CHAPTER II

LITERATURE SURVEY

2.1 Malaria

2.1.1 Introduction (Tracy and Webster, 1996)

Malaria remains the world's most devastating human infection with 300 to 500 million clinical cases and nearly 3 million deaths each year. In India, recent decades have seen an alarming increase in the cases of malaria caused by lethal parasites.

Effective and safe drugs, insecticides and vaccines still are needed to combat malaria, In the 1950s, attempts to eradicate this scourge from most parts of the world failed, primarily because of the development of resistance to insecticides and antimalarial drugs. The chemotherapy of malaria requires a thorough understanding of the biology of malarial infection.

2.1.2 Biology of Malarial Infection (Tracy And Webster, 1996).

Human malaria is caused by four species of obligate intracellular protozoa of the genus *Plasmodium viz. Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae.* Although malaria can be transmitted by transfusion of infected blood, human beings are infected more commonly by sporozoites injected by the bite of infected female mosquitoes (genus *Anopheles*). The parasites rapidly leave the circulation and localize in hepatocytes where they transform, multiply and develop into tissue schizonts. This primary asymptomatic tissue (pre-erythrocytic to exo-erythrocytic) stage of infection lasts for 5 to 16 days, depending on the *Plasmodium* species. The tissue schizonts then rupture, each releasing thousands of merozoites; these enter the circulation, invade erythrocytes and initiate the erythrocytic stage or cycle of infection. Once the tissue schizonts burst in *P. falciparum* and *P.malariae* infections, no forms of the parasite remain in the liver. But in *P.vivax* and *P. Ovale* infections, some tissue parasites persist and are capable of producing relapses of erythrocytic

infection months to years after the primary attack. The origin of such latent tissues forms is unclear. Once plasmodia enter the erythrocytic cycle, they cannot invade other tissues; thus, there is no tissue stage of infection for human malaria contracted by transfusion. In erythrocytes, most parasites undergo asexual development from young ring forms to trophozoites and finally to mature schizonts. Schizont containing erythrocytes rupture, each releasing 6 to 24 merozoites, depending on the plasmodia species. It is this process that produce the febrile clinical attack. The released merozoites invade more erythrocytes to continue the cycle, which proceeds until death of the host or modulation by drugs or acquired immunity. The periodicity of parasitemia and febrile clinical manifestations thus depend on the timing of schizogony of a generation of erythrocytic parasites. For *P. falciparum, P. Vivax* and *P. Ovale* it takes about 48 hours to complete the process of erythrocytic schizogony. Synchronous rupture of infected erythrocytes and release of merozoites into the circulation leads to typical febrile pattern is less regular in falciparum malaria due to a combination of asynchronous release of parasites and segregation of infected parasites in the periphery. In *P.malariae* infection, schizogony requires about 72 hours, resulting in malarial attacks on day 1 & 4 or "quartan malaria".

Some erythrocytic parasites differentiate into sexual forms known as gametocytes. After infected human blood is ingested by a female mosquito, exflagellation of the male gametocyte occurs and is followed by male gametocytosis and fertilization of the female gametocyte in the gut of the insect. The resulting zygote, which develops in the gut wall as an oocyst, eventually gives raise to the infective sporozoites, which invades the salivary gland of the mosquito. The insect then can infect another human host by taking a blood meal.

Each *Plasmodium* species has distinguishing morphological features in a blood smear. The illness caused by each parasite also is distinctive.

P. falciparum causes malignant tertian malaria, the most dangerous form of human malaria. By invading erythrocytes of any age, this species can produce an overwhelming parasitemia and culminating infection in non-immune persons that, if untreated, may rapidly cause death. Delay in treatment until after demonstration of parasitemia may lead to an irreversible state of shock and death may ensue even after the peripheral blood is free of parasites. If treated early, the infection usually responds within 48 hours to appropriate antimalarial drugs. If treatment is inadequate, however, recrudescence of infection may result from multiplication of parasites that persist in the blood.

P.vivax causes begin tertian malaria and produces milder clinical attacks than those of *P. falciparum*. *P vivax* infection has a low mortality rate in untreated adults and is characterized by relapses caused by latent tissue forms.

P.ovale causes a rare malarial infection with a periodicity and relapses similar to those of *P.vivax* but it is milder and more readily cured.

P.malariae causes quartan malaria, an infection that is common in localized areas of the tropics. Clinical attacks may occur years after infection but are much rarer than after infection with *P. Vivax.*

2.1.3 Immunity to Malaria (Rang et al., 1999)

Immunity to malaria occurs and can protect many individuals living in malarious areas. It involves mostly cell medicated reactions, the details of which are now gradually being elucidated. The immunity is lost if the individual is absent from the area for more than six months. There is hope that it may be possible to make vaccine for immunization against malaria.

2.1.4 Classification of Antimalarial Agents (Tracy And Webster, 1996)

Antimalarials can be classified by the stage of the parasite that they affect and the corresponding clinical objective as follows:

2.1.4.1 Tissues schzonticides used for causal prophylaxis : These agents act on primary tissue forms of plasmodia within the liver that are destined within a month or less to initiate the erythrocytic stage of infection. Invasion of erythrocytes and further transmission of infection are thereby prevented. An example is chloroguanide (proguanil) which is used primarily for causal prophylaxis of falciparum malaria.

2.1.4.2 Tissue schizonticides used to prevent relapse: These compounds act on latent tissue forms of *P. Vivax & P. Ovale.* They are used for terminal prophylaxis and for radical cure of relapsing malarial infections. For terminal prophylaxis, regimens with such a drug are initiated shortly before or after a person leaves an endemic area. To achieve radical cure, this type of drug is taken either during the long-term latent period of infection or during an acute attack. In the latter case, the agent is given together with an appropriate blood schizontocide, usually chloroquine, to eradicate erythrocytic stages *P. Vivax & P. Ovale.* An example of this class is primaquine.

2.1.4.3 Schizontocides (blood schizontocides) used for clinical and suppressive cure: Blood schozontocides act on asexual erythrocyteic stages of malaria parasites to interrupt erythrocytic schizogony and thereby terminate clinical attacks (clinical cure). Such drugs also may produce suppressive cure, which refers to complete elimination of parasites from the

body by continued therapy. Inadequate therapy with blood schizontocides may result in recrudescence of infection due to erythrocytic schizogony.

These agents can be divided into two groups:

(A) The rapid acting blood schizontocides: include classical antimalarial alkaloids like chloroquine, quinine and their related derivatives, quinidine, mefloquine and halofantrine as well as the antimalarial endoperoxides such as quighaosu.

(B) Slower acting, less effective blood schizontocides: such as the antimalarial antifolate compounds e.g. pyrimethamine and antibiotic compounds e.g. sulfonamides and tetracyclines. These drugs are commonly used in conjunction with their more rapidly acting counterparts.

2.1.4.4 Gametocytocides: These agents act against sexual erythrocytic forms of plasmodia, thereby preventing transmission of malaria to mosquitoes. Chloroquine and quinine have gametocytocidal activity against *P.vivax, P ovale* and *P. malariae* whereas premaquine displays especially potent activity against *P.falciparum*. However, antimalarials are rarely used clinically just for their gametocytocidal action.

2.1.4.5 Sporontocides : Such drugs able to transmission of malaria by preventing or inhibiting formation of malarial oocysts & sporozoites in infected mosquitoes. Though chloroquine prevents normal plasmodial development within the mosquito, neither this nor other antimalarial agents are used clinically for this purpose.

When WHO launched the Malarial Global Eradication campaign in 1957, there were several safe, effective antimalarial drugs available. During

the past 30 years, malarial parasites, especially *P. falciparum* have rapidly developed resistance to some of these agents notably chloroquine.

2.1.5 Potential new antimalarial drugs

Some new synthetic drugs are Halofantrine, Pyronaridine, Antifolates, Atohraquine, Benflumetol and new 8-aminoquinolines. Halofantrine is more active in-vitro than mefloquine against multi drug resistant falciparum malaria.

Some new Antimalarial agents from plant source include : Qinghaosu (Artemisinin) other formulation of Artemisinin are Artemether(oral), Artesunate(oral), Dihydroartemisinin(oral), Artemether(injectable), Sodium Artesunate (injectable), Artemisinin (suppositories), Arteether (injectable).

Other approaches to the therapy of malaria include the development of new folate antagonists and the possibility of administering antimalarial drug in biodegradable polymer matrices for slow release and thus long action.

2.1.6 References

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2.2 Microspheres

2.2.1 Introduction

Microspheres can be defined as solid, approximately spherical particles ranging in size from 0.5 μm to 1000 μm. (Burgess & Anthony , 1988).

Microspheres are defined as solid spherical particles containing dispersed drug in either solution or microcrystalline form. They range from 1 to 100 μm in size. They are made of polymeric wax or other protective materials like biodegradable, biocompatible synthetic polymers and modified natural products like starch, gum, proteins, fats & peptide. There are two types of microspheres : (Chein 1982)

Microcapsules:- Where the entrapped substance is completely surrounded by a distinct capsule wall.

Micromatrices:- Where the entrapped substance is dispersed throughout the polymer matrix.

Microspheres of biodegradable & non-biodegradable polymers have been investigated for sustained release depending on the final application. In case of non-biodegradable drug carriers, when administered parenterally, the carrier remaining in the body after the drug is completely released poses the possibility of carrier toxicity over a long period of time. Biodegradable, biocompatible carriers which degrade in the body to non-toxic degradation products do not pose the problem of carrier toxicity & are more suited for parenteral application (Jaykrishnan 1997).

Microspheres as a drug carrier :

Microsphere based drug delivery systems have received considerable attention in recent years. The most important characteristic of microspheres is the microphase separation morphology which endous it with a controllable variability in degradation rate & also drug release.

2.2.2 Desirable characters of colloidal carriers

- Biocompatible with environment at the injection site.
- Biodegradable with non-toxic degradation products.
- Injectable with good syringebility.
- Able to sterilize.
- Pharmaceutically stable, with adequate shelf life.
- Compatible with diluents.
- Able to incorporate drug with good efficiency.
- Colloidal drug carrier should maintain its integrity, with no loss of drug until reached to target tissue.
- The colloidal carrier must be able to penetrate any biological membrane.

2.2.3. Advantages of microspheres :

- Test and order masking e.g. Butobarbitone, citric acid, cod liver oil, dicloxacillin, disulfiram, doxycycline HCl.
- Conversion of liquids to solids e.g. Castor oil, clofibrate, dimethicone fluid.
- Protection of drugs against environment e.g. Ferrous citrate, levodapa, meclofenoxate HCl.
- Improves the flow property of the powders.
- Have controlled release e.g. Chlorpheniramine maleate, codeine phosphate.
- Have targeting property e.g. 5-Fluorouracil.
- As Microspheres is multiple unit product ready distribution over a large surface area.
- Delocalization of the total dose in the GI tract.

- Reduce side effects.
- The drug release rate will be less dependent on gastric transit time e.g. Phenylbutazone, nitrofurantoin, theophylline, indomethacin.
- The drug loaded Microspheres are more resistant to fracture.

2.2.4. Dis-advantages of microspheres :

- Burst effect.
- Inadequate shelf-life of sensitive pharmaceuticals.
- Non-reproducible.
- Costly.
- Difficult to scale up.

2.2.5 Desirable criteria for preparation of microspheres

The preparation of microspheres should satisfy certain criteria.

1) The ability to incorporate reasonably high concentrations of the drug.
2) Stability of the preparation after synthesis with clinically acceptable shelf life.
3) Controllable particle size & dispersability in aqueous vehicle for injection.
4) Release of active agent with good control over a wide time scale.
5) Biocompatibility with a controllable biodegradability.
6) Susceptibility to chemical modification.

2.2.6 Microsphere preparation methods

A Emulsion solvent evaporation (Atilla et al 2000)

a Oil in water (o/w) emulsion solvent evaporation.

b Water in oil (w/o) emulsion solvent evaporation.

c Water in oil in water (w/o/w) complex emulsion solvent evaporation.

B Emulsion solvent extraction (precipitation).

C Emulsion solvent diffusion.

D Coaservation & Phase separation technique or cross-linking technique.

- a Chemical cross-linking
- b Physical cross-linking

E Polymerization technique

- a Vinyl Polymerization
- b Normal Polymerization
- c Interfacial Polymerization

F Spray drying & spray congealing

G Freeze drying

H Congealable disperse phase encapsulation technique

In all these methods drug is incorporated in either aqueous phase or organic phase depending on the solubility of drug in that phase.

A In Emulsion Solvent Evaporation Technique, the polymer & drug must be soluble in an organic solvent. The solution containing polymer & the drug may be dispersed in the dispersed phase (aqueous / oil) to form a droplet. Continuous mixing & elevated temperature may be employed to evaporate the more volatile organic solvent & leave the polymer drug particles in aqueous medium & the particles are finally filtered from the suspension.

O/w emulsion solvent evaporation is used for hydrophobic polymers (e.g. PLGA, PLA, Ethyl Cellulose, Eduragit (RS, RL, SL)

W/o emulsion technique is used for hydrophilic polymers (e.g. casein, albumin, chitosan, gelatin starch etc.)

B Emulsion Solvent Extraction or Precipitation Technique is same as evaporation except that the emulsion consists of polar phase dispersed in a non-polar medium. Solvent may be removed from the droplets by the use of a co-solvent. The resulting increase in the polymer drug concentration causes precipitation forming a suspension & particles are recovered by filtration from the suspension. (Atilla et al 2000).

C In Emulsion Solvent Diffusion Method, the organic solution of drug & polymer is dispersed into aqueous medium with constant stirring. During the process, the solvent first diffuses out & then evaporate from the coaservate into the aqueous medium, this method is simple, quicker & does not require heat or any other harmful solvents.

D In Coaservation Phase Separation technique, an aqueous solution of a polymer is emulsified in an organic / oil phase. The polymer particles are then crosslinked physically / chemically using suitable agents such as glutaraldehyde, formaldehyde, glyoxal, borax, epichlorhydrin, etc or they are hardened by heat (thermally crosslinked).

E In Polymerization Technique, Vinyl monomers are polymerized. The microspheres are formed using technique such as suspension, emulsion, soapless emulsion, dispersion, precipitation, seeding or support polymerization. Drug is to be incorporated within the monomers in the initial stage.

F In the Interfacial Polycondensation method, two complementary monomers are taken in a two phase system & one of the phase is dispersed as droplets in an another phase. The drug being incorporated

in any one phase & microspheres are obtained when condensation of monomers takes place at the interface.

G In Spray Drying, the core substance is dispersed in a solution of the coating material, which is then atomized & the solvent dried off using heated air in a spray drier. Spray congealing is same as spray drying except that no solvent is used for the coating material which has the property of melting at elevated temperature when being atomized & congealing when the droplets formed meet cool air in a spray drier.

H In Freeze Drying Technique, the freezing points of continuous & dispersed phases are important. The continuous phase solvent is usually organic & is removed by sublimation at low temperature & pressure. Finally, the dispersed phase solvent of the droplets is removed by sublimation, leaving polymer drug particles.

2.2.7 Characterization

2.2.7.1 Polymer characterization

1. Molecular weight.
   - Gel permeation chromatography
   - Viscosity
   - Colligative properties (Vapour pressure, osmometry, boiling point elevation, freezing point depression)
   - Refractometry
   - Light scattering measurements
2. Purity
   - Gas chromatography (for residual monomer and solvent)
   - Karl fischer and thermo gravimetric analysis
   - Fourier transform Raman spectroscopy
   - Differential photoelectron spectroscopy
   - X-ray photoelectron spectroscopy
3. Miscellaneous

   Density, crystallinity and film forming properties

2.2.7.2 Microspheres characterization

1. Particle size and size distribution
   - Sieving
   - Microscopy
   - Resistance blockage analysis (coulter analysis)
   - Light blockage techniques
   - Laser diffraction analysis
2. Surface characterization
   - Electron microscopy
   - Scanning electron microscopy

- Scanning tunneling microscopy

3. Surface charge analysis
   - Micro electrophoresis
   - Laser Droppler anemometry
4. Surface area
5. Porosity
6. Hardness and Friability
7. Density
   - Bulk density
   - Tapped density
8. Hydrophobicity
   - Contact angle measurements
9. Flow properties
   - Angle of repose
   - Hausner ratio
10. Drug content
11. Drug release profiles
    - In-vitro
    - In-vivo

2.2.8 Applications of microspheres

1. Microspheres are used for sustained and controlled release. e.g. Drugs like riboflavin, indomethacin, aspirin and steroids like progesterone, testosterone etc. can be incorporated in it, to control their release.
2. It is used in enteric release dosage form. e.g. Drugs like aspirin, salbutamol sulphate which are irritant to the stomach and other side effects can be incorporated in microspheres for their selective release in intestine.

3. It is used for drug targeting. e.g. Casein and gelatin microspheres containing adriamycin were magnetically delivered to the tumor site.
4. It is used as antigens carrier. e.g. Microspheres prepared from poly (lactic acid) (PLA) and its copolymer with glycolic and (PLGA) of varying composition have been used to improve the ability of the antigens to provoke a mucosal immune response.
5. To alter the residence time and to improve the bioavailability. e.g. Albumin and gelatin microspheres containing pilocarpine nitrate (ophthalmic drug delivery) for delivery to eye increase residence time of drug in the eye and provide improved bioavailability.
6. It is used to protect reactive materials against environment. e.g. Vitamins, aspirin.
7. To separate incompatible substances. e.g. the stability of incompatible drugs like aspirin and chlorpheniramine maleate mixture was increased by microspheres of individual components.
8. Administration in solid state and dry handling. e.g. Liquids such as eprazine can be converted to a pseudo-solid by microspheres as an aid to handling and storage.
9. It is used to mask bitter or unpleasant taste of the drug. e.g. Quinidine, clofibrate, paracetamol.
10. It is used as an antidote in the poisoning of heavy metals. e.g. Polymercaptal microspheres as an antidote against mercury poisoning.
11. To facilitate handling of toxic materials. e.g. Microspheres has been used to decrease potential danger in handling toxic substances like pesticides, fertilizers and certain pharmaceuticals.
12. It is used as diagnostic tool. e.g. microencapsulated radiolabelled molecules have been used for diagnostic testing of free drug or hormone concentrations, such as thyroxin estimation.

2.2.9 References

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2.3 Implants

2.3.1 Introduction (Joseph - 1987)

Lafarge pioneered, in 1861, the concept of implantable therapeutic systems for long term, continuous drug administration with the development of a subcutaneously implantable drug pellet. The technique was then rediscovered in 1936 by Deanesly and Parks, who administered crystalline hormones in the form of solid steroid pellets to mimic the steady, continuous secretion of hormones from an active gland for hormone substitution therapy.

Subcutaneous drug administration by pellet implantation is known to have several undesirable drawbacks. The primary one is that release profile of drugs from the pellets is not constant & cannot be readily controlled, in terms of precision of release rate & duration of action. The fact has triggered the research & development of novel, controllable & implantable therapeutic systems to replace pellets for long term.

2.3.2 Advantages

1. Less fluctuation in plasma drug levels during therapy.
2. Minimal harmful side effects of systemic administration through local (physically targeted) therapy.
3. Improved patient compliance.
4. Possible reduction in therapy costs because of reduced patient care and the potentially lower drug dose required (especially in case of local therapy).
5. Administration of drugs with short biological half-life may be greatly facilitated (Bhagat and Langer, 1988).

2.3.3 Dis-advantages

1. The primary difficulty is that the release profile of drugs from the pellets is not constant and cannot be controlled readily, in terms of the precision of the rate of release and the duration of action.
2. Possibilities of tissue and body reaction to implant.
3. Potential toxicity of by-products of biodegradable polymers.
4. Surgical procedures necessary for implantation of some of the systems.
5. Cost of implant therapy.
6. Danger of toxic effects in case of leakage or burst release of drug.
7. Difficulty in terminating drug release, if so desired.

2.3.4 Injectable implants

To avoid the surgical problems associated with large implants, nanoparticles & microparticles which can be injected, present an attractive delivery system. The colloidal particles of submicron size, nanoparticles nanocapsules, nanopellets because of ultrafine size act as drug carrier for parenteral purpose (Venkatesan et al., 1995).

Microparticles of monolithic (microspheres) or capsular (microcapsules) configuration, with their smooth surface, can be dispersed readily in vehicles for injection (Steven et al., 1997). Injectable implant controlled delivery systems made of biodegradable drug carrier, offer several advantages to overcome the problems of conventional implants. Reoperation is not required to remove waste skeleton of polymer, has higher level of patient and physician compliance. The therapeutic benefits of subcutaneous controlled drug administration can be illustrated by comparing the biological utility and duration resulted from the subcutaneous drug administration via a

controlled release drug delivery system with those produced by conventional drug administration through subcutaneous injection (Chien, 1982).

2.3.5 Intramuscular Implants

Intramuscular (i.m.) injection of pharmaceuticals is a well known and frequently used route for drug delivery. Intramuscularly administered drugs are released gradually, providing some degree of sustained release. In order to optimize further controlled release from i.m. injection sites, various drug formulations and drug carrier systems among which liposomes have been developed and are being used.

In a number of studies it has been shown that drug-containing liposomes and liposomal lipids administered intramuscularly are retained at the injection site for a considerable period of time and that drug-containing liposomes release the active ingredient slowly from the site of injection.

I.m. or s.c. administered chloroquine-containing liposomes act as a local depot with a slow release of the drug.

2.3.6 Desirable characters of injectable implants

An ideal implantable parentral system should possess following properties :

(1) Environmentally should not break down under the influence of heat, light, air and moisture.

(2) Bio-stable should not undergo physico-chemical degradation when in contact with bio-fluids (or drugs).

(3) Bio-compatible should neither stimulate immune responses (otherwise the implant will be rejected) nor thrombosis and fibrosis formation.

(4) Non toxic and non-carcinogenic :- Its degradation products or leached additives must be completely safe.

(5) Should have a minimum surface area, smooth texture and structural characteristics similar to the tissue in which it is to be implanted to avoid irritation.

(6) Should release the medicament at a constant predetermined rate for a predetermined period of time.

2.3.7 Development of implantable therapeutic systems

Over the years, a number of approaches have been developed to achieve the controlled administration of biologically active agents via implantation or interaction in the tissue. These approaches are outlined as follows :

A. Controlled drug release by diffusion

1. Membrane permeation controlled drug delivery using.
    a. Non porous membranes
    b. Microporous membranes
    c. Semipermeable membranes
2. Matrix diffusion controlled drug delivery using.
    a. Hydrophilic (swellable) polymers
    b. Lipophilic polymers
    c. Porous polymers
3. Micro-reservior dissolution controlled drug delivery using.
    a. Hydrophilic reservior /lipophilic matrix
    b. Lipophilic reservior / hydrophilic matrix

B. Controlled drug release by activation :

1. Osmotic pressure – activated drug delivery
2. Vapour pressure – activated drug delivery
3. Magnetism – activated drug delivery
4. Ultra sound – activated drug delivery
5. Hydrolysis – activated drug delivery

2.3.8 Medical aspects of implantation

(A) The environment of living tissues.

Animal tissue contains approximately 70% body fluid. This fluid consisted of two major compartments :- intra-cellular fluid and extracellular fluid. bathing all tissues is further subdivided into interstitial and intravascular (which includes plasma and lymph) fluid The interstitial fluid, which the implant mostly encounter on the site of implantation, has the chemical composition shown as the table below.

Chemical composition of interstitial fluid (pH 7.4)

Cations		Anions	
Species	Meqlutic	Species	Meqlutic
Na^+	140	Cl^-	105
K^+	4	HCO_3^-	30
Ca^{2+}	5	HPO_4^{-2}	5
Mg^{2+}	3	SO_4^{-2}	3
		Organic acid	6
		Protein	3
	152		152

Oxygen is freely available and readily replaced by complex biochemical processes and haemostatic controls. CO_2 also exists. However, in certain tissues which have necrosed or are in various types of bacterial infection an anaerobic condition may result. At cellular level, the pH value may be lower the pH 7.4 measured in extracellular fluid.

Many enzymes, which are capable of oxidation, reduction or hydrolysis are present in the environment of living tissues. Since many enzymatic reactions are characterized by various trace metals, thorough investigation is needed to search for early or long-term effects of chemicals or additives that could leach out or result from the degradation of implanted polymeric materials.

2.3.9 References

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2.4 Profile of Chloroquine phosphate

2.4.1 Introduction (CIMS Drug Profiles, 1996) :

Chloroquine is primarily an antimalarial that is still used as a drug of choice for the treatment of malaria in areas where there is no chloroquine resistance. It is also used for prophylaxis again making allowance for resistance. It also finds its use in treating extra intestinal amoebiasis & rheumatoid arthritis.

2.4.2 Description (Hong, 1976)

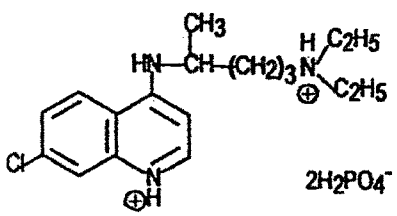
2.4.2.1 Systematic name :

7-chloro–[4-(4-diethylamino-1-methylbutylamino)] quinoline diphosphate.

2.4.2.2 Formula :

(A) Empirical : $C_{18}H_{26}ClN_3.2H_3PO_4$

(B) Structural :



(C) Molecular weight : 515.87

2.4.2.3 Appearance, colour and odour :

Chloroquine phosphate is a white, odorless, crystalline powder having a bitter taste, it discolors gradually on exposure to light.

2.4.3 Physicochemical properties :

Hong (Hong, 1976) has presented a detailed account of the physical properties of chloroquine phosphate, the important properties are described below :

2.4.3.1 Solubility :

Chloroquine phosphate is freely soluble in water, practically insoluble in alcohol, in chloroform and in ether.

2.4.3.2 Ionization constant :

The pKa's for chloroquine phosphate by the titrametric method were found to be 8.10 and 9.94.

2.4.3.3 Melting characteristics :

Chloroquine phosphate exists in two polymorphic forms giving rise to two melting ranges. USP XIX reports one melting between 193°C and 195°C and other between 210°C and 215°C. Mixtures of the forms melt between 193°C and 215°C. It is possible to obtain one form selectively by regulating the rate of crystallization.

2.4.4 Clinical Pharmacology (Therapeutic drugs, 1999, Martindale, 1999 ; Physician's Desk Reference, 2001) :

The exact mechanism of action of chloroquine against malaria parasites is not fully understood. Parasitised red cells accumulate approximately 100-600 times as much chloroquine as plasma whereas uninfected red cells only accumulate 14 times as much chloroquine. The concentration of chloroquine in malaria parasites requires energy and is thought to require a membrane carrier or permeable to transport the drug across the parasite cell membrane. There are three theories on the way in which chloroquine might act once it is inside the parasites :

1. The lysosomal theory states that chloroquine, being a basic compound, is protonated in the lysosomes thus raising lysosomal pH. This rise in intralysosomal pH above a critical level results in loss of lysosomal function. This would reduce the parasite's digestion of hemoglobin and thus prevent its growth.
2. The intercalation theory suggests that chloroquine intercalates into double stranded DNA and inhibits both DNA & RNA synthesis. Chloroquine may be bound with increased affinity by certain parts of the genome and be toxic to the malaria parasite by selective accumulation in specific genes, inhibiting their expression.
3. The ferriprotoporphyrin IX (FP) interaction hypothesis states that chloroquine may form a complex with FP which inhibits sequestration of FP into malaria pigment.

This could impair hemoglobin degradation and permit damage to the food vacuole sufficient to discharge its pH gradient.

Chloroquine is primarily used as a suppressive prophylactic for malaria infections but is also effective in the treatment of acute attacks of malaria caused by chloroquine – sensitive strains. It has been found to the highly active against the erythrocytic forms of *Plasmodium vivax* and *Plasmodium malariae* and most strains of drug sensitive *Plasmodium falciparum*.

Chloroquine may be used as a second-line agent for the treatment of amoebic liver abscess. In rheumatoid arthritis as well, chloroquine is reserved as a second–line agent where it is thought to have disease modifying effect. In this case, dosage duration is long and thus therapy calls for close monitoring to avert retinopathy.

Chloroquine has a depressant effect on cardiac muscle. When given by intravenous bolus injection (which is absolutely contraindicated as opposed

to infusion) or following chloroquine overdose, decreased cardiac output and hypotension have occurred. Chloroquine, like quinidine, reduces excitability and conductivity of cardiac muscle. At toxic concentrations, profound bradycardia with ventricular escape rhythms may develop. Relaxation of smooth muscle in the gut and ciliary body may also occur. Chloroquine may cause pruritis as a side effect and the cause for this is unexplained.

2.4.5 Pharmacokinetics (Therapeutic Drugs, 1999 ; Martindale, 1999 ; Physician's Desk Reference, 2001) :

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract and only a small proportion of the administered dose is found in the stools. Approximately 55% of the drug in plasma is bound to non-diffusible plasma constituents. Chloroquine is deposited in the tissues containing melanin e.g. retina. In animal studies, from 200 to 700 times the plasma concentration may be found in the liver, spleen, kidney and lung; leucocytes also concentrate the drug. The brain and spinal cord, in contrast, contain only 10 to 30 times the amount present in plasma.

Chloroquine undergoes appreciable degradation in the body. The main metabolite is desethyl chloroquine which accounts for one fourth of the total material appearing in the urine. Bisdesethyl chloroquine, a carboxylic acid derivative & other metabolites as yet uncharacterized are found in small amounts in the urine. Slightly more than half of the urinary drug products can be accounted for as unchanged chloroquine. Elimination of chloroquine is slow, with a multi exponential decline in plasma concentration and elimination pattern as equilibrium is reached between the small, central and the large, peripheral compartment. The initial elimination phase has a half-life of 2-6 days while that of terminal elimination is 30-60 days.

2.4.6 Indications (Therapeutic Drugs, 1999; Martindale, 1999 ; Physicians' Desk Reference, 2001) :

Chloroquine is used for :-

a. Malaria : Treatment and prophylaxis
b. Hepatic amoebiasis
c. Rheumatoid arthritis
d. Systemic lupus erythematosus (SLE)
e. Porphyria cutanea tarda.

2.4.7 Dosage & Administration (Therapeutic Drugs, 1999; Martindale, 1999 ; Physicians' Desk Reference, 2001) :

The dosage of chloroquine salt is often expressed or calculated as the base. In infants & children, the dosage is preferably calculated on the body weight.

2.4.7.1 Malaria :

(A) For the treatment of acute attack

Adults : An initial dose of 600mg base followed by 300mg base after 6-8 hours and a single dose of 300mg on each of the two consecutive days. This represents a total dose of 1.5g base in three days.

Children : An initial dose of 10mg base / kg (but not exceeding 600mg base) is followed by 5 mg base / kg (but not exceeding 30mg base) after 6 hours and a single dose of 5mg base / kg on each of the two consecutive days. This represents a total dose of 25mg base / kg body weight in three days.

For radial cure of *P.vivax* and *P.malariae* malaria, concomitant therapy with primaquine is necessary.

Parenteral therapy may be required in patients with falciparum malaria who are seriously ill. Treatment with parenteral chloroquine

must be undertaken with caution, especially with children because of the risk of cardiovascular collapse. The intravenous dose of chloroquine should be 10mg / kg base as a continuous infusion in isotonic fluid over 8 hours followed by 15mg / kg over 24 hours. The intramuscular dose regimen may be 3.5mg base / kg 6 hourly to a total of 25mg base / kg changing to oral route if the patient becomes alright to swallow oral tablets.

(B) For suppressive prophylaxis :

Adults : 300mg base weekly.

Children : 5mg base / kg (not exceeding adult dose regardless of the weight)

If circumstances permit, suppressive therapy should begin two weeks prior to exposure. However, failing this in adults, an initial double dose of 600mg base or in children 10mg base / kg may be taken in two divided doses, 6 hours apart. The suppressive therapy should be continued for 8 weeks after leaving the endemic area.

2.4.7.2 Hepatic amoebiasis :

Adults : 600mg base daily for two days followed by 300mg base daily for atleast 2-3 weeks. Treatment is usually combined with an effective intestinal amoebicide.

2.4.7.3 Rheumatoid arthritis :

Adults : 150mg base daily for 3-6 months

Children : 3mg / kg base for 3-6 months

2.4.7.4 SLE :

Adults : 150mg base daily until maximum improvement is obtained following which a smaller maintenance dosage may be used.

2.4.7.5 Porphyria cutanea tarda :

75mg base 2-3 times per week for 6-18 months.

2.4.8 Contraindications (Therapeutic Drugs, 1999; Martindale, 1999; Physicians' Desk Reference, 2001) :

Chloroquine is contraindicated in

(a) Known or suspected chloroquine resistance in *P. falciparum*

(b) Porphyria, apart from porphyria cutanea tarda

(c) Retinal damage or visual field changes.

(d) Concurrent therapy with hepatotoxic drugs

(e) Previous hypersensitivity to chloroquine.

2.4.9 Adverse drug reactions (Therapeutic Drugs, 1999; Martindale, 1999; Physicians' Desk Reference, 2001) :

2.4.9.1 Ocular reactions :

Irreversible retinal damage in patients receiving long-term or high-dosage 4-aminoquinoline therapy; nyctalopia; scotomatous vision with field defects of paracentral, pericentral ring types and typically temperal scotomas e.g. difficulty in reading with words tending to disappear, seeing half an object, misty vision and fog before the eyes.

2.4.9.2 Neuromuscular reactions :

Convulsive seizures

2.4.9.3 Auditory reactions :

Nerve type of deafness, tinnitus, reduced hearing in patients with pre-existing auditory damage.

2.4.9.4 Gastrointestinal reactions :

Anorexia, nausea, vomiting, diarrhoea, abdominal cramps.

2.4.9.5 Dermatologic reactions :

Pleomorphic skin eruptions, skin and mucosal pigmentary changes: lichen planus-like eruptions, pruritis and hair loss.

2.4.9.6 CNS reactions :

Mild and transient headache, psychic stimulation.

2.4.9.7 Cardiovascular reactions :

Rarely, hypotension, electrocardiograph change.

2.4.10 Warnings & Precautions(Therapeutic Drugs, 1999; Martindale, 1999 ; Physicians' Desk Reference, 2001):

2.4.10.1 In recent years, it has been found that certain strains of *P.falciparum* have become resistant to 4-aminoquinoline compounds (including chloroquine and hydroxychloroquine) as shown by the fact that normally adequate doses have failed to prevent or cure clinical malaria or parasitemia. Treatment with quinine or other specific forms of therapy is therefore advised for patients infected with a resistant strain of parasites.

2.4.10.2 Irreversible retinal damage has been observed in some patients who had received long-term or high-dosage 4-aminoquinoline therapy. Retinopathy has been reported to be dose related.

2.4.10.3 When prolonged therapy with any antimalarial compound is contemplated, initial (base line) and periodic ophthalmologic examinations (including visual acuity, expert slit-lamp, funduscopic and visual field tests) should be performed. If there is any indication (past or present) of abnormality in the visual acuity, visual field or retinal macular areas (such as pigmentary changes, loss of foveal reflex) or any visual symptoms (such as light flashes and streaks) which are not fully explainable by difficulties of accommodation or corneal opacities, the drugs should be

discontinued immediately and the patient closely observed for possible progression. Retinal changes (and visual disturbances) may progress even after cessation of therapy.

2.4.10.4 All patients on long-term therapy with this preparation should be questioned and examined periodically, including testing knee and ankle reflexes, to detect any evidence of muscular weakness. If weakness occurs, the drug should be discontinued.

2.4.10.5 A number of fatalities have been reported following the accidental ingestion of chloroquine, sometimes in relatively small does (0.75g or 1g chloroquine phosphate in one 3 year-old child). Patients should be strongly warned to keep this drug out of the reach of children because they are especially sensitive to the 4-aminoquinoline compounds.

2.4.10.6 Use of chloroquine phosphate tablets, in patients with psoriasis, may precipitate a severe attack of psoriasis. When used in patients with porphyria, the condition may be exacerbated. The drug should not be used in these conditions unless, in the judgment of the physician, the benefit to the patient outweighs the possible hazard.

2.4.10.7 If any severe blood disorder appears which is not attributable to the disease under treatment, discontinuance of the drug should be considered. Since this drug is known to concentrate in the liver, it should be used with caution in patients with hepatic disease or alcoholism or in conjunction with known hepatotoxic drugs.

2.4.10.8 The drug should be administered with caution to patients having G-6-PD (glucose-6-phosphate dehydrogenase) deficiency.

2.4.10.9 Complete blood cell count should be made periodically if patients are given prolonged therapy

2.4.10.10 Because of the potential for serious adverse reactions in nursing infants from chloroquine, a decision should be made whether to discontinue nursing or to discontinue the drug taking into account the importance of the drug to the mother

2.4.10.11 Chloroquine has not been found to have any harmful effects on the fetus when used in the recommended doses for malaria prophylaxis. However, the situation is very different when chloroquine is used in the dosages for treatment of rheumatic diseases. In this situation, the drug has been reported to cause fetal abnormalities like defects in hearing & vision. Given this, pregnancy is not generally a contraindication to malaria prophylaxis with chloroquine whereas for chloroquine regimen in rheumatic diseases, the physician has to pay due consideration to risk benefit ratio.

2.4.11 Drug Interaction (Therapeutic Drugs, 1999, Martindale, 1999, Physicians Desk Reference, 2001):

In-vitro and in-vivo data show that antacids reduce the systemic availability of oral chloroquine. It has been suggested that the administration of chloroquine & antacid preparations should be separated by about 4 hours. Cimetidine inhibits the metabolism of chloroquine resulting in increased plasma concentration.

2.4.12 Qualitative analytical profile of chloroquine phosphate :

Chloroquine phosphate was the result of concerted efforts to develop an antimalarial drug necessitated by World War II (Hong, 1976). Since its synthesis, a great deal of work has been done as regards its analytical profile. A comprehensive review, therefore is not possible and this review, instead,

focuses on the main analytical developments in the past 15 years. Excellent reviews of earlier works are available (Hong, 1976; Tariq and Al. Badr, 1984).

2.4.12.1 High performance liquid chromatography (HPCL):

Enantiomers of chloroquine have been separated by high performance liquid chromatography (Stalcup et al., 1996). HPCL has also been used for photo degradation studies of chloroquine phosphate (Karim et al., 1994).

2.4.12.2 Thin layer chromatography (TLC):

TLC was used to detect chloroquine in human saliva (Ogubona et al., 1986). A low-cost portable TLC method was developed for determining chloroquine quantitatively and semi-quantitatively (Flinn et al., 1992). On-site determination of chloroquine using TLC has been described (Betschart et. al., 1991).

2.4.12.3 Capillary zone electrophoresis (CZE) :

Enantiomeric separation of chloroquine has been achieved using heparin and dextran sulphate (Agyei et al., 1995). Cyclodextrins have also been utilized for the same purpose (Jin and Li, 1998; Dong et al., 1998).

2.4.12.4 Miscellaneous methods

Fluorimetry in conjunction with filter papers has been used to detect chloroquine (Fidanza and Aaron, 1986). High performance thin layer chromatographic field-adapted methods have been developed for detection of chloroquine in urine (Mount et al., 1987) and in finger-stick blood (Mount et al., 1988). Gas chromatography-mass spectrometry has been suggested for quantifying chloroquine in biological fluids (Tapanes et al., 1990).

2.4.13 Quantitative analytical profile of chloroquine phosphate

2.4.13.1 High performance liquid chromatography:

A summary of some of the HPLC methods developed in the past 15 year is presented in Table 2.5

2.4.13.2 Table 2.6 contains the summary of the spectrometric methods developed for analyzing chloroquine phosphate.

2.4.13.3 Miscellaneous methods:

High performance thin layer chromatography (HPTLC) methods have been used to determine chloroquine in biological fluids (Betschart and Steiger, 1986). Non aqueous titration using notromethane-acetic anhydride (Ajiboye and Bark, 1989) and acetals and cyclic ethers as end-point indicators (Greenhow and Kashnipour, 1985) have been used for estimating chloroquine Membrane electrodes have been used for chloroquine determination (Cosofret and Buck, 1985; Saad et. al., 1991; Hassan and Ahmed, 1991). Flourimetry has been investigated for analysis of chloroquine (Rombo et al., 1985; Fidanza and Aaron, 1987; Idowu et al., 1988; Ibrahim et al., 1989). Radio immunoassays for chloroquine have been demonstrated (Escande et al., 1990). Polarography has been used for estimating chloroquine phosphate (Zhan and Mao, 1992). As has gas chromatography (Keller et al., 1998). Capillary zone electrophoresis has been investigated for analysis of chloroquine (Taylor and Reid, 1995).

Table 2.1 SUMMARY OF HPLC METHODS FOR ANALYSIS OF CHLOROQUINE PHOSPHATE

Sr. No.	Column	Mobile phase (flow rate)	Detection	Sample from	Reference
i.	Spherisorb C1 (5μ)	Aqueous 70% methanol containing 7mM-triethylamine (1 ml/min)	Flourescence 380 nm (excitation at 250 nm)	Human liver microsome	Ducharme and Farinotti, 1997
ii.	Bondapak (10μ)	0.2M NaH_2PO_4 methanol/acetonitrile (13:6:1) of pH 3 containing 1ml of perchloric acid/100 ml (1 ml/min)	254nm	Plasma	Walker and Ademowo, 1996
iii.	Nova Pak C18 (4μ)	58mM NaH_2PO_4 buffer containing 6mM sodium heptane sulphonate adjusted to pH 3.1 with H_3PO_4 and methanol; acetonitrile (3:17) gradient elution (1 ml/min)	Diode array 245 and 343 nm	Serum	Volin, 1995
iv.	Spherisorb S5SCX (5μ)	4.705g of NH_4ClO_4/500 ml of 98.5% aqueous methanol (1.5 ml/min)	Flourimetric detection (excitation at 215nm, no emission)	Serum whole blood, filter paper absorbed dry blood	Croes et al., 1994
v.	Spherisorb S5 ODS 1 (5μ)	0.1M phosphate buffer of pH3/acetonitrile (3:2) containing 0.1M $KClO_4$ (ion pairing reagent) 1ml/min)	254nm	Ampoules, tablets	Abdel-rehman et al., 1994
vi.	C8 Lichrospher 60 RP Select B (5 μm)	Water/acetonitrile/methanol (18:7:1) containing 0.5M ammonium formate and 75 mM-perchloric acid and adjusted to pH 4 using 1.1% H_3PO_4 (0.5ml/min)	254 nm	Blood erthro-cytes	Chaulet et al., 1994
vii.	Ultrasphere IP C18 (5μ)	0.02M-Na heptane-1-sulphonate (pH 3-4) containing diethylamine (700μl/litre) acetonitrile (18:4) (1.5ml/min)	343nm	Plasma, whole blood, urine	Houze et al., 1992

Sr. No.	Column	Mobile phase (flow rate)	Detection	Sample from	Reference
viii.	Inertsil (5μ)	Acetonitrile / methanol / aqueous ammonia (57:40:3) (0.9 ml/min)	Flourimetry at 380nm (excitation at 325nm)	Plasma haemoly-sed erythrocy-tes, urine	Chaulet et. al., 1991
ix.	Enantiopak containing α1-acid glycoprotein immobilized on diethylamin o ethylsilica gel (10μ)	Phosphate buffer solution-propan-2-ol (26:1) (0.3 ml.min)	254nm	-	Ibrahim and Fell, 1990
x.	Novapak C18 column	0.06M KH_2PO_4 buffer / methanol / acetonitrile / 60% $HClO_4$ (60:22:13:0:6) (1.2ml/min)	Fluorescence at 380nm (excitation at 325nm)	Plasma, blood and blood spots	Augustiji ns and Verbeke, 1990
xi.	ODS (5μ)	Methanol / water / anhydrous acetic acid (800:200:5) containing 0.5mM sodium dodecyl sulphate (1ml/min)	340nm	Pharma-ceuticals	Sanghi et. al., 1990
xii.	Bondapak C18	Acetonitrile and aqueous 70% heptanesulphonic acid (1.5ml/min)	232nm	-	Williams et. al., 1990
xiii.	Bondapak C18 (10μ)	12.5mM KH_2PO_4 (containing 0.1% of 1N H_3PO_4) : acetonitrile (13:7) (1ml/min)	343nm	Blood	Pos et. al., 1988
xiv.	Bondapak C18 (10μ)	Acetonitrile : 12.5mM KH_2PO_4 containing 0.1% of 1N H_3PO_4 (7:13) (1ml/min)	343nm	Blood	Estadieu et. al., 1989
xv.	Bondapak Phenyl	Methanol - 0.02M KH_2PO_4 (13:37) adjusted to pH 4.3 with anhydrous acetic acid (2.0 ml/min)	257nm	Tablets	Dasgupta, 1986
xvi.	Micropak MCH-10	Methanol-0.2M NaH_2PO_4 (1:1) containing 75mM $HClO_4$ at pH 3.8 (1ml/min)	254nm	Plasma, saliva, urine	Ogubona et al., 1986a

Sr. No.	Column	Mobile phase (flow rate)	Detection	Sample from	Reference
xvii.	Bondapak NH2	Methanol - water - acetonitrile-acetic acid (700:250:49:1) (1ml/min)	254nm	Tablets	Rao et al., 1986
xviii.	Lichrosorb Si 60 (5μ)	Acetonitrile - methanol - diethylamine (160:39:1)	Fluorescence detection at 380nm (excitation at 335nm)	Blood on filter paper	Lindstrom et al., 1985
xix.	Novapak C18 (5μ)	45mM KH_2PO_4 (pH 3) containing 12% of acetonitrile as mobile phase (0.6ml/min)	340nm	Erythrocytes, whole blood, urine	Pussard et al., 1986
xx.	Inertsil (5μm)	(a) Acetonitrile (b) Methanol /aqueous 25% ammonia (0.85 – 0.95ml/min)	Fluorimetric detection at 375nm (excitation at 325nm)	Plasma, haemolysed erthyrocytes, urine	Chaulet et al., 1993

Table 2.2 Summary of spectrophotometric methods for analysis of chloroquine phosphate.

Sr. No.	Method	Reference
i.	Ion pair complex with ammonium reineckate	Ahmed and Elbeshlawy, 1995
ii.	Ion pair extraction with Rose Bengal	Abdel Gawad, 1994
iii.	Using charge transfer complexes	El-Brashy, 1993
iv.	0.1M hydrochloric acid	Raghuveer et al., 1991
v.	Bromothymol Blue, Haskins and Saker-Solomons	Bergqvist et al., 1990
vi.	Treatment with tannic acid	Sastry et al., 1989
vii.	Conventional and derivative spectroscopy using different reagents	Eboka and Adesanya, 1989
viii.	Mixing with tetracyanoethylene in acetonitrile	Ibrahim et al., 1989a
ix.	Complex with iodine	Abdel Salem et al., 1986
x.	Using quinines	Sastry et al., 1986a
xi.	Using Fast Green FCF and Orange II	Sastry et al., 1986b
xii.	Complex with ammonium molybdate	Sastry et al., 1986
xiii.	Treatment with chloranil	Sulaiman and Amin, 1985
xiv.	Derivative spectroscopy in 0.05M H_2SO_4	Singh et al., 1990

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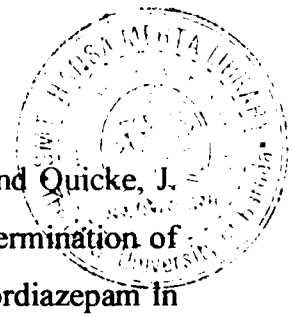

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2.5 Profile of mefloquine hydrochloride

2.5.1 Introduction (Current Index of Medical Specialities)

Mefloquine, a relatively new blood schizonticide, is a 4-quinolinemethanol derivative related to quinine. The antimalarial is effective against all forms of malaria including chloroquine or multidrug resistant strains of *P. falciparum*.

2.5.2 Description (Lim, 1985)

2.5.2.1 Systematic Name

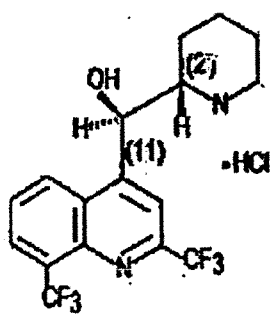
(±Erythro-∝-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline methanol hydrochloride.

2.5.2.2 Formula

(A) Empirical $C_{17}H_{16}F_6N_2O.HCl$

(B) Structural formula:

:



(C) Absolute stereochemistry : Erythro racemate (-) 11S,2'R and (+) 11R, 2'S

(D) Molecular weight : 414.8

2.5.2.3 Appearance, color & odor :

Mefloquine is an odorless, white powder

2.5.3 Physicochemical properties

Lim (Lim, 1985) has presented a detailed account of the physicochemical properties of mefloquine hydrochloride, the salient portions of which are reproduced below :

2.5.3.1 Solubility :

Based on the USP definitions of solubility, mefloquine has been reported to be slightly soluble in water and 0.1M hydrochloric acid, sparingly soluble in methylene chloride and soluble in ethyl acetate.

2.5.3.2 Partition Coefficient (Kp)

The values of Kp for some organic phases is given below. Kp is expressed as the ratio of concentration in the upper phase to that in the lower phase

Organic Phase	Kp
n-butanol	1300
Chloroform	0.1
Ethyl acetate	48
Hexane	3

2.5.3.3 Ionization Constant (pKa)

As mefloquine is only slightly soluble in aqueous media, pKa determinations are carried out in aqueous ethanol. The experimentally found pKa values were 8.54, 8.50 and 8.42 in 30, 50 & 70% aqueous ethanol, the extrapolated value for water was 8.6.

2.5.3.4 Melting Characteristics

Mefloquine melts with decomposition between 253°C and 255°C.

2.5.4 Clinical Pharmacology (Palmer et. al., 1993; Tracy and Webster, Jr. 1996; Martindale, 1999; Physicians Desk Reference, 2001)

Mefloquine acts as a blood schizonticide. It has high affinity for erythrocyte membranes where it preferentially binds with phospholipids. It has been proposed that uptake of mefloquine by infected erythrocytes is electrogenic, with both the proton gradient and the electric potential contributing to the driving force. The concentration attained in infected erythrocytes is usually higher than that attained in non-infected erythrocytes.

This could be due, in part, to the presence of phospholipids in the parasites; also, due to the drug binding with ferriprotoporphyrin IX (heme) which is formed in the course of hemoglobin degradation by plasmodia. It appears that the formation of a toxic mefloquine-ferriprotoporphyrin IX complex is crucial for the antimalarial activity. This toxic complex is believed to interfere with protease and peptidase activity in the acidic parasite food vacuoles.

Mefloquine is a non-competitive inhibitor of cholinesterases, this may be responsible for unwanted efforts like nausea and vomiting and CNS effects like disorientation and hallucinations (observed at high doses).

Emergence of resistance to mefloquine has been reported in areas where mefloquine is routinely used for treating majority of falciparum infections (e.g. Thailand); nevertheless, mefloquine is still a viable antimalarial in most countries where controlled usage is systematically advocated.

2.5.5 Pharmacokinetics (Palmer et. al., 1993; Tracy and Webster Jr., 1996; Martindale, 1999; Physician's Desk Reference, 2001)

After oral dosing of mefloquine, about 75-80% is absorbed from the gastrointestinal tract; time to reach maximal plasma concentration ranges between 2 and 12 hours. Absorption half life has been reported to be 0.36 to 2 hours. The plasma half life varies between 15 and 33 days, with a mean value of about 21 days. The drug is widely and rapidly distributed because of its high lipid solubility. The apparent volume of distribution, about 20 L/Kg, indicates extensive distribution. Mefloquine is highly plasma protein bound (98%), it gets concentrated in erythrocytes, the target cells in malaria, at a relatively constant erythrocyte-to-plasma concentration ratio of about 2. A substantial proportion of mefloquine is metabolized in the liver. Excretion is

chiefly via the bile into the feces, but around 9% of a dose is excreted in breast milk in small amounts.

The pharmacokinetics of mefloquine is reported to be altered by malaria infection, the main effects being reductions in both its volume of distribution and its overall clearance.

2.5.6 Indications (Palmer et. al., 1993; Tracy and Webster Jr., 1996; Martindale, 1999; Physician's Desk Reference, 2001)

Mefloquine is indicated for :

2.5.6.1 Treatment of acute falciparum malaria : Mefloquine is not a first line drug. Presently, mefloquine is recommended for the treatment of acute attacks of falciparum malaria and mixed infections of *P. falciparum*. Infections with *P. Vivax*, *P. Ovale* and *P. malaria* do not require treatment with mefloquine since they respond well to chloroquine.

2.5.6.2 Chemoprophylaxis against chloroquine-resistant falciparum malaria :

Prophylactic use is restricted to visitors to high risk areas of chloroquine resistant plasmodia while prophylactic use by residents of endemic areas is generally discouraged. At present, this is not an approved indication in India. Mefloquine is available in India for hospital use only, this is necessarily required to promote controlled usage and delay the emergence of resistance to mefloquine.

2.5.7 Dosage (Current Index of Medical Specialities)

2.5.7.1 Treatment of acute falciparum malaria :

	Upto 60 Kg	Over 60 Kg
Semi-immune		
Loading dose	750 mg	750 mg
After 6-8 hours		250 mg
Non-immune		
Loading dose	750 mg	750 mg
After 6-8 hours	500 mg	500 mg
After further 6-8 hours		250 mg

The drug should not be taken on empty stomach and should be administered with atleast 8 oz (240 ml) of water. Children under 45 kg irrespective of immunity status, should receive a does of 25 mg/kg body weight.

2.5.7.2 Chemoprophylaxis against falciparum malaria :

Prophylaxis should start 1 week prior to exposure to endemic area and continue weekly until 2 weeks after return from the endemic area. If the stay is the malarious area exceeds 3 weeks, the weekly dose should be halved from the fourth week or the dose interval extended to two weeks maintaining the original dose level.

Adults	> 45 kg	250 mg weekly
Children	15 – 19 kg	62.5 mg weekly
	20 – 30 kg	125 mg weekly
	30 – 40 kg	187.5 mg weekly

2.5.8 Contraindications (Palmer et. al., 1993; Tracy and Webster Jr., 1996; Martindale, 1999; Physician's Desk Reference, 2001)

Mefloquine is contraindicated in

1) Patients hypersensitive to quinine and related compounds.
2) Children under 15 kg body weight or less than 2 years old.
3) Prophylaxis in patients with a history of psychiatric disorders, seizure, severe renal insufficiency, impaired liver function or cardiac conduction disturbances.
4) Epileptic patients.
5) Patients who have been treated with quinine during the preceding 12 hours.

2.5.9 Adverse reactions (Palmer et. al., 1993; Tracy and Webster Jr., 1996; Martindale, 1999; Physician's Desk Reference, 2001)

Side effects such as nausea, vomitting, abdominal pain, diarrhoea, dysphoria and dizziness are encountered frequently; these, however, tend to be dose related and self limiting. Bradycardia, headache, ataxia, alterations

in motor function or the level of consciousness and visual or auditory disturbances are usually mild and self limiting. Severe neuropsychiatric reactions like disorientation, seizures, encephalopathy and a range of neurotic and psychotic manifestations can occur rarely which may require intervention and symptomatic treatment. Other effects less frequently seen include pruritis, urticaria, anorexia, asthenia, insomnia, constipation, pulse irregularities, arthralgia, auditory disturbances and sialorrhea.

2.5.10 Warnings and preacutions (Palmer et. al., 1993; Tracy and Webster Jr., 1996; Martindale, 1999; Physician's Desk Reference, 2001)

Concomitant administration of mefloquine and quinine, quinidine or beta-antagonists may produce ECG changes or cardiac arrest. Further, administration of mefloquine and quinine or chloroquine together may increase the risk of convulsions. Mefloquine administration should thus be delayed atleast 12 hours after the last dose of quinine / quinine related drugs; additionally, monitoring of cardiac and neurological functions is warranted. Since dizziness, associated with mefloquine, may interfere with spatial perception and fine coordination, patients should be warned against driving, piloting airplanes and operating machines during and upto 3 weeks after the use of mefloquine.

Though mefloquine prophylaxis is not an approved indication in India currently, it is advised that periodic evaluation of hepatic function be performed during prolonged prophylaxis.

Mefloquine prophylaxis is contradicted during the first trimester of pregnancy and should be avoided during the second and third trimester, while therapeutic use in pregnancy (particularly the first trimester) should be considered only if expected benefits outweigh potential risk to the fetus.

Nursing mothers should not feed their babies during treatment with mefloquine.

2.5.11 Drug interaction (Palmer et. al., 1993; Tracy and Webster Jr., 1996; Martindale, 1999; Physician's Desk Reference, 2001)

Quinine, quinidine and mefloquine are similar in both chemical structure and their cardiovascular system and central nervous system toxicity. These should not be used concurrently. If necessary, mefloquine administration should be delayed atleast 12 hours after the last dose of quinine or quinidine.

The cardiovascular adverse effects of mefloquine suggest the likelihood of interactions with cardiac drugs like beta blockers. In such a circumstance, as also in cardiac patients, mefloquine should be used with great caution.

Mefloquine is reported to increase the risk of seizures in epileptics controlled by valproate as it lowers blood valproate levels.

It may compromise adequate immunization by live typhoid vaccine.

2.5.12 Qualitative analytical profile of mefloquine hydrochloride (Lim, 1985)

2.5.12.1 Spectral

Mefloquine is most readily identified by its spectral characteristics. Its infrared spectrum shows an abundance of fine structures and offers an excellent means of overall identity.

The proton spectrum of mefloquine easily distinguished the erythro racemate from the threo and delineates the substitution pattern on the quinoline system. Its Electron Impact mass spectrum establishes a molecular weight and a characteristic piperidyl ion. Additional evidence of identity can be found in its ^{13}C- and ^{19}F- NMR spectra. Its UV spectral data, when

recorded as solutions in N/10 HCl, show λmax near 317, 303, 283 and 222 nm. The calculated absorptivities are listed below :

Wavelength	Absorptivity
317 nm	3400
303 nm	4430
283 nm	5740
222 nm	41800

Although less definitive than the aforementioned spectral characteristics, the UV data is nevertheless supportive of the quinoline system.

2.5.12.2 Chromatography

(A) Thin layer chromatography

Different mobile phases and methods of detection have been described and the Rf values of mefloquine and threo racemate have been reported (Lim, 1985).

(B) Gas Chromatography

Being a conjugate acid of a dibasic amine, mefloquine has limited vapor pressure. To enhance its volatility, mefloquine is usually trimethylsilylated prior to being gas chromatographed. However, a satisfactory gas chromatographic profile of mefloquine has not been obtained because of multiple species (Lim, 1985). The O-TMS derivative has been employed in a gas chromatographic procedure to measure mefloquine in whole blood but the formation of other mefloquine-TMS derivatives has not been reported (Nakagawa et. al., 1979).

(C) High performance liquid chromatography

A silica column and methanol : hexane : concentrated ammonium hydroxide (80:19:1) has been found to be a useful qualitative system. A Hamilton PRP-1 column with tetrahydrofuran: 0.8% aqueous concentrated ammonium hydroxide (48:52) and a μBondapak CN column with 0.05 M

potassium dihydrogen phosphate containing 0.1% acetic acid: acetonitrile : tetrahydrofuran (67:24:9) are the other systems which have also been employed (Lim, 1985).

2.5.13 Quantitative analytical profile of mefloquine hydrochloride (Lim, 1985)

2.5.13.1 High pressure liquid chromatography

The most widely used method for analysis of mefloquine is high pressure liquid chromatography. A summary of the various methods is given in Table 2.3.

2.5.13.2 Gas chromatography :

A summary of the methods used for analyzing mefloquine by gas chromatography is given in Table 2.4.

2.5.13.3 Thin layer chromatography :

A portable apparatus has been described for on-line determination of antimalarial drugs like mefloquine and chloroquine in urine. Semi quantitative evaluation is enabled by comparison with standards. (Betschart et. al., 1991).

2.5.13.4 Miscellaneous methods :

Mendenhall and coworkers (Mendenhall et. al., 1979) have reported the use of a plastic ion sensitive electrode for determination of mefloquine from blood. Vanadate oxidation of mefloquine has also been used for determining mefloquine (Assamoi et. al., 1987). Flourimetry with excitation at 340 nm and emission at 380 nm has been investigated for determining mefloquine from tablets (Fidanza and Aaron, 1989). Supercritical fluid chromatography of mefloquine from blood, on a Zorbax-B.P. (7 μm) column at 210°C with pentane containing 0.15% of butylamine as mobile phase (2100 kPa) and electron capture detection, has been attempted (Mount et. al., 1990). Capillary zone electrophoresis has been used to determine the enantiomers of

erythro and threo-mefloquine (Fanali and Camera, 1996). Ultraviolet spectroscopy has been used (Lim, 1985). However, in the last case, no specific conditions of analysis have been reported.

2.5.13.5 Pharmacopoeial methods :

The European Pharmacopoeia gives nonaqueous titrimetry as the official method for determination of mefloquine.

Table 2.3 Summary of high pressure liquid chromatographic methods for analysis of mefloquine hydrochloride

Sr. No.	Column	Mobile phase (flow rate)	Detection	Sample	Reference
i.	μBondapakCN	0.5% acetic acid solution in isopropylether-dioxin (3:2) (2ml/min)	280 nm	Whole blood, plasma	Grindel et. al., 1977
ii.	μBondapakC_{18}	Methanol:0.1M Na_2HPO_4 (3:2) (2ml/min)	280 nm	Urine	Grindel et. al., 1977
iii.	μBondapakC_{18}	Aqueous 70% Methanol that is 5mM in low UV PIC B8 (Waters Association) (1ml/min)	222 nm	Plasma, blood	Kapetano-vic et. al., 1983
iv.	Nucleosil C_{18} (3 μm)	Aqueous 50 mM Na_2SO_2 (adjusted to pH 2.84 with H3PO4) containing 33% acetonitrile (0.5 ml/min)	220 nm	Plasma	Arnold and Stetten, 1986
v.	Spherisorb ODS-1 (5 μm)	Acetonitrile -0.1M phosphate buffer of pH 2.5(21:29) containing 40 mM chlorate and 0.1% of N, N-dimethyl octylamine of pH 2.3 to 2.7 (1 to 1.5 ml)	222 nm	Plasma, whole blood	Bergqvist et. al., 1988
vi.	μBondapak C_{18} (10 μm)	Acetonitrile-0.1M phosphate buffer, pH 3.0 (7:13) (1.5 ml/min)	222 nm	Plasma	Guenzi et. al., (1989)
vii.	(a) Adsorbo-sphere cycano (10 μm)	Hexane-propan-2-ol- methanol (41:2:7) containing 0.005% of trimethylamine (2 ml/min)	285 nm	Plasma whole blood	Gimenez et. al., (1990)

Sr. No.	Column	Mobile phase (flow rate)	Detection	Sample	Reference
	(b) Supelco LC(S)-naphthylurea (5 µm)	Same as (a)	Same as (a)	Same as (a)	
viii.	Spherisorb S3-ODS-1 (3 µm)	0.1M phosphate buffer (pH 3.5) acetonitrile (13:12) (0.5 ml/min)	229 nm	Plasma	Bergqvist et. al., 1991
ix.	C_{18} (5 µm) at 50°C	Aqueous 72% acetonitrile (0.8 ml/min)	Flourescen 475 nm (Excitation at 260 nm)	Whole blood urine	Mount et. al., 1991
x.	Partisil 10 ODS-3 (10 µm)	Aqueous 65% methanol containing 5.25 mM sodium octane-sulphonate (pH 3.4) (2 ml/min)	222 nm	Plasma (for mefloquine)	Edstein et. al., 1991
xi.	(a) Nucleosil cyano-propyl (5 µm)	Hexane-methanol-propan-2-ol (41:7:2) containing 125 µl of triethylamine diluted 1:40 in methanol (2 ml/min)	285 nm	Plasma	Gimenez at. al., 1993
	(b) (s) naphthyl-urea (5 µm)	Hexane-methanol-propan-2-ol (10:9:1) modified as above with triethylamine (1.5 ml/min)	285 nm		
xii.	Ultrasphere IP C_{18} (5 µm)	0.1M phosphate buffer/acetonitrile / 1M sodium perchlorate (173:137:15) at pH 4 (1.4 ml/min)	227 nm	Blood	Bergqvist et. al., 1993

Sr. No.	Column	Mobile phase (flow rate)	Detection	Sample	Reference
xiii.	Porous carbon Hypercarb-S (7 µm)	Acetonitrile methanol : 60 mM acetate buffer of pH 4.6 (12:5:8) and 5mM N-benzyloxycarbonyl glycyl-L-proline (0.8 ml/min)	278 nm	Plasma	Bergqvist et. al., 1993a
xiv.	ODS (5 µm)	Acetonitrile-water-acetic acid (8200:1800:7) (1 ml/min)	263 nm(UV) 475 nm (fluorimetric , excitation at 263 nm)	Plasma Blood	Bergqvist et. al., 1994
xv.	Chiral AGP	Propan-1-ol/0.05M Sodium phosphate buffer of pH 4.85 (1:10) (0.9 ml/min)	222 nm	Plasma, Urine	Wallen et. al., 1994
xvi.	Chirapak A.D. (10 µm)	Hexane : ethanol : diethylamine (960:40:1) (1 ml/min)	285 nm	Tablets	Qiu et. al., 1992

Table 2.4 Summary of gas chromatographic methods for analysis of mefloquine hydrochloride

Sr. No.	Column	Carrier gas (flow rate)	Tempera-ture	Detection	Sample from	Reference
i.	3% of OV-17 on Chromosorb W	-	160°C to 250°C at 1°C/min	Electron capture detection	Whole blood	Nakagawa, et. al., 1979
ii.	Glass column with 3% of SE-30 on Gas-chrom Q (80-100 mesh)	He (30 ml/min)	270°C	Mass spectrome try	Whole blood, plasma, urine	Schwartz and Ranalder, 1981
iii.	3% of SP-2250 on Supelcoport (80 to 100 mesh)	Argon-methane (50 ml/min)	205°C	Electron capture	Plasma	Heizmann and Geschke, 1984
iv.	Glass column of 3% of SE-30 on Gas-Chrom Q (100 to 200 mesh)	Nitrogen (40 ml/min)	180°C for 9 min 280°C for 7 min	Electron capture	Plasma	Dadgar et. al., 1985
v.	Fused silica column coated with CP-Sil8	Nitrogen (60 cm/s)	100°C for 1 min to 280°C at 20°C per min	Flame ionization & mass spectro-metry	-	Gyllenhaal and Vessman, 1987
vi.	Fused silica column coated with DB-5 (0.25 μm)	Helium (3 ml/min)	140°C (held for 1 min) to 290°C at 30°C/min	CH_4 negative ion CIMS detection	Plasma	Neal et. al., 1994

2.5.14 References

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2.6 Profile of Ethyl Cellulose

2.6.1 Chemistry of Ethyl Cellulose

Non-proprietary names:

BP: ethyl cellulose

Ph. Eur: ethyl cellulose

USP: Ethyl cellulose

Synonyms:

Aquacoat, E462, Ethocel, Surelease.

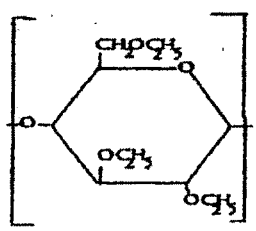
Chemical name and CAS Registry number:

Cellulose ethyl ether [9004-57-3]

Empirical formula:

Ethyl cellulose with complete ethoxyl substitution (DS = 3) is $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$, where n may vary to provide a variety of molecular weights. The cellulose molecule is a chain of β-anhydro-glucose units joined together by acetal linkages.

Structural formula:



2.6.2 Description

Ethylcellulose is a tasteless, free- flowing white to light tan colored powder.

2.6.3 Properties

Density (bulk): $0.4gm/cm^3$

Glass transition temperature: 129-133°C

Hygroscopicity: Ethylcellulose absorbs very little water from humid air or during immersion that small amount evaporates readily at 80% RH-2%

Solubility: Ethylcellulose is practically insoluble in glycerin (propylene glycol), and water. Ethylcellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, tetrahydrofuran & in mixtures of aromatic hydrocarbons with ethanol (95%). Ethylcellulose that contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol and toluene. The addition of 10-20% of a lower aliphatic alcohol to solvents such as ketone, ester & hydrocarbons can improve the solubility.

Specific gravity: 1.12 – 1.15 gm/cm^2

Viscosity: The viscosity of ethyl cellulose is measured typically at 250c using 5% ethyl cellulose dissolved in a solvent blend 80% toluene /20% ethanol (W/W), various viscosity grades of ethyl cellulose are commonly available. Each type based on ethoxyl content there exist low to high viscosity types based on degree of polymerization.

2.6.4 Uses

Coating agent, flavcring fixative, tablet binder, tablet filler, viscosity increasing agent.

Application in pharmaceutical formulation or technology :

Sr. No.	Use	Concentration
1.	Microencapsulation	10.0 – 20.0
2.	Sustained release tablet coating	3.0 – 20.0
3.	Tablet coating	1.0 – 3.0
4.	Tablet granulation	1.0 – 3.0

2.6.5 Stability

It is a stable slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated & to salt solutions. It is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperature. This may be prevented by the use of an antioxidant and chemical additives which absorb light in the 230-240 nm range.

2.6.6 Storage:

It should be stored at a temperature not exceeding 90°f (32°C) in a dry area away from all source of heat. Do not store near to peroxide or other oxidizing agents

2.6.7 Incompatibilities

Incompatible with paraffin wax & microcrystalline wax.

2.6.8 Precautions

Ethylcellulose is not metabolized and therefore is not recommended for parenteral products. It is a nontoxic, non allergenic and nonirritating material. Ethylcellulose powder may be an irritant to the eyes and therefore eye protection should be worn.

2.6.9 Review of work done

Phillip et al (1979) – Reported micro encapsulation of bilateral for controlled release & its effect on bronchodilator and heart rate activities in dog. Spheronized core produced by extrusion to produce beads exhibiting controlled release characteristics. In-vitro dissolution studied indicated that drug released was proportional to the amount of film on the bead. The total increase in heart rate over a 6 hrs period was less than that associated with plain drug powder.

Benita et al (1982) – Studied the effect of polyisobutylene on EC walled microcapsules wall structure and thickness of salicylamide and theophylline microcapsules. Microcapsules were prepared by the EC coacervation process which is based on the differential thermal solubility in cyclohexane. In the presence of a protective colloid concentration on the apparent loss of wall polymer as empty droplets closely parallel its effect on the size of stabilized coacervate droplets when core material was absent. It is proposed that stabilized droplet formation is a side reaction. When core material is present, causing changes in wall thickness.

Fumio et al (1983) – Reported effect of excipients on the tablet properties and dissolution behavior of theophylline tableted microcapsules under different compression forces. Tablet thickness decreased with an increase in particle size. The dissolution of the theophylline from tableted microcapsules without excipient was almost independent of the applied compression force but showed sustained release behavior.

Chowdhary et al (1985) – Studied on a new technique of microencapsulating by EC. A method based on emulsification of the EC solution containing the drug in an immiscible liquid media followed by coacervation by the addition of non-solvent to produce spherical microcapsules. They were reported sulphamethoxazole release from those

microcapsules which was spread over a period of 5 to 6 hrs. linear relationship was observed between percent coat material (EC) & t_{50} values.

Chowdhary et al (1985) – Were reported controlled release through microencapsulation. Nitrofurantion was microencapsulated with EC and gelatin and controlled release was observed with former and found that the release rate of gelatin microcapsules was very fast.

Chowdhary et al (1988) – Were reported comparative evaluation of EC, Gelatin and Ca-alginate microcapsules prepared by complex emulsion method. Diazepam was microencapsulated with EC, Gelatin and ca-alginate by complex emulsion method and the microcapsules was studied controlled drug release with depended % coat material and t_{50} values was observed with EC and Gelatin and also found no such controlled release was observed with Ca-alginate.

Pandey et al (1988) – Were studied buccal mucoadhesive films and mucoadhesive gels of theophylline was prepared using HPMC, EC and carbopol and the drug release pattern and stability of these formulations. The in-vitro drug release was higher with formulations containing carbopol.

Gay et al (1990) – Were reported formulation parameters affecting the preparation of microencapsulated ion-exchange resins containing theophylline. They were found that a method of microencapsulating theophylline ion exchange resins with EC was developed to produces smooth and uniform coats. It was also found that the release rate from coated resins with low cross linking followed a logarithmic plot indicating membrane controlled release. Where as coated resins high cross linking fitted $t_{1/2}$ plot, suggesting particle division control.

Bhalla et al (1991) – Were studied that controlled release matrices for ketoprofen. They reported experimentations with various release retardants to design suitable matrices for controlled release tablet of the drug. Ethyl

cellulose, Guar gum & methocel combination could provide tablets with suitable characteristics & appropriate drug release profile.

Jani et al (1992) – Micro encapsulation of indomethecin by complex emulsification. Micro spheres are prepared by emulsion-solvent evaporation technique. Indomethecin was released at relatively faster rate from small sized micro sphere than the larger micro sphere with core:coat ratio of 1:1, 2:1, 3:1, 4:1 & 9:1.

Alexander et al (1993) – were reported preparation of bio-availability studies of aspirin EC microcapsules. EC walled aspirin microcapsules were compressed into tablet. Showed that the constant plasma concentration of the drug was sustained for about 8-10 hrs & pharmacokinetic of microencapsulated aspirin can be described by one compartment open model comparison of in-vitro drug availability showed that there is a strong correlation between dissolution & absorption for the 1st 6 hours.

Jaiswal et al (1995) – Were reported preparation and evaluation of captopril microsphere by spherical crystallization technique using acrylic polymer and EC as the matrix. The micro spheres were spherical with diameter 150-230 micrometer and incorporation efficiency is 40-65% were obtained and also found increase in the concentration of the polymer decrease the drug release rate.

Shreenivasan et al (1996) – Were reported preparation and evaluation of florbiprofen microcapsules by emulsification solvent evaporation technique. This technique was used for preparation for florbiprofen microcapsules for controlled release mechanism of florbiprofen in pH 7.2 phosphate buffer solution and compared with commercial product.

Biswanath et al (1996) – Were reported release kinetics of drug from EC microcapsules release of sodium benzoate, salbutamol sulphate and caffeine. Microcapsules were prepared by emulsification solvent evaporation

technique. The drug release rate dependence on the different parameters involved in higuchi equation was verified.

Weiss et al (1998) – Were reported micro encapsulation of the rennin inhibitor FK906 (tripeptide) by phase separation of EC in cyclohexane was performed to obtained sustained release of the drug for once a day application. By dissolution tests as a function of the better capacity and the osmolality of the dissolution medium. The buffer capacity was found to be the parameter with greater influence on the release rate.

Chan et al (1998) – were reported effect of polyvinyl pyrrolidone and EC on alginate microsphere prepared by emulsification. Micro sphere prepared with or without polyvinyl pyrrolidone exhibited a better flow property but the drug content was lower and the drug release rate higher. The addition of triethyl citrate which is water-soluble plasticizer was found to increase the rate of drug release while the use of higher viscosity grade of EC produced the opposite effect. EC improved the flow ability of micro sphere to a greater extent than poly vinyl pyrrolidone.

Lin et al (1999) – were reported modification of the initial release of a highly water-soluble drug from EC micro sphere. To develop the micro spherical dosage for a highly water-soluble drug, fenoterol HBR by using the water insoluble, non-biodegradable polymer EC. Three factors, initial amount of drug, the volume of non-solvent (petroleum benzene) and the stirring speed of homogenizer were varied during the preparation. A significant increase in the encapsulation efficiency of fenoterol was observed. When the drug polymer ratio was decreased from 15-5%, particle size range 10-25 micrometer. The initial drug loading and addition of non-solvent significantly effected the initial release of fenoterol from the EC microsphere.

Bhalla et al (1999) – Were studied development and evaluation of controlled release tablets of carbamazepine were prepared using HPMC & EC as release retardants several formulations were tried to achieve the required in-vitro release profile. An accelerated stability study indicated that both, the drug content and in-vitro release profile of the selected EC tablets were satisfactorily maintained.

Handa et al (2000) – Were studied development and evaluation of EC and coated controlled release pellets, pellets of isosorbide, sorbide mononitrate and carbamazepine were prepared by suspension of layering and powder layering techniques, respectively. The drug loaded pellets were coated using EC as release retardant. Different coats weights were applied & pellets were subjected to in-vitro release studies formulation showing similar in-vitro release profile to the innovators product under different condition of pH and agitation were selected for accelerated stability studies and found to be stable under different condition of storage for a period of 6 month.

Yang et al (2000) – were reported an enhanced process for encapsulating aspirin in EC microcapsules by solvent evaporation technique (o/w emulsion) dichloromethane used as the dispersed medium and water as the dispersing medium. The recovered weight particle size distribution aspirin loading efficiency, the aspirin release rate of microcapsules was analyzed. The addition of appropriate amount of non-solvent (n-hexane) prior to the emulsification increases the recovered weight, but decreases the size of the formed micro spheres. The addition of non-solvent also changes the microcapsules characteristics, resulting in a coarser surface & an increased release rate. An increasing the polymer (EC) concentration in the dispersed phase increase in the size of micro spheres, the recovered weight & loading efficiency, but decrease release rate.

Fernandez et al (2000) – Were reported controlled release formulations of alachlor (pesticide) in EC micro sphere were prepared using EC according to the solvent evaporation method.

Manavi et al (2001) – Were studied development and evaluation of ketorolac tromethamine occular films. Microsphere was prepared by solvent casting method using EC and cellulose acetate polymer in various proportions. The occular inserts were evaluated for their uniformly, in-vitro drug release and occular toxicity.

Shivanand et al (2001) – Were studied EC microcapsules of diclofenac potassium. Diclofenac potassium microsphere were prepared by coacervation phase separation induced by temperature change and non-solvent addition method, using EC as coating material in varying coat: core ratio of 1:1, 1:2, 1:3, 1:4 and 1:9. The microcapsules were evaluated for the drug content uniformity particle size distribution, in-vitro drug release characteristics, drug coating polymer interaction.

Anilkumar et al (2001) – Were investigated atomic force microscopy: a novel tool to categories bioadhesive polymer contact mode AFM was used & polymer films were prepared at 2% w/w concentration of polymer alone and in combination with mucin at 1:1 ratio by solvent casting technique. Five polymers HPMC, CMC, sodium alginate, polyvinyl alcohol and EC. Aqueous dispersion were studied using this method and surface roughness of the film was taken as parameter for polymer-mucin interaction. PVP and EC were categorized as non- bioadhesive.

Subrata et al (2001) – Were reported mechanism of in-vitro release kinetics of flurbiprofen to loaded EC micropellets. Micropellets prepared by quasi-emulsion solvent diffusion technique using EC is a matrix-forming polymer. Encapsulation parameters of micro pellets such as actual drug loading. Drug encapsulation efficiency and loss of coating polymer were determined.

Saravanan et al (2001)– were studied formulations and characterization of EC floating micro sphere loaded with ranitidine hydrochloride. The floating microsphere was prepared by solvent evaporation / polymer diffusion technique. The prepared microsphere were characterized by percentage of loading, SEM, particle size distribution and in-vitro release studies.

Smita et al (2001) – Were reported prolonged release pentoxifylline formulation. To prepare prolonged release pentoxifylline tablet using EC as a matrix former. The effect of various formulations and processing variable such as amount of polymer type and concentration of filter and compression force on pentoxifylline release rate was studied.

Dashevsky et al (2001) – Were studied the effect of EC molecular weight on the properties of theophylline micro sphere. Micro sphere of theophylline with both EC of high and low molecular weight and also their mixtures as a coating material were prepared using the solvent evaporation technique. In-vitro dissolution studies exhibited the square root of time (Higuchi model) release characteristics. The size distribution of microsphere was dependent on the ratio of EC mixtures with high and low molecular weight.

Liao et al (2001) – were reported release characteristics of micro sphere prepared by co-spray drying actinobacillus pleuropneoumoniae antigens and aqueous EC dispersion. Using formalin in activated actinobaccillus pleuropneoumoniae antigens and aqueous EC dispersion. Microsphere of oral vaccines were developed by a co-spray drying process. To determine whether the dosage formulation of microsphere could from enteric matrices.

Yang et al (2001) – Were studied encapsulating aspirin into a surfactant free EC microsphere using nontoxic solvents by emulsion solvent evaporation technique. w/o ethanol used as dispersed phase and soyabean oil as the continuous phase. The addition of small amount of non-solvent (water)

prior to the emulsification was found to have a significant impact on the microencapsulation process.

Talele et al (2001) - were reported formulation and evaluation of sustained released dosage form of flurbiprofen using a combined pectin and EC matrix. Combination of pectin and EC were investigated as a sustained release matrix. Flurbiprofen was used as a model drug for evaluating matrix system. Pectin and EC were used in different proportion i.e. 1:1, 2:1, & 3: 1 along with usual tablet additives, starch and talc. Two matrix component of 20% and 30% w/w of total tablet weight were prepared. The granules and tablets were evaluated for physical properties.

The in-vitro release data showed that 30% w/w of total tablet weight matrix component gave extended release of flurbiprofen for 10 hours selected formulations were found to be stable at 37°C and 45°C for a period of one month.

Tsai et al (2001) – were studied preparation of double encapsulated microcapsules for mitigating drug loss and extending release. The double encapsulated microcapsules were prepared by the non-solvent addition phase separation method to form core material and encapsulated with regulate drug release rate. The drug used was theophylline, which is water-soluble. DCM n-hexane were used as the solvent and non solvent respectively. This study investigated how various core material and microcapsules EC/TH ratios affect the drug loss of the double encapsulated microcapsules was 12.8% less then that of the microcapsules prepared by the o/w emulsion non-solvent addition method. The particle size of this double encapsulated micro capsules decreased as the concentration of EC polymers was increased in the second encapsulation process. The roughness of their surface was also in proportion to the concentration of polymers. The dissolution study showed that the t_{20} of the double encapsulated micro capsules range from 2 - 3.54

hours. While that of the o/w emulsion non solvent addition method micro capsules was from 2.7 - 7.7 hours. The greater the level of EC in the polymer solution the slower the release rate of the drug from the microcapsules.

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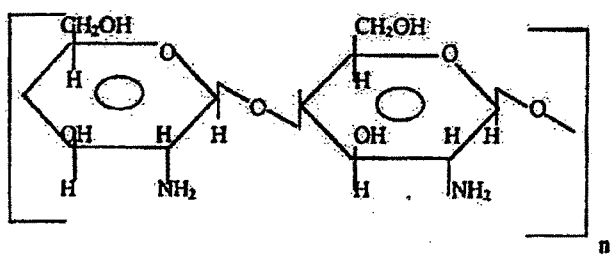
2.7 Profile of Chitosan

2.7.1 Chemistry of Chitosan

Chitosan [α (1→4) 2-amino 2-deoxy β-D glucan], the deacetylated form of chitin, is a muco-polysaccharide having structural characteristics similar to glycosamines with a chemical formula $(C_6H_{11}O_4N)_n$. Chitosan is a biodegradable and biocompatible homo-polysaccharides(i.e. contain single type of monomeric unit) comprising of **GLUCOSAMINE** and **N-ACETYLGLUCOSAMINE** and can be derived by partial deacetylation of chitin. It is marketed under a variety of forms with different molecular weights and degrees of deacetylation, or as chitosan base or salt.

Structural formula :



2.7.2 Description

Chitosan is a tasteless, free flowing, light brown coloured, low density powder.

2.7.3 Properties

Important characteristic of chitosan are its molecular weight, viscosity, degree of deacetylation, crystallinity index, number of monomeric units(n), water retention value, pKa and energy of hydration. Chitosan has a high

charge density, adheres to negatively charged sulfates and chelates metal ions.

Chitosan is insoluble at an alkaline and neutral pH, but forms salts with inorganic and organic acids such as glutamic acid, hydrochloric acid, lactic acid and acetic acid. Upon dissolution, the amine groups of the polymer become protonated with a resultant positively charged soluble polysaccharides (RNH_3^+). However, chitosan salts are soluble in water, the solubility being dependent on the degree of deacetylation. Chitosan with a low degree of deacetylation(40%) has been found to be soluble upto pH 9.0, where as chitosan with a degree of deacetylation of about 85% is soluble only up to pH 6.5. Solubility is also greatly influenced by the addition of salts to the solution. Due to high molecular weight and a linear unbranched structure, chitosan is an excellent viscosity-enhancing agent in an acidic environment.

2.7.4 Uses:

1 As a FLOCULANT in a purification of waste water.

2 As a CHELATING AGENT for harmful metals for detoxification of hazardous waste.

3 In addition, chitosan also has been exploited in the COSMETIC INDUSTRIES, THE DENTAL INDUSTRY, FOR HAIR CARE PRODUCTS and FOR OPTHALMIC APPLICATION, such as for contact lens coating or as the contact lens material itself.

4 In recent years, as a material in the NUTRITIONAL SUPPLEMENT market especially as a WEIGHT LOSS AID and CHOLESTROL LOWERING AGENT.

5 Co encapsulation of other bioactive agents for multiple plasmids.

6 Improvement of bioavailability of DNA due to the protection from serum nuclease degradation by the polymer matrix.

2.7.5 Storage

It is stored in form of dried chitin, so that it can be stored as a stable intermediate for deacetylation to chitosan at a later stage.

2.7.6 Incompatibilities

The pH of chitosan solution must be kept below 6.0 to prevent precipitation of gelatin. Chitosan may best be formulated to the pH 2-3 region. The acid solution of chitosan is compatible with nonionic polymers but is incompatible with sulfates and most anionic water-soluble polymers.

2.7.7 Review of work done

Fwu-Long et al (1997) reported that chitosan microspheres containing oxytetracycline (OTC), an antibiotic agent, were prepared by spray hardening and interfacial acylation methods. The object of this study was to prepare oxytetracycline containing microspheres for oral administration and injection using different molecular weight (Mw 70000 ~ 2000000) of chitosan. The result indicated that the releasing of oxytetracycline from various acylated chitosan microspheres was decreased with increasing the molecular weight of chitosan and would show well sustained-release property.

Wang et al (1996) studied an orthogonal experimental design to optimize the formulation of cisplatin (CDDP)-loaded chitosan microspheres (namely, CDDP-DAC-MS) which were produced by an emulsion-chemical cross-linking technique. Seven factors and three levels for each factor that might affect the formulation of microspheres were selected and arranged in an L_{27} (3^{13}) orthogonal experimental table. The in vitro release of cisplatin from chitosan microspheres in saline was retarded compared with that from saline solution; the release of CDDP from chitosan microspheres was suggested to be controlled by the dissolution and diffusion of the drug from the chitosan matrix.

Lim and Wan (1998) prepared chitosan microspheres using an emulsification-coacervation technique. The DSC analysis data suggested that the magnesium stearate was converted to stearic acid during the preparation process. The release of propranolol hydrochloride from the microspheres was fast, irrespective of the content and magnesium stearate. Drug encapsulation efficiency was enhanced when a greater amount of magnesium stearate was used.

He et al (1999) studied modified spray drying methods, especially a novel w/o/w emulsion-spray drying method, were developed to prepare chitosan microspheres with a sustained drug release pattern. Release of the model drugs cimetidine and famotidine, from the microspheres prepared by the emulsion-spray drying methods, was greatly retarded with release lasting for several hours, compared with drug loaded microspheres prepared by conventional-spray drying or emulsion methods where drug release was almost instant. The slow release of drug was partly due to the poor wetting ability of the microspheres which floated on the surface of the dissolution medium. The addition of a wetting agent increased the release rate significantly. The coating of the microspheres with gelatin decreased the rate of release of drug in the presence of wetting agents.

Kumbar et al (2002) investigated that microspheres of chitosan crosslinked with three different crosslinking agents viz. Glutaraldehyde, sulphuric acid and heat treatment have been prepared to encapsulate diclofenac sodium (DS). Chitosan microspheres are produced in a w/o emulsion followed by crosslinking in the water phase by one of the crosslinking methods. The crosslinking of chitosan takes place at the free amino group in all the cases, as evidenced by FTIR. This leads to the formulation of amine groups or ionic bonds. Polymer crystallinity increases after crosslinking, as determined by x-RD. The method adopted for drug loading into the microspheres is satisfactory, and up to 28-30% w/w loading is observed for the sulphuric acid-crosslinked microspheres, whereas 23-29 and 15-23% of loadings are obtained for the Glutaraldehyde (GA) and heat-crosslinked microspheres, respectively. Among all the systems studied, the 32% GA crosslinked microspheres have shown the slowest release i.e. 41% at 420 min. and a fastest release of 81% at 500 min is shown by heat crosslinking for 3 h. Drug release from the matrices deviates slightly from the Fickian process.

Aiedeh et al (1997) analysed the use the chitosan for the production of surface crosslinked microparticulate systems containing insulin. The microcapsules were prepared by an innovative technique based on interfacial crosslinkage of different amounts of chitosan solubilized in the inner phase of a water/oil emulsion by means of abscorbyl palmitate. The correlation between the main formulation parameters and the functional properties of the microcapsules were analysed. Insulin is incorporated with high efficiency. The peptide release is constant for appreciable periods of time. The content of chitosan modulates the main physico-chemical properties of the matrix.

Helw et al (1998) studied that chitosan microspheres containing phenobarbitone were successfully prepared by Glutaraldehyde cross-linking of an aqueous acetic acid dispersion of chitosan in light liquid paraffin containing sorbitan mono-oleate as a stabilizing agent. Uniform and spherical microspheres, with a loading efficiency up to 57.2% could be prepared depending on the preparation conditions. The main parameters affecting the preparation and the performance of the prepared microspheres were the molecular weight and concentration of chitosan as well as the concentration of the used stabilizing agent. The incorporation of citric acid into the microspheres was found to increase the formation of a water soluble gel when the microspheres come in contact with the dissolution medium increasing the rate of drug release. The particle size was shifted towards smaller diameters with increased concentration of sorbitan mono-oleate, up to 4.0% v/v, by use of a lower concentration of chitosan (1.0% w/v) and chitosan with low molecular weight. Rapid initial drug release (20-30%) of the incorporated drug was exhibited in all the prepared microspheres followed by slow release of the remaining amount of the drug. The release rate of the drug from the microspheres prepared from high molecular weight chitosan was slow in comparison with that prepared from medium and low

molecular weight chitosan. High concentrations of sorbitan mono-oleate increased the rate of drug release.

2.7.8 References

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