate	Aungst&Hussain, 1992
Gonadotrophic hormones	Triptorelin pamoate	Minkov et al., 2001
Local anaesthetic	Lidocaine naphthoate	Nakano et al., 1978
Opioid analgesics	Fentanyl pamoate	Randell et al., 1994
Opioid antagonist	Naltrexone pamoate	Reuning et al., 1981
Anti-inflammatory	Diclofenac resinate	Walter et al., 2001

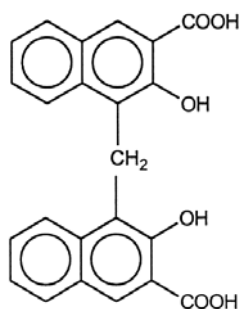


Figure 1: Structure of pamoic acid, which has been widely used as a salt forming agent for preparing poorly soluble salts.

Qualitative guidelines for preparing poorly soluble salts suggest that the counterions selected should have a compact, symmetrical and rigid chemical structure (Anderson 1985; Gould, 1986). Furthermore, as observed by crystallographic analysis of ortho-hydroxy acids, intramolecular hydrogen bonds formed between the ortho-hydroxy group and the oxygen atom of the carboxylate moiety confers stability to the salt and may render the salt more hydrophobic which could partly explain the poor aqueous solubility (Parshad, 2003). These statements make pamoic acid an ideal counterion, but no further investigations have been undertaken to support these suggestions. Thus, the popularity of pamoic acid as counter ion is probably based on previous successful experiences with this molecule.

Based on the above mentioned information and the fact that bacampicillin and pivampicillin as pamoate salts have been reported to be very poorly water soluble and much less bitter compared to their corresponding hydrochloride salts (Saesmaa and Halmekoski, 1987), pamoic acid has been chosen for formulation of a poorly water soluble and consequently tasteless quinine pamoate salt from quinine hydrochloride.

The approach involves a reaction between quinine hydrochloride (water soluble salt) and disodium pamoate to give a quinine pamoate salt as shown in Figure 2.

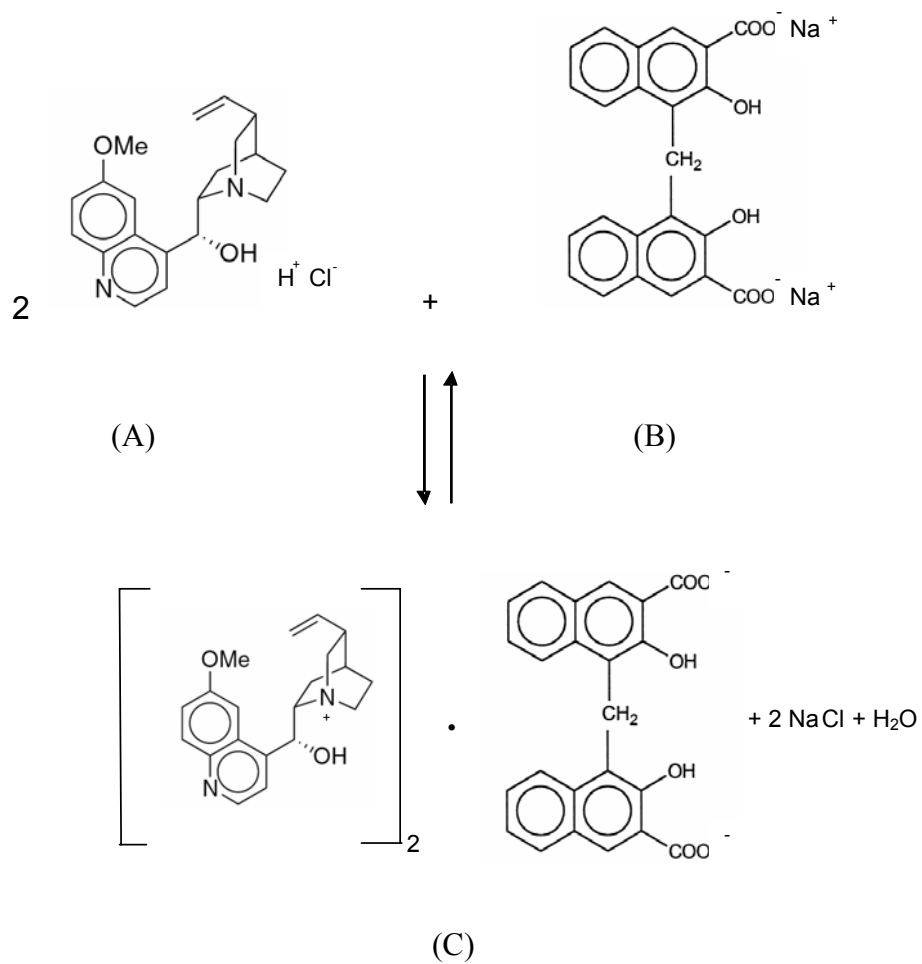


Figure 2: Reaction between quinine hydrochloride (A) and disodium pamoate (B) for the formation of quinine pamoate salt.

II. Preparation of quinine pamoate suspension

Quinine pamoate suspension (QP) was prepared by the in situ precipitation of quinine hydrochloride (QHCl) (Certa, Braine-l'Alleud, Belgium) with pamoic acid disodium salt (PA) (Sigma-Aldrich, Germany). QHCl solutions have been prepared by dissolving 2g of quinine hydrochloride powder in 50 ml demineralized water. Separately, PA samples were prepared by dissolving different amounts of PA in 50 ml demineralized water. A range of quinine pamoate (QP) precipitates was prepared by mixing QHCl and PA solutions in a molar ratio from 2/0.5 to 2/1.2 with a constant quinine concentration of 20 mg/ml (Table 2), aiming to evaluate at which molar ratio complete precipitation of QHCl was reached.

Table 2: Range of quinine pamoate precipitates prepared

	Formulation				
	1	2	3	4	5
QHCl (g/50 ml)	2	2	2	2	2
PA (g/50 ml)	0	0.59	0.95	1.19	1.44
QHCl/PA ratio (mol/mol)	2/0	2/0.5	2/0.8	2/1	2/1.2

For the final QP suspension, two viscosity enhancers were used separately, Avicel[®] RC 581 (FMC, Cork, Ireland) and xanthan gum (Federa, Braine-l'Alleud, Belgium) at concentrations of 1 and 0.2 % (w/v), respectively.

III. Evaluation of quinine pamoate suspension

In vitro evaluation

Precipitation efficiency

The free quinine concentration in the supernatant, as proof of incomplete precipitation, was measured by a reversed-phase HPLC method with fluorescence detection.

Three 5 ml samples were taken from each suspension and centrifuged at 2500g for 10 min. The supernatant was filtered through a 0.2 µm pore diameter cellulose acetate filter (Sartorius, Goettingen, Germany), and injected onto the HPLC system consisting of a solvent pump (L-7110, Hitachi, Tokyo, Japan) set at a constant flow rate of 0.8 ml/min, a variable wavelength detector (L-7480 fluorescence detector, Hitachi, Tokyo, Japan) set at 325 and 375 nm as excitation and emission wavelength, respectively, a C₁₈ reversed phase precolumn and column (Lichrospher 100 RP 18 (5µm), Merck, Darmstadt, Germany) and an automatic integration system (L-7000, Hitachi, Tokyo, Japan). The mobile phase consisted of a filtered and degassed mixture of 0.1 M ammonium acetate, acetonitrile and methanol (40/15/45; v/v/v). The pH was adjusted to 3.0 using perchloric acid. The quinine concentration was calculated by means of a calibration curve (peak area vs. concentration) from 0.4 to 3 mg QHCl /l.

Dissolution testing

Dissolution tests (USP XXVII) were performed using the paddle method at a speed of 100 rpm. Nine hundred ml of 0.1 N hydrochloric acid, pH 5.8 phosphate buffer and

demineralised water ($37 \pm 0.5^\circ\text{C}$) were used as dissolution media to evaluate quinine release from quinine pamoate precipitates. 5 ml was withdrawn at 1 min interval from the dissolution medium over a period of 3 min, a period arbitrary chosen in function of product residence time in mouth of the patient. Then 5ml samples were withdrawn after 5, 10, 20, 30 and 45 min of dissolution test. Samples were injected onto the HPLC system and the amount released calculated by means of a calibration curve as described previously.

Physical properties

Particle size distribution was analysed, in triplicate, by laser diffraction with a Mastersizer-S (Malvern, UK) equipped with 300RF lens and a mixing system set at 1500rpm. *Sedimentation characteristics* of QP suspensions were determined via the sedimentation volume (F) which is defined as the ratio of the final, equilibrium volume of the sediment (V_u), to the total suspension volume (V_o) before settling, as expressed in the following equation: $F=V_u/V_o$ (Tunçel and Gürek, 1992). *Flocculation* and *colour changes* were monitored by visual observation, and *redispersibility* evaluated by manual shaking.

Physical stability of QP suspension

The physical stability of QP suspension was verified after storing the suspension at room temperature and at 40°C for 6 months. They were analysed for sedimentation volume, redispersibility and particle size distribution.

In vivo evaluation

Taste masking efficiency

Five volunteers evaluated the taste of the QP suspension. Referring to the reports by Katsuragi et al. (1997) and Suzuki et al. (2003), aqueous quinine hydrochloride solutions for matching the bitter taste intensity of tested samples were prepared as shown in Table 3. The bitterness of the QP suspensions was evaluated by comparing the taste intensity of QP samples (taken from Table 2) with that of the standard solutions and selecting the standard solution having a taste intensity equivalent to that of the given QP sample. Volunteers compared intensities by placing 1 ml of each formulation on the tongue, tasting it for 10 s, and thoroughly rinsing their mouths with deionized water.

Table 3: Relationship between bitter taste intensity and quinine hydrochloride concentration.

Bitter taste intensity	QHCl concentrations (mg/l)
0	0.0
1	2.3
2	5.0
3	9.4
4	15.7
5	24.1
6	38.8
7	60.8
8	98.5
9	157.2
10	256.8

Quinine bioavailability in dogs

The oral bioavailability of quinine from QP suspensions was evaluated in six fasting dogs without or following pentagastrine pretreatment to lower the stomach pH. Inspired by Akimoto and collaborators (2000), pentagastrine (6 μ g/kg) was injected intramuscularly, one hour before drug intake to be sure that the QP suspension will be delivered in an acidic stomach. The studies were organized in a randomized cross-over design. Each dog was randomly assigned to receive a single dose of quinine pamoate suspension or a freshly prepared quinine hydrochloride solution equivalent to 8.2 mg quinine/kg. A washout period of 1 week separated both drug intakes. Venous blood samples were taken

before and 0.5, 1, 1.5, 2, 3, 4, 8, 12 and 24 h after drug intake. Plasma samples were extracted and analyzed for quinine using a validated HPLC method previously described in Chapter III for quinine quantification in human plasma samples.

IV. Results and discussion

A yellowish QP precipitate was formed immediately upon mixing of QHCl and PA solutions, indicating that a poorly soluble salt was formed (Figure 3).

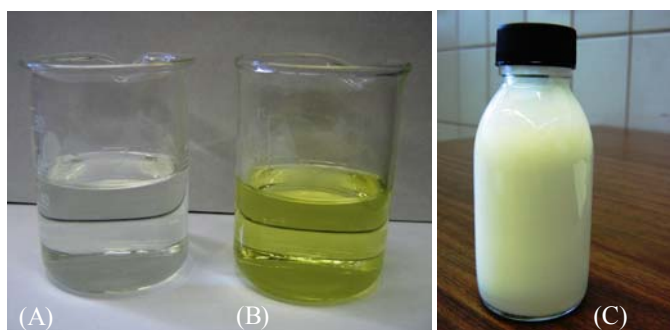


Figure 3: Formation of QP precipitate (C) from precipitation of QHCl solution (A) by PA solution (B).

QHCl precipitation efficiency (evidenced by the decrease of free quinine in the supernatant) correlated well with the increase of concentration of the PA solution used during the reaction (Table 4).

Table 4: Free quinine (mg/l) in the supernatant following QP precipitation at different QHCl/PA ratios

Formulation nr	QHCl/PA ratio (mol/mol)	Free quinine (mg/l)
2	2/0.5	8.65
3	2/0.8	3.47
4	2/1	0.08
5	2/1.2	0.01

The dissolution profiles of the QP suspension are presented in Figure 4. It was observed that the precipitation had no impact on quinine release as more than 80% of drug was already dissolved within the first 10 min in hydrochloric acid and even in phosphate buffer pH 5.8 (USP XXVII requirements: 75% released within 45 min in 0.1N hydrochloric acid).

As expected, a slow quinine release was observed in demineralised water, only 0.5 mg/l was released within 3 min and a maximum of 1.2 mg/l was dissolved after 45 min. As a delay in the onset of drug release is essential to obtain a taste-masking effect, the dissolution profile of QP suspension in water (pH 6.8) suggested that the bitter taste of quinine can be sufficiently delayed in saliva (pH 6.5 - 7.4) when using the poorly soluble QP salt.

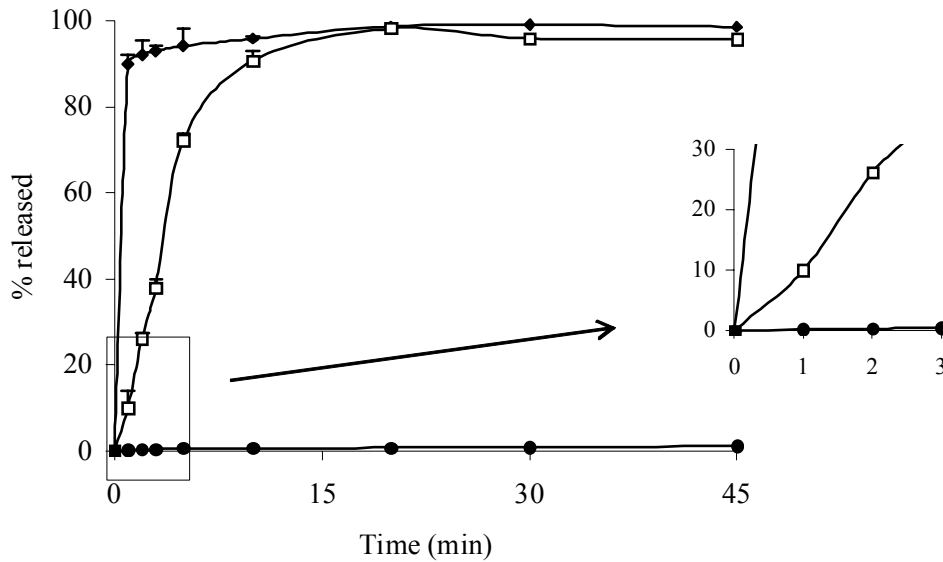


Figure 4: Release of quinine from QP suspension (n=3) in hydrochloric acid 0.1 N (◆), pH 5.8 phosphate buffer (□) and demineralized water (●).

The bitter taste sensation correlated well with the free quinine concentration in the suspension. All volunteers recognized a slight bitter taste in the formulation n° 2 (QHCl/PA molar ratio: 2/0.5 corresponding to 8.65 mg/l free quinine) and no bitter taste was detected in the formulation n° 4 (molar ratio: 2/1, 0.08 mg/l free quinine). The bitter scores of the two formulations were situated around 3 and 0, respectively. However, volunteers were not able to discriminate formulation 2 (8.65 mg/l free quinine, score ~ 3) and formulation 3 (3.47 mg/l free quinine, score between 1 and 2). This finding correlated well with observations by Suzuki and collaborators (2003) as volunteers could easily discriminate the taste of quinine solutions having a bitterness intensity of 3 and higher. Although the suspension made at QHCl/PA ratio of 2/1 was declared tasteless by the volunteers, a limited excess of PA was preferred to push the reaction equilibrium towards

the formation of quinine pamoate, hence a 2/1.2 molar ratio between QHCl and PA has been used for future experiments.

Particle size distribution, sedimentation properties and physical stability

The in-situ precipitation of QP (using a QHCl and PA solution) resulted in a suspension of fine particles ($< 20 \mu\text{m}$) and the size distribution of the final formulation (QP + viscosity enhancer) is presented in Table 5 upon storage at room temperature (RT) and 40°C for 6 months. The sedimentation characteristics (expressed as F value) are presented in Figure 5.

The use of xanthan gum (XG) as viscosity enhancer did not affect the particle size distribution of the QP precipitates. Upon stability test no major changes were observed in particles size distribution whatever the storage conditions. Particle size of the precipitates in the Avicel[®] RC581 suspension was higher since the microcrystalline cellulose particles in Avicel[®] RC581 also contributed to the particle size distribution. According to FMC BioPolymer (manufacturer of Avicel[®]), about 35 % of Avicel[®] RC 581 particles are larger than $75 \mu\text{m}$ diameter. However, despite larger particles compared to XG, the particles size distribution was not affected upon stability test.

Table 5: Particle size distribution of QP suspension upon 6 months of stability testing

Viscosity enhancer	Storage conditions		Size (μm) distribution		
			D(v, 0.1) ^a	D(v, 0.5) ^b	D(v, 0.9) ^c
Xanthan gum	Before		0.5 ± 0.0	9.3 ± 0.1	19.8 ± 0.5
	3 months	RT	0.4 ± 0.0	7.3 ± 0.3	15.8 ± 1.8
		40°C	0.4 ± 0.1	8.4 ± 0.3	18.0 ± 2.3
	6 months	RT	1.2 ± 0.0	6.2 ± 0.1	17.3 ± 0.2
		40°C	3.7 ± 0.1	10.9 ± 0.2	22.3 ± 0.2
	Avicel [®] RC 581	Before		5.7 ± 0.8	21.5 ± 1.5
3 months		RT	6.2 ± 0.9	24.1 ± 3.1	78.4 ± 2.3
		40°C	5.3 ± 0.4	26.4 ± 0.4	86.2 ± 9.7
6 months		RT	7.2 ± 0.2	26.0 ± 0.4	85.8 ± 2.6
		40°C	3.3 ± 0.4	28.8 ± 0.8	86.6 ± 2.8

^a D (v, 0.1): particle size at which 10% of the sample (volume based) is smaller than this size.

^b D (v, 0.5): particle size at which 50% of the sample is smaller and 50% is larger than this size.

^c D (v, 0.9): particle size at which 90% of the sample (volume based) is smaller than this size.

The sedimentation volume was smaller with Avicel[®] RC581 at 40°C, where F values of about 0.3 were measured. However, this sedimentation did not result in caking; the particles were redispersible by low intensity shaking. A slow particle settling was observed when XG was used as viscosity enhancer. According Gabriël and Plaizier (2004), no changes in viscosity are observed when XG dispersions are exposed to higher temperatures, which is an interesting characteristic for suspensions used in tropical conditions. Furthermore, a preservative should be added to the final formulation to

maintain the microbial integrity of the product as suspensions are susceptible to microbiological degradation on prolonged storage.

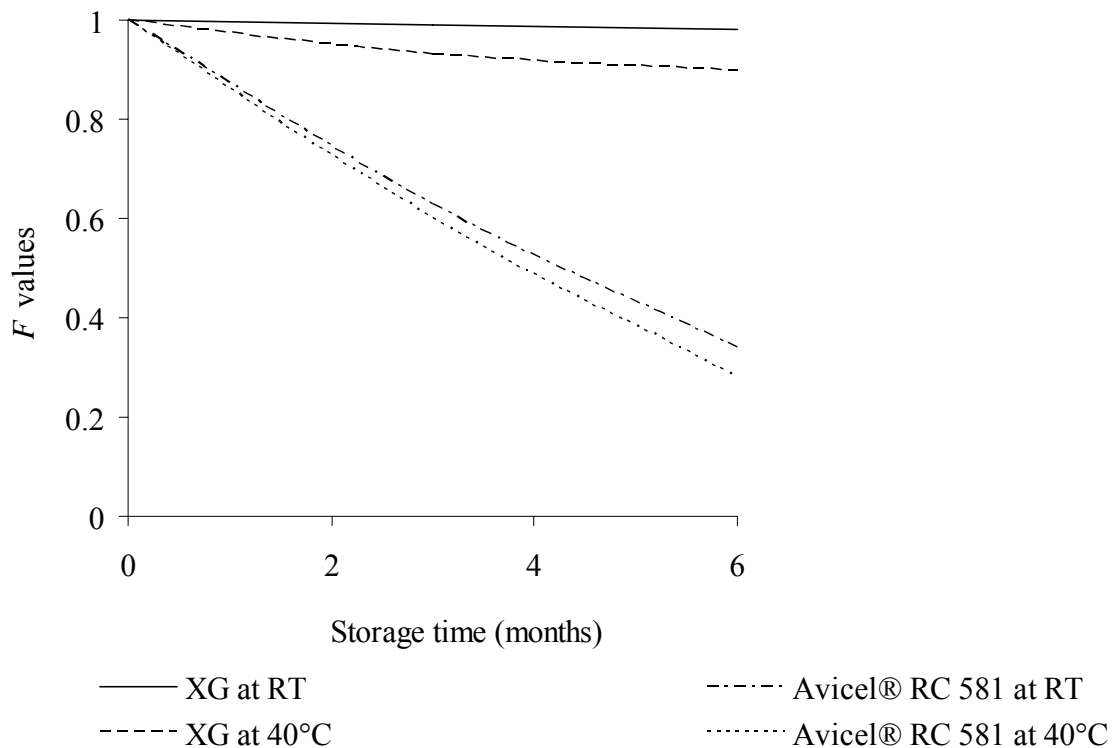


Figure 5: Sedimentation characteristics (expressed as F value) during 6 months storage at RT and at 40°C.

Bioavailability of quinine from QP suspension in dogs

The pharmacokinetic parameters in dogs are presented in Table 6, and the mean ($n=6$) plasma concentration-time profiles after oral administration of 8.2 mg quinine/kg as QHCl solution and QP suspension with XG to dogs is shown in Figure 6.

The evaluation of the quinine bioavailability in dogs showed that without pentagastrine pre-treatment, C_{max} and AUC_{0-24h} of the suspension were significantly lower compared to

the solution. Akimoto and collaborators (2000) investigating pH profiles of beagle dogs found that the majority of animals had a basal pH of around 7 and reported that dogs are poor acid secretors. This low bioavailability in dogs (basal pH) is correlated with a slow quinine release observed in demineralised water (Figure 4). An acidic pH in the stomach is required for a full quinine release from the suspension.

Table 6: Mean pharmacokinetic parameters (\pm S.D.) (n=6) after oral administration of 8.2 mg/kg quinine to dogs as quinine hydrochloride solution and as quinine pamoate suspension

Formulation	Parameter			
	C_{max} ($\mu\text{g/ml}$)	T_{max} (h)	AUC_{0-24} ($\mu\text{g/ml.h}$)	Relative bioavailability (%)
Solution	1.2 ± 0.3	1.5	6.8 ± 2.2	
Suspension				
Without pentagastrine	$0.7 \pm 0.4^*$	2.0	$3.7 \pm 2.1^*$	53.5 ± 21.5
With pentagastrine	1.1 ± 0.4	2.0	5.5 ± 1.9	81.2 ± 10.6

* suspension significantly different from solution ($p < 0.05$, paired t-test)

Following intramuscular injection of dogs with pentagastrine ($6\mu\text{g/kg}$) (analogue of gastrin that reproducibly stimulates gastric acid production), Akimoto and collaborators (2000) observed that stomach pH values from 1.7 to 2.2 were attained within 0.5 - 1.5 h after pentagastrine administration.

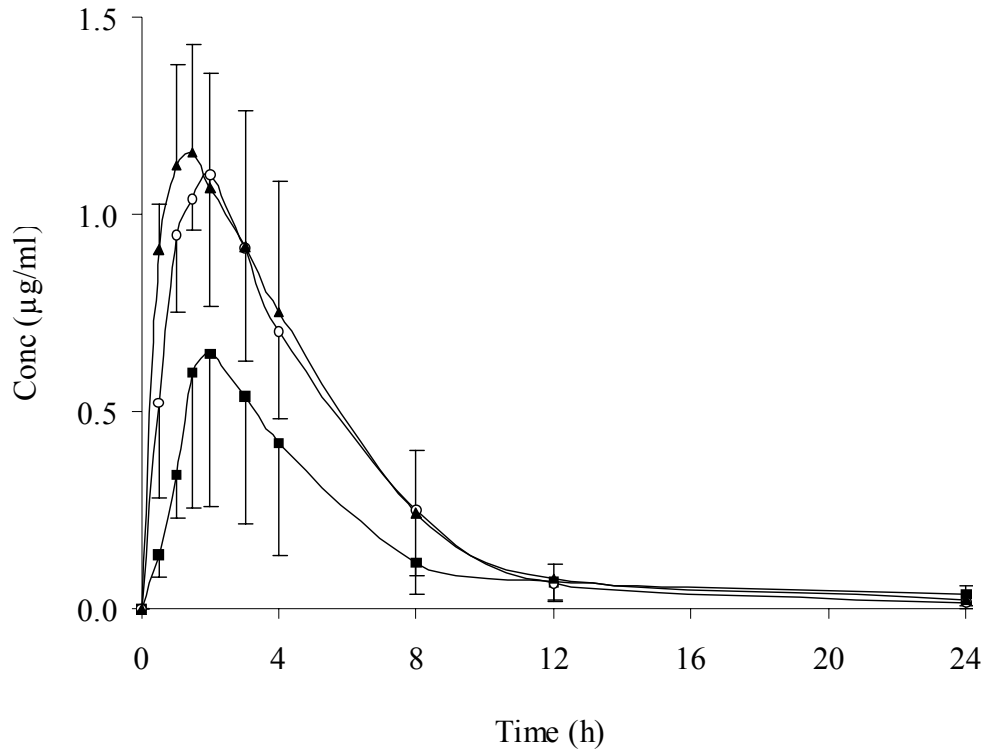


Figure 6: Mean (n=6) plasma concentration-time profiles after oral administration of 8.2 mg/kg quinine to dogs as quinine hydrochloride solution (▲) and as quinine pamoate suspension without (■) and with stomach acidification by pentagastrine (○).

The change in gastric pH was correlated with the increase of pharmacokinetics values observed in the present study. Individual C_{max} and AUC_{0-24h} values increased 1.5- and 1.8-fold, respectively. After gastric acidification the mean relative bioavailability of quinine in the QP suspension compared to the solution increased from 53.5 ± 21.5 to 81.2 ± 10.6 %. However, further investigations need to be conducted in humans to confirm the bioavailability data in dogs.

V. Conclusion

A taste-masked and stable QP suspension has been developed by precipitating quinine hydrochloride by pamoic acid. According to the dissolution profiles, no effect of precipitation on quinine release in the stomach is expected. For viscosity enhancement, xanthan gum is preferred over Avicel[®] RC 581. Quinine pamoate bioavailability in dogs was significantly increased following pentagastrine pre-treatment and no difference was found in pharmacokinetics values between a quinine pamoate suspension and a quinine hydrochloride aqueous solution.

However, further bioavailability investigations are required in humans to confirm the pharmacokinetics parameters found in dogs.

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General conclusion and recommendations

The lack of pediatric formulations is a big concern all over the world. Most of the existing dosage forms are not flexible for dose adaptation to the child bodyweight. Tablet breaking is a frequent method to obtain the desired dose. Despite that quinine is the last chance for treatment of multi-resistant malaria, it has no pediatric formulation. In the present work it was demonstrated that breaking of quinine sulphate tablets (proposed as means for dose adaptation to children) resulted in a weight variation which may compromise the clinical outcome. Another challenge in the development of pediatric quinine formulations is the unpleasant taste that can lead to the poor compliance. Quinine sulphate pellets were proposed as alternative to tablets breaking, as they offer more flexibility for dose adaptation to a child's body weight. They were produced via extrusion-spheronisation and successfully coated with Eudragit[®] E PO polymer for taste-masking purposes. Based on dissolution and the Astree electronic tongue data, the taste masking efficiency of pellets was achieved with a 20% (w/w) Eudragit[®] E PO coat. Via in vivo studies, immediate release of quinine from the pellets and a higher bioavailability compared to tablets were observed. The efficacy of quinine sulphate formulated as taste-masked pellets was demonstrated in children less than five years age suffering from uncomplicated *Plasmodium falciparum* malaria. Administered in combination with food at a dose varying between 10 and 11.4 mg/kg, all patients were cured within 7 days treatment. However, in the present work pellets were packed as single-dose capsules of 2 different volumes (capsules nr 4 and nr 0) and drug was adjusted in function of body weight by combining both packaging volumes.

Since this may complicate drug delivery and is an error prone system, future work must include the design of a dosing instrument that can accurately deliver a specific amount of pellets.

A quinine taste-masked suspension has been formulated by precipitation of quinine hydrochloride after combination with pamoic acid. The precipitation resulted in a stable suspension of fine particles ($< 20 \mu\text{m}$) easily resuspendable by simple shaking. This suspension was declared tasteless by volunteers. A quinine pamoate suspension is a promising option for quinine formulation as liquid dosage form, however further investigations are recommended:

- The choice of additives (conservatives, colorants, sweeteners etc.) should be based on their impact on quinine release and their safety in the pediatric population.
- Quinine bioavailability and efficacy should be determined in humans to verify results from dogs.

Summary

Most licensed medicines are intended for adults. The lack of pediatric drug formulation for the existing as well as new molecules is a worldwide concern. As a consequence, care givers are forced into the situation where they have to use unlicensed or licensed medicines in ways not covered by the license, despite that safety, efficacy or quality of these drugs have not been tested for the indications and methods by which they are used. Despite that malaria is a public health concern worldwide, especially in tropical Africa where children are the most vulnerable group (75% of all malaria deaths being children below 5 years of age) pediatric formulations are lacking for most of antimalarial drugs. Children are treated by fragmenting formulations which are designed for treatment of adults. For quinine, only tablets are listed on the WHO essential drug list and they are commercially available in a dose of 200 or 300 mg quinine sulphate per tablet. The treatment of children is based on the breaking of tablets to adapt the dose to the children's body weight.

Chapter I focused on the accuracy of dose administered to children when quinine sulphate tablets are broken. Large weight variations were observed when quinine sulphate tablets were broken into halves and quarters. Those deviations were related to the presence and position of the score line. Considering the variability in doses administered, the question rises if the same therapeutic effect will be obtained during the entire treatment period. Pellets have been proposed as alternative to tablet breaking since they offer more flexibility for the dose adaptation to the child body weight.

Chapter II details the development of taste-masked quinine sulphate pellets. The first part focused on the production of quinine sulphate pellets via extrusion spherulization. The influence of formulation and process parameters on the quality of pellets was evaluated. A pellet size range of 300 – 700 µm was selected as larger particles had less acceptable mouth feel, whereas smaller particles tended to remain in the mouth. Although pellets having a high quinine sulphate fraction (up to 70%) could be formulated, the amount to be loaded was fixed to 20% as at high quinine sulphate concentration the total amount of pellets per dosing would be too small. The extrusion screen with 400 µm screen perforations was more suitable to produce the targeted pellets size. It has been observed that formulating quinine sulphate as pellets did not affect the drug dissolution profile, as drug release from pellets was complete within only 10 min in 0.1N hydrochloric acid. As quinine sulphate is very bitter and the produced pellets were intended for pediatric application, bitter taste-masking is the second goal to be reached for the formulation acceptability in children. The second part of the chapter focuses on the taste masking of quinine sulphate pellets. An aqueous-based dispersion of Eudragit® E PO (11.4% w/w) was used for coating of quinine sulphate pellets. Sodium lauryl sulphate (SLS, 10% w/w based on dry polymer weight) was used as emulsifier and two plasticizers (10-15% w/w based on dry polymer weight), stearic acid (StA) or dibutyl sebacate (DBS) were evaluated. Magnesium stearate (35% w/w based on dry polymer weight) was added as antisticking agent. Coating was performed via a fluid bed used in the bottom-spray mode with the Wurster setup and coating conditions were optimised. Coating of the 300-700µm pellet fraction using a Eudragit® E PO-based dispersion was possible. However, using 15% dibutyl sebacate (based on polymer weight) as a plasticizer in the formulation

caused pellet agglomeration after one week storage at 40°C and 75% relative humidity. Reducing the plasticizer concentration to 10% did not prevent pellet agglomeration during storage and in addition at this plasticizer concentration the taste-masking properties of the formulation were lost. Pellets were less sensitive to sticking when dibutyl sebacate as a plasticizer was substituted by stearic acid (15% based on polymer weight). The dissolution data in water (pH ~ 7) suggested that Eudragit[®] E PO can sufficiently delay the release of quinine sulphate in saliva, and thereby reduce the bitter taste of the pellets. The delay of the onset of quinine sulphate release was correlated with coating thickness. The bitterness score of quinine sulphate from taste-masked pellets was evaluated in function of time using the bitterness prediction module of the electronic tongue. The results showed that quinine sulphate pellets coated with 20% (w/w) Eudragit[®] E PO is sufficient to delay bitterness appearance and allows patients to swallow the pellets without experiencing any discomfort.

The bioavailability of quinine sulphate from taste-masked pellets was described in Chapter III. The oral bioavailability of quinine sulphate was evaluated in 12 adult healthy volunteers. In a randomized cross-over study, pharmacokinetics of a single dose of 600 mg quinine sulphate administered either as taste-masked pellets or as commercially available tablets have been determined. Quinine plasma concentrations were determined with a validated HPLC-fluorescence method. Administration of taste-masked quinine sulphate pellets resulted in higher plasma concentrations compared to tablets (4.7 ± 0.8 vs. 3.7 ± 0.8 $\mu\text{g/ml}$). The extent of absorption from the pellets was also higher as evidenced by the respective $\text{AUC}_{0-24\text{h}}$ values (63.5 ± 13.9 vs. 54.2 ± 14.4 $\mu\text{g.h.ml}^{-1}$).

Despite difference in pharmacokinetic parameters, the values of both formulations (pellets and tablets) were within the ranges reported in the literature.

In Chapter IV the efficacy of quinine sulphate formulated as taste-masked pellets was assessed in 56 children under five years' age and suffering from uncomplicated *Plasmodium falciparum* malaria, each of them was followed for 14 days. A specific amount of taste-masked quinine sulphate pellets was mixed with a small amount of soft food (Sorghum pudding) and administered under direct observation of trial nurse. The patients were hospitalized for 4 days, parasitaemia and body temperature (as signs of therapeutic response) were daily monitored. Before discharge from the hospital (4th day), one blood sample was taken per child to determine the plasma concentration/time profile of quinine sulphate under steady-state conditions via population kinetics.

Taste-masked quinine sulphate pellets were well accepted by the children and all of them completed the full 14 days follow-up. After 72 h treatment the axillary temperature returned to normal ($36.5 \pm 0.5^{\circ}\text{C}$) in 91% (51/56) of the children. No temperature increase was observed on the 7th and 14th day. The mean time for temperature to fall to the normal level was 60.7 ± 17.8 h.

Within 24 h treatment (3 doses), the parasitaemia fell to about 24% of the initial parasitic charge. The mean estimated time for a 50% decrease of initial parasitaemia (PCT₅₀) was 16.7 ± 5.5 h. After 72 hours treatment, 75% of the children were a parasitic and 25% had a low parasitaemia between 0.1 and 1.1% of the initial baseline. At discharge from the hospital on the 4th day, all patients were free of parasites and no parasite recrudescence was found at the 7th and 14th day check-up. The mean Parasitaemia Clearance Time (PCT) was estimated at 72.8 ± 16.5 h. The peak concentration during steady state ($14.9 \pm$

1.0 µg/ml) was detected 3 h after dose administration. Eight hours after dose administration, the plasma concentration decreased to 10.4 ± 2.1 µg/ml (range: 8.6-13.3 µg/ml), still in the therapeutic range. No treatment failure was observed, making thereby taste-masked quinine sulphate pellets a means for flexible dose adaptation to the body weight of children during malaria treatment.

The development of a taste-masked quinine-based suspension is described in [Chapter V](#). A water insoluble quinine pamoate salt was formed following precipitation of quinine hydrochloride by disodium pamoate salt. Fine quinine pamoate precipitates (< 20 µm) were formed and two viscosity enhancers (Avicel[®] RC 581 and xanthan gum) were used to stabilize the suspension. A range of quinine pamoate precipitates was prepared by mixing quinine hydrochloride and disodium pamoate solutions in different molar ratios. A suspension made at quinine hydrochloride / disodium pamoate molar ratio of 2/1 was declared tasteless by volunteers. Only 1.2 % quinine was released from the suspension after 45 min in water, while more than 80% drug was dissolved within the first 10 min in hydrochloric acid. This suggested that a fast quinine release in the stomach is expected. The quinine pamoate suspension was stable and easily redispersible after 6 month storage at room temperature and 40°C. However, particle sedimentation was faster in the Avicel[®] RC 581 formulation. The oral bioavailability of quinine after administration of the quinine pamoate suspension was evaluated in six fasting dogs (using a quinine hydrochloride solution as reference). Upon lowering the stomach pH with pentagastrine, the mean relative bioavailability of quinine in the quinine pamoate suspension (compared to quinine hydrochloride solution) was increased and no significant difference was found

between both formulations for quinine extent of absorption. However, further investigations in humans are necessary to verify the results obtained in dogs.

Samenvatting

De meeste geneesmiddelen worden specifiek gecommmercialiseerd voor volwassenen waardoor het gebrek aan pediatrische geneesmiddelvormen specifiek ontwikkeld voor kinderen een wereldwijd probleem is. Bijgevolg zijn zorgverstrekkers vaak genoodzaakt om voor pediatrische toepassingen geneesmiddelen te gebruiken op een wijze die niet gedekt wordt door de licentie, ondanks het feit dat de veiligheid, doeltreffendheid en kwaliteit van deze geneesmiddelen niet getest werden voor deze pediatrische indicaties en/of toedieningswijzen.

Een specifiek voorbeeld hiervan is malaria aangezien pediatrische formulaties van de meeste antimalaria geneesmiddelen ontbreken ondanks het feit dat malaria een grote bedreiging vormt voor de volksgezondheid in grote delen van de wereld. In tropisch Afrika zijn kinderen nochtans de meest kwetsbare groep aangezien 75% van alle sterfgevallen ten gevolge van malaria kinderen jonger dan 5 jaar zijn. Zij worden momenteel meestal behandeld door tabletten (ontwikkeld voor volwassenen) te breken in kleinere delen om de dosis aan te passen in functie van het lichaamsgewicht.

Hoofdstuk I onderzoekt de gewichtsvariaties die optreden bij het breken van tabletten in kleinere delen. Voor kininesulfaat-tabletten die commercieel beschikbaar zijn op de Rwandese markt werden grote gewichtsschommelingen vastgesteld bij het breken van de tabletten in twee of vier stukken. Deze variaties waren afhankelijk van de aanwezigheid en de positie van een breuklijn. Aangezien deze gewichtsschommelingen een grote variatie van de toegediende dosis tot gevolg hebben, stelt zich de vraag of tijdens de hele

behandelingsperiode hetzelfde therapeutische effect bereikt wordt. Om dit probleem te omzeilen werden pellets voorgesteld als alternatief voor het breken van tabletten aangezien deze multiparticulaire vormen een accurate dosering toelaten en een grote flexibiliteit bieden voor het aanpassen van de dosis in functie van het lichaamsgewicht van kinderen.

Hoofdstuk II bespreekt de ontwikkeling van kininesulfaat-pellets. Het eerste deel gaat in op de productie van kininesulfaat-pellets via extrusie-sferonisatie, waarbij de invloed van formulatie- en procesparameters op de pelletkwaliteit geëvalueerd werd. Pellets met een diameter van 300-700 μm werden geselecteerd omdat grotere partikels minder aangenaam aanvoelden in de mond, terwijl kleinere partikels de neiging hadden om in de mond te blijven kleven. Hoewel het mogelijk was om pellets met een hoge kininesulfaat-concentratie (tot 70%) te formuleren, werd de fractie aan kininesulfaat beperkt tot 20% aangezien bij hogere geneesmiddelconcentraties het totale aantal pellets per dosis te klein werd. Een extrusiescherm met perforaties van 400 μm liet toe pellets te produceren met een hoog rendement binnen de gewenste pelletfractie (300-700 μm). Het formuleren van kininesulfaat in pellets beïnvloedde het dissolutieprofiel van het geneesmiddel niet: in 0.1 N zoutzuur werd de volledige geneesmiddeldosis binnen de 10 minuten vrijgesteld.

Aangezien kininesulfaat een zeer bittere grondstof is en de formulatie bedoeld is voor pediatrische toepassingen, is het maskeren van de smaak van de kininesulfaat-pellets de tweede doelstelling van dit hoofdstuk. Als polymeer voor het omhullen van de kininesulfaat-pellets werd gebruik gemaakt van een waterige dispersie van Eudragit[®] E PO (11.4% w/w). Als emulgator en antikleefmiddel werden respectievelijk natriumlaurylsulfaat (10% w/w gebaseerd op droog polymeer gewicht) en

magnesiumstearaat (35% w/w gebaseerd op droog polymeer gewicht) toegevoegd. Daarnaast werden twee weekmakers (10-15% w/w gebaseerd op droog polymeer gewicht) geëvalueerd: stearinezuur en dibutyl sebacaat. Het coaten werd uitgevoerd in een wervelbed via de Würster methode en met behulp van bottom-spray, waarbij de procesparameters tijdens coating werden geoptimaliseerd. Het was mogelijk om de 300-700 µm pelletfractie door middel van een Eudragit® E PO dispersie te omhullen, maar het gebruik van 15% dibutyl sebacaat als een weekmaker in de formulatie veroorzaakte reeds na één week bewaring bij 40°C en 75% relatieve vochtigheid aggregatie van de pellets. Het reduceren van de weekmakerconcentratie tot 10% kon dit probleem niet voorkomen. Bovendien gingen bij deze concentratie aan weekmaker de smaakmaskerende eigenschappen van de formulatie verloren. De pellets waren minder gevoelig aan kleven indien stearinezuur werd toegevoegd als weekmaker. De dissolutiedata in water (pH ~7) toonden aan dat Eudragit® E PO de vrijstelling van kininesulfaat in speeksel voldoende kan vertragen, en op die manier de bittere smaak van de pellets kan reduceren. De vertraagde vrijstelling van kininesulfaat was gecorreleerd met de dikte van de polymeerfilm. De bitterheidscore van kininesulfaat geformuleerd in smaakgemaskeerde pellets werd geëvalueerd in functie van de tijd met behulp van een 'elektronische tong'. Deze resultaten toonden aan dat het coaten van kininesulfaat-pellets met 20% (w/w) Eudragit® E PO volstaat om de vrijstelling voldoende te vertragen en zodoende aan de patiënt toe te laten om de pellets zonder enig ongemak in te slikken.

De biologische beschikbaarheid van kininesulfaat in de ontwikkelde pellets werd beschreven in Hoofdstuk III. De orale biologische beschikbaarheid van kininesulfaat werd geëvalueerd bij 12 gezonde volwassen vrijwilligers. In een gerandomiseerde cross-

over studie werd de farmacokinetiek bepaald van een enkele dosis van 600 mg kininesulfaat, toegediend als smaakgemaskeerde pellets of als commercieel beschikbare tabletten. De plasmaconcentraties aan kinine werden bepaald via een gevalideerde HPLC-fluorescentie methode. Het toedienen van smaakgemaskeerde kininesulfaat-pellets verhoogde de plasmaconcentratie in vergelijking met tabletten (4.7 ± 0.8 vs. 3.7 ± 0.8 $\mu\text{g/ml}$) en de totale absorptie van kininesulfaat uit de pellets lag hoger ($\text{AUC}_{0-24\text{u}}$: 63.5 ± 13.9 vs. 54.2 ± 14.4 $\mu\text{g.u.ml}^{-1}$). Ongeacht het verschil in farmacokinetische parameters vielen de waarden van beide formulaties (pellets en tabletten) binnen de grenzen die in de literatuur gemeld werden.

In Hoofdstuk IV werd de doeltreffendheid van kininesulfaat, geformuleerd als smaakgemaskeerde pellets, geëvalueerd bij 56 kinderen jonger dan 5 jaar die lijden aan ongecompliceerde *Plasmodium falciparum* malaria. Elk kind werd gedurende 14 dagen opgevolgd. Een hoeveelheid kininesulfaat-pellets werd gemengd met een kleine hoeveelheid voedsel (sorghum pudding) en deze werden toegediend onder directe observatie van een verpleegster, verbonden aan de klinische studie. De patiënten werden gedurende 4 dagen gehospitaliseerd en dagelijks werden de parasitemie en lichaamstemperatuur (als parameters voor therapeutische respons) opgemeten. Om het plasmaconcentratie/tijdsprofiel van het kininesulfaat onder 'steady-state' omstandigheden te bepalen door middel van populatie-kinetiek werd één bloedstaal per kind genomen voor het ontslag uit het ziekenhuis (4^{de} dag).

De kininesulfaat-pellets werden goed getolereerd door de kinderen en allen voltooiden de volledige follow-up na 14 dagen. Na een behandeling van 72 u was de temperatuur gedaald tot een normaal niveau ($36.5 \pm 0.5^\circ\text{C}$) bij 91% (51/56) van de kinderen. Op de

7^{de} en 14^{de} dag werd geen temperatuurstijging opgemerkt. De gemiddelde tijd nodig om de temperatuur te doen dalen tot een normaal niveau was 60.7 ± 17.8 u.

Na 24u behandeling (3 geneesmiddeldosissen) daalde de parasitemie tot ongeveer 24% van de initiële parasitemie. De geschatte gemiddelde tijd voor een daling met 50% van de aanvankelijke parasieten besmetting (PCT₅₀) was 16.7 ± 5.5 u. Na 72 u behandeling was 75% van de kinderen aparitair en 25% had een lage parasitemie tussen 0.1 en 1.1% van de aanvankelijke besmettingsgraad. Bij ontslag uit het ziekenhuis (op de 4^{de} dag) waren alle patiënten parasietvrij en er werd geen nieuwe opstoot van parasieten vastgesteld bij een controle op de 7^{de} en 14^{de} dag. De gemiddelde parasitemie klaringstijd (PCT) werd geschat op 72.8 ± 16.5 u. De piekconcentratie tijdens steady state (14.9 ± 1.0 µg/ml) werd gemeten 3 u na de toediening van de dosis. 8 u na de geneesmiddeltoediening daalde de plasmaconcentratie tot 10.4 ± 2.1 µg/ml (range: 8.6-13.3 µg/ml), maar nog steeds binnen de therapeutische range. Er werd geen falen van de behandeling vastgesteld, waardoor de kininesulfaat-pellets een geschikte vorm zijn voor een flexibele aanpassing van de dosis in functie van het lichaamsgewicht bij de behandeling van malaria.

De ontwikkeling van een smaakloze suspensie op kininebasis wordt beschreven in Hoofdstuk V. Een niet-wateroplosbaar kininepamoaat-zout werd gevormd na neerslag van kinine hydrochloride met een dinatriumpamoaat-zout. Verschillende kininepamoaat-precipitaten werden bereid door het mengen van kinine hydrochloride en dinatriumpamoaat in verschillende molaire verhoudingen. Er werd een fijn kininepamoaat-neerslag (<20 µm) gevormd en twee viscositeitsverhogers (Avicel[®] RC 581 en xanthaamgom) werden gebruikt om de suspensie te stabiliseren. Een suspensie gemaakt met kinine hydrochloride/dinatrium pamoaat in molaire verhouding van 2/1

werd door vrijwilligers geïdentificeerd als smaakloos. In water werd na 45 min slechts 1.2% kinine vrijgesteld uit de suspensie, terwijl de afgifte in zoutzuur meer dan 80% bedroeg na 10 min. Dit suggereerde dat een snelle vrijstelling van kinine in de maag te verwachten is. De kininepamoaat-suspensie was stabiel en gemakkelijk opschudbaar na 6 maanden bewaren bij kamertemperatuur en 40°C, hoewel de partikelsedimentatie sneller was in de Avicel[®] RC 581 formulatie. De orale biologische beschikbaarheid van kinine in honden na toediening van de kininepamoaat-suspensie werd geëvalueerd, gebruik makend van een kinine hydrochloride-oplossing als referentie. Na het verlagen van de pH in de maag met pentagastrine verhoogde de gemiddelde relatieve biologische beschikbaarheid van kinine ten opzichte van de kinine hydrochloride-oplossing. Een verdere in-vivo studie met humane vrijwilligers is echter noodzakelijk om de resultaten bereikt bij honden te verifiëren.

Résumé

La plupart des médicaments autorisés sur le marché pharmaceutique existent sous des formes conçues pour les adultes. Le manque de formes pharmaceutiques à usage pédiatrique est un problème mondial. Par conséquent, le personnel de santé est obligé d'utiliser des médicaments soit non autorisés, soit autorisés mais selon des modalités d'administration non prévues par l'autorisation de mise sur le marché. Cette pratique est extrêmement dangereuse puisque les risques liés au fait que la sécurité, l'efficacité ou la qualité de ces médicaments n'ont pas été évalués.

Bien que la malaria soit un problème mondial, principalement en Afrique tropicale où les enfants constituent le groupe le plus vulnérable (en effet 75% de la mortalité observée chez les enfants de moins de 5 ans est attribué à la malaria), les formes médicamenteuses à usage pédiatrique font défaut pour la plupart des antipaludéens. Les enfants sont traités par fragmentation des médicaments développés pour les adultes. Pour la quinine, seuls les comprimés sont mentionnés sur la liste de l'Organisation Mondiale de la Santé (OMS) et existent sous des doses de 200 ou 300 mg par comprimé. L'adaptation posologique au poids corporel de l'enfant se fait par utilisation de comprimés sécables.

Le premier Chapitre de ce travail se base sur l'exactitude de la dose quand les comprimés de sulfate de quinine sont cassés. Une grande variabilité de poids liée à la présence et la position de la ligne de division a été observée pour les demis et quarts de comprimés. Cette variabilité de la dose administrée, pourrait avoir des répercussions au niveau de l'effet thérapeutique obtenu durant le traitement. Comme les formes galéniques pluriparticulaires (sphéroïdes) offrent plus de flexibilité à l'adaptation posologique au

poids corporel de l'enfant, elles sont proposées comme alternative aux comprimés sécables.

Le Chapitre II détaille le développement de sphéroïdes à base de sulfate de quinine dont le goût amer a été masqué. La première partie est basée sur la production de sphéroïdes par extrusion et sphéronisation. L'influence de la formulation et des paramètres de fabrication sur la qualité des sphéroïdes a été étudiée. Les sphéroïdes dont la taille est comprise entre 300 et 700 μm ont été choisis, car les sphéroïdes de taille inférieure étaient mal perçus dans la bouche avec une tendance à se coincer entre les dents. Pour des facilités d'administration, la quantité de substance active incorporée dans les sphéroïdes a été fixée à 20%, même s'il était possible d'incorporer jusqu'à 70% de substance active dans la formulation galénique. En effet, lorsque le taux de charge des sphéroïdes est élevé, la quantité de sphéroïdes à administrer est plus petite et donc plus difficile à prélever avec précision.

Pour l'extrusion, un tamis de 400 μm a été choisi pour la production de sphéroïdes de la taille désirée. Formuler le sulfate de quinine sous forme de sphéroïdes n'a pas affecté le profil de dissolution du médicament car la dissolution était complète après 10 min en milieu acide (HCl 0.1N). Comme la quinine est très amère et que les sphéroïdes étaient destinés à être utilisés chez l'enfant, le second objectif de ce chapitre a été consacré au masquage du goût. Une dispersion aqueuse à base du polymère Eudragit[®] E PO (11.4% w/w) a été utilisée pour l'enrobage des sphéroïdes. Le lauryl sulfate de sodium (10% w/w par rapport au polymère) a été utilisé comme émulsifiant. Deux plastifiants, à savoir le dibutyl sebacate et l'acide stéarique (10-15% w/w par rapport au polymère), ont été évalués. Le stéarate de magnésium (35% w/w par rapport au polymère) a été utilisé pour

éviter les problèmes de collage. Le pelliculage a été effectué à l'aide d'un équipement en lit d'air fluidisé via la méthode Würster avec bottom-spray et les conditions d'enrobage ont été optimisées. Des sphéroïdes de taille comprise entre 300 à 700 µm ont été pelliculés avec succès à l'aide de la dispersion aqueuse à base du polymère Eudragit® E PO mais, après une semaine de conservation à 40°C et 75% d'humidité relative, une agglomération des sphéroïdes a été observée. Ce problème a été attribué à la présence du dibutyl sebacate. La réduction de la concentration du dibutyl sebacate à 10% n'a pas résolu le problème d'agglomération et a entraîné la perte du masquage de goût. Ce problème d'agglomération a été résolu par remplacement du dibutyl sebacate par l'acide stéarique (15% w/w par rapport au polymère). Le profil de dissolution (pH~7) a démontré que l'Eudragit® E PO peut suffisamment retarder la libération de la quinine dans la salive, et par conséquent réduire le goût amer. Ce retard de libération est fonction du degré d'enrobage. Le degré d'amertume en fonction du temps a été évalué en utilisant la langue électronique. Les résultats ont montré qu'un pourcentage d'enrobage pouvant aller jusqu'à 20% (w/w) peut suffisamment retarder l'apparition de l'amertume et permettre au patient d'avaler les sphéroïdes sans aucun problème.

L'étude de biodisponibilité du sulfate de quinine formulé sous la forme de sphéroïdes pelliculés à goût masqué est décrite au Chapitre III. La biodisponibilité orale du sulfate de quinine a été évaluée chez 12 volontaires sains au cours d'une étude randomisée. Les paramètres pharmacocinétiques ont été déterminés après prise d'une dose unique de 600 mg de sulfate de quinine administrée soit sous forme de sphéroïdes pelliculés ou sous forme de comprimés disponible sur le marché. Les concentrations de quinine ont été déterminées à l'aide d'une méthode analytique validée utilisant la chromatographie

liquide à haute performance avec détecteur de fluorescence. Les pics plasmatiques (C_{\max}) obtenus pour les sphéroïdes et les comprimés étaient respectivement de 4.7 ± 0.8 et 3.7 ± 0.8 $\mu\text{g/ml}$.

La biodisponibilité du sulfate de quinine administré sous la forme de sphéroïdes à goût masqué est significativement plus élevée que celle obtenue après administration de comprimés ($\text{AUC}_{0-24\text{h}}$: 63.5 ± 13.9 vs. 54.2 ± 14.4 $\mu\text{g.h.ml}^{-1}$).

Dans le Chapitre IV l'efficacité du sulfate de quinine formulé sous forme de sphéroïdes, a été évaluée, chez 56 enfants de moins de cinq ans souffrant du paludisme à *Plasmodium falciparum* non compliqué. La durée de l'étude a été fixée à 14 jours. La quantité adéquate de sphéroïdes de sulfate de quinine à goût masqué a été mélangée avec une petite quantité de nourriture (bouillie de sorgho) et administrée sous la supervision directe de l'infirmière impliquée dans l'étude. Les patients étaient hospitalisés durant 4 jours, la parasitémie et la température corporelle (comme signe de réponse thérapeutique) étaient évaluées chaque jour. Pour la détermination du profil des concentrations plasmatiques, un échantillon de sang par enfant a été prélevé avant la sortie de l'hôpital (4^{ème} jour). La formulation (sphéroïdes de sulfate de quinine à goût masqué) a été bien acceptée par les enfants et tous ont terminé le suivi de 14 jours. Après 72 heures de traitement, la température axillaire est retournée à la normale ($36.5 \pm 0.5^\circ\text{C}$) chez 91% des patients (51/56). Aucune élévation de température n'a été observée au 7^{ème} et 14^{ème} jour. Le temps nécessaire pour que la température retourne à la normale était de 60.7 ± 17.8 h. Durant les premières 24 heures de traitement (3 doses), la parasitémie a chuté à 24% de la charge parasitaire initiale. Le temps moyen pour que la parasitémie initiale chute de 50% (PCT_{50}) était de 16.7 ± 5.5 h. Après 72 heures de traitement, 75% d'enfants étaient sans

parasites et 25% avaient une parasitémie variant entre 0.1 et 1% de la parasitémie initiale. A la sortie de l'hôpital au 4^{ème} jour, tous les patients n'avaient plus de parasites dans le sang et aucune recrudescence de la parasitémie n'a été observée aux contrôles du 7^{ème} et 14^{ème} jour. Le temps requis pour la clairance totale de la parasitémie a été estimé à 72.8 ± 16.5 heures. Le pic de concentration en phase d'équilibre (14.9 ± 1.0 µg/ml) a été atteint en 3 heures après l'administration du médicament. Huit heures après l'administration, la concentration plasmatique était descendue à 10.4 ± 2.1 µg/ml (intervalle: 8.6-13.3 µg/ml) se situant toujours dans l'intervalle thérapeutique. Aucun échec du traitement n'a été enregistré. La formulation galénique sous la forme de sphéroïdes à goût masqué s'est donc révélée être un outil flexible à l'adaptation posologique au poids corporel de l'enfant durant le traitement de la malaria.

Le développement d'une suspension de quinine à goût masqué est décrit au Chapitre V. Un sel de pamoate de quinine insoluble dans l'eau a été formé après précipitation du chlorhydrate de quinine par le pamoate disodique. Un précipité de pamoate de quinine (<20 µm) a été obtenu et deux agents viscosifiants (Avicel[®] RC 581 et gomme xanthane) ont été utilisés pour stabiliser la suspension. Une série de précipités de pamoate de quinine a été préparée par mélange des solutions de chlorhydrate de quinine et du pamoate disodique dans des proportions molaires différentes. Une suspension issue de la précipitation du chlorhydrate de quinine par le pamoate disodique dans des proportions molaires de 2/1 était déclarée sans goût amer par les volontaires. Seulement 1.2% de quinine étaient dissous dans l'eau pendant 45 minutes de test, alors que plus de 80% du médicament étaient dissous pendant les premières 10 minutes en milieu acide chlorhydrique. Cela suggère une libération rapide de la quinine dans l'estomac. Après 6

mois de stockage à température ambiante et à 40°C, la suspension de pamoate de quinine était stable et facilement redispersible. Cependant une sédimentation rapide des particules a été observée dans la formulation à base d'Avicel[®] RC 581 comme agent viscosifiant. La biodisponibilité de la quinine après administration de la suspension du pamoate de quinine a été évaluée chez six chiens à jeun (utilisant la solution de quinine chlorhydrate comme référence) après réduction du pH stomacal avec la pentagastrine. La biodisponibilité relative moyenne de quinine administrée sous la forme de la suspension (comparée à la solution de quinine chlorhydrate) est augmentée et aucune différence significative en termes d'absorption n'a été observée entre les deux formulations. Afin de vérifier la corrélation des résultats obtenus chez le chien, des investigations ultérieures réalisées chez l'homme sont néanmoins nécessaires.