Taste-masked and Sustained-release Formulations of Chloroquine

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by

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ABSTRACT

Chloroquine remains the treatment of choice for malaria in many countries despite the appearance of drug resistance. Even with children, chloroquine is normally administered orally, despite its bitter taste which is not masked by sweetening agents. The aim of this work was to formulate a taste-masked, sustained release preparation of chloroquine microcapsules using a multiple emulsion/solvent extraction technique.

In the first part of this work, surface rheological measurements were used to establish the film forming properties of different types of acacia, which is the most common polymer used in this process. Of the three types investigated, acacia tears were found to exhibit the highest surface viscoelasticity and were therefore used for all subsequent studies.

Direct addition of chloroquine diphosphate to aqueous solutions of anionic polyelectrolytes such as carrageenan and Eudragit® L100, resulted in precipitation due to an acid-base reaction occurring between the cationic drug and the anionic polymer. It was therefore not possible to incorporate the drug in the inner aqueous phases of multiple emulsions containing these polymers (the original method of preparation). This was overcome by using a modified method which incorporated the drug in the middle oily layer of the multiple emulsion. Diffusion studies demonstrated the partitioning of chloroquine from an oily phase to an aqueous phase, the amount of which was enhanced in the presence of anionic polyelectrolytes in the aqueous phase, which bound to the basic chloroquine molecules and acted as a "sink".

Scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) were used to study the physical state of the drug present in the polymer matrix after solvent extraction. This was done by investigating cast chloroquine-ethylcellulose films. Although chloroquine crystals were present in the films as shown by SEM, DSC was unable to detect the presence of such an entity.

In order to establish a manufacturing procedure, the following manufacturing parameters were investigated using a model formulation: (1) the phase volume ratios of both the primary and secondary emulsions, (2) concentration of polymers and (3) the different methods used for solvent extraction and drying.
The effects of these parameters on the surface morphology and particle size distribution of the microcapsules were determined. The rate of drug release was found to be dependent on the pH of the dissolution medium and was also found to increase with a decrease in the particle size distribution of the microcapsules.

The combination consisting of Eudragit® L100 as the inner aqueous phase polymer, Ethylcellulose N10 NF as the oily phase polymer and acacia as the outer aqueous phase polymer was chosen for further development. The chloroquine content of these microcapsules increased with the starting amount of chloroquine (theoretical content) and the Eudragit® L100 concentration in the inner aqueous phase. Increasing the Eudragit® L100 concentration in the inner aqueous phase improved the sustained release characteristic of the formulation. The rate of drug release was decreased by increasing the ethylcellulose concentration in the middle oily phase of the multiple emulsion. Annealing these microcapsules decreased the rate of drug release. Stability studies were then performed on the final formulation selected for the in vivo study.

The formulation of chloroquine (30.7% w/w) microcapsules (0.75% w/v Eudragit® L100 / 10% w/v Ethylcellulose N10 NF / 8% w/v Acacia) was submitted for clinical study with six healthy volunteers. There were no significant differences in the bioavailabilities of the microcapsule formulation and the chloroquine diphosphate tablets (reference dosage form). The elimination half lives, the maximum plasma concentrations and the time at which they occur, were however significantly different, showing sustained release. The inter-subject variation was notably smaller with the microcapsule formulation compared with the tablets. Stored microcapsules showed similar rate of drug release to the fresh microcapsules but a higher peak concentration was observed probably due to experimental error.
To my family

with love and grateful thanks.
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INTRODUCTION
Chapter One

Malaria and Chloroquine
Malaria in man is caused by infection with one or other of four species of *Plasmodia* - *Plasmodium falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. The diseases that these four organisms produce were previously referred to in terms of their characteristic clinical feature, which is the type of febrile cycle. The cycle may be referred to as quotidian, tertian and quartan fevers, denoting 24, 48 and 72 hour cycles of fever respectively. Other names were then designated according to the additional clinical features of the disease. The modern tendency is to refer to the various types of malaria by the names of the causative agents.

The clinical features of malaria, namely the febrile cycle, is a result of the life cycle of the *Plasmodia* in the human host. Most antimalarial drugs have their main effects at particular stages of the development of the malarial parasite. Therefore, a knowledge of its life cycle is important for the understanding of the etiology of the disease and the proper use of antimalarials for its treatment.

### 1.1.1 The Life Cycle of Plasmodium

The life cycle and transmission patterns of all four species, *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*, are fundamentally similar (Figure 1.1) though there are differences which are important in relation to pathogenicity and treatment. Various species of anopheline mosquitoes are definite hosts of the malarial parasites. Of 200 known species, approximately 60 are considered to be vectors of malaria (Markell *et al.* 1986).

When a female mosquito bites an infected person, she draws into her stomach, blood which may contain both male and female gametocytes. The male or microgametocyte undergoes a process of maturation, resulting in the extrusion of spindle-shaped microgametes through a process termed exflagellation. At the same time, the female or
Figure 1.1 Life cycle of the *Plasmodia* protozoa.
(From Cruickshank, Duguid, Marmion and Swain (1973).)
macrogametocyte matures to become a macrogamete. It may then be fertilized by a microgamete, forming a zygote which becomes elongated and active and is called an ookinete. The ookinete penetrates through the stomach wall of the mosquito and ends up beneath the outer covering of the stomach to become an oocyst. Growth and development of the oocyst result in the production of a number of thread-like sporozoites. This occurs over about 14 days, depending on the external temperature, the mosquito host involved and the specie of the parasite (Warhurst 1987). The sporozoites will then travel to the duct of the secretory glands of the mosquito and may be inoculated into the next person bitten.

Sporozoites injected into the blood stream leave the host's vascular system within 40 minutes and invade the parenchymal cells of the liver. Subsequent development of *P. falciparum* and *P. malariae* differs from that observed in *P. ovale* and *P. vivax* in that asexual multiplication (exoerythrocytic schizogony) occurs immediately in the two former species whereas the latter two species undergo a resting stage. The resting stage of the parasite is known as the hypnozoite (Krotoski 1985) and is thought to bring about relapses characteristics of *P. ovale* and *P. vivax* infections. *P. falciparum* and *P. malariae* do not possess this dormant phase (Panisko and Keystone 1990) and relapses do not occur in diseases caused by these species.

Asexual multiplication (exoerythrocytic schizogony) of the mature sporozoites, known as exoerythrocytic schizonts during the stage of multiplication in the parenchymal cells of the liver, results in the production thousands of uninucleate merozoites. These are released into the circulation when the infected liver cells rupture. The number of merozoites released by the infected liver cells varies with species. *P. falciparum* releases 40 000 merozoites per schizont; *P. ovale*, 15 000; *P. vivax*, 10 000 while *P. malariae* releases only 2000 per schizont (Black and Craig 1986). The exoerythrocytic cycle in the liver takes 5.5 to 7 days in *P. falciparum*, 6 to 8 days in *P. vivax*, 9 days in *P. ovale* and 13 to 16 days in *P. malariae* (Garnham 1966). No symptoms appear during this stage which is why it is known as the minimum incubation period. However, the actual incubation period usually observed are 12 days for *P. falciparum*, 13 to 17 days for *P. vivax* and *P. ovale* and 28 to 30 days for *P. malariae* (Bruce-Chwatt 1985).
Merozoites released into the blood stream by the exoerythrocytic schizonts may have two destinations. They may invade other parenchymal cells of the liver and initiate secondary and continuing cycles of exoerythrocytic schizogony or invade erythrocytes, initiating erythrocytic schizogony. Secondary and subsequent cycles of exoerythrocytic schizogony do not occur with *P. falciparum* infections and therefore symptomatic malaria following apparent resolution of the primary attack of falciparum malaria (recrudescences) seldom occur more than 1 year after the primary attack of *P. falciparum*. Recrudescences in falciparum malaria was considered to be a result of persistant erythrocytic forms alone. *P. malariae* may persist in the blood for many (20 to 50) years because of numerous exoerythrocytic cycles as well as persistant erythrocytic forms (Garnham 1981, 1984). This is unlike relapses of *P. vivax* or *P. ovale* malaria which is due to the dormant hypnozoites present in the liver and may continue at intervals for 3 to 4 years. True relapses do not occur in falciparum malaria.

Malarial paroxysms occur at intervals related to the timing of the erythrocytic division cycle of the species concerned. It results from the invasion and rupture of erythrocytes by the merozoites. These merozoites ingest 25% to 75% of the red cell content and develop into mature trophozoites. The trophozoites then undergo asexual multiplication (erythrocytic schizogony) during which they are known as erythrocytic schizonts. The erythrocyte ruptures and each schizont releases about 4 to 36 merozoites within 48 to 72 hours, depending on the species involved. These merozoites in turn invade more erythrocytes thus propagating the infection.

Rupture of the erythrocytes liberates products of metabolism of the parasites and of the red cells. It is thought that if large numbers of erythrocytes were to rupture simultaneously, the volume of toxic material passed into the blood stream could be sufficient to bring about malarial paroxysm. Generally, in the initial stages of infection, rupture of the erythrocytes is not synchronous so that fever may be continuous or remittent rather than intermittent in nature (Gilles 1989). A cycle of erythrocytic schizogony takes 48 hours for *P. falciparum, P. vivax* and *P. ovale* and therefore these give rise to febrile attacks every three days (tertian fever). In *P. malariae*, fever occurs every fourth day (quartan
fever) as the erythrocytic schizont takes 72 hours to mature. However, the degree to which attacks follow the tertian or quartan patterns depends on the immunological history of the patient and whether two or more 'broods' of the parasite are present (Warhurst 1987). In mixed infections with two or more species, or in the early stages of infection with one species, there may be daily (quotidian) paroxysms or even double paroxysms in one day.

After one or more cycles of erythrocytic maturation, usually not until the patient has become clinically ill, some of the merozoites differentiate into the sexual forms or gametocytes (gametogenesis) in the red blood cells. This is probably induced by environmental factors such as the immune status of the host, sub-curative drug level and metabolic stress (Carter and Miller 1979). Gametocytes continue to circulate in the bloodstream for some time and if ingested by the female anopheles mosquito, undergo the sexual cycle of gametogony and subsequently sporogony in the mosquito.

1.1.2 Symptomatology

Each febrile cycle or malarial paroxysm, is typically ushered in with a sudden chill or rigor. This may last between 10 to 15 minutes or even longer. During this period of chill, the patient complains of extreme cold despite an elevation of body temperature throughout this stage. This is followed immediately by a hot stage where the patient becomes flushed suddenly. The hot stage lasts from 2 to 6 hours in vivax and ovale malaria, 6 hours or more in malariae malaria and considerably longer or even continuous for falciparum malaria. After the hot stage, the patient begins to perspire profusely for several hours and usually feels quite well until the onset of the next cycle or paroxysm. This may be a matter of only a few hours in the case of falciparum malaria (Markell et al. 1986).

The primary attack of malaria may be preceded by prodromal symptoms of a non-specific sort: headache, malaise, photophobia, muscular aches and pains, anorexia, nausea, dizziness and sometimes vomiting. It is therefore commonly diagnosed incorrectly as influenza or viral hepatitis.
(Gilles 1989). These symptoms may be seen in all types of malaria but are at times absent in vivax malaria and mild in ovale malaria.

Cerebral malaria is a rapidly progressive encephalopathy with up to 50% mortality (WHO 1986). It is often seen as a complication of *P. falciparum* infection but it has been reported to occur in the other types of the disease (Markell *et al.* 1986). Severe *P. falciparum* infection resulting in cerebral malaria is caused by the sequestration of parasitised erythrocytes in deep vascular beds in the brain. The initial symptoms are similar to uncomplicated malaria but it is usually followed by altered consciousness within a day or two. Coma may also develop suddenly in patients exhibiting no clinical symptoms. Unless prompt treatment is instituted, this condition is usually fatal, especially in children (Phillips and Solomon 1990). Many adult patients do make a full neurological recovery despite profound and sometimes prolonged coma (Harinasuta and Bunnag 1988). Neurological sequelae of cerebral malaria in children is more severe and may be an important cause of neurological handicap in the tropics (Brewster *et al.* 1990).

Blackwater fever is most frequently seen in falciparum malaria although it sometimes develops in other types of the disease. It is thought to be due to intravascular haemolysis resulting in haemoglobin in the urine. Destruction of erythrocytes may be rapid leading to anaemia, which may be exacerbated by the depletion of iron store due to haemozoin formation in the metabolism of haemoglobin by the parasites. Quinine sensitivity has been considered by some as a factor inducing haemolysis. It has also been suggested that invaded erythrocytes may act as antigens, giving rise to antibodies which act as haemolysins.

Algid malaria may develop during the course of an apparently uncomplicated falciparum attack. The body temperature falls rapidly and the patient may become delirious. Symptoms of vascular collapse and shock develop quickly. There may be severe abdominal pain, vomiting and diarrhoea and muscular cramps. Death may ensue rapidly or the disease can pursue a less acute course. Patients having recovered from algid malaria may however develop signs resembling Addison's disease. It is generally considered that adrenal failure is basic to the development of the algid form of malaria.
Acute renal failure may result from an attack of blackwater fever but it may also occur due to acute tubular necrosis brought on by renal anoxia. The nephrotic syndrome due to acute glomerulonephritis has also been reported with falciparum malaria. Proteinuria, renal lesions and other complications due to tissue anoxia such as hyperpyrexia, bilious remittent fever, dysentric malaria and fluid retention resulting in pulmonary oedema have also been reported as a result of malaria (Markell et al. 1986).

1.1.3 Epidemiology.

Figure 1.2 shows the world-wide distribution of malaria in 1988 which spreads over some 100 countries or areas (WHO 1990a) and Gilles (1989) estimated that over 50% of the world's population is at risk from malaria. In 1979, it was estimated that malaria was responsible for 1.2 million deaths annually (Walsh and Warren 1979) but the true figure is probably higher since in Africa alone, the disease is considered to be responsible for the death of 1 million children each year (Bruce-Chwatt 1979). Other data suggests that there are 200 to 300 million cases of malaria with more than 2 million deaths each year (Wyler 1983). Statistics showed that in 1987, 103 million cases were reported worldwide and an estimated 264 million people were infected with malaria parasites (WHO 1988b). It has also been estimated that in Africa alone, 90 million clinical malaria cases may occur and that the prevalence of infection in Africa may be in the order of 250 million parasite carriers (WHO 1990a).

Falciparum and ovale malaria are primarily diseases of the tropics whereas malariae malaria is seen in the subtropics and temperate zones as well. Vivax malaria, on the other hand, is common in all endemic areas. *P. falciparum* and *P. vivax* are considered highly infectious strains
Figure 1.2 Epidemiological assessment of the status of malaria, 1988 (World Health Organisation 1990a).
while the other two are much less so (WHO 1987). Of the four, *P. falciparum* poses the greatest threat and is responsible for the high mortality in children and non-immune adults in endemic areas. This is due to its ability to invade erythrocytes of any age and its short incubation time which produce overwhelming parasitemias (Field 1949). The other three species invade only young (*P. ovale* and *P. vivax*) or old (*P. malariae*) erythrocytes (Neva 1977) and have longer incubation time. The propensity of *P. falciparum* to develop resistance to antimalarial agents such as chloroquine and to an increasing extent to quinine (Payne 1987) makes it an even greater threat as a result of treatment failure.

1.1.4 Antimalarials

Antimalarial drugs can be categorised by the stage of plasmodia against which they have therapeutic efficacy (Webster 1985).

**Tissue schizonticides** act on the initial hepatic tissue form of the malarial parasite to prevent the development of the erythrocytic stage of the disease. They are therefore used as prophylactics and also as anti-relapse agents by acting on the latent hypnozoites in the liver. The prophylactics comprise of the biguanides (e.g. proguanil), the diaminopyrimidines (e.g. pyrimethamine and trimethoprim) and the sulphonamides (e.g. sulphalene and suphadoxine). The only anti-relapse agent used currently is primaquine, an 8-aminoquinoline (Panisko and Keystone, 1990). It causes adverse reactions in patients with hereditary nicotinamide adenine dinucleotide (NAD) methaemoglobin reductase deficiency due to methaemoglobinaemia which results in cyanosis and marked haemolysis in those with glucose-6-phosphate (G-6PD) deficiency.

**Blood schizonticides** are the largest group of antimalarials. They act on the asexual forms of the parasites in the red blood cells and hence produce clinical cure. They include the cinchona alkaloids (e.g. quinine and quinidine), the 4-aminoquinolines (e.g. chloroquine and amodiaquine), the 4-quinoline methanols (e.g. mefloquine) and the sesquiterpene lactones (e.g. qinghaosu). The tetracyclines (e.g. tetracycline and
doxycycline) and clindamycin which are slow-acting blood schizonticides are normally administered sequentially with quinine.

**Gametocytocides** destroy the sexual forms of the parasite which are necessary to perpetuate the next stage of the cycle in the mosquito vector. Examples are the 8-aminoquinolines (anti-relapse agents) which act on all four species and the sulphonamides (prophylactics) which do not act on gametocytes of *P. falciparum*.

**Sporontocides** inhibit the formation of oocysts and sporozoites in the mosquito vector. To be effective, they must be taken up into the vector when it is feeding. The biguanides and the aminopyrimidines which are prophylactics, also act as sporontocides.

The latter two groups of antimalarials do not confer any benefit on the infected persons but could limit the spread of the disease in endemic areas.

### 1.1.5 Drug resistance

Drug resistance has been defined by the World Health Organisation as 'the ability of a parasite strain to multiply or to survive in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their multiplication. Such resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding maximum doses tolerated by the host) (WHO 1963). Three grades of resistance were described, ranging from RI, in which administration of the drug achieves apparent clinical cure only to be followed by recrudescences, to RIII, in which therapy is completely ineffective.

The first recorded instance of drug-resistant malaria occurred in Brazil early this century with the discovery of quinine resistant *P. falciparum*. Despite the widespread use of quinine before the advent of synthetic antimalarials, significant quinine-resistance is limited mainly to the
South East Asia and the situation remains the same today (Panisko and Keystone 1990). Quinidine, the dextrorotatory isomer of quinine, appears to be just as susceptible to resistance if not more (Phillips et al. 1985; Rudnitsky et al. 1987; White et al. 1981).

Chloroquine resistance has not been reported for P. ovale and P. malariae infections but chloroquine-resistant P. falciparum is widespread (Spencer 1986). Cases of chloroquine-resistant P. vivax infection has also been reported recently in the Southwest Pacific (Peters 1990). Chloroquine-resistant P. falciparum (CRPF) will be discussed in greater detail in Section 1.2.3.

With the worldwide spread of chloroquine-resistant falciparum malaria, the combination of pyrimethamine/sulphadoxine has been used extensively. Widespread pyrimethamine/sulphadoxine resistance in P. falciparum is now established in South East Asia, Bangladesh, Oceania, the Amazon basin and parts of East Africa (Doberstyn 1984; Farraroni et al. 1983; Pinichpongse et al. 1982; Weniger et al. 1982). P. vivax is also often resistant to this combination drug. When used on their own without chloroquine, pyrimethamine/sulphadoxine combination often results in treatment failure against P. vivax infection. However, resistance to these drugs occur sporadically, always in areas where they have been used extensively. If the drugs are withdrawn from an area, susceptibility to the compounds returns slowly (Cowman and Foote 1990).

Mefloquine is the newest established antimalarial in the armamentum. However, definite resistance has already been reported in the South East Asia where it is used primarily against multiple-resistant P. falciparum infection (Boudreau et al. 1982; Hoffman et al. 1985). In an attempt to 'protect' the efficacy of the drug and prevent the further development of drug resistance, the WHO has recommended that mefloquine should only be administered in cases where chloroquine or sulphadoxine/pyrimethamine are ineffective (WHO 1990c). However, findings of decreased susceptibility of P. falciparum to mefloquine, parallel to that of quinine in Thailand between 1982 and 1984 (Suebsaeng et al. 1986), and in West Africa before the drug was introduced there (Oduola et al. 1987), raise the question of primary mefloquine drug resistance. In fact, the increasing number of reports of multidrug resistant
*P. falciparum* is causing a major crisis in the selection and use of chemotherapeutic drugs for the treatment and prophylaxis of malaria.

Data on resistance to other drugs used to treat severe falciparum malaria are limited. However, it has become increasingly clear that no antimalarial drug can escape the problem of drug resistance. It must also be emphasized at this point that comprehensive patterns of resistance cannot be mapped out on a countrywide or regional basis and are restricted to discrete study locations (Phillips-Howard 1989).

1.1.6 Chemoprophylaxis

Travelers can decrease their risk of malaria by taking small oral doses of antimalarials although no drug guarantees protection against the disease (Spencer 1985). Chloroquine is the mainstay of antimalarial chemoprophylaxis and should be taken by virtually all travellers at risk of acquiring malaria (Herwaldt *et al.* 1988). Amodiaquine is no longer recommended for chemoprophylaxis even though it has greater activity than chloroquine against some *P. falciparum* isolates with low-level chloroquine-resistance (Spencer *et al.* 1984; Watkins *et al.* 1984). It has been found to cause severe hepatitis (Larrey *et al.* 1986; Neftel *et al.* 1986) and agranulocytosis (Centers for Disease Control 1986; Rhodes *et al.* 1986).

The chemoprophylaxis of chloroquine-resistant falciparum malaria is difficult because the pyrimethamine/sulphadoxine combination (Fansidar) may produce fatal reactions such as erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis (Miller *et al.* 1986). Travelers are advised to take weekly chloroquine chemoprophylaxis and carry a therapeutic dose of pyrimethamine/sulphadoxine (3 Fansidar tablets) for presumptive treatment where clinical aid is not readily available (Centers for Disease Control 1988). Weekly chloroquine-pyrimethamine/sulphadoxine chemoprophylaxis may be used in prolonged, extensive exposure in high risk areas for chloroquine-resistant falciparum malaria where medical care is not readily available. However, resistance to this combination has
been reported in East Africa (Miller et al. 1986) and resistance to these drugs is widespread in rural Thailand.

Important considerations in selecting a chemoprophylactic regimen include the geographic areas to be visited, the prevalence of chloroquine-resistant *P. falciparum* infection in those areas, the time of year and extent of exposure (Centres for Disease Control, 1985, 1986, 1987, 1988, 1989; WHO 1984). Warhurst (1987), Keystone (1990), and many others reviewed the current chemoprophylactic regimens for malaria and gave recommendations according to geographical locations. However, no set of recommendations for malaria is uniformly accepted by those who are responsible for giving this advice (WHO 1988a).

1.1.7 Diagnosis

The cornerstone of treatment of malaria is an accurate diagnosis. Empiric treatment is often used as a public health strategy in the tropics. However, interpretation of symptoms in individual patients in the developing world is difficult because the symptoms in the initial stage of a primary attack of malaria is non-specific. As a result, it is commonly diagnosed incorrectly as influenza or viral hepatitis.

A high index of suspicion is therefore necessary for the diagnosis of malaria and parasitological confirmation should be obtained (Gilles 1989). This is done by means of well-stained thick and thin blood films which allows identification of parasites (Markell et al. 1986). Of similar importance is the timing of blood examination. Depending on the stage of the developmental cycle at which the blood sample is taken, different stages of the parasite will appear in the blood film. Merozoites are impossible to identify by species. However, gametocytes may be present and readily identified. If parasites are not present in the first blood film, additional blood films should be taken every 6 to 12 hours for as long as 48 hours if necessary for specific diagnosis. For the purpose of ruling out malaria in a patient with fever of unknown origin, serologic tests may be used. These tests are, however, not widely available.
1.1.8 Chemotherapy.

The widespread drug-resistant strains of *P. falciparum* has made the treatment of malaria more complicated. To obtain appropriate treatment, it is important that the clinician has identified the infecting species, estimated the degree of parasitaemia, determined where the infection was probably acquired and assessed whether or not drug-resistance is prevalent in that area. The clinical status of the patient will determine whether oral or parenteral therapy is appropriate and whether ancillary support measures are required. An example is the exchange blood transfusions which has been reported to be efficacious, especially for the treatment of cerebral, hepatic and renal complications of falciparum malaria under selected circumstances (Markell et al. 1986). It has been recommended that parenteral therapy should be replaced by oral therapy as soon as possible, especially when chloroquine is used.

The three separate stages of the plasmodial life cycle that occur in man cannot be treated successfully with any single drug. Thus, a combination therapy should be devised, bearing in mind the spectrum of activity of the various drugs and their modes of action.

Chloroquine is the drug of choice for treating infections caused by all malarial species except *chloroquine-resistant P. falciparum* (Krogstad et al. 1988). There is no evidence that severe infections caused by chloroquine-susceptible strains of *P. falciparum* are treated more effectively with quinine (Chongsuphajaisiddhi 1986). However, in multidrug-resistant areas, quinine remains the cornerstone of treatment for *P. falciparum* infection (Gilles 1988). It is normally combined with tetracycline or pyrimethamine/sulphadoxine in countries where the latter is still effective. Recently, mefloquine has also been used in combination with quinine against multidrug-resistant *P. falciparum* infection.
1.1.9 New Drugs

Halofantrine, a 9-phenanthralenemethanol, is a blood schizonticide. Recent clinical data indicated that it is effective for the treatment of chloroquine-resistant falciparum malaria. The WHO has recommended that halofantrine should only be used against multidrug-resistant P. falciparum infection even though there is some concern about the variations in the bioavailability of the currently available oral formulation (WHO 1990b).

The artemisinine (qinghaosu) derivatives, such as artemether, sodium artesunate are very promising alternatives for the treatment of severe and complicated malaria. However, further formulation, toxicity and clinical studies are necessary as very little information about these is available (WHO 1990b). These blood schizonticides are not available commercially.

1.1.10 Other Preventive Methods

The spread of malaria continues today despite chemotherapeutic intervention and vector control programmes. This is due to the rapid spread of single or multidrug-resistant P. falciparum infections and the insecticide-resistance of the mosquito vector. Mosquito control measures have been further handicapped by the increasing awareness of the potentially harmful effects of insecticides. Methods which are useful for the individual are measures of proper screening such as the use of mosquito repellents and the wearing of long-sleeved shirts and long trousers in the evening hours when anopheline mosquitoes bite. One other control measures which has been under investigation is a malaria vaccine.

Nussensweig et al. (1969) and Rieckmann et al. (1979) have shown that immunisation with x-irradiated sporozoites in humans and animals is possible. It was shown that immunised individuals develop antibodies
which are directed against the circumsporozoite (CS) protein which covers the parasite surface (Vanderbreg et al. 1969). However, Ballou et al. (1987) carried out a human study using a formulation of falciparum sporozoite vaccine and found it to be unsuitable for use in the field.

Synthetic peptides corresponding to fragments of falciparum merozoite-specific proteins were shown to induce protection in animals experimentally infected (Pattarayo et al. 1987). The development of parasitaemia was significantly delayed or suppressed when a vaccine based on these peptides was used in humans (Pattaryo et al. 1988). However, due to the high degree of antigen polymorphism, strong protective immunity is achieved only after exposure to a large number of variant forms.

Unlike most candidate antigens of malaria vaccines, Kaslow et al. (1991) described a surface protein of the falciparum zygote, Psf25, which is not limited by immunological recognition. Transmission of infection from the host to the mosquito can be blocked by monoclonal antibodies to this surface protein which blocks the formation of oocysts and hence sporozoites. This is therefore an altruistic vaccine which does not protect the individual but the community as it only serves to block further transmission of the disease. This vaccine could start clinical trials in the United States of America within two years (Brown 1991).
1.2 CHLOROQUINE

1.2.1 Introduction

Chloroquine remains the treatment of choice for malaria despite the appearance of chloroquine-resistance. It was the first of the 4-aminoquinolines to be synthesized as substitutes for quinine (James and Gilles 1985). Its spectrum of activity is similar to that of quinine, being an effective blood schizonticide as well as having gametocidal activity. Its action is however, more rapid than that of quinine.

Chloroquine is the most widely used antimalarial because it is cheap, widely available and well tolerated. In addition, it is effective against the acute, life-threatening form of the disease, cerebral malaria due to its rapid onset of action. Chloroquine is also useful in the treatment of intestinal forms of amoebiasis and has other pharmacological properties at doses in excess of those required for antiparasitic activity. Some of these may appear as side effects or toxic effects in some individuals undergoing malaria chemotherapy using high doses of chloroquine. The other effects of chloroquine that have some therapeutic significance are its anti-inflammatory action and its quinidine-like cardiac effects.

1.2.2 Mechanisms of Action

Chloroquine has no effect against the exoerythrocytic stage of the Plasmodium parasite. It is highly effective and acts rapidly against the asexual erythocytic forms of P. ovale, P. vivax, P. malariae and chloroquine-sensitive P. falciparum. It is also effective against the gametocytes of P. ovale, P. vivax, P. malariae and immature forms of P. falciparum. The mode of action of chloroquine has yet to be defined but there are two prevailing views.
Chloroquine is a weak base and is therefore able to permeate biomembranes in the unionised form. As a result, the molecules would tend to localise in acidic vesicles such as the lysosome due to de-protonation (weak base effect). Vander Jagt et al. (1987) found that proteases necessary for the metabolism of haemoglobin have acidic pH optima and are concentrated in the lysosome. They postulated that chloroquine may inhibit the actions of these proteases and thereby starve the parasites. But on the other hand, their studies also indicated that the proteases have broad pH optima and therefore the activity of the proteases may only be inhibited modestly. In addition, they also found no differences between the proteases of chloroquine-resistant and sensitive strains of the parasites and therefore could not account for chloroquine resistance.

Alternative explanations for the antimalarial action of chloroquine include the potential effects of raising intravesicular pH on the intracellular transport of macromolecules and membranes (Stahl and Schwartz (1986) and phospholipase activity (Ginsburg and Geary 1987). In mammalian cells, intracellular transport of macromolecules and membranes is necessary for differentiation and development and is inhibited by raising intravesicular pH (Bonner et al. 1986; Kent 1982; Lot and Bennet 1982; Sly and Fischer 1982). If the parasite is dependent on the same processes for differentiation and development, it is likely to be inhibited by an increase in the pH of intracellular vesicles as well. As noted by Ginsburg and his colleagues, phospholipase activity may be necessary for the transfer of haemoglobin from endocytic vesicles to the food vacuoles of the parasites (Krugliak et al. 1987; Yayon et al. 1983, 1984; Zarchin et al. 1986). Thus raising the intravesicular pH may kill the parasite by inhibiting intracellular targeting and membrane movement sufficiently to inhibit maturation irreversibly, by inhibiting the transfer of haemoglobin to the food vacuole of the parasite or by other related mechanisms. To support this postulation further, Cain and Murphy (1988) demonstrated the increase in pH of endocytic vesicles of fibroblasts due to accumulation of chloroquine and also showed a decrease in the acidity of fibroblasts in chloroquine-resistant strains of _P. falciparum_ which resists accumulation of chloroquine. This increase in concentration of chloroquine within acidic vesicles is, however, toxic to most cells. It is therefore difficult to understand why malaria parasites...
should be more sensitive to the effects of chloroquine if the mechanism of action is similar to those which lyse mammalian cells. There is indeed some disagreement in the literature as to whether chloroquine actually affects the lysosomal pH of the parasites at therapeutic doses.

Krogstad et al. (1987a, 1987b) observed that the concentration of chloroquine accumulated in the parasite vesicles is more than a 100-fold greater than that predicted by its properties as a diprotic weak base. To explain this "non-weak base effect", Krogstad et al. suggested that vesicles of susceptible parasites possess a chloroquine-concentrating mechanism. Meanwhile, others (Diribe and Warhurst 1980; Warhurst and Thomas 1978) have postulated this special mechanism to be a membrane-located specific carrier protein. In contradiction, Yayon et al. (1985) presented data to show that at the effective concentration of chloroquine, there is no alkalinization of the lysosome. They also presented evidence confirming the theory that chloroquine is trapped in the lysosome by a pH gradient as raising the pH of the lysosome using ammonium chloride effectively made the parasite resistant to chloroquine.

It has been recognized that chloroquine is active only against malarial parasites that produce the pigment haemazoin from the metabolism of haemoglobin (Cowman and Foote 1990). In this digestive process in malaria parasites, the toxic residue haem (ferriprotoporphyrin IX) is sequestered as an inert complex, haemazoin (malarial pigment), by a protein synthesized for this purpose (haembinder) (Fitch 1983; Homewood et al. 1975; Yamada and Sherman 1979). It has been shown that chloroquine binds with high affinity to ferriprotoporphyrin IX and its oxo-dimer (Cohen et al. 1964). This reaction appears to prevent the ferriprotoporphyrin IX from being detoxified by binding to the haembinder. Ferriprotoporphyrin IX-chloroquine complex is known to be as damaging to membranes as ferriprotoporphyrin IX alone and was postulated to lyse the parasitic cells (Fitch 1986). Strongly supportive of this theory is the similarity in the dissociation constant (Kd) of chloroquine for malaria-infected erythrocytes and that for chloroquine binding to ferriprotoporphyrin oxo-dimer (Chou et al. 1980). But on the other hand, there are several problems for this model which have yet to be resolved. Yayon et al. (1985) and Schlesinger et al. (1988) pointed out that no appreciable amounts of free ferriprotoporphyrin IX have been
found in the phagolysosomes of the malaria parasites and if free ferrisprotoporphyrin IX is available to bind chloroquine, why is it not toxic to the cell per se? Yayon et al. (1985) have also noted that ammonium chloride is capable of preventing the concentration of chloroquine by the parasitic cells. Ammonium chloride raises the intracellular pH of the parasites in the same way chloroquine does (weak base effect). If ferrisprotoporphyrin IX is the receptor for chloroquine, this increase in pH should not affect the binding of chloroquine to ferrisprotoporphyrin IX.

The exact mechanism of action of chloroquine is therefore not clear. There are parts of each of the two main postulated mechanisms which require further clarification. Perhaps the final answer will be that both mechanisms have some validity.

1.2.3 Chloroquine Resistance

The first continent to report chloroquine resistance in *P. falciparum* was South America where Mabarti (1960) reported chloroquine resistance in Venezuela. A similar observation was made in Thailand in 1962 but these data were retrospective, dating back to cases reported in the mid- to late-1950s (Harinasuta et al. 1962). From these two foci, chloroquine resistance spreaded around the world and today, no country with endemic malaria is totally free of chloroquine resistance. Figure 1.3 shows the world-wide distribution of chloroquine resistance in 1988 (WHO 1990a).

Chloroquine resistance is thought to occur because the parasite is able to acquire a mechanism by which it can pump chloroquine out of intracellular acidic vesicles. The drug therefore cannot accumulate in the malaria parasite and consequently loses its effect (Krogstad et al. 1988a). It has been discovered that this resistance can be reversed by verapamil, daunomycin and vinblastine (Krogstad et al. 1987c). However, the concentrations required to achieve this effect in humans may be toxic (Krogstad et al. 1988b).
Figure 1.3 Areas where chloroquine-resistant *Plasmodium falciparum* has been reported (World Health Organisation 1990a).
The mode of action of antimalarial drugs such as quinine, amodiaquine and mefloquine seems to be similar to chloroquine and there is some evidence of cross resistance between them. Draper et al. (1988) followed the resistance status of parasites in a small community in East Africa over a period of seven years. The trial started before the appearance of chloroquine resistance and extended well into the chloroquine resistance era. Chloroquine was the only drug used in the area to treat malaria. They tested parasites for sensitivity to chloroquine, quinine, amodiaquine and mefloquin and found a rise in the average MIC (minimum inhibitory concentration) of chloroquine over this period of time. Interestingly, there was also a gradual increase in the MIC for both quinine and amodiaquine with sporadic cases of mefloquine resistance despite the fact that these drugs were not used in this area. Brasseur et al. (1986) found that on the whole, quinine resistance is often associated with resistance to chloroquine but not vice versa. The reason for this was not discussed.

1.2.4 Drug Interactions

As a consequence of increasing drug resistance, chloroquine is being used more frequently in combination with other antimalarial drugs. The synergy or antagonism with these drugs have not been extensively studied.

In a brief report, Stahel et al. (1988) found in vitro antagonism between chloroquine and each of the following drugs: quinine, mefloquine, amodiaquine, artimisinine, and pyrimethamine/sulphadoxine against chloroquine-sensitive and chloroquine-resistant strains of P. falciparum. Chloroquine and each of the drugs were combined, in varying concentrations and added to culture media. The effects on the in vitro growth of the two strains of P. falciparum were observed. All combinations of the antimalarial drugs and chloroquine proved to be antagonistic. However, the clinical relevance of this finding and its possible contribution to the reduction of drug efficacy have not been established (Panisko and Keystone 1990).
1.2.5 Pharmacokinetics

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract when administered orally, with only 8-10% of the drug recovered in the faeces (McChesney and Fitch 1984). Healthy male adults given 500mg chloroquine diphosphate in a single dose attained a peak plasma concentration of $73 \pm 12 \mu g/L$ in a mean time of three hours (Gustafsson et al. 1983).

Oral administration of chloroquine is, however, not always possible, especially in patients who are vomiting or comatose. Intramuscular and intravenous chloroquine administration were considered dangerous because of reports of significant toxicity and death (Harris 1955; Scott 1950; Tuboku-Metzger 1964). In such cases, either large intramuscular doses were administered or the intravenous doses were administered too rapidly, resulting in high plasma concentrations and subsequent toxicity. More recently, kinetic studies have been performed in healthy volunteers and they have demonstrated the tolerability of parenteral chloroquine administration. Gustafsson et al. (1983) observed frequent side effects such as fatigue and difficulty with swallowing and accommodation after intravenous administration of 300mg base over initially 12 then 23.5 minutes but no cardiovascular side effects were observed. The average peak plasma concentration was $837 \pm 248 \mu g/L$, ten times higher than those observed after oral administration of the same amount of chloroquine base.

Although chloroquine is metabolised, half of the drug absorbed is excreted unchanged in the urine. Desethylchloroquine constitutes 25% of the metabolites appearing in the urine while others are found in smaller quantities. These metabolites have varying degrees of antimalarial activity (Webster 1985).

Chloroquine is widely distributed throughout the body and is tightly bound to tissues such as the spleen, kidney, heart, lung and liver. The drug has a high affinity for the melanin-containing tissues of the skin and eye. 55% of the drug is bound to unidentified, nondiffusible constituents in the plasma. Due to its extensive tissue binding, chloroquine has a large
volume of distribution (100 to 1000 L/Kg) and its terminal half-life has been variably estimated from 75 hours to 2 months (Frisk-Holmberg et al. 1983; Gustafsson et al. 1987; Walker et al. 1983).

As a result of high tissue binding, the plasma concentration profile of chloroquine during treatment is governed by the rate at which it distributes between tissue and plasma rather than elimination mechanisms (White 1988). Earlier pharmacokinetic studies suggest that the drug distribution follows a 2-compartmental model following intravenous administration of chloroquine. However, with the development of a more sensitive HPLC (high performance liquid chromatography) technique of measuring chloroquine concentration in plasma, it was found that a multicompartamental model of chloroquine disposition is probably necessary for adequate description of the plasma concentration profile.

Frisk-Holmberg et al. (1979,1984) provided evidence of dose-dependent kinetics in that the bioavailability progressively increased with increasing doses. However, in a similar study, Gustafsson et al. (1983) found no such evidence.

There appears to be no difference in chloroquine pharmacokinetics between normal, malnourished and infected individuals (Panisko and Keystone 1990). The pharmacokinetic properties of chloroquine are also similar in children and adults.

1.2.6 Toxicity

Oral chloroquine is very well tolerated. However, it can produce nausea, vomiting, blurred vision, diarrhoea, abdominal discomfort, headache, lightheadedness and fatigue. Pruritus, particularly in individuals with dark-pigmented skin has been documented in 50-60% of Africans (Krogstad et al. 1988b). Rare, but more severe adverse effects of therapeutic doses have been reported and include dyskineasias, hearing loss, neuromuscular disorders and even cardiovascular collapse (Salako
1.2.7 Physical and Chemical Properties

Chloroquine is a 4-aminoquinoline and has the following structure (structure I in Figure 1.4):

![Chemical structure of chloroquine](image)

Figure 1.4 Chemical structure of chloroquine
The dissociation constants of chloroquine are $pK_{a1}=10.8$ and $pK_{a2}=8.4$ ($20^\circ C$) (The Pharmaceutical Codex 1979). The singly charged cation would therefore be expected to correspond to the structure II (Figure 1.4). However, Rosenderg and Schulman (1978) postulated that because the difference between the two $pK_a$'s is small, it is probable that some dissociation occurs from the alkylamino side chain grouping of the dication, producing a second monocation (structure IV in Figure 1.4) tautomeric with the first. Schulman and Young (1974) showed that the ionisation of the aromatic amino grouping influences the ultraviolet absorption spectra and the fluorescence spectra of the drug and that the alkylamino side-chain grouping has no effect. These analytical methods are normally used together with other more sensitive techniques such as gas chromatography or high performance liquid chromatography to quantify blood or plasma chloroquine levels in kinetic studies and drug-malarial sensitivity testing. As ethanol, sugars and glycerol were found to affect the ultraviolet spectra chloroquine (Takla and Dakas 1987), the presence of these substances in blood or plasma would affect the results obtained in clinical studies.

Chloroquine discolors on exposure to light and has been reported to give toxic reactions which may be ascribed to the photochemical degradation of the substance (Moore and Hemmens 1982). Nord et al. (1991) studied the effects of light on solutions of chloroquine phosphate in isopropanol and in buffer solutions. Seven of the photochemical degradation products were isolated and identified. The main photodecomposition products were also likely to be the normal metabolites formed \textit{in vivo} and so phototoxicity can be ascribed to the drug itself or the metabolites formed.

Yahya \textit{et al.} (1985) investigated the binding of chloroquine to soda and borosilicate glass. Buffered solutions of chloroquine phosphate at varying pH and concentrations were studied. They found that the highest binding in soda glass occurs at physiological pH and at low concentration (7.8 ng/ml). In contrast, there is no appreciable binding of chloroquine to borosilicate glass. In another study, Yahya \textit{et al.} (1986) reported the sorption of chloroquine by plastics, such as cellulose propionate, ethylvinyl acetate, methacrylate butadiene styrene, polyethylene, propylene and polyvinyl chloride but not polystyrene. In some cases, as
much as 85\% of the drug was lost. It is therefore important to avoid contacts with the chloroquine-sorbing materials outlined during laboratory estimation of chloroquine.
Chapter Two

Taste-masked, Sustained-release Dosage Form
2.1 INTRODUCTION

The means to overcome or modify the undesirable (usually bitter) taste of drugs are problems for the pharmaceutical scientist. Conventional methods of taste-masking, such as the use of hard gelatin capsules and macro-coating, are suitable for adult formulations. However, for some patient groups (e.g. infants, children and the elderly), liquids may be preferred because of the ease of swallowing and the flexibility in the administration of range of doses.

Sweetening or flavouring agents and beverages such as fruit juices, are usually used to improve the palatability of liquid preparations (Greene and Seime 1987; Bagger-Sjoback and Bondesson 1989). These, however, may be inadequate for some drugs. Water-insoluble prodrugs have been used successfully for taste-masking but they frequently possess pharmacokinetic properties different from the parent drugs (Bechtol et al. 1981). As new chemical entities, they would require extensive independent evaluation. Other methods, such as complex formation and encapsulation by suitable carriers, have also been used. These methods delay the diffusion of drug molecules into the lipid bilayer of the gustatory membrane by controlling the rate at which the drug is released. Such novel methods include ion-exchange technology, formulation of multiple-emulsions, formation of cyclodextrin inclusion complexes and microencapsulation. Although some controlled release is essential for taste masking, the drug must be released in the gastrointestinal tract to afford its absorption.

Methods used for taste-masking are usually also used to prepare sustained release dosage forms. Sustained release, prolonged release, timed release and controlled release dosage forms are terms used to identify drug delivery systems that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of a single dose (Lordi 1986).
2.2 ADVANTAGES AND DISADVANTAGES OF A SUSTAINED-RELEASE DOSAGE FORM

Besides taste-masking the drug, several other therapeutic advantages can be gained by using sustained release formulations. One such advantage is in prolonging the duration of action of the drug beyond that normally achieved with conventional dosage forms. Consequently, the frequency of dosing can be reduced leading to improved patient convenience and compliance.

Another important benefit is the attainment of a relatively constant steady state blood level with reduced peak-trough fluctuations. A better efficacy/toxicity ratio with the drug is thereby achieved (Theeuwes 1983; Urquhart 1982). This is especially important with drugs of narrow therapeutic indices. A smaller fluctuation will eliminate periods of supra-therapeutic levels associated with systemic toxicity and avoid sub-therapeutic levels resulting in loss of therapeutic efficacy.

Sustained release dosage forms have also been reported to eliminate local irritation and erosion due to the exposure of gastric mucosa to high drug concentrations. Examples of such drugs are aspirin (Levy and Hayes 1960) and Potassium supplements (Livingstone and Livingstone 1988). For drugs with long half-lives (in excess of 12 hours), sustained release offers little benefit since the conventional dosage form requires infrequent administration but it may still be advantageous if the therapeutic index of the drug is narrow (e.g. lithium).

Problems may be experienced with sustained release systems because they often contain a larger quantity of drug than would normally be administered as a single dose. If for some reason the system fails and rapid release occurs, then toxic effects are possible. Problems with absorption may be experienced in individuals with abnormal gastrointestinal motility or if the drug is predominantly absorbed in the upper portion of the gastrointestinal tract. Drugs which undergo extensive first-pass metabolism may be entirely metabolised before they reach the systemic circulation if the release rate is slow. In addition, the complexity of the system often makes them expensive to produce.
Oral sustained release preparations may be classified as single or multi-unit dosage forms (Bechgaard and Nielsen 1978). In single unit devices, the drug is often incorporated into a porous wax or plastic matrix tablet. A multi-unit system may consist of coated pellets or granules of drug contained in a gelation capsule or compressed into a tablet. It may also consist of a suspension of microparticles.

Based on this difference, the multi-unit dosage form appears to have certain advantages over the single unit system. It allows the flexibility of dose adjustment by varying the amount of microparticles administered. There is also a better statistical assurance of drug release since each dose consists of many subunits (Lordi 1986). The risk of dose dumping is equally subdivided (Beckett 1985) and so is the risk of ineffective treatment due to incomplete release of the drug (Murphy et al. 1983).

Dispersion of the individual particles over a large area in the gastrointestinal tract would tend to yield a more predictable drug release profile by reducing local differences in the gastrointestinal environment (Murphy et al. 1983; Beckett 1984). In addition, local effects of an irritant drug could be reduced as a result of the dispersion of the particles over a wide area (Rowe 1983). This is, however, disputed by Hunter et al. (1982), Wilson et al. (1983) and Devereux (1987), who suggested that these particles actually agglomerate in the gastrointestinal tract.

The gastric emptying process of a single unit dosage form is a random process and would inherently result in a large intra and inter-subject variation (Bechgaard 1982). In contrast, a multi-unit system is emptied gradually, giving more predictability to the system. However, Hunter et al. (1982) and Devereux (1987) suggested that gastric emptying of a multi-unit system may occur as a single bolus.
There are numerous methods available for the purpose of taste-masking and sustained release. A few of these methods, relevant to this work, will be described as follows.

### 2.4.1 Multiple Emulsions

An emulsion is a heterogeneous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, whose diameters, in general, exceed 0.1μ. Such systems possess a minimal stability, which may be accentuated by such additives as surface active agents, finely divided solids, etc. (Becher 1965). Multiple emulsions are emulsions whose disperse phase contains droplets of another emulsion. The formulation and stability of multiple emulsions have been reviewed by Florence and Whitehill (1982). There are two main types of multiple emulsions: water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o). It is mainly the w/o/w emulsion that is used for sustained release purposes (Brodin et al. 1978a, b; Garti et al. 1983; Omotosho et al. 1986, 1989; Omotosho 1990).

Sustained release is achieved by isolating the drug in the inner aqueous phase of the w/o/w emulsions. However, the entrapped drug may diffuse across the middle oily layer, termed the membrane phase, into the outer aqueous phase. The loss of drug to the outer aqueous phase lowers the yield of the multiple emulsion (Matsumoto et al. 1976). But on the other hand, it may be used as a single dose system in which the loading dose is incorporated in the outer aqueous phase and the maintenance dose in the internal aqueous phase (Omotosho 1990).

The use of multiple emulsions as sustained release liquid formulations have been limited mainly because of its inherent instability resulting in a short shelf-life. Attempts to improve the stability have also been limited due to its complex nature.
2.4.2 Ion Exchange Technology

Ion exchangers are solid and suitably insolubilized high molecular weight polyelectrolytes that can exchange their mobile ions of equal charge with the surrounding medium (Rághunathan et al. 1981). The resulting ion exchange is reversible and stoichiometric, with the displacement of one ionic species with another on the exchanger (Dorfner 1972). Ionic exchangers can either be cationic or anionic.

Cationic exchangers contain acidic ionisable groups such as sulphonic, carboxylic or phenolic groups. They are normally presented as the sodium, potassium or ammonium salts. Weak cationic exchangers have pKa values of about 5 to 6. They are weak acids and are significantly ionised only at pH values greater than two units below their pKa values. Consequently, their exchange capacities are also affected by pH. On the other hand, strong cationic exchangers are highly dissociated at all pH's due to their low pKa values which are around 1 or 2. Therefore, their exchange capacities tend to be independent of pH. An example of a weak cationic exchanger is polymethacrylic acid which has a pKa value of 6.0. Polystyrene sulphonyl acid which has a pKa of 1.3, is a strong cationic exchanger.

Keating (1961) listed the following advantages of adsorbing basic nitrogen-containing drugs onto sulphonyl acid cation-exchange resins and using them in dosage forms. First of all, availability is prolonged by releasing the drug from the complex over 12 hours in the gastrointestinal tract. As a result of slowing down the rate of release and therefore absorption, toxicity is reduced. Stability of the drug is also increased as the drug is protected from hydrolysis or other degradative changes in the gastrointestinal tract. It improves palatability of the drug and also enables formulation of both liquid and solid sustained-release dosage forms.

Adsorbates of cationic drugs with carboxylic ion-exchange resins for sustained release and taste coverage have been prepared though to a lesser extent than complexes with sulphonyl acid resins (Borodkin and Yunker 1970). This is because sulphonyl acid resins are more efficient than polycarboxylic acid exchangers since their lower pKa (approximately
2 versus 6 for carboxylic acid resins) yields a stronger drug-resin bonding (Borodkin and Sundberg 1971). However, the exchange capacities of the sulphonic acid resins are usually lower than those of carboxylic acid resins (4 to 5 meq./g as compared to greater than 10 meq./g). As a result, lower quantities of drug are adsorbed onto sulphonic acid resins.

Schlichting (1962) evaluated six cationic exchangers (sulphonic and carboxylic) with carbinoxamine and reported retardation of dissolution with all complexes made from resins having pKa less than 5.2. Polystyrene sulphonic acid which has a pKa of 1.3 (the lowest pKa of the six) was found to be the most effective. Schlichting also reported a reduction in dissolution rate due to increased crosslinking of the resins which resulted in a decrease in the accessibility of exchanging ions. Dissolution rate was also affected by pH or salt formation but the particle size of the complexes had little effect on it.

Anionic exchangers contain basic ionisable groups such as amine or quarternary ammonium and are usually presented as the chloride salt. Like cationic exchangers, their pKa values determine the effect of pH on their binding capacities. An example of an anionic exchanger is Dowex 1-X8, a strongly basic ion-exchanger which is not affected by pH.

Although ion exchangers form a useful drug delivery system, improvements can be effected in some cases by coating the resins with various pharmaceutical adjuvants. Coating enhances the taste of the ion exchangers, further decreases the rate of release, allows greater saturation of the resin with the drug and decreases the elution rate. It minimizes elution of drug in liquid preparations of ion exchange resin-drug complexes and interaction of drugs with resins used as tablet disintegrants (Motycka and Nairn 1979).

Koff (1964) described the use of wax coatings to improve the palatability and stability of a highly acidic cation exchange resin complexed with amotropine. Motycka and Nairn (1978) evaluated the effects of seven different wax coatings on strongly basic anionic exchanger and found the dissolution rate to be dependent on the wax to resin ratio and the characteristics of the waxes. Macek et al. (1970) improved the gritty and astringent taste of finely divided polystyrene divinylbenzene copolymer
by coating the particles with acrylic polymer cross-linked with allyl sucrose. To improve the taste-masking property, Borodkin and Sunberg (1971) coated particles of polymethacrylic acid ion exchange complexes of basic drugs with an ethylcellulose-hydroxypropylmethylcellulose (4:1) mixture using an air suspension coater. Clark (1971) applied the enteric coating agent cellulose acetate phthalate on pellets of cation exchange adsorbate of drugs in a coating pan but no dissolution data was given. Jungmann (1975) spray dried a slurry of codeine-resin complex in a solution of the coating agent, a copolymer of acrylic acid esters and methacrylic acid esters modified with ammonium groups. In an attempt to coat divinylbenzenesulphonic acid resin-drug complex with ethylcellulose, Raghunathan et al. (1981) found it necessary to pretreat the particles with polyethylene glycol 4000. This was to retard water uptake which in turn affects the integrity of the ethylcellulose coat. Lu et al. (1991) further improved the taste-masking property of polyacrylic acid-macrolide antibiotic complex by coating the adsorbate particles with hydroxypropylmethylcellulose phthalate using a fluidised-bed air-suspension coater.

Besides macro-coating, the ion exchange resin-drug complex can be "coated" by means of microencapsulation. Motycka and Nairn (1979) microencapsulated strongly anionic ion exchange resin in the benzoate form, using complex coacervation, phase separation by temperature changes and non-solvent addition techniques. They found that the dissolution rates could be controlled over a wide range, depending on the encapsulating material and the technique used to prepare the microcapsules. Using a solvent evaporation technique, Sprockel and Price (1989) microencapsulated the resin-drug complexes with cellulose acetate butyrate. They compared the sustained release properties of sulphonic and carboxylic anionic exchange resins and found the former to be superior, both coated and uncoated.
2.4.3 Microencapsulation

Microencapsulation embodies a series of techniques for the entrapment of solids within polymer coats or matrices. These techniques have been used for the improvement of drug stability, taste masking, providing sustained release, producing targetable drug carriers and for converting liquids to solids (Deasy 1984; Luzzi and Palmier, 1984; Tomlinson, 1983).

The most common structure of a microcapsule is mononuclear spherical. Microcapsules can also take on the shape of the crystals from which they are produced. For some authors however, "microcapsules" are made up of aggregates of small microcapsules (Deasy et al. 1980; Oya Alpar and Walters 1981). The particle size range of microcapsules is between one to a few thousand microns.

2.4.3.1 Coacervation/Phase Separation

The oldest and perhaps the most widely used microencapsulation technique has been coacervation/phase separation. This process involves the deposition of polymer from solution around the material to be coated. This is brought about by changing the temperature of the polymer solution; by adding a salt, a nonsolvent, an incompatible polymer to the polymer solution, or by inducing a polymer-polymer interaction, for example by altering pH. Simple coacervation involves the deposition of a single polymer coating whereas complex coacervation involves more than one polymer for the coating.

Phase separation of ethylcellulose by temperature changes was described by Miller et al. (1967) and Jalsenjak et al. (1976). In some works, aggregates of small microcapsules were produced (Deasy et al. 1980; Oya Alpar and Walters 1981). For others, the addition of protective colloids such as polyisobutylene (Benita and Donbrow 1980, 1982) or polyethylene (John et al. 1979) allowed the formation of individual microcapsules. In general, water soluble and insoluble solids, water insoluble liquids, solutions and dispersions of solids in liquids can be microencapsulated using this method.
2.4.3.2 Polymerization

Another approach that has been studied extensively is polymerization. These techniques involve the preparation of emulsions using monomers and/or monomeric solutions, followed by polymerization either in the disperse phases or the interfaces of the emulsions. Polymerization techniques can generally be classified as vinyl polymerization or polycondensation (Arshady 1988, 1989a, 1989b).

Vinyl polymerization processes can occur in many ways depending on the solubility of the monomers and initiators. Of all the methods used, emulsion polymerization has the greatest potential for producing colloidal-sized delivery systems required for drug targeting applications (Couvreur et al. 1979; El-Samaligy and Kohdewald 1982; Tomlinson 1983). The other methods, namely dispersion and suspension polymerization, produces particles above the submicron range and can be used for other sustained release applications. The main disadvantage of this technique is the removal of contaminants such as the unreacted monomers and the initiators.

Polycondensation techniques can be divided into two main categories:

1. Normal polycondensation processes in which the monomers are present and react in only one phase and
2. Interfacial polycondensation processes in which two complementary monomers are present separately in two immiscible phases; the reaction begins necessarily at the interface.

Many polycondensates, such as polyesters, polyamides and polyurethanes are biocompatible and/or biodegradable. As such, they are well suited for biomedical and pharmaceutical applications.

2.4.3.3 Albumin Microspheres

Albumin microspheres are biodegradable particles, synthesized in the size range of 1 to 200 microns. Generally, the method of preparation involves the formation of a water-in-oil emulsion from an aqueous
solution of the protein and a suitable vegetable oil. This is followed by
heat denaturation, which precluded the encapsulation of heat sensitive
compounds, or chemical hardening using suitable cross-linking agents
such as formaldehyde or glutaryldehyde which may be toxic.

The use of albumin microspheres in drug delivery was first suggested by
Kramer (1974). Since then, extensive work has been made towards its
development, especially as carriers of anti-cancer drugs (Sugibayashi *et al.*
1979; Morimoto *et al.* 1981; Willmott *et al.* 1985; Gupta *et al.* 1989) because
of its lysosomotropic nature (Widder *et al.* 1983). Radiolabelled albumin
microspheres are now routinely used in the diagnostic screening of
abnormalities of body organs and measurements of blood flow rate
(Gupta and Hung 1989a). The physico-chemical characteristics of albumin
microspheres and its application to drug delivery have been reviewed by
Gupta and Hung (1989a, b).

2.4.3.4 Melt Dispersion Technique

Due to their lipophillic character, waxes have been used as carrier or
coating in a variety of sustained release dosage forms. The method of
microencapsulation used is the melt dispersion technique. It has the
advantage that it does not require the use of an organic solvent due to the
processability of the the low viscosity melts (Bodmeier *et al.* 1992a). Most
microencapsulation processes involving water insoluble polymers
require the use of an organic solvent (Kondo 1979). This method is,
however, unsuitable for thermolabile substances.

2.4.3.5 Emulsification/Solvent Evaporation

One of the most recently developed techniques that first appeared in the
literature over a decade ago is emulsification/solvent evaporation (Beck
*et al.* 1979). It involves, first, the emulsification of a polymer solution
containing the drug (either dissolved or dispersed) into a second
immiscible liquid phase containing an emulsifier to form a dispersion of
drug-polymer-solvent droplets. This may be an oil-in-water (o/w), a
water-in-oil (w/o) or an oil-in-oil (o/o) emulsion. The solvent of the
disperse phase is then removed from the droplets by application of heat, vacuum, or both, or by allowing evaporation at room temperature to leave a suspension of drug-containing microspheres.

This technique can be tailored to produce microspheres over a wide size range from less than 200nm to several hundred microns. By choice of suitable solvent systems, drugs can be encapsulated, irrespective of their water-solubility, into a wide range of polymers. Drugs insoluble in water are well encapsulated using o/w emulsions but water-soluble drugs are usually encapsulated using w/o or o/o emulsions due to the solubility of the drug in the outer aqueous phases of o/w emulsions. However, using a w/o or o/o emulsion may lead to problems with the final clean-up requirements of the final product, recovery of the oil phase and agglomeration of the microparticles (Watts et al. 1990).

2.4.3.6 Multiple Emulsion Techniques

Several multiple emulsion techniques have been reported, most of which differ in the way the microcapsules are formed from the multiple emulsions. Most of these methods are in fact modifications of their respective classical methods of microencapsulation such as polymerization (Mac et al. 1989; Ganguly et al. 1989), the melt dispersion technique (Bodmeier et al. 1992b) and emulsification/solvent extraction (Alex and Bodmeier 1990; Bodmeier et al. 1991; Ogawa et al, 1988).

Besides producing single-walled microcapsules, multiple emulsion techniques have also been used to prepare multi-walled microcapsules. Nozawa et al. (1979) and Yoshida et al. (1980) prepared three-walled microcapsules while Nozawa and Fox (1981) prepared two-walled microcapsules.

Warburton (1981) described a method of preparing three-ply-walled microcapsules almost similar to the modified emulsification/solvent extraction technique. The two methods differ in that the method described by Warburton does not require the presence of additional emulsifiers to stabilize the multiple emulsion system. The multiple emulsion is stabilized by the formation of solid films of the polymers at
The multiple emulsion method of manufacture produces reservoir-type microcapsules (Ogawa et al. 1988) in contrast to the matrix microspheres produced by most single emulsification processes. The microcapsules formed can either be unicored or multicored, depending on the type of multiple emulsion (Florence and Whitehill 1982) from which they are produced. It has the advantage of microencapsulating water-soluble drugs which are not well encapsulated in techniques using oil-in-water emulsions due to the solubility of the drug in the external aqueous phase.

2.4.3.7 Other Methods

The term "microencapsulation" has also been applied to the formulation of controlled release oral dosage forms produced by extrusion-spheronisation (Li et al. 1988). In this method, extruded drug-excipient strands are chopped and rotated on a spinning disc to form millimeter-sized pellets that are then film-coated. The major advantage of the process is said to be its suitability for use with a wide range of drug concentrations.

The extrusion-spheronization technique and others, such as spray coating (Li et al. 1988) and spray drying (Bodmeier and Chen, 1988), are especially applicable to industrial scale production. The resultant microspheres however, are primarily suited to oral drug delivery due to their particle size distribution except for those prepared by spray drying.
2.5 OBJECTIVES AND SCOPE OF THE PRESENT STUDY

The main objective of this study was to develop a taste-masked, sustained release formulation of chloroquine microcapsules to be administered as extemporaneous suspensions.

The rapid spread of chloroquine-resistant *Plasmodium falciparum* malaria has led to an increase in the minimum inhibitory concentration of chloroquine. In some cases, the parasite was totally resistant to chloroquine, leading to treatment failure. In others, an increase in the dose of chloroquine was sufficient to overcome the resistance (partial resistance). This increase in dosage of chloroquine might however lead to toxicity due to high chloroquine blood levels as a result of rapid absorption and slow distribution of the drug. It is therefore ideal to have a sustained release preparation of chloroquine, despite its long half-lives (5 days initially but up to 2 months in the terminal phase), in order to delay its release in the gastrointestinal tract. This would decrease the peak-trough fluctuations of chloroquine blood levels, thus improving the efficacy/toxicity ratio. In addition, the inherent delayed release characteristic of the microcapsules would also serve to taste-mask the drug.

Experimentally, chloroquine has been incorporated into emulsions (Okor 1990), multiple emulsions (Omotosho 1990) and liposomes (Peeters *et al.* 1989a, b) to achieve sustained release property. In addition, liposomal chloroquine has also been observed to be effective against chloroquine-resistant *Plasmodium berghei* infections in mice (Peeters *et al.* 1989a). However, due to their colloidal nature, liposomes are more suited for parenteral use rather than oral, which remains the principal route by which drugs are administered. Emulsions and multiple emulsions would appear to be suitable oral dosage forms because liquid dosage forms have the advantages of flexibility in administration of range of doses and ease of swallowing. However, they have limited stability besides being bulky which makes them difficult to transport and store.
In this work, the development of a taste-masked, microparticulate sustained release formulation of chloroquine, which can be administered as a suspension, was undertaken. This required the study to be carried out in various stages with the following objectives:

(1) To select suitable polymeric systems for the preparation of chloroquine microcapsules;
(2) To evaluate the effects of preparative conditions on the microcapsules;
(3) To characterize the microcapsules;
(4) To develop the selected polymeric system for microencapsulation to obtain a high drug content and an acceptable drug release profile;
(5) To evaluate the *in vivo* release profile and extent of absorption of the final formulation in healthy human volunteers;
(6) To correlate the *in vitro* and *in vivo* results;
(7) To study the effect of storage on the drug release profile of the microcapsules.
EXPERIMENTAL
Chapter Three

Preformulation Studies
3.1 SURFACE RHEOLOGY

3.1.1 Introduction

It has been established that there is a close correlation between the interfacial film characteristics of the protective colloid and the physical stability of emulsion systems (Warburton 1976). This has been attributed to the formation of a "solid film" at the interface which provides a physical barrier to coalescence. Interfacial rheological studies have also shown the formation of a rigid bipolymer film at the interface between an aqueous solution of a water soluble polymer and a non-aqueous solution of an oil soluble polymer (Warburton 1976). In a water-in-oil-in-water (w/o/w) emulsion, such an interface would be present both at the w/o and o/w interfaces.

Based on this idea, Warburton (1981) described a technique of microencapsulation which forms spherical bodies of reservoir microcapsules from w/o/w emulsion by the removal of organic solvents from the oily phases of these emulsions. The organic solvents used have to be volatile and partially miscible with water (eg. ethyl acetate) to permit diffusion into the outer aqueous phases followed by evaporation. As the organic solvents are removed, the outer walls contract and eventually come into contact with the inner walls, forming three-ply-walled microcapsules. This process of microencapsulation will be discussed further in Chapter 4.

Different polymers have been used for different layers of the microcapsules but acacia has been used most extensively as both the inner and outer aqueous phase polymer of the w/o/w multiple emulsions (Morris 1982; Morris and Warburton 1982, 1984; Duquemin 1987; Odidi 1990). The surface rheological properties of acacia have been well established (Sherriff and Warburton 1974; Kerr and Warburton 1985; Moules and Warburton 1990; Nithiananthan 1992). However, inter-laboratory and inter-sample variations are large and mainly due to acacia being a natural product. Recent reports have shown the presence
of varying amounts of proteinaceous compounds in specimens from
different sources of gum acacia with variable lability to heat and
ultraviolet radiation (Anderson et al. 1987a, b, c, 1988). Acacia is also
commercially available in different forms, namely the acacia tears and
powdered acacia.

The aim of this part of the study was to compare the surface
viscoelasticity of three different types of acacia as indication of their
ability to stabilize an emulsion system and hence the microcapsules.
The three acacia samples investigated were: acacia tears (AT), acacia
powder (AP) and another acacia powder with a higher protein content
(APHP).

3.1.2 Materials

<table>
<thead>
<tr>
<th>Acacia (AT)</th>
<th>Merck Ltd. (BDH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia powder (AP)</td>
<td>Merck Ltd. (BDH)</td>
</tr>
<tr>
<td>Acacia (APHP)</td>
<td>Exudate Ltd.</td>
</tr>
<tr>
<td>Ethyl acetate GPR</td>
<td>Merck Ltd. (BDH)</td>
</tr>
</tbody>
</table>

3.1.3 Method

The acacia solution was prepared by first dissolving an appropriate
amount of acacia in deionised water which had been saturated with
ethyl acetate. This was necessary as the aqueous solutions used
subsequently for the preparation of multiple emulsions would also be
saturated with the respective organic solvents in order to improve their
stability (Morris 1982). The solution was then centrifuged at 10 000 rpm
and decanted to remove the natural contaminants present.
Surface rheological measurements were made at the air/aqueous solution interface using the normalised resonance oscillating ring surface rheometer (Sherriff and Warburton 1975). The experimental technique consisted of positioning a platinum DuNuoy ring, with the plane of the ring horizontal, so that it just wets the surface of the solution contained in a little petri dish (Figure 3.1). In all cases, 1 ml of the 4%w/v solutions was used.

Driven by a data transfer function analyser connected to a moving coil galvanometer, the ring oscillated at a fixed frequency (4.0Hz) about a vertical axis through the centre of the ring. The amplitude of the motion of the ring was measured by a proximity probe transducer. Automated analysis of the signal generated gave both the dynamic surface viscoelasticity modulus and the dynamic surface viscosity concurrently. The equation of motion for the apparatus and the associated theory have been described by Warburton (1978).

![Fig. 3.1 Diagram of the oscillating ring surface rheometer](image-url)
3.1.4 Results and Discussion

Figure 3.2 shows the surface viscoelasticity of 4% w/v acacia solutions as a function of time. The shape of the curves supports the previously postulated theory for the formation of non-stationary surface films (Warburton 1972). This theory describes the time dependent adsorption of macromolecules from the bulk to subsurface, followed by adsorption from the subsurface to the surface and finally rearrangement of the macromolecules at the surface.

The results obtained for the surface viscoelasticity of the acacia powders were low compared to those reported by other workers (Moules & Warburton 1990; Duquemin 1987; Nithiananthan 1992). This could be a result of the method used to prepare the solutions. As mucilaginous residues were obtained after centrifugation, it is possible that centrifuging the acacia solutions at high speed desolubilised the higher molecular weight acacia molecules, resulting in a decrease in concentration which would in turn give lower surface viscoelasticity. It has been reported that surface viscoelasticity is dependent on concentration (Moules and Warburton, 1990; Duquemin 1987; Nithiananthan 1992). The presence of ethyl acetate has also been shown to decrease the surface viscoelasticity of acacia solutions (Duquemin 1987).

By means of elemental analysis, the sample of acacia tears was found to have the highest nitrogen content (0.68%) compared to the powdered high protein acacia (0.58%) and the ordinary acacia powder (0.37%). This could imply a higher content of surface active proteinacious compounds in the sample of acacia tears resulting in its higher film forming ability compared to the two samples of powdered acacia (Figure 3.2). However, the surface viscoelasticity of the two types of powdered acacia did not differ significantly (Figure 3.2) even though one has a higher nitrogen content than the other. Therefore, the difference in surface viscoelasticity is unlikely to result from differences in protein content.

As both the samples of powdered acacia have quite similar surface viscoelasticity, a possible explanation to the above observation is the
Figure 3.2 Surface viscoelasticity of 4% w/v acacia solutions.
process of pulverizing acacia. This includes milling or spray drying which involves heat and would result in degradation of the thermolabile acacia molecules, thus altering its highly branched structure which is related to its film forming ability. In support of this postulation is the effect of heat on gum acacia solution. Heat has been shown to decrease the emulsifying property of acacia solutions (Anderson and McDougall 1987b) which is dependent on their surface viscoelasticity.

3.1.5 Conclusion

The above results indicate differences in the surface viscoelasticity of different forms of acacia. It is therefore necessary to be consistent in the choice of types of acacia for the purpose of microencapsulation using the multiple emulsion technique which is dependent on surface viscoelasticity. In this project, acacia tears was used for all subsequent work.
3.2 DRUG-POLYMER COMPATIBILITY

3.2.1 Introduction

In a solution containing a polymeric solute and a low molecular weight solute (cosolute), specific interactions may occur between the polymer-cosolute pair. In most cases, the cosolute binds reversibly to the polymer. Examples of this are the Michaelis complex between the enzyme and its substrate and the binding of drugs by serum protein.

The strength of the binding between the polymer-cosolute pair depends markedly upon the types of bonds involved. These may include van der Waals forces of dispersion, dipolar interaction, induced dipolar interaction or hydrogen bonding (Martin et al. 1983). Polymers which undergo ionisation in aqueous solutions to produce polyanions or polycations will bond ionically to molecules of the opposite charge. The number of cosolute molecules bound per polymer molecule is dependent on the number of charged groups per polymer molecule and the conformation of the polymer molecule in solution. This interaction is dependent on the pH of the solution.

The simplest case of interaction between a drug and a polymer involves the reversible binding of one drug molecule to one polymer molecule.

\[
\text{Polymer} + \text{Drug} \rightleftharpoons \text{Polymer-drug complex}
\]

\[
[P] \quad [D] \quad [PD]
\]

The binding equilibria of such an interaction can be described by a form of the Langmuir adsorption isotherm (Martin et al. 1983)

\[
r = \frac{[PD]}{[Pt]}
\]

or

\[
r = \frac{k[D]}{1 + k[D]}
\]
where $k$ = association constant

$[D]$ = concentration of unbound cosolute

and $r$ = the number of moles of cosolute bound, $[PD]$, per mole of total polymer $[Pt]$.

Figure 3.3 shows a plot of $r$ against $[D]$ where $r$ is a measure of the binding capacity of one mole of the polymer. The plot shows the saturability of the binding sites on the polymer which in most cases is more than one. An increase in total drug concentration will increase the amount of drug bound to the polymer. This increases the hydrophobicity of the polymer and may result in the precipitation of the polymer-drug complex when the solution is saturated with respect to the complex.

In the multiple emulsion technique of microencapsulation where the drug is incorporated in the inner aqueous phase of the w/o/w emulsion (Morris 1982), it is necessary for the drug to be compatible with the aqueous polymer used. Polyanions such as the polymethacrylates, carrageenan and acacia are usually used in the inner aqueous phases of the w/o/w multiple emulsions (Odidi 1990). The addition of a drug such as chloroquine diphosphate, which is cationic, may therefore result
in precipitation thereby destabilising the emulsion. In such cases, it might be necessary to encapsulate the drug, preferably a water-insoluble form such as the chloroquine base, in the middle organic phase of the \textit{w/o/w} multiple emulsion (Odidi 1990). However, incorporating the drug in the organic phase of the \textit{w/o/w} emulsion does not necessarily prevent polymer-drug interaction. Drug molecules are able to diffuse into the aqueous phases, the extent of which, in the absence of drug-binding polymer, depends on their partition coefficient

\[
K = \frac{[D(o)]}{[D(a)]}
\]

where \([D(o)]\) and \([D(a)]\) represent the concentration of the unbound drug in the organic and aqueous phase respectively. In the presence of an aqueous polymer of opposite charge in the inner aqueous of the \textit{w/o/w} multiple emulsion, the partition of drug molecules from the organic phase to the inner aqueous phase is enhanced due to the binding of drug molecules to the polymer in the inner aqueous phase. This acts as a "sink" and causes a greater amount of drug to pass into the inner aqueous phase (Duquemin 1987; Odidi 1990).

The aim of this part of the study was to investigate the compatibility of chloroquine with various anionic polymers. The study was carried out in two parts. First, to study the compatibility of the drug chloroquine diphosphate with various anionic polymers and secondly, to demonstrate the effect of anionic polymers on the partitioning of chloroquine from the organic phase to the aqueous phase.

### 3.2.2 Materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td>Merck Ltd. (BDH)</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>FMC Corporation</td>
</tr>
<tr>
<td>Eudragit® L100</td>
<td>Rhom Pharma (GMBH)</td>
</tr>
<tr>
<td>Eudragit® S100</td>
<td>Rhom Pharma (GMBH)</td>
</tr>
<tr>
<td>Ethyl acetate GPR</td>
<td>Merck Ltd. (BDH)</td>
</tr>
<tr>
<td>Chloroquine diphosphate</td>
<td>Sigma Chemical Company Ltd.</td>
</tr>
</tbody>
</table>
3.2.3 Method

3.2.3.1 Compatibility of chloroquine diphosphate with anionic polymers

The following polymeric solutions were all prepared using water and buffered solutions saturated with ethyl acetate. They were:

1. 4% w/v acacia solution.
2. 8% w/v acacia solution.
3. 0.25% w/v carageenan solution.
4. 0.5% w/v carageenan solution.
5. 0.5% w/v Eudragit L100 solution in pH 7.0 phosphate buffer USP.
6. 1% w/v Eudragit L100 solution in pH 7.0 phosphate buffer USP.

500mg aliquots of chloroquine diphosphate were then added to 100ml of the aqueous polymeric solution until precipitation occurred. The solution was stirred continuously using a magnetic stirrer at constant speed.

3.2.3.2 Effect of anionic polymers on the o/w partitioning of chloroquine

Using the pH complexation method (Martin et al. 1983), chloroquine base was precipitated from 50ml 10% w/v chloroquine diphosphate solution using molar sodium hydroxide solution. The precipitate was then extracted using 4X100ml ethyl acetate and the chloroquine extract washed with 100ml deionised water to remove any excess sodium hydroxide. The amount of chloroquine in the remaining solution and the wash liquid was then determined by ultraviolet spectroscopy.

10ml of the aqueous polymeric solution to be investigated was placed in a 50ml separating funnel with 10ml of the chloroquine extract layered on top of it. The mixture was then shaken lightly and allowed to stand for 24 hours. Samples of the aqueous phase were then analysed for its chloroquine content using ultraviolet spectroscopy. Aqueous polymeric solutions used were prepared with 0.1M hydrochloric acid (pH1.2), deionised water or pH7.4 phosphate buffer USP.
3.2.4 Results and Discussion

3.2.4.1 Compatibility of chloroquine diphosphate with anionic polymers

<table>
<thead>
<tr>
<th>Polymeric Solution</th>
<th>Concentration of chloroquine diphosphate at which precipitation occurred (%w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td>No precipitation observed. Solubility of chloroquine diphosphate equivalent to solubility in water (1 in 4 parts water).</td>
</tr>
<tr>
<td>(4%w/v, 8%w/v)</td>
<td></td>
</tr>
<tr>
<td>Carrageenan</td>
<td>&lt;0.5%w/v</td>
</tr>
<tr>
<td>(0.25%w/v, 0.5%w/v)</td>
<td></td>
</tr>
<tr>
<td>Eudragit L100</td>
<td>1.5%w/v to 2.0%w/v</td>
</tr>
<tr>
<td>(0.5%w/v)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.5%w/v</td>
</tr>
<tr>
<td>(1.0%w/v)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1 Concentration of chloroquine diphosphate at which precipitation occurred in various polymer solutions.

The results in Table 3.1 shows carrageenan to be highly incompatible with chloroquine diphosphate even at the lower concentration of 0.25%w/v. Eudragit® L100 exhibits limited compatibility whereas the presence of acacia in the aqueous solution did not appear to affect the solubility of chloroquine diphosphate in water. These observations agree with those of Odidi (1990) who observed the binding capacities of the polymers to be of the following order

Carrageenan > Eudragit® L100 > Acacia.

The difference in binding capacities between the three polymers is a result of the differences in the acidity of the anionic binding sites and the number of binding sites per polyelectrolyte molecule.
Carrageenan is a sulphonic acid base polyelectrolyte with pKa between 1 and 2 and has an approximate sulphate content of 32% (Glicksman 1969). It is more acidic than carboxylic acid based polyelectrolytes such as Eudragit® L100 which has pKa's between 5 and 6, even though Eudragit® L100 consists of 50% methacrylic acid (Handbook of Pharmaceutical Excipients 1986). Acacia, which is the least acidic of the three, is also a carboxylic acid based polyelectrolyte consisting of only 13% glucuronic acid. Due to its greater acidity, carrageenan would precipitate as the polymer-drug complex at lower concentrations of both the drug and polymer, followed by Eudragit® L100 and finally acacia.

3.2.4.2 Effect of anionic polymers on the o/w partitioning of chloroquine

<table>
<thead>
<tr>
<th>Polymer solution</th>
<th>pH 1.2</th>
<th>Deionised water</th>
<th>pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of polymer</td>
<td>94.8</td>
<td>9.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Acacia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% w/v</td>
<td>94.8</td>
<td>13.7</td>
<td>precipitation</td>
</tr>
<tr>
<td>8% w/v</td>
<td>95.1</td>
<td>15.8</td>
<td>precipitation</td>
</tr>
<tr>
<td>Carrageenan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25% w/v</td>
<td>precipitation</td>
<td>precipitation</td>
<td>precipitation</td>
</tr>
<tr>
<td>0.5% w/v</td>
<td>precipitation</td>
<td>precipitation</td>
<td>precipitation</td>
</tr>
<tr>
<td>Eudragit L100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5% w/v</td>
<td>polymer</td>
<td>polymer</td>
<td>75.9</td>
</tr>
<tr>
<td>1.0% w/v</td>
<td>insoluble</td>
<td>insoluble</td>
<td>82.3</td>
</tr>
<tr>
<td>Eudragit S100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5% w/v</td>
<td>polymer</td>
<td>polymer</td>
<td>55.4</td>
</tr>
<tr>
<td>1.0% w/v</td>
<td>insoluble</td>
<td>insoluble</td>
<td>60.9</td>
</tr>
</tbody>
</table>

Table 3.2 Percentage of chloroquine partitioned into aqueous polymer solution in 24 hours.
### Partition coefficient of chloroquine (K_{o/w})

<table>
<thead>
<tr>
<th>pH 1.2</th>
<th>Deionised water</th>
<th>pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>12.13</td>
<td>57.47</td>
</tr>
</tbody>
</table>

Table 3.3 Partition coefficient of chloroquine at different pH values.

<table>
<thead>
<tr>
<th>Polynomial solution</th>
<th>pH 1.2</th>
<th>Deionised water</th>
<th>pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4%w/v</td>
<td>3.9e-4</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>8%w/v</td>
<td>2.1e-3</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>Eudragit L100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5%w/v</td>
<td>polymer</td>
<td>polymer</td>
<td>4.651</td>
</tr>
<tr>
<td>1.0%w/v</td>
<td>insoluble</td>
<td>insoluble</td>
<td>2.526</td>
</tr>
<tr>
<td>Eudragit S100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5%w/v</td>
<td>polymer</td>
<td>polymer</td>
<td>3.366</td>
</tr>
<tr>
<td>1.0%w/v</td>
<td>insoluble</td>
<td>insoluble</td>
<td>1.855</td>
</tr>
</tbody>
</table>

Table 3.4 Binding capacities of various polymers to chloroquine.
As shown in Table 3.3, the oil-water partition coefficient of chloroquine ($K_{O/w}$) was found to increase with pH values. This is due to chloroquine being a weak base ($pK_{a1} 8.4$ and $pK_{a2} 10.8$). At lower pH values, chloroquine would partition into the aqueous phase by receiving protons from the relatively acidic aqueous solution.

In the presence of the polyanionic polymers, the partition coefficient of chloroquine changes due to polymer-drug interaction. This polymer-drug interaction resulted in the precipitation of the highly acidic carrageenan at the drug levels investigated but not for Eudragit® L100, Eudragit® S100 or acacia (Table 3.2).

The binding capacity of each polymer is indicated by its $r$ value. The greater the binding capacity of the polymer, the greater is its $r$ value. Even though the total amount of drug partitioned increased with an increase in polymer concentration (Table 3.2), the $r$ values decreased (Table 3.4). This is because of the disproportionate increase in the amount of drug bound, to the increase in polymer concentration.

Eudragit® S100, a polymethacrylate which is 30% methacrylic acid as compared to Eudragit® L100 which is 50% methacrylic acid, was included in the study to demonstrate further the effect of binding sites on the binding capacity of the polymer. An increase in the number of anionic binding sites per mole of polymer from Eudragit® S100 to Eudragit® L100 increased the binding capacity.

The almost total partitioning of chloroquine to the aqueous phase at pH 1.2 was due to salt formation and was not affected by the presence of acacia which was found to have the lowest binding capacity of the four polymers investigated. Therefore, the order of polymers in terms of their binding capacities, which in this case can be indicated by their acidities, is:

\[
\text{Carrageenan} > \text{Eudragit® L100} > \text{Eudragit® S100} > \text{Acacia}
\]
3.2.5 Conclusion

The above study showed the incompatibility of carrageenan and Eudragit® L100 with chloroquine diphosphate. Therefore, these polyelectrolytes cannot be used as the inner aqueous phase polymer in the preparation of chloroquine diphosphate microcapsules where the drug is incorporated in the inner aqueous phase of the multiple emulsion during preparation. In formulations where these polymers are used, the drug would have to be incorporated in the middle organic phase of the multiple emulsion.

The partitioning of chloroquine from the organic phase to the aqueous phase has been demonstrated. This was found to be enhanced by the presence of anionic polyelectrolytes in the aqueous phase, as a result of drug-polymer interaction. This polymer-drug interaction resulted in the precipitation of the highly acidic carrageenan, but not Eudragit® L100, Eudragit® S100 or acacia. When used as the inner aqueous phase polymer in the preparation of chloroquine microcapsules where the drug is incorporated in the middle organic phase of the multiple emulsion during preparation, these polyelectrolytes might delay drug release from the resulting microcapsules due to the formation of strong ionic bonds.
3.3 FILM STUDIES

3.3.1 Introduction

In the manufacture of chloroquine microcapsules where the drug is incorporated in the middle organic phase, two important factors to consider are:

1. the choice of organic solvent and
2. the drug-polymer compatibility.

The main criterion in the choice of an organic solvent for the process is its volatility and partial miscibility with water. This is necessary so as to facilitate the removal of organic solvent by diffusion into the outer aqueous phase followed by evaporation. Of all such organic solvents, the three which are used most frequently for the purpose of microencapsulation are: ethyl acetate, methylene chloride (dichloromethane) and chloroform.

It has been shown in Section 3.2 that chloroquine is soluble in ethyl acetate. Ethyl acetate was the solvent of choice in this work mainly because it is the least toxic of the three organic solvents listed above (Martindale 1989). This is especially important in view of the fact that large quantities of organic solvent can remain in the microcapsules even after drying. In one instance, thermogravimetric analysis recorded a weight loss of between 1.8 and 4.7% that was attributed to methylene chloride evaporation, this being subsequently confirmed by chlorine content analysis (Benoit et al. 1986).

Another important factor is the effect of the organic solvent on the tendency of the drug to form crystals in the matrix wall of the microcapsule and on its surface. This is important as the presence of crystals in the wall of the microcapsule or on its surface, would result in rapid release of the drug. Bodmeier and Chen (1989, 1990) showed that the formation of indomethacin crystals in ethylcellulose microspheres prepared using chloroform as the organic solvent, was a result of drug precipitation before polymer precipitation during solvent extraction. At the same time, they found that when methylene chloride or ethyl
acetate was used in place of chloroform, polymer precipitation occurred first and the resulting microspheres were crystal free. However, at higher drug loadings, only ethyl acetate produced crystal free microspheres. They attributed this to the faster rate of solvent extraction and hence polymer precipitation in the order

\[
\text{ethyl acetate} > \text{methylene chloride} > \text{chloroform.}
\]

The rate of solvent diffusion in the aqueous phase and hence the rate of polymer precipitation are related to the water solubility and the rate of evaporation of the organic solvent. The water solubilities (20 °C) of ethyl acetate, methylene chloride and chloroform are 8.80 (Stephen and Stephen 1963), 1.03 and 0.55% w/w (Horvarth 1982) and the molar heats of vaporization are 8.4, 6.8 and 7.3 kcal (Cox and Pilcher 1970) respectively. These data could explain the decrease in the rate of polymer precipitation and increase in drug crystallization in the order of ethyl acetate > methylene chloride > chloroform. In addition, water immiscible solvents might form boundary layers around the microspheres. The thickness of the boundary layer would increase with a decrease in water solubility of the solvent. This results in the formation of drug crystals on the surface of the microspheres due to the precipitation of drug in this boundary layer after solvent evaporation. In view of this fact as well as the clinical acceptability of the organic solvent, ethyl acetate was chosen for use in this work.

To establish drug-polymer compatibility, Bodmeier and Chen (1990) cast films of the drug-polymer solutions in methylene chloride on cover glass and observed the drug concentration at which drug crystallization occurred using scanning electron microscopy. The physical state of the drug in the polymer matrix was also established by differential scanning calorimetry (Bodmeier et al. 1989, 1990, 1991) as the drug could either be dispersed in the polymer matrix, dissolved in it, or both. The presence of a melting endotherm implies the existence of particulate drug in the microsphere but the absence of a thermal event does not necessarily imply that the drug is molecularly dispersed in the polymer matrix (Watts et al. 1990). If the polymer melts at a lower temperature than the drug, the drug may dissolve in the melted polymer and escape detection by differential scanning calorimetry. It is often possible to determine
whether the drug is molecularly dispersed in the polymer by measuring its glass transition temperature. If the drug interacts with the polymer to form a true solution, it will plasticise the polymer and lower its glass transition temperature.

In this part of the study, ethylcellulose films, with and without chloroquine, were cast using ethyl acetate as the organic solvent. The aim was to establish the physical state of chloroquine in the ethylcellulose matrix on solvent evaporation by scanning electron microscopy and differential scanning calorimetry. Cast films were used in place of microcapsules for this study because the presence of other ingredients found in the microcapsules were found to interfere with the thermal events of chloroquine and ethylcellulose.

3.3.2 Materials

<table>
<thead>
<tr>
<th>Materials</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine diphosphate</td>
<td>Sigma Chemical Company Ltd.</td>
</tr>
<tr>
<td>Ethylcellulose</td>
<td>Hercules Ltd.</td>
</tr>
<tr>
<td>Ethyl acetate AnalR</td>
<td>Merck Ltd. (BDH)</td>
</tr>
<tr>
<td>Sodium hydroxide pellets AnalR</td>
<td>Merck Ltd. (BDH)</td>
</tr>
</tbody>
</table>

3.3.3 Method

Chloroquine base was precipitated from three 20ml chloroquine diphosphate solutions containing 1, 2 and 4g chloroquine diphosphate respectively. This was done using molar sodium hydroxide solution (pH titration method). The precipitate in each case was then extracted using 4 x 5ml ethyl acetate which was saturated with deionised water. The resulting chloroquine extract was then washed with 2 x 10ml deionised water, saturated with ethyl acetate, to remove any excess sodium hydroxide. The three chloroquine extracts would then have theoretical concentrations of 3, 6 and 12%w/v chloroquine base.
2g of ethylcellulose was then dissolved in 20ml of each of the above chloroquine extract and also to 20ml of ethyl acetate (saturated with deionised water) to give three chloroquine-ethylcellulose solutions, each containing 10%w/v ethylcellulose, and a 10%w/v ethylcellulose solution. 10ml of each of these solutions was then poured into a pyrex glass dish (10cm in diameter). Each of the remaining 10ml of the above organic solutions was poured slowly into a pyrex glass dish containing 20ml deionised water which had been saturated with ethyl acetate. All the glass dishes were left in an oven at 50 °C under reduced pressure and protected from light for 48 hours to allow the evaporation of the organic solvent. After 48 hours, the films cast over deionised water were removed from the dishes and dried for another 24 hours. Additional chloroquine extract in ethyl acetate was prepared as before from 20%w/v chloroquine diphosphate solution. The solvent was then evaporated in an oven at 50 °C under reduced pressure and protected from light for 72 hours to obtain the chloroquine (base) crystals. The resulting chloroquine crystals and the films, which were prepared in duplicate, were then analysed using scanning electron microscopy and differential scanning calorimetry. The analyses using differential scanning calorimetry were repeated after two months. All samples were stored in evacuated dessicators containing silica gel.

**Scanning Electron Microscopy**

Small segments of the films were cut out and attached to the stage, on which they were then coated with gold and observed with a Philips XL20 scanning electron microscope.

**Differential Scanning Calorimetry**

The process of differential scanning calorimetry has been reviewed by McNaughton and Mortimer (1975). In this process, small quantities of the samples (5mg) were placed in aluminium pans which were then sealed by normal lid-crimping. The pan containing the sample and the reference were then heated at a rate of 20 °C per minute from 30 to 300 °C in nitrogen gas. The differential scanning calorimetry system (Perkin-Elmer DSC-7) measures any difference in either heat liberated or heat absorbed between the sample and the reference. In this study, only comparative qualitative analyses of the samples were carried out.
3.3.4 Results and Discussion

Scanning electron micrographs of the films cast on glass are shown in Plate 1. The films had relatively smooth surfaces both at the air-film and glass-film interfaces. Films with a theoretical chloroquine concentration of 3% w/v appeared similar to the ethylcellulose film (not shown). At the theoretical chloroquine concentration of 6% w/v, the films were shown to contain some needle-like structures when observed from the glass film interface (Plate 1(d)). This was confirmed by the presence of drug crystals on the surfaces of the air-film interfaces (Plate 1(c)) of the same films. A greater concentration of these structures at the higher theoretical chloroquine concentration of 12% w/v supports the postulation that these are actually chloroquine crystals. In addition, these crystals were also similar to the chloroquine crystals obtained from the chloroquine extract in ethyl acetate.

Films cast on water appeared different to those cast on glass (Plate 2). The water-film interfaces were corrugated (Plate 2(b), (d) and (f)) and it was not possible to see the presence of drug crystals in the films when observed from the water-film interfaces of the chloroquine-ethylcellulose films even though they were present when observed from the air-film interfaces (Plate 2(c) and (e)). Drug crystals were again not observed at the theoretical chloroquine concentration of 3% w/v. Even though chloroquine crystals were observed in the films with theoretical chloroquine concentrations of 6 and 12% w/v, they were not as numerous and concentrated as the respective films cast on glass. In fact, drug crystals were not observed on the surfaces of the films with theoretical chloroquine concentration of 6% w/v even though they were present on the films of similar theoretical chloroquine concentration which were cast on glass. This is probably due to a lower actual concentration of the drug in the films cast on water as a result of the partitioning of drug molecules into the aqueous phase (section 3.2).
Plate 1. Scanning electron micrographs of films cast on glass.
Plate 2. Scanning electron micrographs of films cast on water.
Differential scanning calorimetry (DSC) was also used to investigate the physical state of chloroquine in the ethylcellulose films. Theoretically, if chloroquine crystals are present in the film, an endotherm corresponding to the melting point of chloroquine crystals should be observed in its DSC thermogram. But if chloroquine is dissolved in the film matrix, the endotherm would be absent. The dissolved chloroquine would also alter the glass transition temperature of the ethylcellulose film.

DSC thermograms of the freshly prepared samples are shown in Figure 3.4. The numerous thermal events observed in all the fresh samples (ethylcellulose films, chloroquine-ethylcellulose films and chloroquine crystals) were absent after two months (Figure 3.5). The thermal events observed in the fresh samples could be a result of the effect of residual solvents on chloroquine and ethylcellulose. Ramtoola et al. (1991) observed the effect of residual solvents (acetone, methylene chloride and chloroform) in poly-DL-lactide and poly-DL-lactide-co-glycolide micromatrices. These endotherms were observed in the temperature range of 70-80 °C. In this study, no thermal events were observed below 100 °C for the fresh samples. The solvent-effect observed in the DSC thermograms of the fresh samples is probably due to residual water as the ethyl acetate used was saturated with distilled water. The four peaks observed for chloroquine (Figure 3.4) were probably endotherms of various polymorphs of chloroquine containing bound water molecules. This explains why the melting point of these polymorphs were higher than the "aged" chloroquine crystals (Figure 3.5). The presence of residual water probably catalysed the decomposition of ethylcellulose in the ethylcellulose and chloroquine-ethylcellulose films. The reaction involved was probably hydrolysis of the ethoxy substituents of the ethylcellulose molecules.

An absence of the melting transition of the drug has been interpreted as drug being either dissolved or molecularly dispersed in the polymer matrix (Benoit et al. 1986). However, in this study, chloroquine crystals were present in the chloroquine-ethylcellulose films (Plate 1 and 2) but the endotherm for chloroquine crystals were not observed in the DSC thermograms. This apparent discrepancy can be explained as follows. During DSC analysis, temperature was increased at a constant rate.
Figure 3.4 DSC thermogram of fresh samples.
Figure 3.5 DSC thermogram of aged samples.
Ethylcellulose probably melted before the chloroquine crystals, which subsequently dissolved in the polymer melt, causing the melt transition to disappear (Bodmeier and Chen 1989). It is therefore misleading to assume that the drug was dissolved in the original polymeric film because of the absence of an endotherm at the drug melting point.

3.3.5 Conclusion

This study showed that at higher chloroquine concentrations, chloroquine crystals are formed in the ethylcellulose matrix and also on the surface of the chloroquine-ethylcellulose film. Differential scanning calorimetry however, did not show the presence of these chloroquine crystals. Such results may be misinterpreted as the absence of drug crystals. In addition, differential scanning calorimetry has also been shown to be very sensitive to the presence of small amounts of contaminants, such as residual solvents. Results obtained from such contaminated samples may also be misleading.

The presence of crystals in, and on the ethylcellulose wall matrix of microcapsules would result in rapid rates of drug release. This can be overcome by decreasing the concentration of chloroquine in the ethylcellulose matrix. In the preparation of microcapsules, this may be achieved by the use of an anionic polyelectrolyte in the inner aqueous phase of the multiple emulsion (section 3.2). The anionic polyelectrolyte in the inner aqueous phase would increase the partitioning of chloroquine from the oily phase containing ethylcellulose, into the inner aqueous phase, thus decreasing the chloroquine concentration in the subsequent ethylcellulose wall formed.
Chapter Four

Development of a Manufacturing Procedure for Microencapsulation
4.1 INTRODUCTION

The original multiple emulsion method of microencapsulation described by Warburton (1981) and developed further by Morris (1982) and Duquemin (1987) gives three-ply-walled microcapsules which may be obtained as a free flowing powder.

In this technique, a stable water-in-oil (w/o) primary emulsion is first prepared, with the drug encapsulated in the aqueous phase. This w/o emulsion is stabilised by the presence of adsorbed films at the water/oil interface which provides a physical barrier to coalescence (Morris and Warburton 1982). The w/o emulsion is then re-emulsified to form a water-in-oil-in-water (w/o/w) secondary emulsion. The fresh water/oil interface formed is again stabilised by adsorbed films. The two aqueous phases of the secondary emulsion can be different so that three different wall materials are present.

The organic solvent used in the oily phase of the multiple emulsion has to be volatile and partially miscible with water. Microcapsules are formed by its diffusion into the outer aqueous phase, followed by evaporation. Being denser than the surrounding medium, the microcapsules will sediment and subsequently, a free flowing powder can be obtained by drying the wet microcapsules.

To develop a procedure for the microencapsulation of chloroquine using the multiple emulsion technique, some of the manufacturing parameters were investigated. It was necessary to be selective in choosing the parameters for investigation because of the large number of variables involved. To simplify the study, it was carried out using drug-free microcapsules.

This study was carried out in two main parts:

(1) to study the effects of the two emulsification processes and
(2) to study the effects of the solvent extraction and drying processes on microcapsule characteristics.
The multiple emulsion technique of microencapsulation involves the formation of a primary water-in-oil (w/o) emulsion which is then re-emulsified to form a secondary water-in-oil-in-water (w/o/w) emulsion. It was therefore necessary to study the emulsification processes in two stages:

(1) the formation of the primary w/o emulsion and
(2) the formation of the secondary w/o/w emulsion.

Due to the complexity of the system, a factorial design was applied to study the effect of a few selected independent variables. The dependent variable investigated was the particle size distribution of the microcapsules. The model formulation used was the acacia ($w_1$) / ethylcellulose (o) / acacia ($w_2$) combination with ethyl acetate as the organic solvent used in the middle oily phase (Duquemin 1987).

### 4.2.1 Factorial Design

Factorial designs are experimental designs which allow the simultaneous determination of the effects of several factors and their interactions (Bolton 1990). In such an experimental design, all possible combinations of the levels of the factors are investigated. Several examples of the application of factorial designs to pharmaceutical problems have appeared in the literature and proved to be extremely useful for screening purposes or to help interpret complex systems (Bolton 1983; Devay et al. 1984; Dincer and Ozdurmus 1977; Gupta et al. 1989; Waltersson 1986; Oner et al. 1988). It offers precision and the possibility of detecting interaction between factors, giving more information at lower cost.

A factor is an assigned variable such as concentration or temperature. The levels of a factor are the values or designations assigned to the factor.
For example, the factor concentration may have two levels, 0.5 molar and 1.0 molar. The lower concentration of 0.5 molar is referred to as the low level of the factor and the higher concentration of 1.0 molar is referred to as the high level of the factor. Therefore, a $2^k$ factorial design is an experimental design consisting of $k$ number of factors at two levels each. The minimum number of trials (experiments) which is equal to the number of treatment combination is therefore $2^k$. The total number of trials would be $n2^k$, where $n$ is the number of replicates.

In a factorial design, the factors are usually denoted by capital letters of the alphabet. As an example, consider the $2^3$ factorial design which has three factors at two levels each. The three factors would be denoted by A, B and C. The levels of each factor in a treatment combination is denoted by the absence (low) or presence (high) of the small letter of the alphabet representing the factor. For example, when factor A is present at its high level and the other factors are present in their low levels, the notation a is used for the treatment combination. If factors A and B are present at their high levels, the treatment combination would be denoted by ab and so on. The notation (1) is used for the treatment combination where all the factors are present in their low levels. The notations used for the various treatment combinations and the low or high levels of each factor are listed in Tables 4.1 and 4.2 for the $2^3$ factorial design and the $2^4$ factorial design respectively.

The effect of a factor is the change in response caused by varying the level(s) of the factor. The main effect is the effect of a factor averaged over all levels of the other factors. An interaction of factors occurs when the effect of a factor is different at different levels of another(other) factor(s). For example, consider a system where factor A increases the particle size distribution of the system at the low level of factor B. If the effect of factor A at the high level of factor B is different from its effect at the low level of factor B, an AB interaction has occurred. This interaction may be synergistic, that is, the effect of factor A is greater at one level of factor B compared to the other. It may also be antagonistic where a change in the level of factor B actually decreases the particle size distribution. The significance of the effects and interactions of factors are usually analysed by the analysis of variance.
Table 4.1 Eight treatment combinations of the $2^3$ factorial design.

<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>a</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td>b</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>ab</td>
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<td>+</td>
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<tr>
<td>c</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ac</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>bc</td>
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<td>+</td>
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</tr>
<tr>
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</tr>
</tbody>
</table>

* -, factor at low level; +, factor at high level.

Table 4.2 Sixteen treatment combinations of the $2^4$ factorial design.

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<tr>
<td>b</td>
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<td>+</td>
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<td>-</td>
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<tr>
<td>ab</td>
<td>+</td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
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<td>+</td>
<td>-</td>
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<td>-</td>
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</table>

*-, factor at low level; +, factor at high level.
4.2.2 Analysis of Variance

The analysis of variance or ANOVA, is a general method of analyzing data from designed experiments, whose objective is to compare two or more group means. The name itself is derived from a partitioning of total variability into its component parts, represented by a statistical model. For example, consider the analysis of variance model used for the analysis of results from a $2^3$ factorial design experiment which has three factors, A, B and C, with two levels each. The model is represented as

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$$

where $i, j, k = 1$ or $2$ (first or second of the two levels),

and

- $\mu$ is the overall mean effect,
- $\alpha_i$ is the effect of the $i^{th}$ level of factor A,
- $\beta_j$ is the effect of the $j^{th}$ level of factor B,
- $\gamma_k$ is the effect of the $k^{th}$ level of factor C,
- $(\alpha\beta)_{ij}$ is the effect of the interaction between $\alpha_i$ and $\beta_j$,
- $(\alpha\gamma)_{ik}$ is the effect of the interaction between $\alpha_i$ and $\gamma_k$,
- $(\beta\gamma)_{jk}$ is the effect of the interaction between $\beta_j$ and $\gamma_k$,
- $(\alpha\beta\gamma)_{ijk}$ is the effect of the interaction between $\alpha_i$, $\beta_j$ and $\gamma_k$ and
- $\varepsilon_{ijkl}$ is a random error component.

For a fixed effect model, the total sum of squares

$$SS_T = \sum_{i=1}^{2} \sum_{j=1}^{2} \sum_{k=1}^{2} \sum_{l=1}^{n} Y_{ijkl}^2 - \frac{Y_{....}^2}{2^3n}$$

where $Y_{....} = \text{sum of all responses}$, is used as a measure of overall variability. It can also be expressed as
\[ SST = SS_A + SS_B + SS_C + SS_{AB} + SS_{AC} + SS_{BC} + SS_{ABC} + SS_E \]

where \( SS_A, SS_B, SS_C, SS_{AB}, SS_{AC}, SS_{BC}, \) and \( SS_{ABC} \) are sums of squares due to the respective effects (factor or interaction) and \( SS_E \) = sum of squares due to error.

To estimate an effect or to compute the sum of squares for the effect, the contrast associated with the effect must first be determined. First of all, a table of contrast constants for the effects and interactions (Table 4.3) has to be established. This is obtained by assigning a positive sign to the combination where the factor concerned is present at a high level and a negative sign where it is present at a low level, as in Tables 4.1 and 4.2. Contrast constants for interactions are products of the contrast constants of the main effects involved. A contrast for an effect is the sum of the products of the respective contrast constant and their corresponding observed response. For example, the contrast for the effect of factor A is:

\[ \text{Contrast}_A = -(1) + a - b + ab - c + ac - bc + abc \]

<table>
<thead>
<tr>
<th>Treatment Combination</th>
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<th>Interactions**</th>
</tr>
</thead>
<tbody>
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<tr>
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</tr>
</tbody>
</table>

* -, factor at lower level; +, factor at higher level.
** Multiply signs of factors to obtain signs for interaction terms in combination

\[ \text{e.g. AB at (1)} = (-) \times (-) = (+) \]

Table 4.3 Contrast constants of the \( 2^3 \) factorial design.
Once the contrasts for the effects and interactions have been computed, the estimates of the effects and interactions and their sums of squares can be calculated according to

\[ AB......K = 2 \left( \text{Contrast}_{AB...K} \right) / n^{2k} \]

and

\[ SS_{AB...K} = \left( \text{Contrast}_{AB...K} \right)^2 / n^{2k} \]

where \( n \) = total number of replicates.

The sum of squares due to an effect, \( SS_{\text{effect}} \) (all except \( SS_{\epsilon} \)), divided by its number of degrees of freedom (D.F.) gives an estimate of the sample variance termed the mean square of the effect (\( MS_{\text{effect}} \)). The number of degrees of freedom is the number of independent elements in the sum of squares. If the effect of a particular treatment is insignificant, its mean square would be equal to the mean square of error (\( MS_{\epsilon} \)), which is an estimate of the population variance, \( \sigma^2 \). However, if the mean square of treatment is greater than the mean square of error, we would have reason to suspect that the effect is significant. The ratio

\[ F_0 = \frac{MS_{\text{effect}}}{MS_{\epsilon}} \]

is therefore used as the test statistic for the hypothesis of no difference in treatment means. If \( F_0 \) is too large

\[ F_0 > F_{\delta,u,v}, \]

where \( \delta \) is the level of significance, \( u \) is the D.F. of the numerator, \( MS_{\text{effect}} \), and \( v \) is the D.F. of the denominator, \( MS_{\epsilon} \), we can say with \( (100-\delta)\% \) confidence that the effect of the treatment is significant.

For a \( 2^k \) factorial design, the total number of treatment combination is large even for a moderate number of factors. Frequently, the availability of resources or the nature of the experiment restricts the number of replicates employed. When only a single replicate is employed (\( n=1 \)), it is
impossible to estimate the mean square error. Thus it seems that the hypotheses cannot be tested. However, by assuming that certain high order interactions are negligible, their mean squares may be combined to estimate the experimental error since any variability in insignificant effects will be due to experimental error (Bolton 1990; Montgomery 1984). The smallest design for which this procedure is recommended is the $2^4$ factorial design.

As a general rule, it is probably unwise to assume two factor interactions to be zero without prior information. If most two factor interactions are small, then it seems likely that all higher order interactions will be insignificant also. But occasionally, real high order interactions occur and the use of an error mean square obtained by pulling high order interactions is inappropriate. A simple way to overcome this problem is to plot the estimates of the ordered effects (in decreasing or increasing order of magnitude) against cumulative probability points:

$$P_k = \frac{(k - 0.5)}{N}$$

where $P_k$ = the cumulative probability point,

$k$ = the order of the effect or interaction (1....N),

$N$ = the number of effects or interactions ($2^k - 1$)

This is a check of normality (Montgomery 1984). Effects that are negligible are normally distributed with mean zero and variance, $\sigma^2$. The normal probability plot will yield a straight line for insignificant effects. Effects that are significant will not lie along the straight line as they have non zero means. In this way, the insignificant high order interactions can be isolated.

From this method of analysis, another interpretation is possible. If $h$ number of main effects ($h<k$) and all interactions involving them are negligible, they can be dropped and the unreplicated $2^k$ factorial can be projected into a full replicated $2^{k-h}$ factorial design with $2^h$ replicates. For example, if the main effects of any two factors of a $2^4$ factorial design are negligible ($h=2$), the experimental design can be projected into a $2^2$ factorial design with 4 replicates. The conclusions that can be drawn from this analysis are essentially unchanged from the $2^k$ factorial design.
but now, we have an estimate of the error based on replication which should agree closely to that obtained from the estimates of the high order interactions based on the $2^k$ factorial design.

### 4.2.3 Model Adequacy Checking

The use of the analysis of variance to test for no differences in treatment means require certain assumptions to be satisfied. Specifically, for the $2^3$ factorial design, these are that the observations are adequately described by the model

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$$

where $i, j, k = 1$ or $2$ (first or second of the two levels),

1 = 1......n, where n is the number of replicates,

and

- $\mu$ is the overall mean effect,
- $\alpha_i$ is the effect of the $i^{th}$ level of factor A,
- $\beta_j$ is the effect of the $j^{th}$ level of factor B,
- $\gamma_k$ is the effect of the $k^{th}$ level of factor C,
- $(\alpha\beta)_{ij}$ is the effect of the interaction between $\alpha_i$ and $\beta_j$,
- $(\alpha\gamma)_{ik}$ is the effect of the interaction between $\alpha_i$ and $\gamma_k$,
- $(\beta\gamma)_{jk}$ is the effect of the interaction between $\beta_j$ and $\gamma_k$,
- $(\alpha\beta\gamma)_{ijk}$ is the effect of the interaction between $\alpha_i$, $\beta_j$ and $\gamma_k$ and $\varepsilon_{ijkl}$ is a random error component,

and that the errors are normally and independently distributed with mean zero and constant but unknown variance, $\sigma^2$. If these assumptions are valid, then the analysis of variance procedure is an exact test. Validity of these assumptions and adequacy of the model can be investigated by the examination of residuals which is defined as

$$e_{ijkl} = Y_{ijkl} - \hat{Y}_{ijkl}$$

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where $\hat{Y}_{ijkl}$ are estimates (fitted values) of the corresponding observations $Y_{ijkl}$ calculated from the assumed model. If the model does describe the observations, a normal probability plot of the residuals should yield a straight line (Montgomery 1984).

4.2.4 Particle Size Analysis

Methods for the particle size analysis of w/o emulsions are not as established as those for the o/w emulsions. This is probably due to the wide application of o/w emulsions as compared to w/o emulsions. In this experiment, the 2600 series Particle Sizer (Malvern Instruments), based on Fraunhoffer laser diffraction, was used to size the emulsion droplets because of its numerous advantages over other methods of analysis. It is (1) non-intrusive, (2) faster than mechanical techniques, (3) it does not require a conducting media, (4) it does not subject the particles to large shearing forces apart from possible effects of sample handling and (5) it does not require calibration (Weiner 1984). In addition, this method of analysis can also be used for sizing multiple emulsion droplets and solid particles of microcapsules and so it allows a better comparison of particle size distributions throughout the three manufacturing stages of the study.

The main problem encountered in the analysis was the presence of droplets in the submicron range. To analyse these submicron droplets accurately, it would be necessary to use photon correlation spectroscopy as Fraunhoffer diffraction is utilized mainly for particles larger than 1µ. The lowest size band of the 63mm lens of the Particle Sizer extends to 1.2µ. However, due to sub-size measurement compensation, the sub-size class gives an estimate of the less than 1.2µ contribution and is sensitive down to 0.5µ as long as not more than 20% of the material is in this range. Since all the preparations meet this requirement, the Particle Sizer was considered adequate for particle size analysis in all three stages of manufacture.
4.2.5 Materials

Acacia
Ethylcellulose N10NF
Ethyl acetate GPR

Merck Ltd. (BDH)
Hercules Ltd.
Merck Ltd. (BDH)

4.2.6 Methods

4.2.6.1 Droplet size distribution of the primary w/o emulsion

In this experiment, a $2^4$ factorial design was applied, that is, four factors were investigated at two levels each. The factors and levels were chosen from prior experience in initial formulation work. The aim of this experiment was to examine the effects of (A) concentration of disperse phase (acacia), (B) concentration of continuous phase (ethylcellulose), (C) mixing time and (D) primary phase volume ratio (w/o) on the particle size distribution of the primary w/o emulsion. The four factors and their levels investigated in this study are listed in Table 4.4. The sixteen treatment combinations and the levels of each factor in each combination have already been listed in Table 4.2.

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>LOW LEVEL</th>
<th>HIGH LEVEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Acacia concentration (%w/v)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>(B) Ethylcellulose concentration (%w/v)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>(C) Mixing time (min)</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>(D) Primary phase volume ratio (w/o)</td>
<td>0.25</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Table 4.4 Factors investigated in the primary emulsification process.
The acacia solution was added dropwise to the ethylcellulose solution and mixed at maximum shear rate using a Silverson mixer-emulsifier. The rate of addition of the acacia solution was kept constant by means of a peristaltic pump (48rpm). The fresh emulsion was then analyzed for its size distribution using the Particle Sizer. It was then allowed to stand in an air-tight container for 24 hour, after which, the emulsion was analysed again. The procedure was consistent for all 16 emulsions (trials or treatment combinations).

4.2.6.2 Droplet/Particle size distribution of the secondary w/o/w emulsion/microcapsules

In this experiment, the effects of (A) primary phase volume ratio (w/o), (B) secondary phase volume ratio (w/o/w) and (C) concentration of the outer aqueous phase polymer (acacia) on the size distribution of the multiple emulsion droplets were investigated. A $2^3$ factorial design with 2 replicates was used. The factors and their respective levels are listed in Table 4.5. The eight treatment combinations and the levels of each factor in each treatment combination are listed in Table 4.1.

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>LOW LEVEL</th>
<th>HIGH LEVEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Primary phase volume ratio (w/o)</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>(B) Secondary phase volume ratio (w/o/w)</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>(C) Acacia concentration (%w/v)</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 4.5 Factors investigated in the secondary emulsification process.
Based on the results of experiment 1, the primary w/o emulsion composed of 4% w/v acacia in 4% w/v ethylcellulose, mixed for 0.5 hour, was chosen for this experiment. The primary w/o emulsion formed with either a primary phase volume ratio of 0.25 or 0.50 was added dropwise to the acacia solution (4 or 8% w/v). The amount added depended on the secondary phase volume ratio (0.25 or 0.50) and the mixture was mixed in an ice-bath, at a pre-calibrated speed. The resulting secondary w/o/w emulsion was analysed using the Particle Sizer and then rotary evaporated at 30°C for 1 hour. The resulting suspension was allowed to stand overnight. The microcapsules formed were isolated by filtration and the slurry was oven-dried at 50°C for 24 hours. The dried microcapsules were then suspended in deionised water and analysed using the Particle Sizer.
4.2.7 Results and Discussion

4.2.7.1 Droplet size distribution of the primary w/o emulsion

The acacia-ethylcellulose w/o emulsions were found to have log-normal droplet size distribution. They can therefore be described using the geometric mean diameter, $X$, and the geometric standard deviation, $N$ (Table 4.6). Figure 4.1 shows a typical size distribution profile of the primary emulsions.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>G. MEAN DIAMETER ($\mu$)</th>
<th>G. STANDARD DEVIATION ($\mu$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMBINATION</td>
<td>FRESH</td>
<td>AGED</td>
</tr>
<tr>
<td>(1)</td>
<td>0.88</td>
<td>*</td>
</tr>
<tr>
<td>a</td>
<td>1.04</td>
<td>*</td>
</tr>
<tr>
<td>b</td>
<td>4.06</td>
<td>*</td>
</tr>
<tr>
<td>ab</td>
<td>5.11</td>
<td>*</td>
</tr>
<tr>
<td>c</td>
<td>0.88</td>
<td>*</td>
</tr>
<tr>
<td>ac</td>
<td>0.91</td>
<td>*</td>
</tr>
<tr>
<td>bc</td>
<td>3.10</td>
<td>*</td>
</tr>
<tr>
<td>abc</td>
<td>3.18</td>
<td>*</td>
</tr>
<tr>
<td>d</td>
<td>1.31</td>
<td>1.39</td>
</tr>
<tr>
<td>ad</td>
<td>1.86</td>
<td>1.73</td>
</tr>
<tr>
<td>bd</td>
<td>1.19</td>
<td>1.14</td>
</tr>
<tr>
<td>abd</td>
<td>1.35</td>
<td>1.24</td>
</tr>
<tr>
<td>cd</td>
<td>1.33</td>
<td>1.28</td>
</tr>
<tr>
<td>acd</td>
<td>2.42</td>
<td>2.52</td>
</tr>
<tr>
<td>bcd</td>
<td>1.29</td>
<td>1.26</td>
</tr>
<tr>
<td>abcd</td>
<td>1.42</td>
<td>1.39</td>
</tr>
</tbody>
</table>

* unstable

Table 4.6 Geometric mean diameters and geometric standard deviations of the primary w/o emulsions.
Figure 4.1 Droplet size distribution of primary w/o emulsion (treatment combination bc).

The two parameters of the log-normal distribution, $X$ (geometric mean diameter) and $N$ (geometric standard deviation), can be described by the expressions (Smith and Jordan 1964)

$$
log X = \frac{\sum_{i=1} n_i \log x_i}{\sum_{i=1} n_i}
$$

and

$$(log N)^2 = \frac{\sum_{i=1} n_i (\log x_i - \log X)^2}{\sum_{i=1} n_i}
$$

where $n_i =$ number of particles within size interval $\Delta x_i$

The geometric standard deviation, $N$, therefore relates to the spread of diameters about the geometric mean diameter, $X$ (Walstra 1983).
When only a single replicate is employed, as in this case, it is impossible to estimate the mean square error and therefore the hypotheses of equality of treatment means cannot be tested. However, by assuming that certain high order interactions are negligible, their mean squares may be combined to estimate the experimental error. A simple way to isolate these insignificant effects is to plot the estimates of the ordered effects against cumulative probability points. As effects that are negligible are normally distributed with mean zero and variance, $\sigma^2$, the plot will yield a straight line for insignificant effects. Effects that are significant will not lie along the straight line as they have non-zero means. To isolate the insignificant effects, the estimates of the ordered effects are plotted against probability points in Figure 4.2 for the geometric mean diameter and Figure 4.3 for the geometric standard deviation.

On examining Figure 4.2, we see that all of the small effects on geometric mean diameter lie approximately on a straight line while the large effects do not (BD, B and D). From this method of analysis, another interpretation is possible. Since the main effect of factor C (mixing time) and all interactions involving it were negligible, it can be removed from the design and the unreplicated $2^4$ factorial design can be projected into a full replicated $2^3$ factorial design with two replicates ($n=2$). The conclusions that can be drawn from this analysis are essentially unchanged from the $2^4$ factorial design but now, we have an estimate of the error based on replication which should agree closely to that obtained from the estimates of the high order interactions in the $2^4$ factorial design. In this particular case, the analysis may be reduced further to a $2^2$ factorial design with four replicates as the effect of factor A (acacia concentration in the aqueous phase disperse) also appear to be insignificant. However, only the analysis of variance of the results analysed based on a $2^3$ factorial design are presented here (Tables 4.7).
Figure 4.2 Probability plot of estimated ordered effects on geometric mean diameters of primary w/o emulsions.

Figure 4.3 Probability plot of estimated ordered effects on geometric standard deviations of primary w/o emulsions (r = 0.97).
<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>$Fo$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.66</td>
<td>1</td>
<td>0.66</td>
<td>2.11</td>
</tr>
<tr>
<td>B</td>
<td>6.34</td>
<td>1</td>
<td>6.34</td>
<td>20.29a</td>
</tr>
<tr>
<td>D</td>
<td>3.05</td>
<td>1</td>
<td>3.05</td>
<td>9.76b</td>
</tr>
<tr>
<td>AB</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>AD</td>
<td>0.02</td>
<td>1</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>BD</td>
<td>11.24</td>
<td>1</td>
<td>11.24</td>
<td>35.97a</td>
</tr>
<tr>
<td>ABD</td>
<td>0.33</td>
<td>1</td>
<td>0.33</td>
<td>1.056</td>
</tr>
<tr>
<td>ERROR</td>
<td>2.50</td>
<td>8</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>24.15</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Significant at the 1% level  
*b* Significant at the 5% level

Table 4.7 Analysis of variance of the geometric mean diameters of the primary w/o emulsions based on a $2^3$ factorial design.

Figures 4.5 to 4.12 show the effects of the various factors on the geometric mean diameter and geometric standard deviation of the primary w/o emulsions. The various treatment combinations are denoted as before but exclude the factor which was being investigated. For example, when examining the effect of factor A, treatment combinations (1) and a will be denoted by (1) only in Figures 4.5 and 4.6. The point at the lower level of factor A corresponds to combination (1) whereas the point at the higher level of A will correspond to combination a.

From the analysis of variance (Table 4.7), the significance of the effects of the various factors on the geometric mean diameter of the primary w/o emulsion are, in descending order,

$$BD > D > B$$
where \( B \) and \( D \) represent the effects of the ethylcellulose concentration (\( B \)) and the phase volume ratio (\( D \)) respectively and \( BD \) represents the interaction between the effects of the two factors. That is, the effects of factor \( B \) and factor \( D \) are different at different levels of the other factors. The antagonistic effect of the interaction is shown as the inter-sectioning of the graphs in Figures 4.7 and 4.11.

From Figures 4.5 and 4.9, it is clear that factor \( A \) (acacia concentration) and factor \( C \) (mixing time) do affect the geometric mean diameter of a few of the the primary w/o emulsions but not as significantly as the above three effects (\( B \), \( D \) and \( BD \)). Unless precautions, such as the selection of suitable levels of the factors and visual screening of effects (by graphical methods) are taken, underestimation of an effect can occur in factorial experiments. This is where one or more dominating factor(s) cover(s) the smaller effect(s) of other factor(s). Therefore, in the projection of a \( 2^k \) factorial design to a \( 2^{k-h} \) factorial design (\( h = \) number of insignificant effects; \( h<k \)), precautions such as visual screening should be taken in addition to the normal probability plot.

The usual diagnostic check was applied by plotting a normal probability plot of the residuals based on a \( 2^3 \) factorial design on the geometric mean diameter (Figure 4.4). The points on this plot lie reasonably close to a straight line (correlation coefficient, \( r = 0.965 \)), lending support to the conclusions of the analysis of variance based on a \( 2^3 \) factorial model and that the underlying assumptions of the analysis are valid.

Due to the linearity of all the points on the probability plot of the ordered effects on geometric standard deviation (correlation coefficient, \( r = 0.97 \)) (Figure 4.3), it is not possible to assume that any of the effects of the factors are insignificant. It would also be inappropriate to assume all high order interactions to be insignificant as this would lead to an underestimation of the true experimental error. Therefore, the hypotheses concerning the main effects and interactions cannot be tested by the analysis of variance. It is however possible to screen the effects visually by graphical methods (Figures 4.6, 4.8, 4.10 and 4.12).
Figure 4.4 Probability plot of ordered residuals of geometric mean diameters of primary w/o emulsions based on $2^3$ factorial design ($r = 0.965$).
Figure 4.5 Effect of acacia concentration on geometric mean diameters of primary w/o emulsions.

Figure 4.6 Effect of acacia concentration on geometric standard deviations of primary w/o emulsions.
Figure 4.7 Effect of ethylcellulose concentration on geometric mean diameters of primary w/o emulsions.

Figure 4.8 Effect of ethylcellulose concentration on geometric standard deviations of primary w/o emulsions.
Figure 4.9 Effect of mixing time on geometric mean diameters of primary w/o emulsions.

Figure 4.10 Effect of mixing time on geometric standard deviations of primary w/o emulsions.
Figure 4.11 Effect of primary phase volume ratio on geometric mean diameters of primary w/o emulsions.

Figure 4.12 Effect of primary phase volume ratio (w/o) on geometric standard deviations of primary w/o emulsions.
A problem encountered by most workers investigating the factors governing the size distribution of emulsion droplets is that the variables often do not affect the result in the same way under different conditions (Walstra 1983) as it is seen here. At the lower phase volume ratio of 0.25 (1, a, c and ac in Figures 4.7 and 4.8), an increase in ethylcellulose concentration led to an increase in both the geometric mean diameter and standard deviation. However, at the higher phase volume ratio of 0.50 (d, ad, cd and acd in Figure 4.7; d and cd in Figure 4.8), a decrease in both the geometric mean diameter and standard deviation was observed with the same increase in ethylcellulose concentration. The decrease in the geometric standard deviation was however reversed when acacia was at the higher concentration (ad and acd in Figure 4.8). These interactions are shown as the intersectioning of the graphs in Figures 4.7 and 4.8 and we see the same interactions again in Figures 4.11 and 4.12.

At the higher ethylcellulose concentration of 4% w/v (b, ab, bc and abc in Figures 4.11; b and ab in Figure 4.12), an increase in the phase volume ratio decreased both the geometric mean diameter and standard deviation. This time however, the decrease in geometric standard deviation occurred only when the emulsion has been mixed for a shorter period of time (low level of factor C). That is when factor C was at the higher level (bc and abc in Figure 4.12), an increase in geometric standard deviation was observed. Possible explanations of the above observations lie in the mechanisms involved in the formation of emulsion.

Flow conditions during emulsification are mostly turbulent where predominant inertial forces lead to droplet breakup. A spectrum of eddy sizes also exist which results in a spread in droplet size. Turbulent flow is usually characterized by the local flow velocity \( u \) in respect to its time average value \( v \). The velocity \( u \) fluctuates in a chaotic way. The deviations are characterized by a root mean square time average

\[
u' = \{ (u - v)^2 \}^{1/2}\]

In the direction of flow, \( v \) equals the overall flow velocity but in the direction perpendicular to flow direction, \( v = 0 \). Usually \( u' \) depends on direction. When \( u' \) is the same in any direction, turbulent flow is described as isotropic.
Presumably because the presence of polymer depresses turbulence, an increase in ethylenecellulose concentration impairs disruption in turbulent flow by removing the smaller eddies from the spectrum. This occurs when the ratio of the stretch length of the polymer to the size of the smallest eddies is less than 0.5 (Walstra 1983) and results in an increase in the average size of the droplets formed. Theoretically, it should also decrease the distribution width of the droplets which is contrary to the results obtained here. A possible explanation is that an increase in ethylenecellulose concentration also increases the viscosity of the continuous phase which prevents isotropic turbulence. This widens the relative eddy spectrum resulting in a wider spread in droplet size.

In addition to inertial forces, the mechanism of droplet breakup may also depend on viscous drag which decreases turbulence. Viscous drag increases with the viscosity of the continuous phase and the phase volume ratio of the emulsion. An increase in the viscosity of the continuous phase also increases the shear stress on dispersion globules at a fixed shear rate resulting in a decrease in droplet size. Droplet size may also be the resultant of disruption and coalescence and if coalescence is the size-determining factor, both the phase volume ratio and the viscosity of the continuous phase would influence the result as indicated by the DLVO theory (Martin et al. 1983).

In view of the above factors, it is possible that when both the ethylenecellulose concentration and the phase volume ratio were at the higher level, a lower average size and size distribution were observed as a result of less coalescence due to increased hydrodynamic interaction. Another explanation could be the increase in temperature resulting from the kinetic energy dissipated by the smaller eddies as they are removed from the spectrum. Smaller droplets are normally formed at higher temperatures. For polymers, temperature may also affect solvent quality and this may in turn influence polymer conformation and hence its surface excess. The increase in temperature may also decrease the viscosity of both the continuous and disperse phases resulting in more isotropic turbulence and hence a smaller size distribution. In his review of the subject, Walstra (1983) concluded that general rules cannot be provided.
An increase in acacia concentration resulted in an increase in size and size distribution of a few of the emulsion systems (Figures 4.5 and 4.6). This is probably due to an increase in deformation time as a result of an increase in the viscosity of the disperse phase. Deformation time becomes increasingly larger with higher disperse phase viscosity and as a result, larger eddies become responsible for the droplet deformation process. Flow is hardly isotropic on the scale of the larger eddies and hence a higher geometric size distribution. This also explains the counter effect of a higher acacia concentration on the decrease in geometric standard deviation of systems with the higher phase volume ratio in Figure 4.8. As shown in Figure 4.9, mixing time appeared to affect only systems with the low phase volume ratio but high ethylcellulose concentration (b and ab).

Macroscopic examination of the aged emulsions after 24 hours showed some to be unstable. In these emulsions, sedimented aqueous layers were observed. Generally, only the emulsions with the lower phase volume ratio of 0.25 separated within 24 hours. Sedimentation was expected as the disperse phase had a higher density than the continuous phase. The stability of the emulsions with a higher phase volume ratio was probably due to the increased hydrodynamic interaction. Viscosity of the emulsion system was observed to increase dramatically with primary phase volume ratio. Viscous emulsions may be more stable than mobile ones by virtue of the retardation of flocculation and coalescence (Martin et al. 1983).

Differences in the droplet size distributions of the fresh and aged emulsions were tested using paired t-tests. The probabilities obtained for the geometric mean diameter and the geometric standard deviation were 0.37 and 0.54 respectively, with seven degrees of freedom each. That is, the geometric mean diameters and the geometric standard deviations of the fresh and aged emulsions were not significantly different (0.01% significance level).
4.2.7.2 *Droplet/Particle size distribution of secondary w/o/w emulsion/microcapsules*

The droplet size distribution of the acacia-ethylcellulose-acacia w/o/w secondary emulsions and the microcapsules formed subsequently, were also found to be log-normal (Figures 4.13 and 4.14). Morris (1982) and Duquemin (1987) reported bimodal distributions for both the secondary w/o/w emulsions and the resulting microcapsules in a few of the systems. They attributed the bimodal distribution to the presence of both unicored and multicored droplets or microcapsules. However, in this study, it has been observed that most of the microcapsules formed were multi-cored (therefore monodisperse) as were the multiple emulsion droplets. The multiple emulsions were mostly composed of large multiple droplets with a large number of internal droplets. This could imply that the primary w/o emulsion encapsulated was probably a flocculated system (Florence and Whitehill, 1982). These findings were in agreement with the results obtained by Odidi (1990).

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>G. MEAN DIAMETER (µ)</th>
<th>G. STANDARD DEVIATION (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMBINATION</td>
<td>2º EMULSION MICROCAPSULES</td>
<td>2º EMULSION MICROCAPSULES</td>
</tr>
<tr>
<td>(1)</td>
<td>4.81</td>
<td>8.1</td>
</tr>
<tr>
<td>a</td>
<td>9.96</td>
<td>11.98</td>
</tr>
<tr>
<td>b</td>
<td>5.15</td>
<td>5.62</td>
</tr>
<tr>
<td>ab</td>
<td>8.33</td>
<td>9.62</td>
</tr>
<tr>
<td>c</td>
<td>5.17</td>
<td>7.11</td>
</tr>
<tr>
<td>ac</td>
<td>7.83</td>
<td>8.91</td>
</tr>
<tr>
<td>bc</td>
<td>4.25</td>
<td>4.88</td>
</tr>
<tr>
<td>abc</td>
<td>6.82</td>
<td>7.89</td>
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</table>

Table 4.8 Geometric mean diameters and geometric standard deviations of the secondary w/o/w emulsion.

122
Secondary w/o/w emulsion: 4% w/v Acacia / 4% w/v Ethylcellulose / 8% w/v Acacia

Figure 4.13 Droplet size distribution of secondary w/o/w emulsion (treatment combination bc).

Microcapsules: 4% w/v Acacia / 4% w/v Ethylcellulose / 8% w/v Acacia

Figure 4.14 Particle size distribution of microcapsules (treatment combination bc).
<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>$F_o$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>45.80</td>
<td>1</td>
<td>45.80</td>
<td>248.16 a</td>
</tr>
<tr>
<td>B</td>
<td>2.59</td>
<td>1</td>
<td>2.59</td>
<td>14.04 a</td>
</tr>
<tr>
<td>C</td>
<td>4.33</td>
<td>1</td>
<td>4.33</td>
<td>23.44 a</td>
</tr>
<tr>
<td>AB</td>
<td>1.23</td>
<td>1</td>
<td>1.23</td>
<td>6.64 b</td>
</tr>
<tr>
<td>AC</td>
<td>2.57</td>
<td>1</td>
<td>2.57</td>
<td>13.91 a</td>
</tr>
<tr>
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<td>1</td>
<td>0.10</td>
<td>0.55</td>
</tr>
<tr>
<td>ABC</td>
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<td>1</td>
<td>0.72</td>
<td>3.90</td>
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<tr>
<td>ERROR</td>
<td>1.48</td>
<td>8</td>
<td>0.18</td>
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<tr>
<td>TOTAL</td>
<td>58.81</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Significant at the 1% level  
*b* Significant at the 5% level

Table 4.9 Analysis of variance of the geometric mean diameters of the secondary w/o/w emulsions based on a $2^3$ factorial design.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>$F_o$</th>
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<td>1</td>
<td>0.0004</td>
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<tr>
<td>B</td>
<td>0.0009</td>
<td>1</td>
<td>0.0009</td>
<td>0.48</td>
</tr>
<tr>
<td>C</td>
<td>0.0030</td>
<td>1</td>
<td>0.0030</td>
<td>1.59</td>
</tr>
<tr>
<td>AB</td>
<td>2.5e-5</td>
<td>1</td>
<td>2.5e-5</td>
<td>0.01</td>
</tr>
<tr>
<td>AC</td>
<td>0.0009</td>
<td>1</td>
<td>0.0009</td>
<td>0.49</td>
</tr>
<tr>
<td>BC</td>
<td>0.0004</td>
<td>1</td>
<td>0.0004</td>
<td>0.23</td>
</tr>
<tr>
<td>ABC</td>
<td>0.0012</td>
<td>1</td>
<td>0.0012</td>
<td>0.64</td>
</tr>
<tr>
<td>ERROR</td>
<td>0.0151</td>
<td>8</td>
<td>0.0019</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>0.0220</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Significant at the 1% level  
*b* Significant at the 5% level

Table 4.10 Analysis of variance of the geometric standard deviations of the secondary w/o/w emulsions based on a $2^3$ factorial design.
Figure 4.15 Effect of primary phase volume ratio on the geometric mean diameters of the secondary w/o/w emulsions.

Figure 4.16 Effect of primary phase volume ratio on the geometric standard deviations of the secondary w/o/w emulsions.
Figure 4.17 Effect of secondary phase volume ratio on the geometric mean diameters of the secondary w/o/w emulsions.

Figure 4.18 Effect of secondary phase volume ratio on the geometric standard deviations of the secondary w/o/w emulsions.
Figure 4.19 Effect of outer acacia concentration on the geometric mean diameters of the secondary w/o/w emulsions.

Figure 4.20 Effect of the outer acacia concentration on the geometric standard deviations of the secondary w/o/w emulsions.
The mechanism for the formation of a secondary emulsion is again turbulent flow. The factors affecting droplet size in the primary emulsion are therefore also likely to affect the droplet size of the secondary emulsion. The significance of the effects on the geometric mean diameter (Table 4.9) of the secondary w/o/w emulsions are, in descending order

$$A > C > B > AC > AB$$

Factor A, the primary phase volume ratio had the most significant effect on the droplet size distribution of the secondary w/o/w emulsion, followed by factor C, the outer acacia concentration and then factor B, the secondary phase volume ratio. None of the factors were found to affect the geometric standard deviation significantly (Table 4.10).

An increase in the geometric mean diameter was observed with an increase in the primary phase volume ratio (Figure 4.15). This again is probably due to the increase in viscosity of the disperse phase which in this case is the primary w/o emulsion. The relative viscosity of an emulsion is known to increase exponentially with its phase volume ratio (Becher 1965). As in the case of a primary emulsion, an increase in the viscosity of the disperse phase in a secondary emulsion should also increase the deformation time of the primary emulsion droplets. This means that larger eddies are responsible for droplet formation and hence larger droplet sizes.

The decrease in geometric mean diameter as a result of an increase in the outer acacia concentration was independent of the secondary phase volume ratio (Figure 4.19). This is unlike the effect of ethylcellulose concentration on the primary emulsions which was probably due to changes in the viscosity of the ethylcellulose solution with changes in concentration. Acacia is unusual in that it produces solutions of high viscosity only at high concentrations. Variations in viscosity at the concentrations used were not noticeable. A possible explanation for the decrease in droplet size is a decrease in coalescence as a result of an increase in the concentration of the acacia macromolecule which protects the globules formed. The decrease in droplet size as a result of an increase in the secondary phase volume ratio (Figure 4.17) is probably also due to a decrease in coalescence.
The document contains two tables, Table 4.11 and Table 4.12, which represent the analysis of variance for the geometric mean and geometric standard deviations of the microcapsules based on a $2^3$ factorial design. The tables provide the source of variation, sum of squares, degrees of freedom, mean square, and the corresponding $F$-values. The significance levels are marked with superscript $a$ and $b$, indicating significance at the 1% and 5% levels, respectively.
Figure 4.21 Effect of primary phase volume ratio on the geometric mean diameters of microcapsules.

Figure 4.22 Effect of primary phase volume ratio on the geometric standard deviations of the microcapsules.
Figure 4.23 Effect of secondary phase volume ratio on the geometric mean diameters of microcapsules.

Figure 4.24 Effect of the secondary phase volume ratio on the geometric standard deviations of the microcapsules.
Figure 4.25 Effect of outer acacia concentration on geometric mean diameters of microcapsules.

Figure 4.26 Effect of outer acacia concentration on the geometric standard deviations of the microcapsules.
In general, we see that the microcapsules are larger in size than their respective multiple emulsion droplets (Table 4.5). The geometric standard deviations are also greater and more varied. This is possibly due to the greater volume of the porous membrane formed on solvent extraction as compared to the organic solution of the polymer. It could also be due to the swelling of the dry microcapsules in deionised water during particle sizing. Duquemin (1987) found the particle size distribution of the microcapsules to be dependent on the method of solvent extraction. In addition, the organic solvent used and the ethylcellulose concentration in the middle organic phase were also reported to affect the particle size distribution of the microcapsules. These two factors were kept constant in this study.

From the results, it is clear that the size of the microcapsules is dependent on the size of the secondary emulsion droplets. The effects of the various factors on microcapsule size are similar to that on the multiple emulsion droplets as Figures 4.21, 4.23 and 4.25 show. The main difference is probably the order of the significance of the secondary phase volume ratio to the outer acacia concentration. The outer acacia concentration is likely to affect the size of the final microcapsules more as it forms a physical layer around the microcapsule on drying.

In contrast to the secondary w/o/w emulsion, both the primary and secondary phase volume ratio were found to affect the geometric standard deviation of the microcapsule size significantly. The variabilities in the size distributions of the systems were probably enhanced in the process of solvent extraction.

The usual diagnostic check of the residuals based on a $2^3$ factorial design was applied to the geometric mean diameter and geometric standard deviation of both the multiple w/o/w emulsion and the microcapsules. The residuals (differences between the observed and calculated data) were zero for all observations. That is, the calculated data were exactly the same as the observed data. This confirms the validity of the results obtained by the analysis of variance based on a $2^3$ factorial design.
After the formation of a stable secondary w/o/w emulsion, the organic solvent used in the middle organic phase can be removed in several ways. Duquemin (1987) investigated several methods of solvent extraction namely dialysis, stirring at atmospheric pressure, spray drying and rotary evaporation under reduced pressure. The first two methods were found to be unsuitable mainly because they require several days to complete the process. This may result in bacterial contamination and may also affect the stability of the drug encapsulated. Spray drying and rotary evaporation were found to be the more efficient methods of solvent extraction. Both methods were rapid and easy to scale up. As the organic solvent waste can be collected from the condenser of the rotary evaporator, rotary evaporation has the added advantage of being more environmentally friendly.

Figure 4.26 shows a flow diagram of the microencapsulation process investigated here. After most of the organic solvent has been removed from the secondary w/o/w emulsion by rotary evaporation under reduced pressure, the suspension of microcapsules formed may be spray dried to give a free flowing powder. The microcapsules may also be centrifuged or sedimented overnight. The slurry of wet microcapsules can then be dried in the oven to give a free flowing powder. Alternatively, the secondary w/o/w emulsion may be spray dried directly to give a free flowing powder. In this case, a safer volatile organic solvent, such as dichloromethane, has to be used in place of ethyl acetate which is highly inflammmable.

In this study, the surface morphology of the microcapsules formed was used to assess the various pathways of solvent extraction and drying. This was done using the Philips XL20 scanning electron microscope.
Figure 4.27 Flow diagram of microencapsulation process using the multiple emulsion / solvent extraction technique.
4.3.1 Materials

Acacia (tears)  Merck Ltd. (BDH)
Eudragit® L100  Rhom Pharma (GMBH)
Ethylcellulose N10 NF  Hercules Ltd.
Dichloromethane GPR  Merck Ltd. (BDH)
Ethyl acetate GPR  Merck Ltd. (BDH)

4.3.2 Method

Three formulations of empty microcapsules were investigated in these experiments. The solvents were varied as explained below. The polymeric content of each phase of the multiple emulsion is listed in place of the phase it is in, in the notation w1/o/w2, where w1 denotes the inner aqueous phase, o denotes the oily phase and w2 denotes the outer aqueous phase. The three formulations investigated were:

**Formulation 1**
4% w/v Acacia / 4% w/v Ethylcellulose / 4% w/v Acacia

**Formulation 2**
8% w/v Acacia / 8% w/v Ethylcellulose / 8% w/v Acacia

**Formulation 3**
0.5% w/v Eudragit® L100 / 8% w/v Ethylcellulose / 8% w/v Acacia

The secondary emulsions were prepared

(1) with a primary and secondary phase volume fraction of 0.5 and
(2) mixed at maximum shear rate for 0.5 hour and at the calibrated rate of 5 for 1 hour for the primary and secondary emulsification stages respectively.
4.3.2.1 Rotary evaporation

The multiple emulsions were prepared using ethyl acetate. They were rotary evaporated using a Buchi R110 rotary evaporator under reduced pressure at (1) 30 °C for one hour or (2) 40 °C for half hour to remove as much of the organic solvent as possible under the respective temperatures. The resulting microcapsule suspensions were then centrifuged at 6000 rpm immediately or left to sediment overnight and then filtered. The slurry was then dried in the oven at 50 °C overnight to constant weight.

4.3.2.2 Spray drying

The secondary w/o/w emulsions were prepared using dichloromethane instead of ethyl acetate because its vapour is not inflammable when mixed with air. The secondary w/o/w emulsions were spray dried using a Buchi 190 Mini Spray Dryer fitted with a 0.7mm nozzle. The inlet temperature used was 140-150 °C; outlet temperature ranged from 70-80 °C; pump setting was varied at 5 or 6 with maximum aspirator setting to maintain both the inlet and outlet temperatures.

4.3.2.3 Rotary evaporation-spray drying

Multiple emulsions prepared using ethyl acetate were rotary evaporated at 30 or 40 °C as in Experiment 1. The resulting suspensions were then spray dried as in Experiment 2.
4.3.3 Results and Discussion

Microcapsule formation using the multiple emulsion / solvent extraction technique is a phase separation process. The microcapsules are formed as a result of polymer precipitation at the oil-water interfaces. This occurs as the organic solvent is removed by diffusion into the outer aqueous phase followed by evaporation. The rate of solvent extraction is therefore of crucial importance in determining the nature of the microcapsule membrane and so indirectly, its sustained release property.

In order to investigate the rate of solvent extraction on the surface morphology of the microcapsules, the three formulations were rotary evaporated under reduced pressure at either 30 °C or 40 °C. Microcapsules prepared from Formulation 1 had perforated walls (Plate 3(a)). Embleton and Tighe (1992) explained this effect in terms of the conformational changes in the polymer as it contracts on precipitation. These conformational changes act as a deforming force on the developing wall which, besides forming porous walls could deform the shape of the microcapsule if the mechanical properties of the wall is weak. Another possible explanation is the perforation of the thin walls formed at the droplet interfaces as a result of the diffusion of the remaining solvents in the microcapsules. The fact that an increase in ethylcellulose concentration (Formulation 2 and 3) gave non-perforated microcapsule walls (Plate 3(c) and (d)) lends support to this theory.

Although large perforations in the walls of the microcapsules can be prevented by increasing the concentration of the wall forming polymer (ethylcellulose concentration), porous membranes may still be obtained. If the polymer is precipitated rapidly with large amounts of solvent still present in the microcapsules, the diffusion of solvents will form pores in the walls already formed. This is especially so for microcapsules prepared using the multiple emulsion technique as compared to the primary emulsion technique due to the presence of the internal aqueous phase in addition to the organic solvent (Bodmeier et al, 1991). It also explains the decrease in the size of the perforations (Plate 3(b)) on the walls of the microcapsules (Formulation 1) when the rate of solvent extraction was decreased.
Plate 3. Scanning electron micrographs of microcapsules prepared by rotary evaporation at 30 or 40 °C, followed by sedimentation.
The "wrinkled" appearance of the microcapsules prepared could also be due to a high rate of solvent extraction. Once the polymer has precipitated at the droplet interface forming a solid membrane, the remaining solvents will diffuse out resulting in a reduction of volume in the core. This decreases the actual surface area of the microcapsule which is smaller than the surface area of the membrane already formed thereby causing it to "wrinkle". This effect is not observed at the lower rate of solvent extraction for Formulation 3 (Plate 3(f)). However, a lower rate of solvent extraction would result in the formation of a more fluid and tacky membrane before it solidifies (Moldenhauer and Nairn 1991). This could result in slightly deformed microcapsules but in this case probably resulted in aggregation as shown in Plate 3(f). The aggregation of microcapsules may be advantageous in the preparation of a sustained release dosage form in that it decreases the effective surface area for drug release and hence decreases the rate of drug release. The presence of tiny beads around the microcapsules has been postulated to be deposits of excess polymer which appears when the concentration of ethylcellulose used was very high (Ganguly et al. 1989).

Centrifuging the microcapsules immediately after rotary evaporation caused them to cake completely. This is probably because the walls of the microcapsules were not completely solidified after rotary evaporation. In addition, as shown in section 3.1, the formation of a highly viscoelastic acacia film is a time dependent process. Time is therefore required for the acacia molecules to build up around the microcapsules and re-orientate, in order to give mechanical protection against aggregation.

The use of a spray drying technique both for solvent extraction or drying appears to be attractive in that it is rapid and easy to scale-up. At the operating temperatures, the solvents are removed almost instantaneously. The resulting product is however, not exposed to high temperatures as it is sucked out of the drying chamber almost immediately.

Plate 5(a), (c) and (e) show the microcapsules prepared by direct spray drying. These microcapsules appear to be smaller in size than the ones prepared by rotary evaporation (Plate 3) which is in agreement with the
Plate 4. Scanning electron micrographs of microcapsules prepared by rotary evaporation at 30 or 40 °C followed by centrifugation.
Plate 5. Scanning electron micrographs of microcapsules prepared by spray drying or rotary evaporation at 30 °C followed by spray drying.
results obtained by Duquemin (1987). This could be due to the effect of dichloromethane on the formation of microcapsules. More probably, this has occurred because most of the microcapsules have actually collapsed, ruptured or fragmented. It could also be due to a reduction in the size of the droplet prior to the formation of a solid wall as a result of the simultaneous extraction of water and dichloromethane at the high temperatures used.

When the multiple emulsion droplets first come into contact with the hot air which is above the boiling point of water, the organic solvent would evaporate first, forming the ethylcellulose wall. This is followed by the rapid outward movement of water vapour across the porous ethylcellulose wall which results in a vacuum within the core of the microcapsule causing the wall to collapse.

The spheres observed in these preparations were most likely acacia spheres which dried around the air bubbles that were incorporated in the feed prior to or during atomization (Masters 1976). These expand regularly due to the semi-plastic particle crust, resulting in smooth round particles. In cases where the hard crust cannot withstand the rise in inner pressure, the particles may rupture or fragment.

Microcapsules which were rotary evaporated prior to spray drying have similar distorted structures to those directly spray dried (Plate 5(b)). However, no satisfactory microcapsules were observed for Formulations 2 and 3 (Plate 5(d) and (f)) as these two formulations tend to clog the nozzle of the spray dryer. The resulting products were masses of polymers with no distinctive structures albeit the presence of the occasional microcapsule.
From the results obtained in sections 4.2 and 4.3, a few conclusions can be made regarding the procedure for the manufacture of microcapsules. These are as follows.

(1) The higher primary phase volume ratio of 0.5 should be used to ensure the stability of the primary emulsion. This is especially necessary in the subsequent preparation of chloroquine microcapsules where the primary emulsion would have to be kept for sometime before secondary emulsification to allow the diffusion of chloroquine into the inner aqueous phase of the primary emulsion (section 3.2). In addition, a higher primary phase volume ratio would also increase the yield of the microcapsules.

(2) A higher secondary phase volume ratio of 0.5 should also be used as it would give a higher yield of microcapsules. An added advantage is the smaller variability in the particle sizes of the microcapsules formed. However, these microcapsules will be smaller than those produced at the lower secondary phase volume ratio.

(3) Perforations on the walls of the microcapsules should be avoided by using higher ethylcellulose concentrations. This would increase the thickness of the ethylcellulose wall and hence its mechanical property. It would also decrease the rate of drug release by increasing the diffusional pathway.

(4) An extended mixing time did not affect the droplet size distribution of the primary emulsions significantly. Therefore, it is possible that the same would apply to the secondary emulsions.

(5) Changes in the acacia concentration in the inner aqueous phase did not affect the droplet size distribution of the primary emulsion significantly. However, smaller microcapsules were observed at the higher acacia concentration in the outer aqueous phase. A higher acacia concentration in the outer aqueous phase might also decrease the rate of solvent extraction and give microcapsules with less porous walls.
Of the processes of solvent extraction investigated, only rotary evaporation produced spheres with smooth walls. Direct spray drying produced irregularly shaped microcapsules, many of which were fractured. Rotary evaporation, followed by spray drying did not produce satisfactory microcapsules.

Time is required for the microcapsule wall to solidify before the microcapsules can be extracted successfully. Immediate centrifugation caused the microcapsules to cake completely.

The rate of solvent extraction is crucial to the porosity of the microcapsule wall. A slower rate of solvent extraction at 30 °C under reduced pressure gave spherical microcapsules with smooth walls. However, due to the formation of fluid and tacky membranes during solvent extraction, a high degree of aggregation was observed.

For the manufacture of a taste-masked, sustained release preparation of chloroquine microcapsules, the most important factor to consider would be the porosity of the microcapsule wall. Porous walls would be leaky and result in rapid drug release. The particle size distribution of the microcapsule is also important as smaller microcapsules release drug at a faster rate due to increased effective surface area. Therefore, it would be ideal to use a slow rate of solvent extraction as this gives microcapsule walls that are less porous. The resulting aggregation of microcapsules might actually be advantageous as the increase in "particle size" might decrease the rate of drug release.
Chapter Five

Choice of Polymeric Systems
5.1 INTRODUCTION

Formulations prepared using the original method of multiple emulsion / solvent extraction technique of microencapsulation (Morris 1982; Duquemin 1987) had the drug incorporated in the inner aqueous phase of the system. This is however not possible in some cases due to the incompatibility between the drug and the inner aqueous phase polymer.

Instead of incorporating the drug quinine in the inner aqueous phase of the multiple emulsion system, Odidi (1990) encapsulated the drug in the organic phase using a modified method. Due to the polymer-drug interaction, the modified method was found to

1. improve the drug content of the microcapsules
2. improve its sustained release property and
3. overcome the incompatibility between the drug and the anionic polyelectrolyte when both were incorporated in the inner aqueous phase.

Transfer of chloroquine from the organic phase into the aqueous phase and subsequent interaction between the cationic drug and the anionic aqueous phase polymer has been shown to occur (section 3.2). In addition, direct addition of chloroquine to some of the aqueous phases containing anionic polyelectrolytes has also been shown to result in precipitation. The modified method described by Odidi (1990) therefore appears to be suitable for microencapsulating chloroquine.

A stable primary emulsion and a corresponding multiple emulsion of reasonable degree of stability are two of the pre-requisites for the formation of three-ply-walled microcapsules using the multiple emulsion technique (original and modified). Various combinations of polymers with the drug were prepared to determine which of these would give stable primary w/o and secondary w/o/w emulsions. Ethyl acetate, the organic solvent used in the middle organic phase, was removed by rotary evaporation. The resulting suspensions of
microcapsules were sedimented, then dried to see if free flowing powders may be obtained.

In this work, only anionic polyelectrolytes were investigated as the inner aqueous phase polymer. This was to enhance the drug concentration in the core of the microcapsules through the interaction between the chloroquine and the anionic polyelectrolyte molecules. Due to this same drug-polymer interaction, only weak anionic polyelectrolytes were considered for the outer aqueous phase so as to minimise drug loss.

5.2 WALL FORMING MATERIALS

**Hydrophillic Polymer**

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia (tears)</td>
<td>Merck Ltd. (BDH)</td>
</tr>
<tr>
<td>iota-Carrageenan</td>
<td>Sigma Chemical Company Ltd.</td>
</tr>
<tr>
<td>Collagen</td>
<td>Gattefosse Cie</td>
</tr>
<tr>
<td>Dextran T500</td>
<td>Pharmacia</td>
</tr>
<tr>
<td>Eudragit® L100</td>
<td>Rhom Pharma, (GMBH)</td>
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<tr>
<td>Eudragit® S100</td>
<td>Rhom Pharma, (GMBH)</td>
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</tbody>
</table>

**Hydrophobic Polymer**

<table>
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<th>Supplier</th>
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<tr>
<td>Eudragit® RS100</td>
<td>Rhom Pharma, (GMBH)</td>
</tr>
</tbody>
</table>
5.3 METHOD

The original method of microencapsulation using multiple emulsions, described by Morris (1982) and Duquemin (1987), encapsulates a cosolution of a drug and a polymer, usually an anionic polyelectrolyte, in the inner aqueous phase of the multiple emulsion during preparation. As such, only water soluble compounds may be encapsulated. In the modified method, the drug is dissolved in the middle oily phase of the multiple emulsion during preparation. It may therefore be used to encapsulate water insoluble drugs.

In this study, microcapsules were prepared using the above two methods. Chloroquine diphosphate was encapsulated using the original method of preparation while its water insoluble base, chloroquine, was encapsulated using the modified method. It was also possible to encapsulate both forms of chloroquine in the microcapsules by encapsulating the salt form (chloroquine diphosphate) in the inner aqueous phase and the base (chloroquine) in the oily phase simultaneously. The various polymeric systems investigated are listed in Tables 5.1 and 5.2 for the original and the modified method respectively.

5.3.1 The Original Method of Preparation

In this method, the drug, which was chloroquine diphosphate, was first dissolved in an aqueous solution of an anionic polyelectrolyte in deionised water saturated with the organic solvent, ethyl acetate (inner aqueous phase). A primary water-in-oil (w/o) emulsion was then prepared by the dispersion of one part of the aqueous drug-polymer solution in one part of a solution of the hydrophobic polymer (middle oily phase) in water-saturated ethyl acetate. The aqueous solution was added dropwise by means of a peristaltic pump (48 rpm) and mixed using a Silverson mixer-emulsifier at maximum shear rate. One part of the resulting primary w/o emulsion was then added dropwise to one part of another aqueous polyelectrolyte solution (outer aqueous phase) and
mixed using the same Silverson mixer-emulsifier at a lower shear rate (speed setting 5) to form the secondary water-in-oil-in-water (w/o/w) emulsion. Ethyl acetate was then removed by means of a rotary evaporator under reduced pressure at 30 °C for one hour. The resulting suspension of microcapsules was then left overnight to sediment, filtered and finally dried in an oven at 50°C.

5.3.2 The Modified Method of Preparation

The only difference between the original method and the modified method was the preparation of the primary w/o emulsion. In the modified method, the drug, which was chloroquine base, was first precipitated from its diphosphate salt using sodium hydroxide solution. The chloroquine precipitate was then extracted using ethyl acetate (in four aliquots) and washed twice with an equal volume of deionised water to remove any excess sodium hydroxide. This was necessary as excess sodium hydroxide can affect the film forming property of some polyelectrolytes, such as acacia. The hydrophobic polymer (middle oily phase polymer) was then dissolved in the washed extract of chloroquine in ethyl acetate to give an organic co-solution of chloroquine and the hydrophobic polymer. One part of an aqueous solution of an anionic polyelectrolyte (inner aqueous phase) was then added to one part of the organic co-solution (middle oily phase), and mixed using a Silverson mixer-emulsifier to give a primary w/o emulsion. The resulting primary w/o emulsion was then left to stand for at least twelve hours to allow the transfer of drug molecules from the oily phase to the inner aqueous phase. The subsequent steps for the preparation of the secondary w/o/w emulsions and microcapsules were similar to the original method. All other conditions and equipment settings were also similar.
5.3.3 In-vitro Dissolution Studies

Besides assessing the various systems macroscopically by their stability and ability to form free flowing powders, the in vitro release profiles of suitable formulations were also assessed. This was carried out using the paddle unit (method 2) of the USPXXI dissolution test apparatus (model PTWS, Pharma Test Apparatebau, W. Germany). All tests were conducted in 900ml of the dissolution medium maintained at 37± 0.5 °C with a paddle rotation speed of 100rpm.

The microcapsules were also sieved to specific particle size ranges to study the effect of particle size distribution on the dissolution profile of the microcapsules. 100mg of microcapsules were used in each case to ensure sink conditions. Fixed volumes of samples were withdrawn at various predetermined time intervals by means of an automated sampler (Pharma test Apparatebau Type PTFC1, W. Germany). Different dissolution media were used and they included 0.1M hydrochloric acid (pH 1.2) and distilled water (pH 6.2).

The drug concentrations of the samples were determined by direct measurement of the ultraviolet absorbance at 343nm using a Perkin-Elmer 554 UV-Vis. spectrophotometer. Preliminary experiments have established a linear relationship between drug concentration and absorbance values from which the drug concentration of the samples were calculated.
5.4 RESULTS AND DISCUSSION

Of the various polymeric systems used to fabricate chloroquine diphosphate microcapsules using the original method of preparation, only two combinations gave free flowing microcapsules. They were systems a1 (Acacia / Ethylcellulose N10 / Acacia) and f1 (Eudragit® L100 / Ethylcellulose N10 / Acacia) (Table 5.1).

With acacia as the inner aqueous phase polymer, large amounts of chloroquine diphosphate can be incorporated in the inner aqueous phase without causing precipitation (section 3.2). The microcapsules formed were however, irregular in shape and highly porous (Plate 6(a)). At higher drug loadings (Systems a2 and a3), microcapsules also tend to aggregate, forming a gritty powder.

It is of interest to note that at lower drug loadings (System a1), such irregular structures were only observed after drying the microcapsules in the oven and not after rotary evaporation. A possible explanation is the formation of chloroquine diphosphate crystals in the core of the microcapsules as the water content was removed. Using a multiple emulsion / solvent extraction technique, Bodmeier et al. (1991) observed drug crystals of pseudoephedrine hydrochloride within poly(methyl methacrylate) microcapsules using dichloromethane as the organic solvent. However, most of their microcapsules were not deformed because they were unicored, with thick, uniform capsular walls. Unlike these poly(methylmethacrylate) microcapsules, the microcapsules prepared here were multi-cored and as a result, have walls of uneven thickness. In addition, ethyl acetate has also been reported to form thinner ethylcellulose walled microcapsules of similar particle size as compared to dichloromethane (Sheorey et al. 1991). However, due to its higher boiling point, ethyl acetate produced microcapsules with better surface characteristics and therefore better sustained release property. Aggregation at higher drug loadings (System a2 and a3) is probably a result of a less stable emulsion or multiple emulsion system which will be discussed in the next chapter.
<table>
<thead>
<tr>
<th>System</th>
<th>*Inner Phase Composition</th>
<th>Middle Phase Composition</th>
<th>w/o Emulsion Stability</th>
<th>Outer Phase Composition</th>
<th>w/o/w Emulsion Stability</th>
<th>Rotary Evaporation</th>
<th>Microcapsule Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Acacia</td>
<td>Ethylcellulose N10</td>
<td>stable</td>
<td>Acacia</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td>1</td>
<td>8% w/v (5g)</td>
<td>10% w/v</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Coarse suspension</td>
<td>Some aggregates</td>
</tr>
<tr>
<td>2</td>
<td>8% w/v (10g)</td>
<td>10% w/v</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Coarse suspension</td>
<td>Some aggregates</td>
</tr>
<tr>
<td>3</td>
<td>8% w/v (10g)</td>
<td>12% w/v</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>Acacia</td>
<td>Ethylcellulose N10</td>
<td>stable</td>
<td>Collagen</td>
<td>unstable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8% w/v (5g)</td>
<td>10% w/v</td>
<td>stable</td>
<td>0.03% w/v</td>
<td>unstable</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>8% w/v (5g)</td>
<td>10% w/v</td>
<td>stable</td>
<td>0.06% w/v</td>
<td>unstable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8% w/v (5g)</td>
<td>10% w/v</td>
<td>stable</td>
<td>0.1% w/v</td>
<td>unstable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>Acacia</td>
<td>Ethylcellulose N10</td>
<td>stable</td>
<td>Dextran T500</td>
<td>unstable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8% w/v (5g)</td>
<td>10% w/v</td>
<td>stable</td>
<td>10% w/v</td>
<td>unstable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>Acacia</td>
<td>Eudragit® RS100</td>
<td>stable</td>
<td>Acacia</td>
<td>stable</td>
<td>Very fine suspension</td>
<td>Hard resinous cake</td>
</tr>
<tr>
<td>1</td>
<td>8% w/v (5g)</td>
<td>8% w/v</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>Acacia</td>
<td>Eudragit® RS100</td>
<td>stable</td>
<td>Collagen</td>
<td>unstable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8% w/v (5g)</td>
<td>8% w/v</td>
<td>stable</td>
<td>0.1% w/v</td>
<td>unstable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>Eudragit® L100</td>
<td>Ethylcellulose N10</td>
<td>stable</td>
<td>Acacia</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td>1</td>
<td>0.5% w/v (1.5g)</td>
<td>10% w/v</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>Eudragit® L100</td>
<td>Eudragit® RS100</td>
<td>stable</td>
<td>Acacia</td>
<td>stable</td>
<td>Very fine suspension</td>
<td>Hard resinous cake</td>
</tr>
<tr>
<td>1</td>
<td>0.5% w/v (1.5g)</td>
<td>8% w/v</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses refer to the amount of chloroquine diphosphate dissolved in 100ml of the inner aqueous phase.

Table 5.1 Polymeric systems investigated using the original method of preparation.
Microcapsules prepared from System f1 (Plate 6(b)) were also irregular in shape but not as porous and irregular in shape as System a1. Due to the incompatibility of the drug, chloroquine diphosphate and the inner aqueous phase polymer, Eudragit® L100, only a limited amount of drug can be incorporated in the inner aqueous phase of these microcapsules. Its lower drug content probably explains the less irregular shape of these microcapsules in comparison to system a1 as there would be fewer crystals present.

Even though a very dilute solution of collagen shows good viscoelastic film forming properties (Nithiananthan 1992), it cannot support the formation of stable multiple emulsions when used in place of acacia in the outer aqueous phase (System b1, b2, b3 and e1). Similarly, the use of Dextran T500, a neutral polysaccharide, as the outer aqueous phase polymer did not produce a stable multiple emulsion (System c1). A possible explanation is the lack of interaction between the ethylcellulose molecules and the Dextran T500 molecules as reported by Duquemin (1987). This could also explain unstable emulsions formed with collagen.

Duquemin (1987) prepared Dextran T500 / Ethylcellulose / Acacia microcapsules but found no interaction between the ethylcellulose molecules and the dextran molecules through interfacial studies. This implies that interactions between the middle and the outer aqueous phase polymers are essential for the stabilization of the multiple emulsion whereas it is not necessary for the primary emulsion. Reports of the formation of a stable w/o emulsion, of an aqueous solution of pseudoephedrine hydrochloride in an organic solution of ethylcellulose in dichloromethane, without the presence of a stabilizer (Bodmeier et al. 1991), lends support to the above postulation.

Replacing ethylcellulose N10 with Eudragit® RS100, an acrylic polymer, did not produce any microcapsules even though both the primary and secondary emulsions were stable (System d1 and g1). The inability of the microcapsule slurry to form a free flowing powder could be due to its small particle size. It was observed that the suspension formed after rotary evaporation was colloidal in nature. The dried microcapsules would therefore be more cohesive.
Another possible reason could be the aggregating effect of residual acacia or the insufficiency of acacia as a non-adhesive surface coating agent, even though it is a good stabilizing agent. This is especially so since there is a large increase in surface area due to the decrease in particle size of the microcapsules. In a study using o/w fabrication procedure, forty-two drugs were investigated for their ability to be encapsulated by Eudragit® RS using magnesium stearate as a stabilizing and separating agent. However, of these forty-two, only three produced acceptable microspheres (Watts et al. 1990). It has been claimed that more acceptable microspheres (less swelling and fragility) could be prepared using an o/o fabrication procedure (Kawata et al. 1986).

Watts et al. (1991) investigated the effect of surfactants (sodium dodecyl sulphate and Tween 20) on Eudragit® RS microspheres containing 5-aminosalicylic acid using a o/w emulsion/solvent extraction technique. Microspheres prepared in the presence of surfactants were found to have higher rates of drug release. They attributed this to the more porous matrix formed in the presence of surfactants. The mechanism of pore formation was postulated to be the deposition of polymer drug matrix on aqueous droplets which entered the unformed microspheres as a result of stabilization by the surfactants. In another study, Benita et al. (1985) reported the stabilization of individual microcapsules by the addition of polyisobutylene, an anti-aggregating agent, using a coacervation technique (addition of non-solvent). Polyisobutylene was found to adsorb only to the surface of the smooth walls of the microcapsules without affecting the polymeric matrix formed.
Plate 6. Scanning electron micrographs of chloroquine microcapsules prepared from various polymeric systems.
<table>
<thead>
<tr>
<th>System</th>
<th>*Inner Phase Composition</th>
<th>**Middle Phase Composition</th>
<th>w/o Emulsion Stability</th>
<th>Outer Phase Composition</th>
<th>w/o/w Emulsion Stability</th>
<th>Rotary Evaporation</th>
<th>Microcapsule Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Acacia</td>
<td>Ethylcellulose N10</td>
<td>Acacia</td>
<td>8% w/v (10g)</td>
<td>stable</td>
<td>Coarse suspension</td>
<td>Some aggregates</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10% w/v (10g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Coarse suspension</td>
<td>Some aggregates</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12% w/v (10g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Coarse suspension</td>
<td>Some aggregates</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10% w/v (10g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Coarse suspension</td>
<td>Some aggregates</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12% w/v (10g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Coarse suspension</td>
<td>Some aggregates</td>
</tr>
<tr>
<td>B</td>
<td>Carrageenan</td>
<td>Ethylcellulose N10</td>
<td>Acacia</td>
<td>0.25% w/v</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10% w/v (10g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.50% w/v (10g)</td>
<td>unstable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Carrageenan</td>
<td>Ethylcellulose N50</td>
<td>Acacia</td>
<td>0.25% w/v</td>
<td>too viscous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10% w/v (10g)</td>
<td>too viscous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Eudragit® L100</td>
<td>Ethylcellulose N10</td>
<td>Acacia</td>
<td>0.5% w/v</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8% w/v (1.5g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12% w/v (1.5g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10% w/v (10g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10% w/v (15g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10% w/v (15g)</td>
<td>stable</td>
<td>8% w/v</td>
<td>stable</td>
<td>Fine suspension</td>
<td>Free flowing powder</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>8% w/v (5g)</td>
<td>too viscous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Eudragit® L100</td>
<td>Ethylcellulose N50</td>
<td>Acacia</td>
<td>0.5% w/v</td>
<td>too viscous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10% w/v (10g)</td>
<td>too viscous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses refer to the amount of chloroquine diphosphate dissolved in 100ml of the inner aqueous phase.

** Numbers in parentheses refer to the amount of chloroquine diphosphate from which chloroquine was precipitated to give 100ml of the organic extract.

Table 5.2 Polymeric systems investigated using the modified method of preparation.
Polymeric systems investigated using the modified method of preparation are listed in Table 5.2. System A which has the same combination of polymers as System a1 (Acacia / Ethylcellulose N10 / Acacia) also formed gritty microcapsules (Plate 6(c)). The walls of the microcapsules were perforated and rough, even though drug crystals could not be seen on them. This again is probably due to the presence of drug crystals in the core of the microcapsules.

Spherical microcapsules with fairly smooth walls were observed for System B1 with carrageenan as the inner aqueous phase polymer, System D3 and System D4, both with Eudragit® L100 as the inner aqueous phase polymer (Plate 6(d), (e) and (f)). These polymers have been shown to bind significantly to chloroquine, which partitions from the organic phase into the aqueous phases containing these polymers. It is therefore likely that minimal crystalization has occured in these formulations resulting in less distortion in the shape of the microcapsules. Direct addition of chloroquine diphosphate to the inner aqueous phase of the system, in addition to chloroquine in the oily phase (System D5), again resulted in slight irregularity in the shape of the microcapsules (not shown).

Another problem associated with Systems B1, D3 and D4 is aggregation. As mentioned in section 3.3, addition of chloroquine to the ethylcellulose film gives a less brittle film which is more tacky during the process of drying. This "tackiness" of the membrane is exacerbated by the slow rate of solvent extraction which gives a more fluid membrane as the organic solvent is slowly removed. Therefore, as the unformed microcapsules collide during the process of rotary evaporation, more will coalesce to form aggregates. The microcapsules discussed here are therefore not only free microcapsules, but also consist of aggregates.

An increase in concentration of carrageenan from 0.25% w/v to 0.50% w/v destabilized the primary emulsions (System B2). This is probably a result of the precipitation of carrageenan in the inner aqueous core due to the polymer-drug interaction (section 3.2). An increase in Eudragit® L100 concentration from 0.5% w/v to 1.0% w/v gave primary emulsions which were too viscous to be re-emulsified at shear rates which did not destabilize the multiple emulsions.
<table>
<thead>
<tr>
<th>System</th>
<th><strong>Inner Phase Composition (%w/w)</strong></th>
<th>Chloroquine Content (%w/w)</th>
<th>Distilled Water</th>
<th>0.1M HCl</th>
<th>0.1M HCl</th>
<th>Distilled Water</th>
<th>0.1M HCl</th>
<th>Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acacia (8%w/v (5g))</td>
<td>4.0</td>
<td>5 min</td>
<td>1 h</td>
<td>&gt; 6 h</td>
<td>2 min</td>
<td>2 min</td>
<td>&gt; 2 min</td>
</tr>
<tr>
<td>1</td>
<td>Eudragit® L100 (10%w/v (1.5g))</td>
<td>1.4</td>
<td>2 min</td>
<td>15 min</td>
<td>10 min</td>
<td>6 min</td>
<td>6 min</td>
<td>6 min</td>
</tr>
<tr>
<td>A</td>
<td>Acacia (8%w/v)</td>
<td>15.6</td>
<td>2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
</tr>
<tr>
<td>1</td>
<td>Ethylcellulose N10 (10%w/v)</td>
<td>12.6</td>
<td>2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
</tr>
<tr>
<td>2</td>
<td>Ethylcellulose N10 (10%w/v)</td>
<td>8%w/v Acacia</td>
<td>19.2</td>
<td>5 min</td>
<td>2 min</td>
<td>2 min</td>
<td>2 min</td>
<td>&lt; 2 min</td>
</tr>
<tr>
<td>3</td>
<td>Ethylcellulose N10 (10%w/v)</td>
<td>19.1</td>
<td>5 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
</tr>
<tr>
<td>4</td>
<td>Carrageenan (0.5%w/v (15g))</td>
<td>24.3</td>
<td>10 min</td>
<td>1 h</td>
<td>&gt; 6 h</td>
<td>10 min</td>
<td>&lt; 6 h</td>
<td>&lt; 6 h</td>
</tr>
<tr>
<td>B</td>
<td>Eudragit® L100 (10%w/v (15g))</td>
<td>2.9</td>
<td>10 min</td>
<td>10 min</td>
<td>&gt; 6 h</td>
<td>10 min</td>
<td>&gt; 6 h</td>
<td>&gt; 6 h</td>
</tr>
<tr>
<td>D</td>
<td>0.5%w/v (1.5g)</td>
<td>2.3</td>
<td>2 min</td>
<td>15 min</td>
<td>&lt; 2 min</td>
<td>2 min</td>
<td>&lt; 2 min</td>
<td>&gt; 6 h</td>
</tr>
<tr>
<td>2</td>
<td>2%w/v (10g)</td>
<td>16.8</td>
<td>2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 2 min</td>
<td>2 min</td>
<td>&lt; 2 min</td>
<td>&lt; 6 h</td>
</tr>
<tr>
<td>3</td>
<td>5%w/v (10g)</td>
<td>25.0</td>
<td>30 min</td>
<td>1h</td>
<td>&gt; 6 h</td>
<td>30 min</td>
<td>&gt; 6 h</td>
<td>&gt; 6 h</td>
</tr>
<tr>
<td>4</td>
<td>5%w/v (10g)</td>
<td>20.9</td>
<td>22.5 min</td>
<td>30 min</td>
<td>&gt; 6 h</td>
<td>22.5 min</td>
<td>&gt; 6 h</td>
<td>&gt; 6 h</td>
</tr>
</tbody>
</table>

Table 5: Chloroquine content and in vitro drug release parameters of microcapsules.
A common method for reducing drug release rate from microcapsules is to lengthen the diffusion pathway through the polymer membrane. This can be achieved by decreasing the drug to polymer ratio and also by increasing the tortuosity of the membrane by increasing the molecular weight of the polymer (Ghorab et al. 1990). Ethylcellulose N50 was therefore used in two of the polymeric systems (C1 and E1) for the purpose of comparing with Ethylcellulose N10. The resulting primary emulsions were too viscous to be re-emulsified at shear rates which did not destabilize the multiple emulsions. A decrease in Ethylcellulose N50 concentration could however, have decreased the viscosity of the primary emulsions.

Drug release from the systems a1 and f1 showed little, if any sustained release property in both distilled water and 0.1M hydrochloric acid (Table 5.3). The drug release profile of System a1 is characterized by a burst effect followed by a very gradual increase in drug concentration (Figure 5.1). The burst effect is probably due to the rapid influx of the dissolution medium through the porous membranes of the microcapsules which is followed by rapid efflux of chloroquine through the same porous membranes. The rate of drug release decreased with microcapsule drug concentration. Both t50% and t90% were higher for system a1 than system f1 because of the greater drug concentration in the former.

The dissociation constants of chloroquine are pK\textsubscript{a1} = 10.8 and pK\textsubscript{a2} = 8.4 (20 °C) (The Pharmaceutical Codex, 1979). Therefore, the drug is slightly soluble in the distilled water used which has a pH of 6.2, approximately two units below the lower pK\textsubscript{a} of chloroquine. In 0.1M hydrochloric acid which has a pH of 1.2, chloroquine dissolves rapidly by forming the chloride salt. Drug release from chloroquine microcapsules prepared using the modified method is therefore dependent on pH as the drug is incorporated as chloroquine base in the microcapsule wall. In addition, extraction of the drug from the anionic polyelectrolytes to which it is bound in the microcapsules would also be pH dependent.

Even though high chloroquine contents were achieved by encapsulating the drug both in the core and in the ethylcellulose wall of the microcapsules in Systems A1, A2, A3 and A4, no sustained release property was observed (Figure 5.2). This is probably due to the formation...
of crystals of the unbound drug in the ethylcellulose wall of the microcapsule which was observed in the film studies (section 3.3). As the dissolution medium diffuses into the microcapsules, the drug crystals in the ethylcellulose wall dissolved, creating more pores. This explains the lower t50% and t90% in these systems as compared to the porous microcapsules of Systems A1 and f1 which do not have the drug encapsulated in the ethylcellulose layer. It also explains the increase in rate of drug release with increase in drug loading in Systems A1, A2, A3 and A4 due to the greater number of crystals present in the ethylcellulose wall of the microcapsules at higher drug loading. The fact that t50% and t90% were higher in other microcapsules with an even higher drug loading (e.g. System D4) implies that the effect of the concentration gradient across the microcapsule wall is not rate determining.

Microcapsules prepared using carrageenan (System B1) as the inner aqueous phase polymer showed the highest t50% of 4.5 hours in distilled water (Table 5.3). This is because of all the anionic polyelectrolytes investigated, carrageenan binds most strongly to chloroquine. This is shown again in the content of the microcapsules where with the same amount of starting material (chloroquine obtained from 10g chloroquine diphosphate), System B1 ended up with a higher drug loading of 24.3%w/w as compared to System D3 which has a drug loading of 16.8%w/w. It was, however, impossible to increase the drug content of the microcapsules by increasing the amount of starting material due to the instability of the primary emulsions. This is probably a result of the drug-polymer interaction in the inner aqueous phase.

Another possible reason for the slower rate of release in System B1 is the structure of the microcapsules (Plate 7 and 8). Microcapsules prepared from System B1 were larger than those of System D3 probably a result of increase coalescence during the secondary emulsification process. This would imply a smaller effective surface area for drug dissolution. The absence of spherical aqueous cores in these microcapsules again points to the relatively unstable primary emulsion of System B1 compared to System D3. The microcapsules consist mainly of the polymeric matrix. This would indicate a more tortuous diffusion pathway in microcapsules prepared using System B1 compared to System D3.
Plate 7. Photomicrographs of semi-thin sections of chloroquine microcapsules prepared from (a) System B1 and (b) System D3.
Plate 8. Transmission electron micrographs of ultra-thin sections of chloroquine microcapsules prepared from (a) System B1 and (b) System D3.
Sustained release behaviour was also observed in the systems containing Eudragit® L100 as the inner aqueous phase polymer (System D) (Figure 5.4). An increase in drug content (System D3 to D4) increases the t₅₀% of the microcapsules even though the initial rate of drug release appears to be higher. This implies that the majority of the additional drug incorporated in System D4 is probably bound to the anionic polyelectrolyte, Eudragit® L100, which retards its release. The apparent partition coefficient of chloroquine in the presence of Eudragit® L100 in the aqueous phase (section 3.2) supports this postulation.

Incorporating chloroquine diphosphate in the inner aqueous phase increased the drug content of the microcapsules from 16.8% w/w (System D3) to 20.9% w/w (System D5). The initial rate of drug release was however increased as reflected by the decrease in t₅₀% from 30 minutes to 22.5 minutes. This could be a result of the increased porosity of the microcapsule wall as a result of the formation of crystals in the inner aqueous core. It could also be due to the increase in the increased rate of influx of water molecules due to the higher ionic concentration in the core of the microcapsules, resulting in a higher rate of dissolution.

Generally, the drug release profile is depicted by a burst followed by a gradual increase in drug concentration in the dissolution medium, which is dependent on the type of anionic polyelectrolyte used in the inner aqueous phase and not so much the drug content of the microcapsules (Figure 5.5). This is indicated by the similarity of the gradients of the later parts of the dissolution profiles of the systems containing the same concentration of the same anionic polyelectrolyte in the inner aqueous phases. Drug release at the later stages of the in vitro dissolution tests is probably due to the release of bound drug molecules. The rate of release is slow in distilled water (pH 6.2) but increased dramatically at the lower pH of 1.2 as the hydrogen ions rapidly displace the drug from the anionic binding sites. The increase in rate of drug release at the lower pH of 1.2 has also been postulated to be a result of an increase in the solubility of ethylcellulose in the acidic medium (Ghorab et al. 1990).

The drug content of microcapsules prepared from Systems f1, D1 and D2 were very low. Their drug release profiles consisted of only a burst effect. However, it is of interest to note that with the same amount of starting
material (1.5g of chloroquine diphosphate), drug loading is higher with
the modified method (Systems D1 and D2) compared to the original
method (System f1). This was despite the fact that some of the drug
would have been lost in the process of precipitation and extraction in the
modified method of preparation.

All the \textit{in vitro} release profiles obtained so far were based on both free
and aggregated microcapsules. It was decided then to investigate the effect
of particle size on the drug release profile of the microcapsules. Figures
5.6, 5.7 and 5.8 show the particle size distribution (by weight) of the
microcapsules and its aggregates prepared from Systems B1, D3 and D4
respectively. Most of the particles in the size range greater than 45\(\mu\) were
actually aggregates. Aggregation could be the result of a more fluid
membrane formed during rotary evaporation due to a slow rate of
solvent extraction (section 4.3). In addition, chloroquine-ethylcellulose
matrices have also been found to be more tacky than pure ethylcellulose
matrices in the process of drying, as indicated by the cast films (section
3.3).

From Figures 5.9, 5.10 and 5.11, we see that a decrease in particle size
increased the rate of drug release. This is probably a result of an increase
in the effective surface area for drug dissolution.
Figure 5.1 *In vitro* release profile of System a1.

Figure 5.2 *In vitro* release profile of System A.
System B1: 0.25%w/v Carrageenan / 10%w/v Ethylcellulose N10 (10g) / 8%w/v Acacia

Figure 5.3 In vitro release profile of System B1.

System D3: 0.5%w/v Eudragit® L100 / 10%w/v Ethylcellulose (10g) / 8%w/v Acacia
System D4: 0.5%w/v Eudragit® L100 / 10%w/v Ethylcellulose (15g) / 8%w/v Acacia
System D5: 0.5%w/v Eudragit® L100 (1.5g) / 10%w/v Ethylcellulose (10g) / 8%w/v Acacia

Figure 5.4 In vitro release profile of System D.
System B1: 0.25%w/v Carrageenan / 10%w/v Ethylcellulose (10g) / 8%w/v Acacia
System D3: 0.5%w/v Eudragit® L100 / 10%w/v Ethylcellulose (10g) / 8%w/v Acacia
System D4: 0.5%w/v Eudragit® L100 / 10%w/v Ethylcellulose (15g) / 8%w/v Acacia

Figure 5.5(a)

Figure 5.5(b)

Figure 5.5 *In vitro* release profiles of systems B1, D3 and D4 in distilled water (a) percentage released; (b) amount released.
System B1: 0.25% w/v Carrageenan / 10% w/v Ethylcellulose N10 (10g) / 8% w/v Acacia

![Figure 5.6 Particle size distribution of system B1.]

System D3: 0.5% w/v Eudragit® L100 / 10% w/v Ethylcellulose N10 (10g) / 8% w/v Acacia

![Figure 5.7 Particle size distribution of System D3.]

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Figure 5.8 Particle size distribution of System D4.
Figure 5.9 Effect of particle size on the *in vitro* release profile of System B1 in (a) distilled water and (b) 0.1M hydrochloric acid.
System D3: 0.5% w/v Eudragit® L100 / 10% w/v Ethylcellulose N10 (10g) / 8% w/v Acacia

Figure 5.10(a)

Figure 5.10(b)

Figure 5.10 Effect of particle size on the *in vitro* release profile of System D3 in (a) distilled water and (b) 0.1M hydrochloric acid.
System D4: 0.5% w/v Eudragit® L100 / 10% w/v Ethylcellulose N10 (15g) / 8% w/v Acacia

Figure 5.11(a)

Figure 5.11(b)

Figure 5.11 Effect of particle size on the *in vitro* release profile of System D4 in (a) distilled water and (b) 0.1M hydrochloric acid.

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The encapsulation of the water soluble drug, chloroquine diphosphate, in the inner core of the microcapsules appeared most attractive as the walls of the microcapsules would theoretically act as a barrier to drug release. However, this was not found to be so for the chloroquine diphosphate microcapsules investigated in this study. This was probably due to formation of drug crystals in the core of the microcapsules which affected the formation of smooth-walled, spherical microcapsules.

The use of other polymers, besides ethylcellulose, in the middle oily phase of the multiple emulsion during the preparation of microcapsules were also unsuccessful and so were the attempts to replace acacia with other polymers in the outer aqueous phase.

In formulations where the drug, chloroquine, is encapsulated in the ethylcellulose wall of the microcapsules, burst effects were observed for the microcapsules prepared using acacia as the inner aqueous phase polymer. This was probably due to the formation of drug crystals in the ethylcellulose wall of the microcapsules which form pores in the walls as the crystals dissolve in the dissolution media. The use of Eudragit® L100 and carrageenan, which bind more strongly to chloroquine, decreased the rate of drug release. This is probably due to fewer drug crystals being formed in the ethylcellulose wall. The binding of chloroquine to Eudragit® L100 and carrageenan would also decrease the rate of drug release.

From the results obtained, it is clear that the effect of the inner aqueous phase polymer on the sustained release property of the microcapsules are in the order

Carrageenan > Eudragit® L100 > Acacia

Microcapsules prepared using acacia as the inner aqueous phase polymer did not show good sustained release characteristics. Carrageenan, which is the most acidic polyelectrolyte of the three anionic polymers, bonds with chloroquine and precipitated at lower concentrations of both drug and
polymer. Only formulations consisting of Eudragit® L100 as the inner aqueous phase polymer showed potential for further development work.

The rate of release of chloroquine from the chloroquine microcapsules (Systems B1, D3 and D4) were found to decrease with an increase in particle size. Therefore, small particles would be unsuitable for the purpose of taste-masking due to its rapid rate of drug release. But on the other hand, large particles are gritty to taste and unsuitable for use in an oral suspension which is the intended dosage form of the microcapsules. As a large proportion of the microcapsules have diameters in the size range of 45µ to 355µ (approximately 80%), only microcapsules of this medium size range will be used in subsequent work. Both the fine and large microcapsules may be removed by sieving.
Chapter Six

Development of a Taste-masked, Sustained-Release Dosage Form
6.1 INTRODUCTION

From the earlier *in vitro* studies, the formulation consisting of Eudragit® L100 in the inner aqueous phase, Ethylcellulose N10 in the middle organic phase and Acacia in the outer aqueous phase was used to prepare chloroquine microcapsules using the modified method. It was chosen for further development to increase the chloroquine content of the microcapsules and simultaneously delay its release from the microcapsules. This was to decrease the amount of microcapsules required for each dose of chloroquine and the delayed release would in turn taste-mask the drug.

To achieve the above aims, the efficiency of encapsulation and the *in vitro* release profiles were determined. The three factors investigated were:

1. the increase in the concentration of chloroquine (the theoretical loading of the microcapsules);
2. the change in the Eudragit® L100 concentration in the inner aqueous phase to which chloroquine will bind, and
3. the change in the Ethylcellulose N10 concentration which affects the microcapsule wall thickness and therefore drug release.

In addition, the microcapsules were exposed to further heat treatment in an attempt to delay the drug release.

As the microcapsules were meant to be administered as a suspension, the taste masking ability of the microcapsules would be dependent on the amount of drug released into the suspension vehicle before the dose was administered. Therefore, the amount of chloroquine released from the microcapsules in distilled water within the first half hour was used to assess the taste-masking ability of the various formulations.

Of all the various formulations investigated, one was selected for the *in vivo* study. Stability studies were then performed on the microcapsules prepared with this formulation.
6.2 MATERIALS

Acacia (tears)  Merck Ltd. (BDH)
Ethylcellulose N10 NF  Hercules Ltd.
Eudragit® L100  Rhom Pharma (GMBH)
Ethyl acetate AnalR  Merck Ltd. (BDH)
Chloroquine diphosphate  Sigma Chemicals Company Ltd.

6.3 METHOD

The microcapsules were prepared using the modified method specified in section 5.3.2. The microcapsules were then sieved and the 45-355μ fraction used to determine the chloroquine content of the microcapsules and also its in vitro drug release profile.

6.3.1 The effect of theoretical drug content

To investigate the effect of the theoretical drug content, that is the amount of starting material (chloroquine diphosphate) used to obtain the chloroquine extract, different amounts of chloroquine diphosphate (5, 10, 15 or 20g) were used to obtain each of the 100ml of the chloroquine extract in ethyl acetate. This corresponded to 3.1, 6.2, 9.3 and 12.4%w/v of chloroquine base respectively. The microcapsule formulation used was:

Formulation 1
0.5%w/v Eudragit®L100 / 10%w/v Ethylcellulose N10 / 8%w/v Acacia

6.3.2 The effect of Eudragit® L100 concentration

To study the effect of an increase in Eudragit® L100 concentration in the inner aqueous phase of the multiple emulsion during microcapsule preparation, Experiment 1 was repeated using a second formulation:
Formulation 2
0.75%w/v Eudragit®L100 / 10%w/v Ethylcellulose N10 / 8%w/v Acacia

6.3.3 The effect of ethylcellulose concentration

Using chloroquine extract obtained from 10g of chloroquine diphosphate per 100ml extract (6.2%w/v chloroquine), chloroquine microcapsules were prepared using 8, 10 or 12%w/v Ethylcellulose N10. The general formulation of the microcapsules prepared was:

Formulation 3
0.5%w/v Eudragit®L100 / x%w/v Ethylcellulose N10 / 8%w/v Acacia

6.3.4 The effect of annealing

Selected formulations of microcapsules were further heat treated (annealed) in the oven at 100 °C for one hour before the in vitro dissolution test.

6.3.5 The effect of storage

From the results of Experiments 1 to 4, Formulation 2:

0.75%w/v Eudragit®L100 / 10%w/v Ethylcellulose N10 / 8%w/v Acacia

with a chloroquine content of 30.7%w/w was chosen for the clinical study. Further tests were done on the microcapsules prepared and they were:

(a) a comparison of the in vitro release profiles of the treated and untreated microcapsules in 0.1M hydrochloric acid, distilledwater and pH 7.0 phosphate buffer USP and
(b) storage stability tests were carried out on the microcapsules stored in a dessicator at room temperature after one month. This was done by repeating the tests in (a) after one month.
The theoretical loading of the microcapsules was expressed as the percentage of chloroquine base by mass, to the total mass of the microcapsules (% w/w). It is assumed that the contribution of acacia to the total mass of the microcapsules was negligible. The error introduced by the above assumption would also be constant throughout since the concentration of acacia in the outer aqueous phase was unchanged throughout the experiments. The theoretical amount of chloroquine base in the microcapsules was calculated from the amount of chloroquine diphosphate from which it was precipitated and extracted. This was calculated using the ratio of the molecular masses of chloroquine to its diphosphate salt. The efficiency of encapsulation is therefore a ratio of the actual chloroquine content of the microcapsules to the theoretical content, expressed as a percentage.

6.4.1 The effect of theoretical drug content

In this experiment, an attempt was made to increase the chloroquine content of the microcapsules by increasing the amount of starting material (chloroquine diphosphate) used. The results (Table 6.1) showed a two fold increase in the actual chloroquine content (10.1% w/w to 23.0% w/w) with a similar two fold increase in the theoretical drug content (22.8% w/w to 47.0% w/w). This is reflected in the efficiency of encapsulation which increased only by 4%. That is, the fraction of chloroquine encapsulated in each case was almost constant.

When the amount of starting material (chloroquine diphosphate) used was increased to 20g, the resulting primary emulsion was unstable. This is probably a result of the precipitation of the drug-polymer complex in the inner aqueous phase.

As the microcapsules were prepared from w/o/w multiple emulsions, the concentration of chloroquine in each phase was determined by the degree of drug binding to the anionic polyelectrolytes in the two aqueous phases. This binding of chloroquine base to the anionic polyelectrolytes,
Eudragit® L100 and acacia, will act as a "sink" and cause a greater amount of drug to pass into the aqueous phases. When the polymeric composition of the system remains constant, the proportion of chloroquine in each phase would be dependent on the respective association constants and partition coefficients. This explains why the efficiencies of encapsulation were similar in all three preparations. As Eudragit® L100 has a greater binding capacity than acacia (section 3.2), a greater amount of chloroquine will partition into the inner aqueous phase compared to the outer aqueous phase in this formulation.

As discussed in the previous chapters, the binding of chloroquine to anionic sites on the Eudragit® L100 molecules results in precipitation when the drug was added directly to the inner aqueous phase. This was overcome by incorporating the drug in the organic phase. Diffusion studies however showed that there is still significant binding between the chloroquine molecules which had partitioned into the aqueous phase and the Eudragit® L100 molecules in it. At the drug concentrations investigated, this resulted in a higher partition coefficient but not precipitation.

Although the amount of chloroquine released in the first 0.5 hour (Table 6.2) in distilled water increased slightly (8.7 to 11.6mg) with chloroquine content (Figure 6.1(a)), the percentage of this amount decreased from 86.1% to 50.8% (Figure 6.1(b)). The burst effect is largely a result of the release of unbound chloroquine on the ethylcellulose wall matrix or on the surface of the microcapsules. This implies that a larger proportion of the drug in the microcapsules with a higher drug content was actually bound.

From Figure 6.1, it can be observed that the rates of release, as indicated by the slopes of the release profiles, tend to become constant with time. In addition, the concentration-time profiles of the three preparations tend to become more parallel with time, indicating a similar rate of release (independent of concentration in the microcapsules). As the later part of the release profile is probably due to the release of bound chloroquine molecules, this indicates that the dissociation of chloroquine from the binding sites is the rate determining step.

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**Formulation 1**

0.5%w/v Eudragit® L100 / 10%w/v Ethylcellulose N10 / 8%w/v Acacia

<table>
<thead>
<tr>
<th>Chloroquine diphosphate (g)</th>
<th>Chloroquine content (%w/w)</th>
<th>Efficiency of Encapsulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>22.8</td>
<td>10.1</td>
</tr>
<tr>
<td>10</td>
<td>37.1</td>
<td>16.7</td>
</tr>
<tr>
<td>15</td>
<td>47.0</td>
<td>23.0</td>
</tr>
<tr>
<td>20</td>
<td>(primary emulsion was unstable)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.1 Chloroquine content of microcapsules prepared from varying amounts of starting material using Formulation 1.

<table>
<thead>
<tr>
<th>Chloroquine content (%w/w)</th>
<th>Chloroquine released in 0.5 h in distilled water (%) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.1</td>
<td>86.1 8.7</td>
</tr>
<tr>
<td>16.8</td>
<td>54.8 9.2</td>
</tr>
<tr>
<td>23.0</td>
<td>50.8 11.6</td>
</tr>
</tbody>
</table>

Table 6.2 Effect of chloroquine content of microcapsules prepared using Formulation 1 on chloroquine released in 0.5 hours in distilled water.
Figure 6.1(a)

Figure 6.1(b)

Figure 6.1 *In vitro* release profiles of chloroquine microcapsules (Formulation 1) with varying chloroquine content in distilled water.
6.4.2 The effect of Eudragit® L100 concentration

To investigate the effect of a higher Eudragit® L100 concentration on the properties of the microcapsules, Experiment 1 was repeated using a similar formulation with the exception of a higher Eudragit® L100 concentration of 0.75% w/v instead of 0.5% w/v. This increase in Eudragit® L100 concentration resulted in a two fold increase in the actual loading with only one and a half fold increase in theoretical loading (Table 6.3). This indicates a higher efficiency of encapsulation compared to Formulation 1 as a result of an increase in the total number of binding sites available. In fact, an increase in theoretical loading (amount of starting material) also resulted in an increase in the efficiency of encapsulation (60.8% to 83.9%). A possible explanation to this is the participation of different classes of binding sites on the polymer.

Frequently in drug binding studies, more than one class of binding sites exist, each having its own number of binding sites, with a unique association constant. At the lower polymer concentration of 0.5% w/v in Experiment 1, additional drug-binding due to an increase in the amount of starting material used must have mainly involved the same class of binding sites and therefore similar association constants. Assuming that there exists a class of binding sites which were already saturated at the lowest theoretical drug content in Experiment 1, it is possible that these sites were not saturated at the same theoretical drug content in Experiment 2 due to an increase in the total number of binding sites at the higher polymer concentration of 0.75% w/v. The saturation of these binding sites due to an increase in the amount of drug incorporated could have resulted in the availability of another class of binding sites which were otherwise shielded. This new class of binding sites should, theoretically, have a higher association constant to result in a higher efficiency of encapsulation (from 60.8% to 83.0%).

It is also interesting to note that with the higher Eudragit® L100 concentration, the primary emulsion prepared using 15g chloroquine diphosphate as the starting material was unstable even though it was stable at 0.5% w/v Eudragit® L100. This can be explained in terms of the proportion of bound chloroquine in the two formulations. At the higher
Eudragit® L100 concentration, a greater proportion of the drug would be bound due to an increase in the number of binding sites resulting in a higher concentration of drug-polymer complex. The solubility of the drug-polymer complex is constant. Therefore, saturation occurs at a lower theoretical loading when Eudragit® L100 was at the higher concentration of 0.75%w/v.

In addition to increasing the chloroquine content of the microcapsules, an increase in Eudragit® L100 concentration also improved its sustained release property by increasing the proportion of drug bound. Comparing the results in Table 6.2 with those in Table 6.4, we see that the amount of drug released in the corresponding formulations did not differ significantly (from 8.7mg to 8.1mg and from 9.2mg to 9.4mg). This again indicates that the initial burst effect is not affected by the Eudragit® L100 concentration and therefore is due largely to the unbound drug in the ethylcellulose wall matrix or on the surface of the microcapsules. Figure 6.2 shows the release profile of the microcapsules which are similar to Formulation 1 in that the rate of release tends towards a constant and the release profile of the two formulations tend to become parallel at later times. This again indicates similar rate of release (similar gradient of slope) probably determined by the dissociation of chloroquine from the anionic binding sites on the Eudragit® L100 molecules.
**Formulation 2**

0.75%*w/v* Eudragit® L100 / 10%*w/v* Ethylcellulose N10 / 8%*w/v* Acacia

<table>
<thead>
<tr>
<th>Chloroquine diphosphate (g)</th>
<th>Chloroquine content (%<em>w/w</em>)</th>
<th>Efficiency of Encapsulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>22.4</td>
<td>60.8</td>
</tr>
<tr>
<td>10</td>
<td>36.6</td>
<td>83.9</td>
</tr>
<tr>
<td>15</td>
<td>(primary emulsion was unstable)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>(primary emulsion was unstable)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.3 Chloroquine content of microcapsules prepared from varying amounts of starting material using Formulation 2.

<table>
<thead>
<tr>
<th>Chloroquine content (%<em>w/w</em>)</th>
<th>Chloroquine released in 0.5 h in distilled water (%</th>
<th>(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.6</td>
<td>59.6</td>
<td>8.1</td>
</tr>
<tr>
<td>30.7</td>
<td>30.6</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Table 6.4 Effect of chloroquine content of microcapsules prepared using Formulation 2 on chloroquine released in 0.5 hours in distilled water.
Figure 6.2(a)

Figure 6.2(b)

Figure 6.2 In vitro release profiles of chloroquine microcapsules (Formulation 2) with varying chloroquine content in distilled water.
6.4.3 The effect of ethylcellulose concentration

An increase in ethylcellulose concentration should logically decrease the drug content of the microcapsules due to an increase in the ratio of polymer to drug concentration which remained constant. This is in agreement with the results obtained.

The efficiency of encapsulation again remained almost constant and was similar to that found in Experiment 1. This lends support to the hypothesis that the drug content of the microcapsules is mostly dependent on the Eudragit® L100 concentration. The slight decrease in the efficiency of encapsulation may be due to the effect of ethylcellulose on the diffusion of chloroquine into the inner aqueous phase. This has been reported to be due to the increased viscosity of the organic phase (Duquemin 1987).

As predicted, an increase in ethylcellulose concentration decreased the burst effect. This is probably due to a longer diffusion pathway as a result of increased tortuosity and density of the ethylcellulose wall formed (Ghorab et al. 1990).

The rate of drug release in each of the three formulations, tends to become constant at later times. The concentration-time profiles of the three formulations also tend to become parallel with time as in sections 6.4.1 and section 6.4.2. This again supports the hypothesis that drug release at later times is largely due to the bound chloroquine and the rate determining step being the dissociation of chloroquine from its binding sites.
Formulation 3

0.5%w/v Eudragit® L100 / x%w/v Ethylcellulose N10 / 8%w/v Acacia

<table>
<thead>
<tr>
<th>Ethylcellulose N10 (% w/v)</th>
<th>Chloroquine content (%w/w)</th>
<th>Efficiency of Encapsulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>43.7</td>
<td>45.1</td>
</tr>
<tr>
<td>10</td>
<td>38.3</td>
<td>43.9</td>
</tr>
<tr>
<td>12</td>
<td>34.1</td>
<td>42.9</td>
</tr>
</tbody>
</table>

Table 6.5 Chloroquine content of microcapsules prepared using varying concentrations of Ethylcellulose N10 in Formulation 3.

<table>
<thead>
<tr>
<th>Chloroquine content (%w/w)</th>
<th>Chloroquine released in 0.5 h in distilled water (%)</th>
<th>Chloroquine released in 0.5 h in distilled water (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.1</td>
<td>61.6</td>
<td>12.3</td>
</tr>
<tr>
<td>16.7</td>
<td>54.8</td>
<td>9.2</td>
</tr>
<tr>
<td>14.6</td>
<td>47.5</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Table 6.6 Effect of chloroquine content of microcapsules prepared using Formulation 3 on chloroquine released in 0.5 hours in distilled water.
Figure 6.3(a)

Figure 6.3(b)

Figure 6.3 *In vitro* release profiles of chloroquine microcapsules with varying Ethylcellulose N10 content (Formulation 3) in distilled water.
6.4.4 The effect of annealing

Yolles and Sartori (1980) suggested that microcapsules prepared by the solvent extraction technique leaves pores in the matrices which can be reduced by annealation above the glass transition temperature of the polymer used and hence decrease the rate of drug release. They found this to be true for the progesterone/DL-polylactic acid microcapsules. Zhou and Chang (1988) also found this to be true for prostaglandin/polylactic acid microparticles.

In an attempt to decrease the burst effect, the microcapsules were heat treated at 100 °C for one hour after preparation as in section 5.3.2.. It was not possible to heat the microcapsules above the softening temperature of ethylcellulose (152-162 °C) because of the presence of acacia which decomposes at this temperature range.

The results showed a decrease in the burst effect in all the preparations (Tables 6.7, 6.8 and 6.9) despite the fact that the microcapsules were not heated above the glass transition temperature of ethylcellulose. Jalil and Nixon (1990) found that storing polylactic acid microparticles near the vicinity of the glass transition temperature resulted in a lower rate of drug release. They suggested the reason to be the softening of the thermoplastic polymer which filled the interstices of the matrix. Another possibility could be the lowering of the glass transition temperature of polymers due to the presence of additives, such as water. In this case, the removal of residual solvents in the microcapsules as a result of heat treatment, could have dehydrated the microcapsules and hence resulted in a longer time for drug dissolution to occur.

The similarities of the drug release profiles of the microcapsules in distilled water (Figures 6.4, 6.5 and 6.6), with only a negative shift along the y-axis implies that the effect of heat treatment is mainly on the burst effect which is governed by dissolution of the unbound drug, followed by diffusion across the ethylcellulose wall. The rate of release at later times which is governed by the dissociation of bound chloroquine was not affected by the heat treatment. This is indicated by the similarities of the gradients of the drug release profiles at later times.
In 0.1M hydrochloric acid, only the initial rate of release was affected (Figures 6.7, 6.8 and 6.9) with no difference in drug concentrations after approximately one hour. Therefore, only the drug release profiles of the first hour are presented here. The delay in drug release is probably due to an increase in the time required for the dissolution medium to wet or diffuse into the microcapsules, after which, the drug was rapidly extracted. The slight delay can be the result of a less porous matrix due to annealation or a decrease in the solvent content of the microcapsules.
**Formulation 1**

0.5%<sup>w/v</sup> Eudragit<sup>®</sup> L100 / 10%<sup>w/v</sup> Ethylcellulose N10 / 8%<sup>w/v</sup> Acacia

<table>
<thead>
<tr>
<th>Chloroquine content (%&lt;sup&gt;w/w&lt;/sup&gt;)</th>
<th>Chloroquine released in 0.5 h in distilled water (%)</th>
<th>Chloroquine released in 0.5 h in distilled water (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated  treated</td>
<td>untreated  treated</td>
</tr>
<tr>
<td>10.1</td>
<td>86.1   55.2</td>
<td>8.7    5.6</td>
</tr>
<tr>
<td>23.0</td>
<td>50.6   39.7</td>
<td>11.6   9.1</td>
</tr>
</tbody>
</table>

Table 6.7 Effect of heat treatment on chloroquine released from microcapsules prepared using Formulation 1.

**Formulation 2**

0.75%<sup>w/v</sup> Eudragit<sup>®</sup> L100 / 10%<sup>w/v</sup> Ethylcellulose N10 / 8%<sup>w/v</sup> Acacia

<table>
<thead>
<tr>
<th>Chloroquine content (%&lt;sup&gt;w/w&lt;/sup&gt;)</th>
<th>Chloroquine released in 0.5 h in distilled water (%)</th>
<th>Chloroquine released in 0.5 h in distilled water (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated  treated</td>
<td>untreated  treated</td>
</tr>
<tr>
<td>13.6</td>
<td>59.6   53.5</td>
<td>8.1    7.3</td>
</tr>
<tr>
<td>30.7</td>
<td>30.6   23.5</td>
<td>9.4    7.2</td>
</tr>
</tbody>
</table>

Table 6.8 Effect of heat treatment on chloroquine released from microcapsules prepared using Formulation 2.
### Table 6.9 Effect of heat treatment on chloroquine released from microcapsules prepared using Formulation 3.

<table>
<thead>
<tr>
<th>Chloroquine content (% w/w)</th>
<th>Chloroquine released in 0.5 h in distilled water (%)</th>
<th>Chloroquine released in 0.5 h in distilled water (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>untreated treated</td>
<td>untreated treated</td>
</tr>
<tr>
<td>20.1</td>
<td>61.5 54.7</td>
<td>12.3 10.8</td>
</tr>
<tr>
<td>(8% w/v Ethylcellulose N10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.5</td>
<td>47.5 42.0</td>
<td>6.9 6.1</td>
</tr>
<tr>
<td>(12% w/v Ethylcellulose N10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.4 Effect of heat treatment on \textit{in vitro} release profile of chloroquine microcapsules (Formulation 1) in distilled water.

- **Figure 6.4(a)**
- **Figure 6.4(b)**

*0.5\%w/v Eudragit® L100 / 10\%w/v Ethylcellulose N10 / 8\%w/v Acacia*
Figure 6.5 Effect of heat treatment on *in vitro* release profile of chloroquine microcapsules (Formulation 2) in distilled water.
Figure 6.6 Effect of heat treatment on *in vitro* release profile of chloroquine microcapsules (Formulation 3) in distilled water.
Figure 6.7(a)

Figure 6.7(b)

Figure 6.7 Effect of heat treatment on \textit{in vitro} release profile of chloroquine microcapsules (Formulation 1) in 0.1M hydrochloric acid.
Figure 6.8 Effect of heat treatment on *in vitro* release profile of chloroquine microcapsules (Formulation 2) in 0.1M hydrochloric acid.
Figure 6.9 (a) and (b) show the effect of heat treatment on the in vitro release profile of chloroquine microcapsules (Formulation 3) in 0.1M hydrochloric acid. The graphs illustrate the release of chloroquine over time for different formulations with varying concentrations of Eudragit® L100 and Ethylcellulose N10. The release is measured in both percentage and milligrams.
Chloroquine microcapsules with 30.7%w/w chloroquine content, prepared using Formulation 2 were chosen for further studies in vivo. Therefore stability tests were performed on these microcapsules.

Figure 6.10 shows the in vitro release profiles of these microcapsules at different pH's. Release profiles were found to be pH dependent. Considering the two pKa's of chloroquine, 8.4 and 10.8, and the pKa of the carboxyl groups on the Eudragit® L100 polymer which is 5 to 6, it is logical that drug release is rapid at pH 1.2 in 0.1M hydrochloric acid. This pH dependence was observed again in the slightly lower rate of release at pH 7.0 in comparison to pH 6.2 (distilled water). The results also suggest that drug release was not dependent on the ionic strength of the dissolution medium as the release profile in the pH 7.0 phosphate buffer solution was not significantly different to the release profile in distilled water. This indicates that the microcapsules formed were not reservoir-type microcapsules, with drug encapsulated in the inner aqueous core, as drug release from reservoir-type microcapsules is dependent on the ionic concentration of the dissolution medium. In all three cases, heat treatment resulted in a lower rate of drug release.

The release profiles of the microcapsules stored for one month are presented in Figures 6.11, 6.12 and 6.13. In all three dissolution media, stored microcapsules appear to have lower release rates which could be due to an increase in particle size as a result of aggregation. In contrast, the heat treated microcapsules appear to be more stable on storage than the untreated microcapsules as it showed only a slight decrease in the rates of release, compared to the untreated microcapsules.

Plate 9 shows the scanning electron micrographs of heat treated microcapsules with 30.7%w/w chloroquine content, prepared using Formulation 2, before and after drug release. The presence of a small population of corrugated microcapsules (Plate 9(a)) after heating could be explained by (1) the shrinkage of the ethylcellulose wall, (2) the presence of partially decomposed acacia on the microcapsule surface or (3) the transformation of metastable chloroquine in the ethylcellulose wall to
stable chloroquine crystals. The later is not likely to be so as it would have resulted in an increase in the rate of drug release.

The absence of disintegrated or highly porous microcapsules after drug release (Plate 9(b)) implies that the drug was released either by diffusion from the matrix or through the micropores in the matrix, or both. The mechanisms of drug release from these microcapsules are complex as a result of the drug existing in different forms and the complexity of the microcapsule structure.

In the inner core of the microcapsule, chloroquine may exists in solution or as crystals, giving rise to a first order release mechanism. It may also exists as a molecular dispersion in the ethylcellulose layer of the microcapsule wall and hence have release rates proportional to the square root of time of dissolution (Higuchi 1963). At high chloroquine concentration in the ethylcellulose layer, chloroquine may also form crystals, giving rise to rapid rates of drug release. Bound chloroquine would on the other hand have a zero order release mechanism as the rate of dissolution supercedes the rate of dissociation (Lordi 1986).

In distilled water and pH 7.0 phosphate buffer USP, the mechanism of drug release is probably a combination of the mechanisms described here. It is difficult to characterize the release kinetics of these complex containing microcapsules, where the drug exists in more than one form. Bodmeier et al. (1989a) found similar difficulty with quinidine/poly lactic acid microspheres. However, it is clear from the in vitro release profiles that the rate of drug release, as indicated by the slope of the concentration-time curve, tends towards zero order at later times in both distilled water and pH 7.0 phosphate buffer USP. In 0.1M hydrochloric acid, chloroquine was extracted almost instantaneously, probably as the chloride salt, resulting in rapid drug release.
0.75% w/v Eudragit® L100 / 10% w/v Ethylcellulose N10 / 8% w/v Acacia

Figure 6.10 Effect of pH on the in vitro release profile of treated and untreated microcapsules containing 30.7% w/w chloroquine.
$0.75\%\text{w/v Eudragit® L100} / 10\%\text{w/v Ethylcellulose N10} / 8\%\text{w/v Acacia}$

Figure 6.11 Effect of storage on the in vitro release profile of treated and untreated microcapsules containing $30.7\%\text{w/w chloroquine}$ in distilled water.
$0.75\% w/v$ Eudragit® L100 / $10\% w/v$ Ethylcellulose N10 / $8\% w/v$ Acacia

![Graph showing chloroquine release profile](image)

**Figure 6.12** Effect of storage on the *in vitro* release profile of treated and untreated microcapsules containing $30.7\% w/w$ chloroquine in pH 7.0 phosphate buffer USP.
$0.75\% w/v$ Eudragit® L100 / $10\% w/v$ Ethylcellulose N10 / $8\% w/v$ Acacia

Figure 6.13 Effect of storage on the *in vitro* release profile of treated and untreated microcapsules containing $30.7\% w/w$ chloroquine in 0.1M hydrochloric acid.
Plate 9. Scanning electron micrographs of chloroquine microcapsules (30.7%w/w chloroquine) (a) before and (b) after drug release in distilled water.
The chloroquine content of the microcapsules increased with an increase in the theoretical content of the microcapsules (the initial amount of chloroquine diphosphate used to obtain chloroquine). This increase was greater at the higher Eudragit® L100 concentration in the inner aqueous phase of the multiple emulsion probably due to the binding of chloroquine to Eudragit® L100 which resulted in a lower amount of drug loss to the outer aqueous phase of the same multiple emulsion.

Drug release from the microcapsules was pH dependent due to the basicity of the drug. At pH 1.2 (0.1M hydrochloric acid), chloroquine was extracted rapidly. A more sustained release profile was observed in distilled water where the initial rapid rate of drug release (burst effect) decreased with an increase in drug content, indicating that the additional drug encapsulated was probably bound to Eudragit® L100. The greater decrease in the burst effect, observed in microcapsules prepared using a higher Eudragit® L100 concentration, further supports this hypothesis. The initial rate of drug release from the microcapsules also decreased with an increase in the ethylcellulose concentration used in the oily phase of the multiple emulsion during its preparation. This is probably due to the formation of a more tortuous diffusion pathway for the drug molecules in the microcapsule wall. Annealing the microcapsules also had the same effect (decreasing the initial rate of drug release).

After the initial burst effect, the rate of drug release tends to become constant with time. At later times, drug release was probably from the bound chloroquine molecules, with the dissociation of chloroquine from Eudragit® L100 being rate determining.

Heat treated microcapsules with a chloroquine content of 30.7%w/v, prepared using Formulation 2, was chosen for the clinical study because they had the highest chloroquine content with the smallest burst effect. In addition, heat treated microcapsules were also more stable than the untreated microcapsules on storage, in that the changes in the drug release profile of the treated microcapsules were smaller than those of the untreated microcapsules.
Chapter Seven

In vivo study
7.1 INTRODUCTION

The formulation selected for the in vivo study has been evaluated by earlier in vitro studies. These estimated the dissolution profile of the dosage form. The results showed satisfactory in vitro dissolution characteristics and also demonstrated stability on storage. However, in vitro tests alone cannot be used to predict the in vivo performance of the sustained release dosage form which is dependent on physiological factors related to absorption or disposition of the drug in the human body (Abdou 1989). It would also be difficult to assess the taste masking property of the formulation in vitro even though models have been proposed for such a purpose (Ho 1984).

The most common method used to establish the sustained release characteristics of a sustained release dosage form is to assess both its rate and extent of absorption in human volunteers in comparison to a reference dosage form. In view of the fact that the microcapsules were to be administered as a suspension, the ideal reference dosage form would be a suspension of the crushed tablets. Most investigators support the idea that for a specific drug, the bioavailability decreases in the order: solution > suspension > tablets (Abdou 1989). However, due to the bitter taste of chloroquine suspension, the tablets were chosen as the reference dosage form. In such cases, double blinding should have been provided by using the double dummy technique where both dosage forms are administered to each subject, one of which contains the active dose while the other contains the placebo (Bolton 1990). But due to the difficulty in obtaining placebo tablets, identical to the active tablets available commercially, the idea was abandoned. In this study, no restrictions were placed on the choice of volunteers.

As the microcapsules are intended to be used as substitutes for available liquid preparations of chloroquine, the ideal test for the taste-masking property of the microcapsules would have been a direct comparison with these liquid preparations. However, if the microcapsules prove to be more palatable than the tablets, they should also be more palatable than the corresponding chloroquine solution or suspension. To minimise complications due to the presence of additives, the microcapsules were dispersed in tap water and administered as such.
7.2 MATERIALS

Chloroquine phosphate (250mg) tablets (Avloclor®, ICI).

Chloroquine (30.7% w/w) microcapsules (45μ - 355μ)
(0.75% v/v Eudragit® L100 / 10% v/v Ethylcellulose / 8% v/v Acacia).

7.3 METHODS

7.3.1 In vivo study protocol

Six healthy volunteers whose biodata are listed in Table 7.1, participated in the study with informed consent. The six volunteers fasted overnight for at least nine hours before the doses were administered and fasting continued until three hours after drug ingestion.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender (M/F)</th>
<th>Age (yrs)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>47</td>
<td>1.83</td>
<td>83.0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>40</td>
<td>1.76</td>
<td>75.0</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>59</td>
<td>1.88</td>
<td>95.0</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>25</td>
<td>1.60</td>
<td>49.0</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>26</td>
<td>1.58</td>
<td>48.0</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>32</td>
<td>1.70</td>
<td>62.5</td>
</tr>
</tbody>
</table>

| Mean (standard deviation) | | | |
|---------------------------|-----------------|-----------------|
| Age                       | 38.17 (13.23)   | 1.725 (0.121)   |
| Height                    | 68.75 (18.93)   |                 |

Table 7.1 Biodata of volunteers for the clinical study.
In the first part of the study, each volunteer received two chloroquine diphosphate tablets (reference dosage form) with one glass of tap water (approximately 200ml). The dose was equivalent to 300mg chloroquine, which is the recommended dose for chemoprophylaxis (300mg weekly) (WHO 1990c). Blood samples (5 ml) were taken before dosing at zero hour and at 2, 4, 6, 24, 48 and 72 hours after drug ingestion. The above procedure was repeated with fresh chloroquine microcapsules, equivalent to 300mg chloroquine, after a washout period of 28 days. After another 28 days, three of the volunteers (volunteers 2, 3 and 5) were given another dose of the same microcapsules which have been stored in screw-capped bottles at room temperature. Fasting and blood sampling procedures were as before.

The study protocol was approved by the University College Hospital committee on the ethics of clinical investigation. The first two parts of the study which involved the tablets and the fresh microcapsules, were carried out at the Hospital for Tropical Diseases, St. Pancras Way, London. The last part of the study which involved the stored microcapsules was carried out at the Travel Clinic, Tottenham Court Road, London.

7.3.2 Plasma Drug Analysis

Drug analysis was done at the Robert Gordon's Institute of Technology, Schoolhill, Aberdeen. A high performance liquid chromatographic procedure was used for the assay of the plasma concentration of chloroquine (Taylor et al. 1990).

7.3.3 Plasma Data Analysis

The data was analysed using a computer programme, STRIPE, written in BASIC for the analysis of drug pharmacokinetics (Johnston and Woillard 1983). The pharmacokinetic parameters, such as the elimination rate constant (ke) and hence the elimination half life \((t_{1/2,\text{elim}})\), were estimated by linear regression of the log values of the plasma concentrations against time. The exponentials were then separated by curve stripping and the residuals used to detect the presence
of an absorption phase which triggers other calculations for the pharmacokinetic parameters of the absorption phase, such as the absorption rate constant, \( k_a \) (assuming first order rate of absorption). Area under the curve (AUC\(_{0-t}\)) was calculated by the trapezoidal method. The total area under the curve (AUC\(_{0-\infty}\)) was calculated by adding the value of the last concentration divided by the elimination rate constant, \( k_e \), to AUC\(_{0-t}\). The maximum plasma concentration (C\(_{max}\)) following absorption, and the time at which it occurred (T\(_{max}\)), was found by calculating concentrations at small increments of time until a maximum is reached. Relative bioavailability was calculated manually by dividing the AUC\(_{0-\infty}\) of the microcapsules formulation to the AUC\(_{0-\infty}\) of the tablet formulation (Abdou 1989).

The main advantage of this computer program is its ability to interact with the operator. It offers the investigator the choice of the number of points to fit into each phase of the drug plasma concentration profile, hence giving the operator direct control of the analysis. In this study, the plasma concentrations were analysed using a one compartment disposition model because of the lack of sample points.

### 7.3.4 Taste Evaluation

Taste evaluation of the microcapsules was carried out simultaneously with bioavailability studies. The method was adapted from that used by Sjovall et al (1984). Each volunteer assessed the overall palatability and the bitter aftertaste of the tablets or microcapsules immediately after drug ingestion. Overall palatability was assessed based on a scale of 0 to 4: bad (0), fair (1), good (2), very good (3), excellent (4). The bitter aftertaste was assessed based on a scale of 0 to 3: strong (0), moderate (1), slight (2) and none (3). Therefore, the higher the numerical value associated with the preparation, the better is its palatability.
Figure 7.1  Average plasma concentration-time profile of chloroquine after a single dose of tablets or microcapsules containing 300mg chloroquine.

The results obtained from the pharmacokinetic analysis of the plasma data using the computer program, Stripe, are given in Appendix I. The average plasma chloroquine levels for the chloroquine tablets and microcapsules are shown in Figure 7.1. A qualitative difference is clearly evident. Statistical analysis of the pharmacokinetic parameters using paired t-tests (Table 7.2) also showed significant differences between the two formulations in terms of the peak concentration ($C_{\text{max}}$), time of peak concentration ($T_{\text{max}}$), elimination half-life ($t_{1/2, \text{elim}}$) and the extrapolated area under the plasma concentration-time curve ($\text{AUC}_{\text{trn} \to \infty}$) (from the last sampling time, 72 hours, to infinity). The absorption half lives ($t_{1/2, \text{abs}}$) (p=0.182) and the total area under the plasma concentration-time curve ($\text{AUC}_{0 \to \infty}$) (p=0.322) were not significantly different.
<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Chloroquine Tablets</th>
<th>Chloroquine Microcapsules</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2, \text{abs}}$ (h)</td>
<td>0.65 (0.56)</td>
<td>1.21 (0.65)</td>
</tr>
<tr>
<td>$t_{1/2, \text{elim}}$ (h)</td>
<td>21.05 (9.49)</td>
<td>32.81 (5.31)**</td>
</tr>
<tr>
<td>$\text{AUC}<em>{0\rightarrow t</em>{n}}$</td>
<td>4601.29 (1620.61)</td>
<td>3422.55 (597.82) *</td>
</tr>
<tr>
<td>$t_{n} \rightarrow \infty$ (extrapolated)</td>
<td>884.67 (596.59)</td>
<td>1313.63 (429.28)</td>
</tr>
<tr>
<td>$0\rightarrow \infty$ (ng/ml)h</td>
<td>5485.93 (1849.89)</td>
<td>4736.18 (797.93)</td>
</tr>
<tr>
<td>Extrapolated area as % of $\text{AUC}_{0\rightarrow \infty}$</td>
<td>15.6 (12.0)</td>
<td>27.66 (6.87)**</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>162.21 (62.07)</td>
<td>84.05 (13.02)**</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>2.88 (1.19)</td>
<td>5.79 (1.85)**</td>
</tr>
</tbody>
</table>

Paired t-tests: * significant ($p<0.10$); ** significant ($p<0.05$).

Table 7.2  Pharmacokinetic parameters of chloroquine after a single dose of tablets or microcapsules containing 300mg chloroquine (mean + s.d.) (n=6).
Although chloroquine appeared to be rapidly absorbed, the extent of absorption cannot be determined from this study since the drug was not administered intravenously as well. In this case, $T_{\text{max}}$ and $\text{AUC}_{0->\infty}$ may be used to represent the rate and extent of absorption respectively (Grahnen 1984).

The results obtained for the tablet formulation in this study were comparable to those obtained in other similar single dose studies (Gustafsson et al. 1983; Ette et al. 1989). The results obtained for the microcapsule formulation indicated that the rate of absorption from the microcapsule formulation was significantly slower than the tablets. The time at which the peak concentration occurred ($T_{\text{max}}$) in the tablet formulation was half that of the microcapsules. However, the extent of absorption was not significantly different as suggested by the relative bioavailability of the microcapsule formulation to the tablets ($94.6\pm34.3\%$). $C_{\text{max}}$, which is related to both the rate and extent of absorption (Grahnen 1984), was twice as high in the tablet formulation compared to the microcapsules. In three of the subjects (out of a total of six), administration of the tablet formulation resulted in $C_{\text{max}}$ values greater than 200ng/ml, which had been estimated as the threshold concentration, above which minor side effects such as dizziness and fatigue may be experienced (White et al. 1987). However, no side effects were reported in this study.

In a more detailed study, the plasma concentration of chloroquine had been described using a multi-compartmental model with terminal half-lives as long as 1 to 2 months (Frisk-Holmberg et al 1984). In this study, the elimination half-life of the tablet formulation was found to be approximately 21 hours. The microcapsule formulation had a significantly higher elimination half-life of approximately 33 hours. This finding is consistent with the polyexponential nature of the decline of chloroquine blood plasma levels (Frisk-Holmberg 1979). That is, the rate of decline decreases with time or the elimination half-lives increases with time. Chloroquine microcapsules, which had a slower rate of drug absorption would therefore have a longer elimination half-life than the tablet formulation within the sampling time of this study. At the last sampling time (72 hours), plasma concentration of both formulations were above the minimum therapeutic level of 10ng/ml.

Inter-subject variation for all pharmacokinetic parameters were large due to the large variation in the biodata of the volunteers, especially since the plasma concentration profile of chloroquine is governed by distribution rather than
elimination (White 1988). Generally, the inter-subject variation for all the pharmacokinetic parameters were larger for the tablet formulation compared to the microcapsules.

Due to the lack of data points, the $\text{AUC}_{0->\infty}$ was not determined for the results obtained from the study on stored microcapsules. Neither the absorption half-life ($t_{1/2, \text{abs}}$) ($p=0.544$) or the rate of chloroquine absorption as indicated by $T_{\text{max}}$ ($p=0.594$), was significantly different from the fresh microcapsules (Table 7.3). However, the peak plasma concentration was approximately three times higher than that of the fresh microcapsules and approximately one and a half times greater than the tablet formulation. As haemolysis of blood samples was not reported, a possible explanation could be the release of chloroquine from granulocytes and platelets. Bergquist and Domeij-Nyberg (1983) showed that besides red blood cells, up to 80% of the total amount of chloroquine bound to blood cells may be found in granulocytes and platelets. Centrifugation of blood samples at 2000g for 10 to 15 minutes is necessary for the complete separation of granulocytes and platelets from plasma (White 1985). Since the study on the stored microcapsules was conducted at a different venue with different facilities, differences in sample handling and storage may have contributed to the release of chloroquine from granulocytes or platelets resulting in high plasma levels of chloroquine which did not affect the time at which peak plasma concentration occurred.
Figure 7.2  Average plasma concentration-time profile of chloroquine after a single dose fresh or stored microcapsules containing 300mg chloroquine.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Fresh Chloroquine Microcapsules</th>
<th>Stored Chloroquine Microcapsules</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{1/2, \text{abs}} ) (h)</td>
<td>1.21 (0.65)</td>
<td>2.15 (1.40)</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>84.05 (13.02)</td>
<td>272.32 (98.44)*</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>5.79 (1.85)</td>
<td>5.16 (1.48)</td>
</tr>
</tbody>
</table>

* paired t-tests : significant \((p < 0.10)\); t-tests : significant \((p < 0.01)\).

Table 7.3  Pharmacokinetic parameters of chloroquine after a single dose of fresh or stored microcapsules containing 300mg chloroquine (mean + s.d.) \((n=3)\).
Evaluation of the palatability and bitter aftertaste of the two formulations showed the microcapsules to be significantly more palatable (Table 7.4). Though most of the volunteers found the bitter aftertaste to be less in the microcapsule formulation, the result was not statistically significant in comparison to the tablet formulation (p=0.224).

<table>
<thead>
<tr>
<th></th>
<th>Tablets</th>
<th>Microcapsules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palatability</td>
<td>1.17 (1.17)</td>
<td>2.17 (0.98) *</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>1.17 (1.17)</td>
<td>2.00 (1.27)</td>
</tr>
</tbody>
</table>

paired t-tests: * significant \( p < 0.1 \)

Table 7.4 Palatability and aftertaste of chloroquine tablets and microcapsules.
It can be concluded from this study that the sustained-release preparation of chloroquine microcapsules possesses satisfactory in vivo performance. The rate of drug release and therefore the rate of drug absorption from the gastrointestinal tract was significantly decreased resulting in a lower peak plasma concentration (less peak-trough fluctuation). This improves the safety of the drug especially in the treatment of partially chloroquine-resistant falciparum malaria where the dose of chloroquine has to be increased. In addition, the elimination half-life of the drug at the earlier disposition phases was also prolonged. This indicates a more sustained plasma concentration above the minimum therapeutic level. As the bioavailability of the microcapsules was not significantly different from the tablets, such a dosage form would be especially beneficial for the purpose of prophylaxis where doses are administered weekly.

An added advantage of the microcapsule formulation was its taste-masking property. As it seems to be more palatable than the tablet formulation, it should also be more palatable than other formulations, such as chloroquine diphosphate solution, which is more unpleasant to taste than the tablets due to a greater concentration of the drug in solution. The bitter aftertaste would also be greater after the oral administration of chloroquine diphosphate solution.
GENERAL CONCLUSION
Chapter Eight

Summary and General Conclusion
Due to its film-forming property, acacia is one of the most frequently used water soluble polymers in the multiple emulsion technique of microencapsulation used in this project. Surface rheology was used to investigate the different types of acacia available commercially. Of the three types investigated, acacia tears, acacia powder and a high protein acacia powder, acacia tears was found to have the best film forming ability (surface viscoelasticity) and was therefore used in all subsequent work.

The compatibility between chloroquine and the water soluble polymers used, such as acacia, carrageenan and Eudragit® L100 was investigated. Direct addition of chloroquine to aqueous solutions of carrageenan and Eudragit® L100 resulted in precipitation. Therefore, when these polymers were used as the inner aqueous phase polymer, it was not possible to incorporate the drug in the inner aqueous phase of the multiple emulsion (original method of microcapsule preparation) during the preparation of microcapsules. This was subsequently overcome by incorporating the drug in the middle oily phase of the multiple emulsion (modified method of microcapsule preparation).

Diffusion studies showed an increase in the partitioning of chloroquine from an oily phase to an aqueous phase containing the anionic polyelectrolytes, carrageenan and Eudragit® L100, due to the polymer-drug interaction in the inner aqueous phase which acted as a "sink". This was subsequently found to reduce the loss of chloroquine to the outer aqueous phase during microcapsule formulation and as a result, increase the chloroquine content of the microcapsules.

Ethylcellulose films, with and without chloroquine, were studied using scanning electron microscopy and differential scanning calorimetry to investigate the physical state of chloroquine in the ethylcellulose layer of the microcapsule wall after solvent extraction. Even though scanning electron micrographs showed chloroquine crystals to be present in the film, differential scanning calorimetry was unable to detect the presence of such an entity probably due to the solubilization of the the drug in the polymer melt.

In order to establish a manufacturing procedure, various manufacturing parameters were investigated using a model formulation. The parameters,
such as the phase volume ratios of both the primary and secondary emulsions, concentration of polymers, temperature used for solvent extraction and methods used for drying, were studied for their effects on the surface morphology and particle size distribution of the microcapsules.

Chloroquine microcapsules composed of different polymers were then prepared using the adopted procedure. The drug was incorporated, either in the inner aqueous phase of the multiple emulsion as the diphosphate salt (original method) or in the middle oily phase as the base (modified method). Microcapsules prepared using the original method were generally irregular in shape, highly porous and showed no sustained-release characteristic. With the exception of acacia, only microcapsules of low chloroquine content could be prepared due to the incompatibility of chloroquine with some of the the anionic polyelectrolytes used in the inner aqueous phase, such as carrageenan and Eudragit® L100.

Of all the combinations investigated using the modified method, only microcapsules consisting of acacia, carrageenan or Eudragit® L100 as the inner aqueous phase polymer, Ethylcellulose N10 NF as the oily phase polymer and acacia as the outer aqueous phase polymer, gave satisfactory microcapsules. As chloroquine is basic, the rate of drug release was rapid at the lower pH of 1.2 in 0.1M hydrochloric acid and more sustained in distilled water. Microcapsules prepared using acacia as the inner aqueous phase polymer did not show good sustained release characteristic. Carrageenan, which is the most acidic polyelectrolyte of the three anionic polymers, bonds with chloroquine and precipitated at lower concentrations of both drug and polymer. Only formulations consisting of Eudragit® L100 as the inner aqueous phase polymer showed potential for further development work. In addition, the effect of particle size on the in vitro drug release profile of a few of the selected formulations were investigated. Generally, the rate of drug release was found to increase with a decrease in particle size.

The combination consisting of Eudragit® L100 as the inner aqueous phase polymer, Ethylcellulose N10 NF as the oily phase polymer and acacia as the outer aqueous phase polymer was then chosen for further development. The chloroquine content of these microcapsules, increased with increases in the starting amount of drug (theoretical content) and the Eudragit® L100 concentration in the inner aqueous phase. Increasing the Eudragit® L100
concentration in the inner aqueous phase also improved the sustained release characteristic of the formulation. The rate of drug release was decreased by increasing the ethylcellulose concentration in the middle oily phase of the multiple emulsion which gave microcapsule walls of increased tortuosity. Annealing these microcapsules also decreased the rate of drug release. Stability studies were then performed on the final formulation selected for the in vivo study by repeating the in vitro tests on the same microcapsules which had been stored for one month. The results showed annealed microcapsules to possess greater stability on storage.

The final formulation of annealed chloroquine (30.7%w/w) microcapsules (0.75%w/v Eudragit® L100 / 10%w/v Ethylcellulose N10 NF / 8%w/v Acacia) was then submitted for clinical study with six healthy volunteers. The taste-masking property of the microcapsule formulation seemed significantly better than the tablets although the bitter aftertaste was not statistically different. There were no significant differences in the bioavailabilities of the microcapsule formulation and the chloroquine diphosphate tablets (reference dosage form). The elimination half-lives, the maximum plasma concentrations and the times after drug ingestion at which they occur, were however significantly different. This indicates sustained-release of chloroquine from the microcapsule formulation which also has the added advantage of similar bioavailability to the commercial tablet formulation. The inter-subject variation was also notably smaller in the microcapsule formulation compared to the tablets.

The study on the stored microcapsules in vivo showed similar rate of absorption as indicated by the time at which peak concentration occurred (Tmax) but the maximum plasma concentration (Cmax) was three times higher than the fresh microcapsule formulation and one and a half times greater than the tablet formulation. Such a phenomenon was not observed in the stability studies in vitro. In addition, the taste-masking property of the microcapsule was not found to be affected by storage. As chloroquine is normally concentrated in blood cells such as erythrocytes, granulocytes and platelets, the excess chloroquine in the plasma samples could have been released from blood cells before they were separated from the plasma by centrifugation or due to incomplete separation of blood cells, especially the granulocytes and platelets, from plasma. This could have happened as a
result of differences in sample handling and storage, from the initial study on the tablet and fresh microcapsule formulations.

Despite the discrepancy between the *in vitro* and *in vivo* results of the stored microcapsules, it can be concluded that the microcapsule formulation was able to taste-mask the chloroquine. The fresh microcapsule formulation also showed satisfactory sustained-release property by decreasing the peak plasma concentration and prolonging the elimination half-life of the drug. Even though chloroquine has an inherently long half-life, the sustained plasma concentration could be an added benefit in the prophylaxis and treatment of partially chloroquine-resistant falciparum malaria where the minimum therapeutic level would have increased. A higher dose may also be administered due to a lower peak plasma concentration which would prevent incidences of adverse side effects. As the microcapsules are to be administered as a suspension, an added benefit would be the flexibility in the administration of a range of doses, especially for paediatrics.
Chapter Nine

Suggestions for Further Work
From this work, it is evident that further work in a number of areas would lead to a better understanding and control of the method of microencapsulation using the multiple emulsion / solvent extraction technique.

First of all, interfacial rheological studies of polymeric solutions would lead to a better understanding of the relationship between the water soluble polymer (e.g. acacia, collagen and dextran) in the aqueous phase and the water insoluble polymer (e.g. ethylcellulose) in the organic phase. This would give a better insight into the possible reasons as to why certain water soluble polymers, when used as the outer aqueous phase polymer, do not give satisfactory microcapsules. Different anti-aggregating agents, such as polyisobutylene and magnesium stearate may also be investigated as substitutes for acacia in the outer aqueous phase, especially for the preparation of microcapsules containing Eudragit® RS as the oily phase polymer.

Studies on chloroquine-ethylcellulose films showed the presence of chloroquine crystals in and on the films after solvent extraction. The absence of drug crystals in microcapsules prepared using the multiple emulsion technique has been postulated to be a result of the partitioning of the drug into the inner aqueous phase of the multiple emulsion from the oily phase, followed by binding to the anionic polyelectrolytes present in the inner aqueous phase. This may be confirmed by preparing microcapsules using an o/w emulsion technique instead of the multiple w/o/w emulsion technique. Microcapsules prepared from o/w emulsions should theoretically give microcapsules containing drug crystals. At the same time, film studies using films cast on aqueous solutions of the anionic polyelectrolytes may also be used to confirm the postulation.

The results in this work showed that the rate of solvent extraction has great effects on the properties of the microcapsules. Further work on the method of rotary evaporation would be necessary to control the process better. Factors such as the reduction of atmospheric pressure in the system and the volume of material processed in each batch should be investigated.
The use of carrageenan as the inner aqueous phase polymer has potential for sustained release. Such formulations may also be developed in comparison to the Eudragit® L100 formulations.

As drug release is dependent on the pH of the dissolution medium, further studies on the effect of food on the sustained release characteristic of the microcapsules would be necessary. A more detailed study, with increased frequency and length of sampling time would also be necessary to understand the effect of sustained release on the elimination half-lives of the chloroquine microcapsules. More accurate and detailed storage studies would also be necessary to establish the in vivo stability of the dosage form. This may be supported by in vitro dissolution study and scanning electron microscopy. The taste-masking property of the microcapsules may also be improved by using flavouring agents in the vehicle of the suspension or by incorporating the flavouring agents in the microcapsules. The effect of such flavoring agents on drug release may also be investigated.

In cases where high drug loadings are obtained or where more potent drugs are encapsulated, it may be possible to wash the microcapsules to remove the amount of drug contributing to the burst effect.
APPENDIX I

Pharmacokinetic parameters of chloroquine after a single dose of 300mg chloroquine tablets.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>( t_{1/2, \text{abs}} ) (h)</td>
<td>1.69</td>
</tr>
<tr>
<td>( t_{1/2, \text{elim}} ) (h)</td>
<td>7.12</td>
</tr>
<tr>
<td>( \text{AUC}_0 \rightarrow t_n )</td>
<td>2779.95</td>
</tr>
<tr>
<td>( t_n \rightarrow \infty ) (extrapolated)</td>
<td>1.03</td>
</tr>
<tr>
<td>( AUC_0 \rightarrow \infty ) (ng/ml)h</td>
<td>2780.85</td>
</tr>
<tr>
<td>Extrapolated area as % of ( AUC_0 \rightarrow \infty )</td>
<td>0.04</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>135.68</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>4.74</td>
</tr>
</tbody>
</table>
APPENDIX I
Pharmacokinetic parameters of chloroquine after a single dose of fresh microcapsules containing 300mg chloroquine.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$t_{1/2, \text{abs}}$ (h)</td>
<td>0.89</td>
</tr>
<tr>
<td>$t_{1/2, \text{elim}}$ (h)</td>
<td>5.72</td>
</tr>
<tr>
<td>AUC o -&gt; $t_n$</td>
<td>3112.13</td>
</tr>
<tr>
<td>$t_n$ -&gt; $\infty$ (extrapolated)</td>
<td>1150.35</td>
</tr>
<tr>
<td>AUC o -&gt; $\infty$ (ng/ml)h</td>
<td>4262.48</td>
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<tr>
<td>Extrapolated area as % of AUCo-&gt;$\infty$</td>
<td>26.99</td>
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<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>82.15</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>4.74</td>
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</tbody>
</table>
APPENDIX I

Pharmacokinetic parameters of chloroquine after a single dose of stored microcapsules containing 300mg chloroquine.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Subjects</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>Mean (s.d.)</td>
</tr>
<tr>
<td>$t_{1/2,\text{abs}}$ (h)</td>
<td>3.63</td>
<td>0.84</td>
<td>1.99</td>
<td>2.15</td>
<td>(1.40)</td>
</tr>
<tr>
<td>C$_{\text{max}}$ (ng/ml)</td>
<td>372.82</td>
<td>177.20</td>
<td>267.06</td>
<td>272.32</td>
<td>(98.44)</td>
</tr>
<tr>
<td>T$_{\text{max}}$ (h)</td>
<td>6.05</td>
<td>3.45</td>
<td>5.98</td>
<td>5.16</td>
<td>(1.48)</td>
</tr>
</tbody>
</table>
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