EVALUATION OF SPHERONIZED PELLETS AS POTENTIAL MATRIX SUSTAINED RELEASE DOSAGE FORM

by

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2. PREPARATION OF PELLETS

3. PELLET FORMULATION DEVELOPMENT

APPENDICES

ABSTRACT

Satisfactory spherical pellets were successfully prepared by the process of extrusion/spheronization using the excipient microcrystalline cellulose (MCC) as pellet-forming material. Nonetheless, the preparation of acceptable round spheres is highly dependent upon the attainment of an optimal formulation suited for this method of processing. The earlier part of the work has revealed that the MCC pellet system containing the active ingredient diclofenac sodium generally produced strongly bonded spheroids which remained intact throughout the dissolution test. It may thus be postulated that the pellets behaved as an inert matrix system.

The effect of some excipients on the pellet quality and physical properties of diclofenac sodium-containing pellets was investigated. Certain disintegrants used were found to have a marked influence on the quality of the pellets. In addition, rapid pellet disintegration and dissolution were observed for the soybean oil-formulated pellets, suggesting the potential of soybean oil as a disintegrating agent in pellet dosage form. However, when two other model drugs of varying aqueous solubilities, namely paracetamol and artemisinin, were separately incorporated into the soybean oil-containing formulations and compared, differences in the physical characteristics of the pellets such as disintegration and *in vitro* drug release were observed. The artemisinin-formulated pellets did not disintegrate and at the same time showed a considerably slower dissolution release rate than the pellets containing either diclofenac sodium or paracetamol, which are of higher aqueous solubilities. Since the former preparation exhibited no disintegration and produced an *in vitro* dissolution profile characteristic of sustained release products, it is apparent that the incorporated soybean oil failed to act as a disintegrant in the

evident that both fast- and slow-release spherical pelleted products can be prepared via the extrusion/spheronization technique.

In vivo evaluation of the artemisinin-containing preparation in five healthy human subjects, produced relatively uniform plasma concentration versus time profiles that were reflective of a slow and sustained rate of absorption. Moreover, the preparation showed a comparable extent of absorption than that of the drug administered as an aqueous suspension. Plasma concentrations of the drug were determined using a developed HPLC method. A satisfactory correlation was obtained between the *in vitro* dissolution rate measurements and its *in vivo* pharmacokinetic findings.

ABSTRAK

Pelet-pelet yang berbentuk sfera telah berjaya disediakan melalui proses ekstrusi/sferonisasi dengan menggunakan mikrokristalin selulosa sebagai bahan pembentuk pelet. Walau bagaimanapun, penyediaan sfera yang baik sangat bergantung kepada pemerolehan suatu formulasi optimum yang padan akan proses tersebut. Kajian awal telah menunjukkan bahawa sistem pelet mikrokristalin selulosa yang mengandungi ramuan aktif diclofenac sodium, pada amnya menghasilkan sferoid yang mempunyai ikatan kuat sehingga bentuknya masih kekal semasa proses pemelarutan. Dengan ini, boleh dikatakan bahawa pelet tersebut bertindak sebagai sistem matriks lengai.

Kesan beberapa eksipien ke atas kualiti dan ciri-ciri fizikal pelet yang mengandungi diclofenac sodium telah dikaji. Setengah agen pengecai didapati mempunyai kesan signifikan ke atas kualiti pelet. Tambahan pula, proses pengecaian dan pemelarutan yang cepat telah diperolehi daripada pelet yang mengandungi minyak kacang soya, yang mencadangkan potensi minyak kacang soya sebagai agen pengecai dalam bentuk dosej pelet. Walau bagaimanapun, apabila dua model drug yang berbeza keterlarutan air, iaitu paracetamol dan artemisinin, dimasukkan secara berasingan ke dalam formulasi-formulasi yang mengandungi minyak kacang soya dan dibandingkan, perbezaan dalam ciri-ciri fizikal pelet seperti pengecaian dan pelepasan drug *in vitro* didapati. Pelet-pelet yang mengandungi artemisinin tidak mengecai serta menunjukkan kadar pemelarutan yang lebih perlahan berbanding dengan pelet yang mengandungi diclofenac sodium ataupun paracetamol, yang mempunyai keterlarutan air yang lebih tinggi. Oleh kerana sediaan yang

pemelarutan ciri-ciri pelepasan tertahan, ini membuktikan bahawa minyak kacang soya gagal bertindak sebagai agen pengecai dengan kehadiran artemisinin, iaitu suatu drug yang tidak larut air. Daripada kajian ini, terbukti bahawa kedua-dua produk pelet bersfera konvensional dan pelepasan tertahan boleh disediakan melalui teknik ekstrusi/sferonisasi.

Penilaian *in vivo* sediaan yang mengandungi artemisinin dalam lima subjek manusia yang sihat menghasilkan profil kepekatan plasma lawan masa yang seragam serta menunjukkan penyerapan yang perlahan dan kadar yang tertahan. Lagipun, sediaan tersebut menunjukkan tahap penyerapan yang setara dengan sediaan ampaian berair. Kepekatan drug dalam plasma ditentukan melalui kaedah HPLC. Suatu korelasi yang memuaskan telah diperolehi antara pengukuran kadar pemelarutan *in vitro* dengan keputusan farmakokinetik *in vivo*.

1.1 Oral Sustained Release Dosage Forms

1.1.1 Origins And Developments

Oral administration remains the principal route for systemic delivery of drugs because of its convenience to the patients. The ideal drug product is one which when given via a multiple dose regimen, will attain steady state levels rapidly with minimum fluctuations in peak-trough drug concentration. Unfortunately, conventional dosage forms can only partially fulfil this therapeutic goal.

Frequently, therapeutic failures can be attributed to poor patient compliance. An inconvenient dosing regimen may be a possible cause for the failure of drug therapy. When the regimen is reduced from more than three times daily to twice or once daily, patient compliance may improve. However, once a day dosing with conventional dosage forms tends to produce big fluctuations between peak and trough levels. Therefore, an ideal dosage form will be one that releases its drug gradually, preferably at a constant rate, such that relative uniform drug levels are attained. This is particularly important when the drug possesses a narrow therapeutic index. Thus, sustained release dosage forms were designed to deliver drug in this manner to maintain a uniform drug blood levels within the therapeutic range for prolonged periods of time (Wilson & Washington, 1985). This may also help to reduce the frequency of dosing, thereby improving patient convenience and compliance. A variety of terms has been used to describe these dosage forms, such as sustained release, controlled release and prolonged release (Longer & Robinson, 1985).

One of the earliest attempts to control drug release in the gastrointestinal tract was the use of enteric coating. The concept of enteric coating for pharmaceutical products was introduced more than a century ago (Ellis *et al.*, 1976, Helfand &

primarily to delay drug release until emptying from the stomach had occurred. In contrast, modern sustained release or controlled release preparations are designed to release the drug gradually over a great length of the gastrointestinal tract.

Controlled release preparations have a number of therapeutic advantages over conventional dosage forms. One of the major advantages of using sustained release products is in reducing the frequency of dosing leading to better patient compliance and convenience. Such dosage forms will be most useful for drugs that are used chronically and must be administered several times a day. It has been shown that sustained release products help to minimize or eliminate the possibility of forgotten doses since less frequent dosing is required (Longer & Robinson, 1985). Another advantage of sustained release products, as mentioned previously, is in attaining smaller fluctuations in peak-trough levels within the therapeutically desirable range and thus improves treatment efficiency, especially with drugs having narrow therapeutic indices (Mutschler & Langguth, 1985). Some sustained release devices such as those that are microencapsulated may be suitable for drugs (example indomethacin) which irritate the gastrointestinal mucosa (Rowe, 1983). Reduction in gastrointestinal irritation and toxicity is also made possible by controlling the rate of drug release, thereby avoiding high drug concentrations with the mucosal wall. From studies with lithium, it has revealed that conventional preparations generally produced more nausea (gastrointestinal irritation) than the slow-release forms (Gibaldi, 1977).

Since the 1950s and 1960s, a variety of sustained release dosage forms were developed. Many methods were used to control the drug release, such as coating of drug pellets with varying thicknesses, embedding the drug in a porous plastic matrix, binding the drug to ion-exchange resins and using drug complexes with colloidal materials. Nevertheless, many problems were encountered with these

assessed. Thus, bioavailability studies were added as one of the criteria for effective drug product development.

The development of improved analytical techniques together with advancement in biopharmaceutics and pharmacokinetics in the 1960's and 1970's have led to improvements in the design and evaluation of sustained release dosage forms. As a result, a modern generation of sustained release products were developed. The use of chemical modifications for sustained drug delivery, such as using a prodrug, is another approach to help increase bioavailability. A prodrug is formed by adjusting the parent compound's physicochemical properties so that absorption is increased and is bioreversibly converted to the active drug slowly after absorption. It is this prolongation of the duration of action of the parent compound that results in a sustained-release effect. Example of prodrugs are long-chain esters of steroidal compounds (Kennerley, 1983).

Sustained release products can be formulated as single- or multiple-unit dosage forms. Single-unit systems may be erodable, or non-disintegrating, typically based on matrix release or osmotic delivery. Materials that are commonly used in matrix formulations are insoluble plastics (example methylacrylate - methyl methacrylate), hydrophilic polymers (example sodium carboxymethylcellulose), and fatty/waxy compounds such as glyceryl stearate. Abbott's Gradumet tablet and Ciba Geigy's Lontab tablet are examples of dosage forms utilizing plastic and wax matrices, respectively (Longer & Robinson, 1985).

In the plastic matrix system, the drug is dissolved slowly by the permeating gastrointestinal fluids and leached out from the system along the cracks and capillary channels at a rate that is dependent on several factors including the porosity of the inert matrix and drug solubility. The release rate generally

described by Higuchi (1963). The principle controlling drug release by hydrophilic matrix is that on exposure to aqueous fluids the hydrophilic gum rapidly hydrates forming a gel layer at the tablet surface where soluble drug diffuses through the swollen matrix. When the hydrated matrix is worn away by external agitation, drug is released by matrix erosion (Colbert, 1974). The wax and lipid matrix system is based on surface erosion mechanism whereby the tablet does not disintegrate but simply erode in the gastrointestinal tract. Drug release is due to tablet erosion where the release rate declines with surface area (Notari, 1987).

Oral osmotic dosage form has been introduced relatively recently as a rate-controlled dosage form for many drugs. In this system as described by Theeuwes (1975), the drug reservoir is surrounded by a rigid rate-controlling membrane which is semi-permeable with respect to water. Uptake of water through the rigid membrane at a controlled rate, will cause the device to deliver, via an orifice in the membrane, a volume of saturated drug solution equal to the volume of water imbibed. The release rate of the dissolved drug is constant provided an excess solid remains within the device. Since the mechanism of this system is based on osmotic pressure, the system delivers drug at a rate that is essentially independent of external conditions such as gastrointestinal pH. Examples of some marketed products utilizing osmotic delivery system are Acutrim (phenylpropanolamine), the appetite suppressant from Ciba Geigy, and Osmosin (indomethacin), the NSAID from Merck Sharp & Dohme. However, due to reported serious adverse reactions, the product Osmosin was withdrawn from the market in 1983 (Beckett, 1983; Ganderton, 1985; Notari, 1987).

As controlled-drug delivery systems become popular, multiple-units dosage form design and development also increased substantially. A multiple-units dosage form is a system of numerous pellets or granules of drug filled in a hard gelatin capsule

Theo-Dur tablets from Astra). These subunits combine to give the overall desired controlled drug release of the dose. For example, the pellets in a capsule can be coated with different thicknesses of a slowly soluble substance, such that a relatively constant rate of drug release is produced when the coats are dissolved at different periods of time. This is because the dissolution of the coats is a function of its thickness. Spansule capsules, which was commercially introduced by Smith Kline & French in the 1950's, was based on this principle (Notari, 1987). Alternatively, the drug pellets can be coated with a non-soluble polymer to control the drug release. Both the thickness and the porosity of the coat are important considerations in such a design (Yuen *et al.*, 1993).

1.1.2 Multiple-Units Versus Single-Units Dosage Forms

Oral controlled-release products can be formulated as single-unit or multiple-units dosage form. Single-unit preparations such as matrix tablets, consist of a single nondisintegrating unit which releases drug during passage through the gastrointestinal tract, whereas multiple-unit preparations such as coated pellets are usually filled in a hard gelatin capsule. In the gastrointestinal tract the capsule dissolves and the units are released. Pelletized products, of late, are becoming more popular in the pharmaceutical industry because they appear to have some advantages over the single-unit dosage forms.

In the case of single-unit preparations, gastric emptying is essentially a random process with a large intra- and inter-subject variation, while a multiple-units dosage form has the advantage of emptying gradually from the stomach (Bechgaard, 1982). Pellet dosage forms of diameter less than 1mm are said to be sufficiently small to pass through the pylorus even when the sphincter is closed, and that they can be widely dispersed throughout the gastrointestinal tract, resulting in reduced

al., 1985).

Multiparticulate devices may be well distributed over the intestinal region and since each subunit releases the drug slowly, high local concentrations of drug are seldom produced, thus minimizing the risks of local irritation or damages on the intestinal mucosa (Beckett, 1983). Moreover, if drug release from some subunits is impaired, only a small proportion of the dose is affected. Therefore, the danger of incomplete release of drug content is reduced.

Another potential advantage of multiple-units dosage forms is that pellets containing different active substances can be readily mixed and filled into a capsule. This enables incorporation of chemically incompatible drugs in one single dosage form. Also, in the case of multiple pellet systems, a combination of pellets of different coatings of the same drug can be used to obtain different rates of drug release in order to provide the desired sustained action (Ghebre-Sellasie, 1989).

1.1.3 Physiological Factors Influencing Bioavailability

The gastrointestinal tract may be divided into three major segments : the stomach, the small intestine, and the large intestine or colon. Among the physiological factors that may influence the release of drug from some oral sustained release dosage forms are gastric emptying time, intestinal motility, variations in gastrointestinal pH and surface area, to name a few.

The gastrointestinal tract possesses important regions of variation with respect to absorption. The small intestine has the greatest available surface area and hence represents the segment of the gastrointestinal tract with the highest capacity for drug absorption. Therefore, any factor which affects intestinal motility can affect

drug slowly. In contrast, the available surface area of both the stomach and the colon is significantly small, and for most drugs, absorption through the gastric mucosa and from the colon is far slower than that occurring from the small intestine. The colon may serve as an absorption site for dosage forms which release drug slowly, for example sustained release products or enteric-coated tablets, only if the drug molecules can be absorbed in this region. Generally, if a considerable fraction of a dose reaches this region, it is likely that incomplete absorption of the drug results (Gibaldi, 1977; Mayersohn, 1979; Wilson & Washington, 1985).

Variation in the bioavailability of sustained release formulations can arise due to variable gastrointestinal transit times which affect the time period over which drug can be absorbed. The orocaecal transit time of a dosage form is highly dependent on gastric emptying. Hence, by prolonging the gastric residence, the overall transit time of a dosage form can therefore be extended. If the drug dissolves in the stomach contents, drug solution will then pass in an unimpeded manner to the small intestine for subsequent absorption at the optimal site. Gastric emptying of pharmaceutical dosage forms is a process influenced by numerous factors such as diet, emotional state, posture of the subject and the type of dosage form administered (Kennerley, 1983; Khosla & Davis, 1987). Conversely, small intestine transit is not affected by these factors and it has been shown that the mean transit time of insoluble granules through this region in humans is estimated to be about 4 hours (Gibaldi, 1977; Christensen *et al.*, 1985).

Various attempts have been made to control or influence the gastrointestinal transit time of slow release preparations such as altering particle or pellet size and density, using of bioadhesive polymers and intragastric floating systems (Wilson & Washington, 1985; Khosla & Davis, 1987; Blok *et al.*, 1991).

investigated and conflicting results have been reported. Bechgaard & Ladefoged (1978) revealed that an increase in pellet density from 1.0 to 1.6g/cm^3 significantly delayed average transit times in the small intestine in ileostomy subjects. However, the diameter of pellets, increased from 0.5 to 1.5mm was of minor significance. Similarly, recent studies performed by Devereux *et al.* (1990) reported that the gastric emptying of the heavy pellets of density 2.8g/cm^3 as compared to the light pellets of density 1.5g/cm^3 was extended in both the fed and fasted state. Nevertheless, there was no significant difference in the small intestine transit time. In contrast, studies by Kaus *et al.* (1984) have failed to show significant differences due to density in the transit rate through the small intestine in normal subjects between two specific gravities investigated; one with a range 1.01 to 1.05, whilst the other 1.59 to 1.63. Additionally, no influence from pellet density (densities between 0.94 and 1.96g/cm^3) was observed on gastrointestinal transit in ileostomy subjects by Bechgaard *et al.* (1985).

1.1.4 *In Vitro-In Vivo* Drug Correlation

The development of controlled release dosage forms necessitates the need for appropriate standard quality control tests to evaluate some of the finished drug product specifications in good manufacturing practice. The documented inability of disintegration tests to provide an index of bioavailability has resulted in the introduction of *in vitro* dissolution tests for sustained release products. Since 1960, many satisfactory *in vitro-in vivo* correlations were reported. Thus, dissolution testing became a convenient and reliable *in vitro* method to assess the release characteristics of a drug formulation.

Development of the *in vitro* dissolution test method should take into physiological considerations so that consistent *in vivo-in vitro* correlations are obtained. The

(1982) are based on its ability to discriminate between subtle variability of dissolution characteristics, reproducibility of data, as well as its flexibility to accommodate a wide variety of drug products.

According to Nelson & Miller (1979), currently, there are two distinct types of dissolution test models, the stirred vessel and the flow-through column. The former method is characterized by a relatively large volume of dissolution medium with minimal liquid exchange and agitation is accomplished by stirring the liquid or by motion of the vessel. Conversely, the characteristic of the latter type is a relatively small dissolution cell by which the dissolution medium is replenished with fresh solvent at constant rate and without additional agitation.

There are a number of operating variables that must be considered when performing dissolution methodology, regardless of the type. One of the major factors is the type and intensity of agitation. Stirring rates must be controlled, and specifications differ between drug products. Secondly, the temperature of the dissolution medium must be controlled and temperature variations should be avoided. Most dissolution test are performed and maintained at 37°C ($\pm$0.5°C). A third consideration is the nature of the dissolution medium. The dissolution medium should be aqueous and should not be saturated by the drug in order to simulate the *in vivo* sink condition. Usually, a volume of medium larger than the amount of solvent needed to completely dissolve the drug is used so that the drug concentration in the *in vitro* dissolution medium never exceeds 10-15% of saturation. Commonly employed aqueous systems include phosphate buffer, dilute hydrochloric acid, simulated gastric and intestinal fluid, and distilled water. Surface-active agents may be added to the dissolution medium of specialized drug products to simulate bile function in the gastrointestinal tract (Pernarowski, 1974; Gibaldi, 1977; Abdou, 1985).

The United States Pharmacopeial (USP) provides several official *in vitro* methods for testing controlled release dosage form dissolution. Examples of stirred-vessel types of dissolution apparatus are the rotating basket method (apparatus 1) and the paddle method (apparatus 2). The mode of operation for both methods is easily adapted to automated version. The column method of dissolution testing is not as widely used as the stirred-vessel method, example the flow-through cell dissolution apparatus. This flow-through cell method, which is considered as apparatus 4 in USP 23, can be readily automated as well. The rotating bottle apparatus, a nonofficial method for determining dissolution rate of pharmaceutical preparations with controlled-release characteristics, has been included in the National Formulary (NF) X111. This method, however, has become less popular owing to its manual procedure, a main disadvantage, since automatic testing is highly desirable in sustained release preparations (Nelson & Miller, 1979; Hanson, 1982).

Comparisons between *in vitro* release or dissolution rates and the *in vivo* absorption data are useful to validate *in vitro* dissolution model systems. It is advisable that *in vivo* studies be conducted on human subjects as there are fundamental differences in overall physiology between experimental animals and man. *In vivo-in vitro* correlations can be based on clinical observations or response, plasma or urine concentration data, or absorption kinetics. Most of the bioavailability evaluations are based on drug levels in plasma or urine which give a better objective information on bioavailability of drugs in humans.

When performing an *in vivo* bioavailability study, a reference formulation is necessary against which all other formulations of the drug are compared. The reference formulation should be in its most bioavailable formulation, that is, solution or suspension. The study can also be evaluated relative to intravenous (IV) administration of the drug for which instantaneous and complete bioavailability is

assumed. Alternatively, the use of an established immediate-release formulation which already has valid scientific and clinical data can also be acceptable. The *in vivo* performance of these formulations are compared by examining their corresponding blood-level profiles. In addition, the bioavailability data of the drug product should demonstrate sustained release as claimed without dose dumping. Also, a bioequivalence study is essential to investigate whether the dosage form is bioequivalent to a reference standard sustained release preparation, which may be an original patent or currently marketed drug product, which has undergone extensive clinical testing.

There are cases where *in vitro* release or dissolution rates may not reflect *in vivo* absorption rates. Some dosage forms do not demonstrate bioavailability and dissolution correlation and this may be attributable to several *in vivo* factors such as variable gastric emptying and intestinal transport rate and differences in clinical response shown by sick patients and normal, healthy human volunteers (Swarbrick, 1970). Single-dose studies are still commonly employed to obtain *in vivo* data and is usually performed in healthy human subjects. Nevertheless, in drug product development, dissolution testing is still a very useful tool for evaluating a potential drug formulation prior to performing an *in vivo* bioavailability study.

1.2.1 Introduction

Malaria is a major health problem in many parts of the world, the most hyperendemic malarious areas being in the tropics. The infection occurs through the bite of an infected female anopheles mosquito (the disease vector). The emergence of chloroquine-resistant falciparum malaria has been occurring on a global scale, of late. Chloroquine, the most widely used antimalarial drug, has in the past been considerably effective in the treatment and control of malaria due to *Plasmodium falciparum*, the species which caused 85% of world malaria infection according to World Health Organisation (Luo & Shen, 1987). Recently, this parasite has also developed resistance to other available antimalarial drugs such as quinine, pyrimethamine-sulfadoxine (Fansidar), and even to mefloquine, the effective long-acting quinine analogue (Li *et al.*, 1984). In view of the increasing development of multidrug-resistant strains of *Plasmodium falciparum*, novel antimalarial drugs effective against resistant strains are urgently needed to overcome this potential serious problem.

One such drug is the natural product artemisinin, also called qinghaosu in Chinese, which is now a leading and promising compound of a new class of anti-malarial drugs. Artemisinin is a constituent of the Chinese medicinal plant *Artemisia annua* Linn. (Qinhao), where the genus *Artemisia* belongs to the Compositae family (Asteraceae). The crude extract of this herb has been used for many centuries in China as a treatment for febrile illness. Later, it became evident that its antipyretic property is confined to malaria therapy. The active principle artemisinin was isolated and defined in 1972 by Chinese scientists and showed to have a unique chemical structure, unlike most other antimalarial drugs. It is a sesquiterpene lactone that bears a peroxide group (Figure 1.2), a functionality rare in natural products but which is essential for expressing antimalarial activity (Titulaer *et al.*, 1990; Titulaer *et al.*, 1991). However, extracts from other species

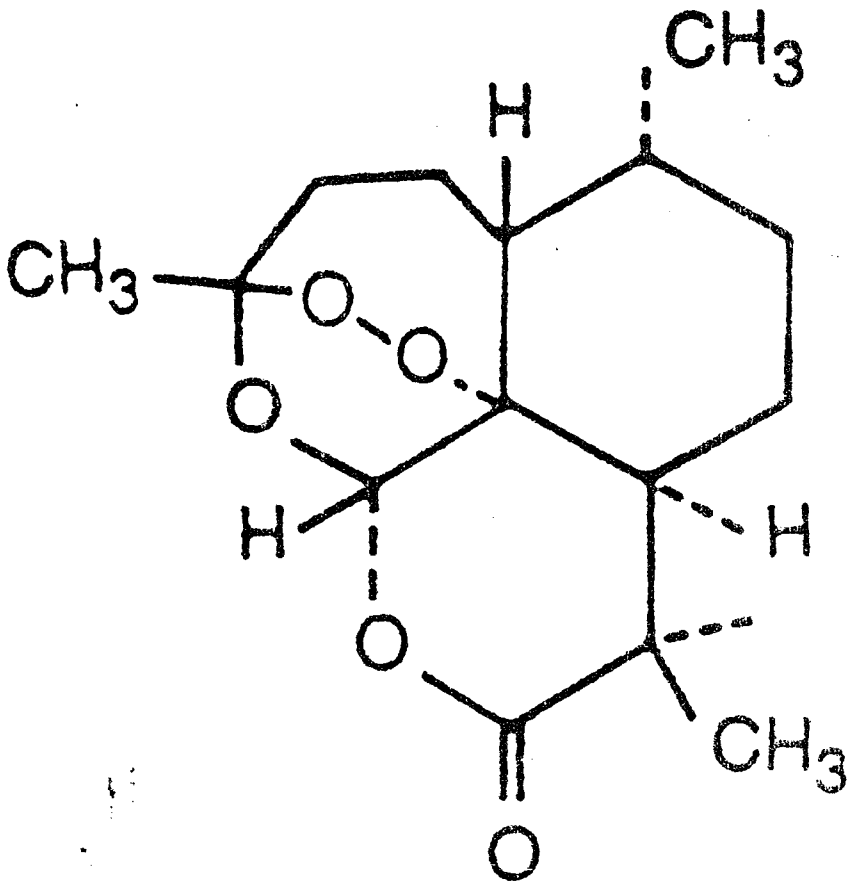

Figure 1.2: Structure of artemisinin

Artemisinin is an effective antimalarial agent against vivax malaria as well as both chloroquine-sensitive and chloroquine-resistant falciparum malaria. It is active against *plasmodia* of the erythrocytic phase, especially in their early development cycle. This rapid acting blood schizontocide destroys asexual parasites, thus arresting trophozoite development at the small ring stage. These characteristics enable it to treat critically ill malaria patients with high parasitaemia, since it has a rapid rate of parasitaemia clearance and inhibitory effect on parasite development which make it a potent and superior drug for the treatment of cerebral malaria resistant to other well-known antimalarials. This fatal complication is an advanced form of *Plasmodium falciparum* malaria, usually presenting as delirium or coma, that can occur when more than 5% of erythrocytes are infected with parasites (Li *et al.*, 1984; Klayman, 1985; Luo & Shen, 1987; Titulaer *et al.*, 1991).

Artemisinin seems to be less liable to induce drug resistance in *Plasmodia*. Until now no resistance to this drug has been described in patients, but to date, it is still not readily available. There has been no marked evidence of cross-resistance between artemisinin and other antimalarial agents. This new compound has relatively low toxicity. The acute toxicity seems to be negligible or considerably less than that of chloroquine. It appears to be remarkably well tolerated in man, even to women in middle and late stage of pregnancy (16-38 weeks) with malaria where they were found to be effectively treated without adverse effects. Nonetheless, safety for use in pregnant women and nursing mothers has not been established. Embryotoxic effects of the drug has been reported in animal studies (Qinghaosu Antimalaria Coordinating Research Group, 1979; Klayman, 1985; Fu *et al.*, 1990; Titulaer *et al.*, 1991).

potentiated the action of artemisinin against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum in vitro* ; whilst primaquine showed potentiation only against the resistant strains. By comparison, antagonism was found for the combination of artemisinin with chloroquine and pyrimethamine. The interactions observed correspond with those found in rodent malaria *in vivo* and may thus be ascribed to a direct action on the parasite and not merely due to effects on the drug pharmacokinetics in the host (Chawira *et al.*, 1987). In order to prevent or delay the emergence of parasite resistance, it is found necessary to use drug combinations. A combination of mefloquine and artemisinin is recommended by World Health Organisation in the treatment of uncomplicated falciparum malaria in cases where all the standard antimalarials fail because of resistance (World Health Organization, 1993). Interactions of artemisinin with other drugs to date, have not been reported (Hien & White, 1993).

1.2.2 Mechanism Of Action

The precise mechanism of action of artemisinin is not fully understood but acts differently in mode and site of action from other antimalarial drugs. The minimal inhibitory concentration for this drug is estimated to be 10^{-7}M *in vitro* (Klayman, 1985). Studies revealed that artemisinin does not interfere with the folic acid metabolism of the malaria parasites (Luo & Shen, 1987; Titulaer *et al.*, 1991).

It has been suggested that the action of artemisinin may involve increase of oxidant stress on the infected erythrocytes. Although the unique peroxide moiety in its structure is vital for its antimalarial activity, no evidence has been obtained on its possible role in the action. Cessation of protein synthesis in *Plasmodium falciparum*, possibly due to oxidative damage to the protein synthesis machinery, may be one of the prime targets of the drug action. According to studies performed