FORMULATION AND CHARECTERIZATION OF ARTEMISININ LIPOSOMES

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In partial fulfillment for the award of degree of

MASTER OF PHARMACY

IN

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Submitted by

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THIRUNINRAVUR-602024

CHENNAI, TAMILNADU.

NOVEMBER – 2019

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND CHARECTERIZATION OF ARTEMISININ LIPOSOMES"** submitted to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** in partial fulfillment of the requirement for the award of the degree of **MASTER OF PHARMACY (PHARMACEUTICS).** It is a bonafide work done by **S.HEMASHREE** (Reg.No-261711360) in our institution during the academic year **2018-2019**, under the guidance of **Dr.B.Senthilnathan.** This dissertation was found to be complete and satisfactory.

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled "**FORMULATION AND CHARACTERIZATION OF ARTEMISININ LIPOSOMES**"is a genuine research work carried out by me under the guidance of **Dr.B.SENTHILNATHAN. M.Pharm., Ph.D**. Professor and Head, Department of Pharmacy Practice, Jaya College of Paramedical Sciences, College of Pharmacy. The work embodied in the thesis is original and has not been submitted on the basis for the award of diploma, degree, associate ship(or) fellowship of any other Institution (or) University.

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EVALUATION CERTIFICATE

This is to certify that the dissertation entitled "**FORMULATION AND CHARACTERIZATION OF ARTEMISININ LIPOSOMES**" submitted to The Tamilnadu Dr.M.G.R Medical University, Chennai, is a bonafide project work of **Ms.S.HEMASHREE (Reg.No-261711360), carried out in the Department of Pharmaceutics. Jaya college of Paramedical Sciences, College of Pharmacy, Thiruninravur, Chennai-24 in partial fulfillment for the degree of MASTER OF PHARMACY (PHARMACEUTICS) under the guidance of Dr.B.SENTHILNATHAN, M.Pharm., Ph.D. Professor and Head, Department of Pharmacy Practice, Jaya College of Paramedical Sciences, College of Pharmacy, Thiruninravur Chennai-24.**

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CONTENTS


Introduction

1. INTRODUCTION

LIPOSOMES :

Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids. Due to their size and hydrophobic and hydrophilic character(besides biocompatibility), liposomes are promising systems for drug delivery. Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphos phatidylcholine) form a rigid, rather impermeable bilayer structure[1]. It has been displayed that phospholipids impulsively form closed structures when they are hydrated in aqueous solutions. Such vesicles which have one or more phospholipid bilayer membranes can transport aqueous or lipid drugs, depending on the nature of those drugs.

Because lipids are amphipathic (both hydrophobic and hydrophilic) in aqueous media, their thermodynamic phase properties and self assembling characteristics influence entropically focused confiscation of their hydrophobic sections into spherical bilayers. Those layers are referred to as lamellae[1].

Generally, liposomes are definite as spherical vesicles with particle sizes ranging from 30 nm to several micrometers. They consist of one or more lipid bilayers surrounding aqueous units, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases. On the other hand self-aggregation of polar lipids is not limited to

conventional bilayer structures which rely on molecular shape, temperature, and environmental and preparation conditions but may self-assemble into various types of colloidal particles.

Liposomes are extensively used as carriers for numerous molecules in cosmetic and pharmaceutical industries. Additionally, food and farming industries have extensively studied the use of liposome encapsulation to grow delivery systems that can entrap unstable compounds (for example, antimicrobials, antioxidants, flavors and bioactive elements) and shield their functionality[2]. Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets.

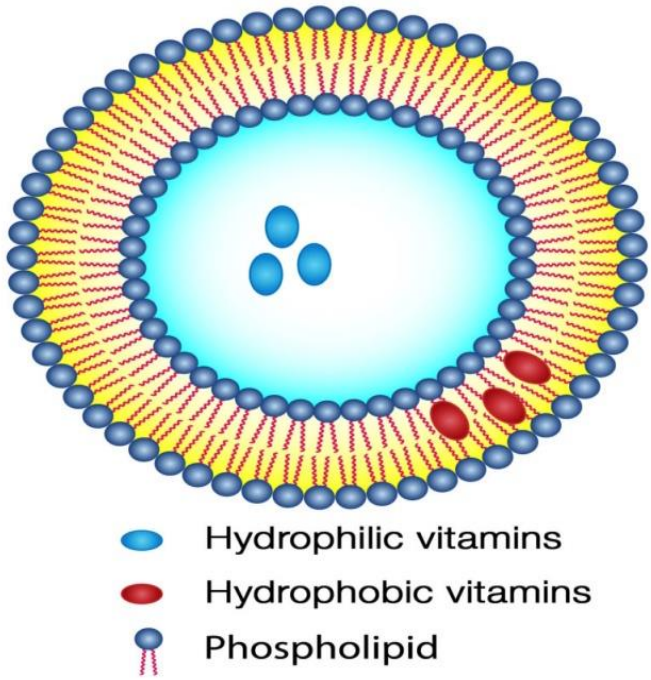
Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues, liposomes have increased rate both as an investigational system and commercially as a drugdelivery system. Many studies have been conducted on liposomes with the goal of decreasing drug toxicity and/ or targeting specific cells. Liposomal encapsulation technology (LET) is the newest delivery technique used by medical investigators to transmit drugs that act as curative promoters to the assured body organs. This form of delivery system proposal targeted the delivery of vital combinations to the body[1, 2].

LET is a method of generating sub-microscopic foams called liposomes, which encapsulate numerous materials. These 'liposomes' form a barrier around their contents, which is resistant to enzymes in the mouth and stomach, alkaline solutions, digestive juices, bile salts, and intestinal flora that are generated in the human body, as well as free radicals. The contents of the liposomes are, therefore, protected from oxidation and degradation. This protective phospholipid shield or barrier remains undamaged until the contents of the liposome are delivered to the exact target gland, organ, or system where the contents will be utilized[3]. Clinical medication keeps an enormously broad range of drug molecules at this time in use,

and new drugs are added to the list every year. One of the main aims of any cure employing drug is to increase the therapeutic index of the drug while minimizing its side effects.

The clinical usefulness of most conservative chemotherapeutics is restricted either by the incapability to deliver therapeutic drug concentrations to the target soft tissue or by Spartan and harmful toxic side effects on normal organs and tissues. Different approaches have been made to overcome these difficulties by providing the 'selective' delivery to the target area; the ideal solution would be to target the drug alone to those cells, tissues, organs that are affected by the disease. Selected carriers, for instance colloidal particulates and molecular conjugates, can be appropriate for this determination. Colloidal particulates result from the physical incorporation of the drug into a particulate colloidal system, for instance reverse micelles, noisome, micro-and nano-spheres, erythrocytes, and polymers and liposomes. Among these carriers, liposomes have been most studied[4]. Their attractiveness lies in their composition, which makes them biodegradable and biocompatible. Liposome involves an aqueous core entrapped by one or more bilayers composed of natural or synthetic lipids. They are composed of natural phospholipids that are biologically inert and feebly immunogenic, and they have low inherent toxicity.

Furthermore, drugs with different lipophilicities can be encapsulated into liposomes: strongly lipophilic drugs are entrapped almost totally in the lipid bilayer, intensely hydrophilic drugs are located entirely in the aqueous compartment, and drugs with intermediary logP effortlessly partition between the lipid and aqueous phases, both in the bilayer and in the aqueouscore. The introduction will briefly explain the characteristics of liposomes and explore the related problems and solutions proposed, with a focus on liposome preparation, characterizations, affecting factors, advantages, and disadvantages. In particular, we return to the literature relating to high-stability, long-circulating liposomes (stealth liposomes), and their field of application[2, 4].



CLASSIFICATION OF LIPOSOMES :

The liposome size can vary from very small (0. 025 μm) to large (2. 5 μm) vesicles. Moreover, liposomes may have one or bilayer membranes. The vesicle size is an acute parameter in determining the circulation half-life of liposomes, and both size and number of bilayers affect the amount of drug encapsulation in the liposomes. On the basis of their size and number of bilayers, liposomes can also be classified into one of two categories: (1) multilamellar vesicles (MLV) and (2) unilamellar vesicles. Unilamellar vesicles can also be classified into two categories: (1) large unilamellar vesicles (LUV) and (2) small unilamellar vesicles (SUV). In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution[1, 5]. In multilamellar liposomes, vesicles have an onion structure. Classically, several unilamellar vesicles will form on the inside of the other with

smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water.

Methods of liposome preparation:

General methods of preparation:

Detergent removal method (removal of non-encapsulated material):

Dialysis :

The detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids. As the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. The detergents were removed by dialysis. A commercial device called LipoPrep (Diachema AG, Switzerland), which is a version of dialysis system, is obtainable for the elimination of detergents. The dialysis can be performed in dialysis bags engrossed in large detergent free buffers (equilibrium dialysis).

Detergent: (cholate, alkyl glycoside, Triton X-100) removal of mixed micelles (absorption)
Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany) and Bio-beads SM2 (Bio-Rad Laboratories, Inc. , Hercules, USA). The great benefit of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.

Gel-permeation chromatography:

In this method, the detergent is depleted by size special chromatography. Sephadex G-50, Sephadex G-l 00 (Sigma-Aldrich, MO, USA), Sepharose 2B-6B, and Sephacryl S200-

S1000 (General Electric Company, Tehran, Iran) can be used for gel filtration. The liposomes do not penetrate into the pores of the beads packed in a column[6]. They percolate through the inter-bead spaces. At slow flow rates, the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; therefore, pretreatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

Dilution:

Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from poly-dispersed micelles to vesicles occurs[7].

Stealth liposomes and conventional liposomes:

Although liposomes are like biomembranes, they are still foreign objects of the body. Therefore, liposomes are known by the mononuclear phagocytic system (MPS) after contact with plasma proteins. Accordingly, liposomes are cleared from the blood stream. These stability difficulties are solved through the use of synthetic phospholipids, particle coated with amphipathic polyethylene glycol, coating liposomes with chitin derivatives, freeze drying, polymerization, micro-encapsulation of gangliosides. Coating liposomes with PEG reduces the percentage of uptake by macrophages and leads to a prolonged presence of liposomes in the circulation and, therefore, make available abundant time for these liposomes to leak from the circulation through leaky endothelium[2, 7].

A stealth liposome is a sphere-shaped vesicle with a membrane composed of phospholipid bilayer used to deliver drugs or genetic material into a cell. A liposome can be

composed of naturally derived phospholipids with mixed lipid chains coated or steadied by polymers of PEG and colloidal in nature. Stealth liposomes are attained and grown in new drug delivery and in controlled release[8]. This stealth principle has been used to develop the successful doxorubicin-loaded liposome product that is presently marketed as Doxil (Janssen Biotech, Inc. , Horsham, USA) or Caelyx (Schering- Plough Corporation, Kenilworth, USA) for the treatment of solid tumors. Recently impressive therapeutic improvements were described with the useof corticosteroid loaded liposome in experimental arthritic models. The concerning on the application of stealth liposomes has been on their potential to escape from the blood circulation.

However, long circulating liposome may also act as a reservoir for prolonged release of a therapeutic agent. Pharmacological action of vasopressin is formulated in long circulating liposome.

Drug loading in liposomes:

Drug loading can be attained either passively (i. e. , the drug is encapsulated during liposome formation) or actively (i. e. , after liposome formation). Hydrophobic drugs, for example amphotericin B taxol or annamycin, can be directly combined into liposomes during vesicle formation, and the amount of uptake and retention is governed by drug-lipid interactions. Trapping effectiveness of 100% is often achievable, but this is dependent on the solubility of the drug in the liposome membrane[9]. Passive encapsulation of water-soluble drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation.

Trapping effectiveness (generally <30%) is limited by the trapped volume delimited in the liposomes and drug solubility. On the other hand, water-soluble drugs that have

protonizable amine functions can be actively entrapped by employing pH gradients, which can result in trapping effectiveness approaching 100%.

Freeze-protectant for liposomes (lyophilization):

Natural excerpts are usually degraded because of oxidation and other chemical reactions before they are delivered to the target site. Freeze-drying has been a standard practice employed to the production of many pharmaceutical products. The overwhelming majority of these products are lyophilized from simple aqueous solutions. Classically, water is the only solvent that must be detached from the solution using the freeze-drying process, but there are still many examples where pharmaceutical products are manufactured via a process that requires freeze-drying from organic co-solvent systems[10].

Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at tremendously low pressures. The process is normally used to dry products that are thermo-labile and would be demolished by heat-drying. The technique has too much potential as a method to solve long-term stability difficulties with admiration to liposomal stability. Studies showed that leakage of entrapped materials may take place during the process of freeze-drying and on reconstitution[11]. Newly, it was shown that liposomes when freeze-dried in the presence of adequate amounts of trehalose (a carbohydrate commonly found at high concentrations in organism) retained as much as 100% of their original substances. It shows that trehalose is an excellent cryoprotectant (freeze-protectant) for liposomes. Freeze-driers range in size from small laboratory models to large industrial units available from pharmaceutical equipment suppliers.

Mechanism of transportation through liposome:

The limitations and benefits of liposome drug carriers lie critically on the interaction of liposomes with cells and their destiny in vivo after administration. In vivo and in vitro studies of the contacts with cells have shown that the main interaction of liposomes with cellsis either simple adsorption (by specific interactions with cell-surface components, electrostatic forces, or by nonspecific weak hydrophobic) or following endocytosis (by phagocytic cells of the reticuloendothelial system, for example macrophages and neutrophils). Fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal content into the cytoplasm, is much rare[6, 12]. The fourth possible interaction is the exchange of bilayer components, for instance cholesterol, lipids, and membrane-bound molecules with components of cell membranes. It is often difficult to determine what mechanism is functioning, and more than one may function at the same time.

Fusogenic liposomes and antibody-mediated liposomes in cancer therapy:

It has been infrequently well-known that a powerful anticancer drug, especially one that targets the cytoplasm or cell nucleus, does not work due to the low permeability across a plasma membrane, degradation by lysosomal enzymes through an endocytosis-dependent pathway, and other reasons[13]. Thus, much attention on the use of drug delivery systems is focused on overcoming these problems, ultimately leading to the induction of maximal ability of anti-cancer drug.

In this respect, a new model for cancer therapy using a novel drug delivery system, fusogenic liposome , was developed. Fusogenic liposomes are poised of the ultraviolet inactivated Sendai virus and conventional liposomes. Fusogenic liposomes effectively and directly deliver their encapsulated contents into the cytoplasm using a fusion mechanism of the

Sendai virus, whereas conventional liposomes are taken up by endocytosis by phagocytic cells of the reticuloendothelial system, for example macrophages and neutrophils.

Thus, fusogenic liposome is a good candidate as a vehicle to deliver drugs into the cytoplasm in an endocytosis-independent manner. Liposomal drug delivery systems provide steady formulation, provide better pharmacokinetics, and make a degree of 'passive' or 'physiological' targeting to tumor tissue available[14]. However, these transporters do not directly target tumor cells. The design modifications that protect liposomes from unwanted interactions with plasma proteins and cell membranes which differed them with reactive carriers, for example cationic liposomes, also prevent interactions with tumor cells. As an alternative, after extravasation into tumor tissue[15], liposomes remain within tumor stroma as a drug-loaded depot. Liposomes ultimately become subject to enzymatic degradation and/or phagocytic attack, leading to release of drug for subsequent diffusion to tumor cells.

The next generation of drug carriers under development features directs molecular targeting of cancer cells via antibody-mediated or other ligand-mediated interactions.

Applications of Liposomes:

Over the last 30 years the field of liposome research has expanded considerably. It is now possible to engineer a wide range of liposome of varying size, phospholipids and cholesterol compositions, surface morphology that is suitable for wide range of applications. Liposomes cause liposomal components to be associated with target cells interacts with cells in many ways[16].

The liposome carrier can be targeted to liver and spleen and distinction can be made by using tomography between normal and tumours tissue. Liposome has a great application in case of transdermal drug delivery systems. Reduction in the toxic effect and enhancement of the effectiveness of drugs is achieved by Liposomal drug delivery system when used to target

the tumour cells. The targeting of the liposome to the site of action takes place by the attachment of amino acid fragment, such as antibody or protein or appropriate fragments that target specific receptors cell[17]. Few of the recent applications of liposome are DNA vaccination and improved efficiency of gene therapy.

Several modes of drug delivery application have been purposed for the liposomal drug delivery system, few of them are as follows:

1. Enhancement of drug solubilisation (Amphotericin-B, Minoxidil, Paclitaxels, and Cyclosporins)

2. Protection of sensitive drug molecules (Cytosine arabinosa, DNA, RNA, Anti-sense oligo-nucleotides, Ribozymes)

3. Enhancement of intracellular uptake (Anticancer, anti viral and antimicrobial drugs)

4. Alteration in pharmacokinetics and bio-distribution (prolonged or sustained released drugs with short circulatory half life)[18]

Several recent applications of liposomal drug delivery system are as follows:

Liposome for Respiratory Drug Delivery System:

Liposome is widely used in several types of respiratory disorders. Liposomal aerosol has several advantages over ordinary aerosol which are as follows:

1. Sustained release.

2. Prevention of local irritation.

3. Reduced toxicity.

4. Improved stability in the large aqueous core[18].

Liposomal drug delivery system for the lung is dependent on the following parameters:

1. Lipid composition.
2. Size.
3. Charge.
4. Drug and Lipid ratio and
5. Method of delivery.

The recent liposome application for the delivery of DNA to the lung means that a greater use in macromolecular delivery via inhalational is now emerging. This new knowledge, including new lipids and analytical techniques, can be used in the development of liposome based protein formulations. The liquid or dry form is taken for the inhalation of liposome and during nebulisation the drug release occurs. Drug powder liposome is produced by milling or by spray drying[18, 19].

Liposome in Nucleic Acid Therapy:

The successful delivery of nucleic acid into cells in vitro and in vivo depends on recombinant DNA technologies and studies of gene function and gene therapy. For the selective delivery of the gene to the malignant cells non viral vectors are developed. The vector attaches itself to the nutrients ligand onto the vector (liposome) and will exploit the increase requirements of rapidly growing cells for more nutrients. The vector enhances nucleic acid binding along with novel pH sensitive surfactants by having passively charged lipid to some of the engineered liposomal and non liposomal versions like pH sensitive cationic and anionic liposome, pH sensitive immuno-liposome, fusagenic liposome, and genosomes[20]. This agent, has membrane disrupting effects and will become activated at endosomal pH and inactivated before reaching the liposome. The novelty of this delivery system stems from soft pH sensitive

surfactants (SPS). The pH sensitive liposomes have been reported as plasmid expression vectors for the cytosolic delivery of DNA. It is also effective carrier for intracellular trafficking of anti sense oligo nucleotides[22].

Liposome in Eye Disorders:

Liposome has been used widely to treat disorder of both anterior and posterior segment. The disease of eye includes dry eyes, keratitis, corneal transplant rejection, endopthelmitis and proliferative vitro retinopathy. Retinal diseases are important cause of blindness[21, 23]. Liposome is used as vector for genetic transfection and monoclonal antibody directed vehicle. Applying of focal laser to heat induced release of liposomal drugs and dyes are the recent techniques of the treatment of selective tumour and neo-vascular vessels occlusion, angiography, retinal and choroidal blood vessel stasis[24].

Liposome as Vaccine Adjuvant:

Liposome has been established firmly as immuno-adjuvant that is potentiating both cell mediated and non cell mediated (humoral) immunity.

Liposome acts as immuno-adjuvant by the following therapeutic points of view:

1. Liposomes as an immunological (vaccine) adjuvant
2. Liposomal vaccines
3. Liposomes as carrier of immuno modulation for
4. Liposomes as a tool in immuno diagnostics

Liposomal immuno-adjuvant act by slow release of encapsulated antigen on intramuscular injection and also by passive accumulation within regional lymph node. The accumulation of liposome to lymphoid is done by the targeting of liposome with the help of

phosphotidyl serine[25]. Liposomal vaccine can be prepared by inoculating microbes, soluble antigen, and cytokinesis of deoxyribonucleic acid with liposome.

Liposomes for Brain Targeting:

The biocompatible and biodegradable behaviour of liposomes have led to their advanced application as brain drug delivery system. Liposomes with a small diameter (100 nm) as well as large diameter undergo free diffusion through the Blood Brain Barrier (BBB)[26]. However small unilamellar vesicles (SUVS) coupled to brain drug transport vectors may be transported by receptor mediated or absorptive mediated transcytosis through the BBB. Cationic liposomes undergo absorptive mediated endocytosis into cells whereas the same undergoing absorptive mediated transcytosis through the BBB has not yet been determined[27]. The addition of the sulphatide (a sulphur ester of galactocerebroside) to liposome composition increases several recent applications and ability to cross BBB. Wang et al. reported that liposomes coated with the mannose reach brain and assist transport of loaded drug through BBB. The neutropeptides, leu-enkephaline and mefenkephalin kyoforphin normally do not cross BBB when given systemically. The anti depressant amitriptylline normally penetrate the BBB, due to versatility of this method[28].

Liposome as Anti-Infective Agents:

The therapeutic agent may be targeted to the organs using liposome as vehicle system. To remove the intracellular pathogen like protozoa, bacteria, and fungi that reside in the liver and spleen. The diseases like leishmaniasis, candidiasis, aspergelosis, histoplasmosis, erythrococosis, gerardiasis, malaria and tuberculosis are targeted by the respective therapeutic agent using liposome as carrier[2, 29].

Liposome in Tumour therapy:

The toxic side effect is caused by the long term therapy of anticancer drug. The liposomal therapy for the targeting to the tumour has revolutionised the therapy with lesser toxic effects[30]. The small and stable liposome is passively targeted to different tumour because they can circulate for longer time. Liposome macrophage uptake by liver and spleen has led to the development of liposome as drug delivery for over 20 years[31].


Literature Reviews

2. LITERATURE REVIEW

DADGAR NEDA *et al* (2013) reported the efficacy of artemisinin nano liposome, artemisinin nano liposome polyethyleneglycol on breast cancer cell line (MCF -7 cell line). In this Liposomes were prepared by reverse phase evaporation method. Phophatidylcholine, cholesterol and artemisinin were combined together at certain concentrations in this method. The stability of the prepared formulation was increased by pegylattion (Polyethyleneglycol 2000). The diameter of the nano liposomes were instrumentally determined by Zetasizer. The results showed that encapsulation and release of artemisinin from only liposomated formulation was more than pegylated form. This study also revealed that the cytotoxicity effect of artemisinin liposome polyethyleneglycol was more than that of artemisinin liposomes.

BENEDETTA ISACCHI *et al* (2011) reported a detailed development and optimization of artemisinin-loaded liposomes having proper physical characteristics as drug carriers for parenteral administration, in terms of particle size, PDI, encapsulation efficacy, and zeta - potential. The in vivo pharmacokinetic studies led to conclude that encapsulation of artemisinin into liposomes is a reasonable method to prolong its circulating time in the blood.

AJAYPATIDAR *et. al (2010)* reviewed the latest development of lipid based nano carriers preparation its several advantages and disadvantages. He also discussed the recent development in the field of sln , nanostructured lipid carriers, and lipid drug conjugates

RABINARAYANPARHI *et. al (2010*) reviewed the production of SLN, release mechanism, colloidal carriers such as liposomes, emulsion , polymeric micro and nano particles and routes of administration s like the pulmonary , topical, oral, parentral, nasal

and mentioned the various techniques for the drug loading , loading capacity , drug incorporation and the factors affecting its loading capacity and drug release especially emphasizing on mechanism of drug release.

GAURAVKANT SAROJI *et. al (2009)* studied the gelatin nano carriers as potential vectors for effective management of tuberculosis. He developed and characterisied rifampicin (rif)loaded gelatin nano particulate delivery system for tuberculosis.

RAINER H. MULLER *et. al (2009)* had reviewed the current status of the sln regarding the production techniques, drug incorporation, loading capacity, drug release mechanisms and different routes of administration

XIAOHCLIPU *et. al (2009)* had studied chemically stable 10 –hydroxycamptothecin nano suspension. In this study, the process parameters like agitation rate of stabilizer solution, homogenization pressure and cycle numbers were investigated and optimized based on the particle size and zeta potential of the nano suspension.

SARAHKUCHLER *et. al (2009)* had studied nano particles for skin penetration enhancement and compared dendritic core multishell – nano transporter and sloid lipid nanoparticles. It showed that incorporaed nile red in to stable dendritic nanoparticle matrix, dye amounts increased eight fold in the stratum corneum and 13 fold in the epidermis compared to Sln. The cream nano transporters can favour the penetration of a model dye in to the skin even more than sln which may reflect size effects.

MEDHA D. JOSHI *et. al (2009)* reviewed the lipid nano articles for parentral delivey and the attempts to in corporate anticancer agents , imaging agents, antiparasites, antiarthritis , gene for transfection agents for liver and the cardiovascular drugs and cns targetting have been summarizied.

SAGAR D. MANADAWGADE *et. al (2008)* studied the formulation of sln from natural lipids by using a simple microemulsion technique and sln was charecterisied for particle size, polydispersityindex, entrapment efficiency and morphology.

XIAOLIN LI *et. al (2008)* studied anti tumour efficiency of cisplatin loaded nano particles by intra tumoural delivery with decreased tumour metabolisim rate has demonstrated extraordinary activites against a variety of solid tumours to reduce the toxicity and enhance the circulation time of cisplatin nanoparticles core prepared from block copolymer.

PATRICKLIMSO *et. al (2008)* studied the drug release mechanism of paclitaxel from a chitosan lipid implant system. The *in vitro* release studies of paclitaxel from the implant were conducted in various fluids with a strong correlation (r^2 = 0. 977) was found in addition, it was found to be good indicator of the *in vivo* release profile.

SUNGHEECHOI *et. al (2008)* studied the novel cationic solid lipid nanoparticles for gene delivery was produced by the melt homogenization method. The result revealed that cationic SLN mediated p53 gene delivery may have potential for clinical application as a non viral vector mediated lung cancer therapy due to its effective induction of apoptosis and tumor growth inhibition.

ANDER ESTELLA –HERMOSODEMENDOZA *et. al (2008)* studied lipid nano particles for alkyl lysophospholipid edelfosine by using the lipid compritol and stearic acid. The drug encapsulation efficiency and drug release kinetics were measured and concluded that compritol presents advantagesas a matrix material for the manufacture of nanoparticles and for the controlled release of edelfosine.

ELEONORAVIGHI *et. al (2007)* studied the redispersible catoionic sln after freeze drying process in the absence of lyo –and cryoprotectors and characterized. He concluded

theat the cationic slns based on stearic acid retain the ability to complex dna even after the freeze drying in the absence of lyo-or cryoprotectors.

HARIVARDHAN REDDY *et. al (2006)* studied tamoxifen citrate loaded solid lipid nano particles and their preparation , characterization , *invitro* drug release , and pharmaceutic evaluation by emulisifier cation and high pressure homogenization technique. and influence factors such as homogenization pressure time , surfactant concentration and the nanoparticle size distribution were investigated and optimized. The formulation with homogenization at 15 , 000psi for 3 cycles was found to be optimum and resulted in small sized nano particles.

NINA PEDERSEN *et. al (2006)* studied solid lipid nanoparticles can effectively bind DNA , streptavidin and biotinylated ligiands. he prepared the SLN by micro emulsion technique using stearylamine and compritol ATO 888. The SLN are able to condense DNA in complexes and displayed low cytotoxicity in cell culture. in addition the SLN:DNA: , streptavidin complexes are stable and capable biding to biotinylated ligand which can inter act with surface receptors. This method allows for development of a fast and simple method of preparing a targeted non viral gene therapy vector.

L. SERPE *et. al (2004)* studied the cytotoxicity of anti cancer drugs incorporated in solid lipid nanoparticles on HT -29 colorectal cancer cell line. The results suggested that solid lipid nanoparticles could be proposed as alternatve drg delivery system.

JIAN-XIN WANG *et. al (2002)* studied the enhanced brain targetting of 3'5' –dictanoyl 5 –fluro 2' deoxyuridine and incorporation in to solid lipid nano particles by thin layer ultasonication technique and central composite design was applied to optimize the formulation. In this study the *invitro* drug release was studied by a bulk equilibrium

reverse dialysis bag technique in phosphate buffere saline pH 7. 4 containing 0. 31% pancreatic enzyme showes the improved ability of the drug to penetrate the BBB and ideal drug targeting system for the treatment of CNS disorders.

ELENAVGAZIO *et. al (2001*) studied the effect of formulation and concentration of cholesteryl butyrate solid lipid nano spheres on nh –h460 cell proliferation. The cells were treated with scalar conc

entrations of cholesteryl butyrate(0. 008-1. 000nm) for each experimental conditions and the cell growth was inhibited in all cases. The best formulation showed the comlete inhibition at 0. 125nm chol-but While the same concentration of sodium butyrate provided only 38% inhibition.


OBJECTIVES

3. AIM & OBJECTIVE

AIM OF THE WORK

The main aim of present study is to prepare and evaluate the liposomes for the selected drug Artemisinin.

OBJECTIVE OF THE WORK

- To improve the poor bioavailability of Artemisinin.
- To achieve controlled release.
- To reduce the dosing frequency.
- To enhance the drug release.
- To achieve increased patient compliance.


PLAN


WORK

4. PLAN OF WORK

1. Preformulation studies of Artemisinin
2. Preparation of standard calibration graph
3. Analysis of Artemisinin
4. Drug-Excipients compatibility study by DSC
5. Preparation of Artemisinin liposomes
6. Optimization of Egg phosphatidyl choline
7. Evaluation of Artemisinin liposomes
    - Particle size
    - Zeta potential
    - Drug content
    - Entrapment efficiency
    - *In vitro* drug release study

DRUG PROFILE

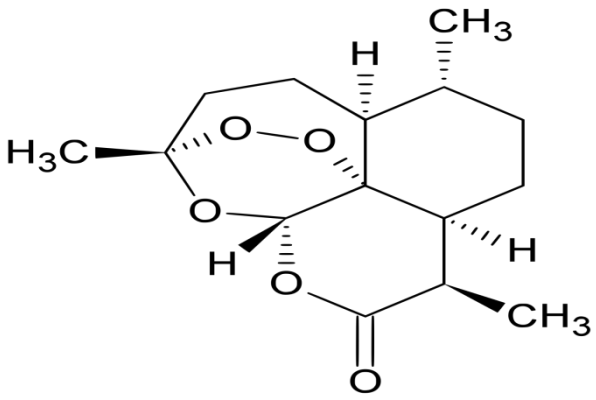
5. DRUG PROFILE

ARTEMISININ

SYNONYMS: Artemisinine, qinghaosu

Artemisinin is a sesquiterpene lactone obtained from sweet wormwood, Artemisia annua, which is used as an antimalarial for the treatment of multi-drug resistant strains of falciparum malaria.

CHEMICAL STRUCTURE:



Formula: $C_{15}H_{22}O_5$

Molar mass: 282. 332 g/mol

ATC code: P01BE01 (WHO)

Melting point: 152 to 157 °C (306 to 315 °F)

IUPAC NAME: (3R, 5aS, 6R, 8aS, 9R, 12S, 12aR)-Octahydro-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano[4, 3-j]-1, 2-benzodioxepin-10(3H)-one

CLASS: Antimalarial

CHEMISTRY:

An unusual component of the artemisinin molecules is an endoperoxide 1, 2, 4-trioxane ring. This is the main antimalarial centre of the molecule. Modifications at carbon 10 (C10) position give rise to a variety of derivatives which are more powerful than the original compound. Because the physical properties of artemisinin itself, such as poor bioavailability, limit its effectiveness, semisynthetic derivatives of artemisinin have been developed. Derivatives of dihydroartemisinin were made since 1976[32]. Artesunate, arteether and artemether were synthesised in 1986. Many derivatives have been produced of which artelinic acid, artemotil, artemisone, SM735, SM905, SM933, SM934, and SM1044 are among the most powerful compounds. There are also simplified analogs in preclinical research. Over 120 other derivatives have been prepared, but clinical testing has not been possible due to lack of financial support.

Artemisinin belongs to a class of sesquiterpene lactones. The solid is poorly soluble in oils and water. Therefore, it is mostly applied through the digestive tract, either by oral or rectal administration. Some chemical modification are suitable for administration by injection. Artesunate is the only artemisinin compound available for all types of administration procedure. A synthetic compound with a similar trioxolane structure (a ring containing three oxygen atoms) named RBx-11160 showed promise in in vitro testing. Phase II testing in patients with malaria was not as successful as hoped, but the manufacturer decided to start Phase III testing anyway[33].

PHARMACOLOGY AND MODE OF ACTION:

As of 2018, the exact mechanism of action of artemisinins was not known because of the complex chemical interactions involved. Artemisinins do not directly attack malarial parasites or cells. They have to undergo chemical changes in the blood. Their functional group endoperoxide ring has to be activated first. Activation is done by cleavage of the endoperoxide ring. As the drug molecules come in contact with the haem (inside the haemoglobin of the red blood cells), the iron(II) oxide breaks the endoperoxide ring. This process produces free radicals that in turn damage susceptible proteins, resulting in the death of the parasite[34]. In 2016 artemisinin was shown to bind to a large number of targets suggesting that it acts in a promiscuous manner. Unlike other antimalarials which are active only on a particular stage of malarial parasite, artemisinin is able to kill all the life cycle stages.

PHARMACOKINETICS:

METABOLISM AND PHARMACOKINETICS:

Once absorbed, the artemisinin derivatives are converted primarily to dihydroartemisinin (DHA) and thence to inactive metabolites via hepatic cytochrome P-450 and other enzyme systems. DHA is itself a potent antimalarial with an elimination half life of about 45 minutes. The extent of conversion to DHA differs between derivatives. Artemisinin itself is not metabolised to DHA but acts as the primary antimalarial, while artesunate is rapidly (within minutes) hydrolysed to DHA and its antimalarial activity is largely mediated by DHA[35]. Artemether and arteether contribute to antimalarial activity, probably to a similar extent as DHA, to which they are converted more slowly. DHA is mostly (90%) bound to plasma proteins.

Pharmacokinetic studies on artemisinins have been limited by difficulties of assay; several techniques with differing accuracies have been used by various groups. Furthermore, studies must necessarily take into account active metabolites (mostly DHA). Bioassay techniques measuring total antimalarial activity account for this and, along with advances in assay methods, have allowed clearer pharmacokinetic profiling to emerge for drug formulations that have often been used empirically for many years. These studies are improving our understanding pharmacodynamic and toxicological aspects of this group of compounds.

In uncomplicated malaria, when artemisinins are used orally, most pharmacokinetic information is now available for artesunate followed by artemether. The absolute bioavailability of antimalarial activity after a single dose of oral artesunate in uncomplicated adult malaria is about 60% although there is greater interpatient variation than in healthy volunteers[36]. Time to maximum DHA concentration is typically one to two hours. Studies suggest that clearance after artesunate is reduced during acute infection compared with recovery, either via disease effects on pharmacokinetics or enzyme autoinduction.

Although absolute bioavailability studies for artemether, artemisinin, and DHA are not possible given lack of intravenous formulations, pharmacodynamic activity (parasite clearance) after oral dosing of these derivatives is satisfactory. When studied, oral formulations show appropriately reliable and rapid absorption in the treatment of uncomplicated malaria. As for artesunate, studies of oral artemether and artemisinin show increasing clearance with multiple dosing and during recovery from acute infection.

In severe malaria, the delayed and variable absorption of the oil soluble derivatives artemether and arteether when given by the intramuscular route is of great potential clinical relevance.

METABOLISM:

In the liver artemisinin is converted to different inactive metabolites such as deoxyartemisinin, deoxydihydroartemisinin, crystal 7, and 9, 10-dihydrodeoxyartemisinin. The metabolites have lost the endoperoxide group and become ineffective. The reaction is catalysed by an enzyme CYP2B6, while another enzyme CYP3A4 acts as a secondary catalyst. In the absence of CYP2B6, CYP3A4 becomes the primary enzyme. These enzymes belong to cytochrome P450 group present in the smooth endoplasmic reticulum. Artemisinin derivatives are metabolised differently. They are first converted to dihydroartemisinin (DHA). DHA itself is a strong antimalarial molecule and is active in the blood circulation for two to three hours[37]. The antimalarial activity of artesunate is actually only through DHA. (Artemisinin, arteether, artemether, etc. are directly antimalarials.) Artesunate is converted to DHA within a minute of its absorption. About 90% of the total DHA normally binds to blood plasma. In the liver, cytochrome P450 enzyme system (including CYP2A6, CYP3A4, and CYP3A5) convert DHA into inactive metabolites. All the metabolites undergo glucuronidation after which they are excreted through the urine or faeces. UDP-glucuronosyltransferases, in particular UGT1A9 and UGT2B7, are responsible for the process. DHA is also removed through bile as minor glucuronides, such as tetrahydrofurano acetate. Due to fast metabolism, artemisinins are relatively safe drugs[37].

LIMITATIONS OF ARTEMISININ:

Putting aside questions of cost, which may be the most important for users of antimalarials but have been comprehensively reviewed in a recent authoritative report from the Institute of Medicine, there are certain inherent problems with current artemisinins that require discussion.

Poor cure rate of monotherapy:

Artemisinins reliably reduce initial malaria parasitaemia by a factor of 104 per 48 hour asexual cycle and modelling studies therefore suggest that six days of treatment should cure parasite burdens of up to 1012 parasites. This model is difficult to reconcile with the high recrudescence rates (10%–15%) seen with artemisinin monotherapy[38]. This poor efficacy of cure (which is not due to resistance) is usually attributed to the intrinsically short half life of artemisinins, which is further shortened by the increased drug clearance that develops during repeat dosing and/or convalescence with various oral artemisinin derivatives Blaming pharmacokinetic factors alone for the poor efficacy of artemisinin monotherapy may not be justified because constant drug levels are not necessary for potent pharmacodynamic effects (at least in the initial, visible phase of parasitaemia). Furthermore, if pharmacokinetic behaviour were a problem, prolongation of treatment course may be predicted to compensate, but this is not generally observed in practice; seven days of monotherapy with artemisinin still only cures 80%–90% of uncomplicated falciparum infections. Parasite reduction ratio models for artesunate derived on data obtained at the start of treatment may not be applicable to the process of eradication of small numbers of residual parasites, which determines eventual cure rates. Other phenomena may exist that permit escape from artemisinin therapy, necessitating a second (albeit less visibly effective) antimalarial. Although it has been strongly argued that, in any case, combination therapy has long term benefit in preventing resistance, the poor efficacy of monotherapy with the current generation of artemisinins remains a troubling and poorly explained phenomenon.

Neurotoxicity:

Despite pre-clinical evidence of brainstem toxicity in animals, millions of doses in various formulations have been given to humans without significant evidence of major toxicity, even when particular attention is given to monitoring for neurotoxicity both clinically and pathologically[40]. This discrepancy between animal and human toxicity has been attributed to the comparatively high and prolonged dosing regimens used in certain animal studies. In addition, pharmacokinetic studies of parenteral artemether and arteether have showed the slow release and consequently long exposure times seen with oil based formulations of these drugs in both animals and humans[37, 38]. It is probably the duration of exposure to artemisinins that determines neurotoxicity rather than the maximum concentrations reached. Prolonged high concentrations of artemisinins are certainly not seen in oral regimens, which constitute the vast majority of artemisinin courses given, and there is no pathological evidence of neurotoxicity in patients exposed to an average of 76. 5 hours of intramuscular artemether. A recent claim that artemether-lumefantrine induces mild but significant hearing loss seems to contradict this view but needs to be reproduced independently and the mechanism dissected, particularly in terms of the time course of hearing loss. Concern with regard to neurotoxicity should also be maintained in the context of children who have more vulnerable neurological systems and where therapeutic experience is more limited[39]. Even taking into account these concerns, artemisinin derivatives have less major toxicity than other available antimalarial drugs[41].

6. DISEASE PROFILE

MALARIA

Malaria is a mosquito-borne infectious disease that affects humans and other animals. The parasite is transmitted to humans through the bites of infected mosquitoes. People who have malaria usually feel very sick, with a high fever and shaking chills. Each year, approximately 210 million people are infected with malaria, and about 440, 000 people die from the disease[43]. Most of the people who die from the disease are young children in Africa.

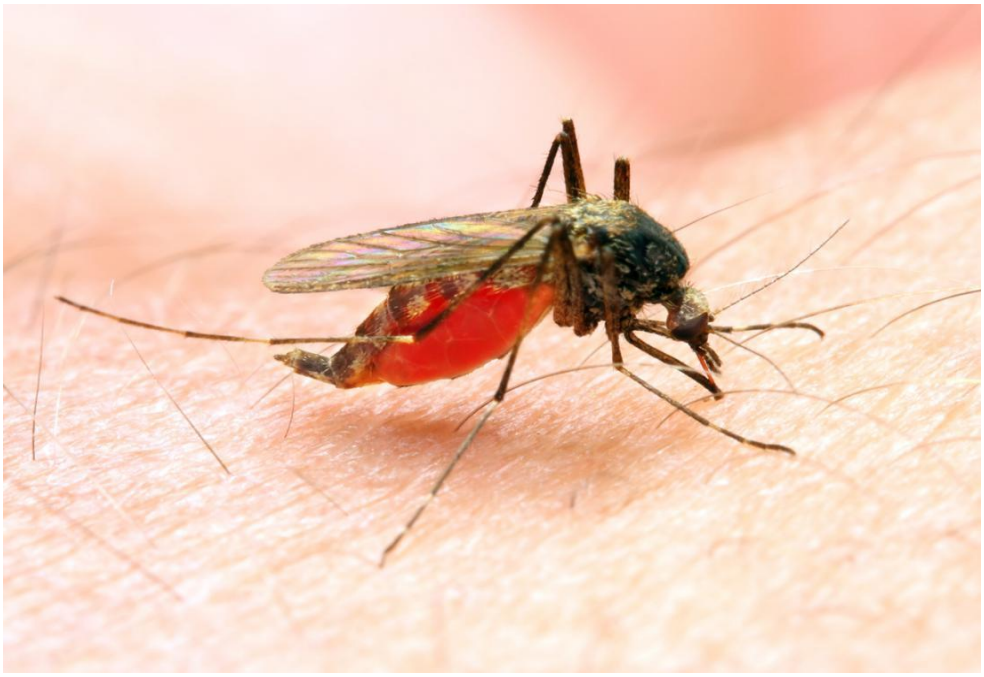
While the disease is uncommon in temperate climates, malaria is still common in tropical and subtropical countries. World health officials are trying to reduce the incidence of malaria by distributing bed nets to help protect people from mosquito bites as they sleep. Scientists around the world are working to develop a vaccine to prevent malaria.

If you're traveling to locations where malaria is common, take steps to prevent mosquito bites by wearing protective clothing, using insect repellants and sleeping under treated mosquito nets[44]. Depending on the area you are visiting and your individual risk factors for infection, you may also want to take preventive medicine before, during and after your trip. Many malaria parasites are now resistant to the most common drugs used to treat the disease.

SYMPTOMS:

Malaria symptoms can be classified into two categories: Uncomplicated and severe malaria.

Uncomplicated malaria:



Malaria is passed on by the Anopheles mosquito.

A doctor would give this diagnosis when symptoms are present, but no symptoms occur that suggest severe infection or dysfunction of the vital organs[45].

- This form can become severe malaria without treatment, or if the host has poor or no immunity.
- Symptoms of uncomplicated malaria typically last 6 to 10 hours and recur every second day.
- Some strains of the parasite can have a longer cycle or cause mixed symptoms.

As symptoms resemble those of flu, they may remain undiagnosed or misdiagnosed in areas where malaria is less common[46].

In uncomplicated malaria, symptoms progress as follows, through cold, hot, and sweating stages:

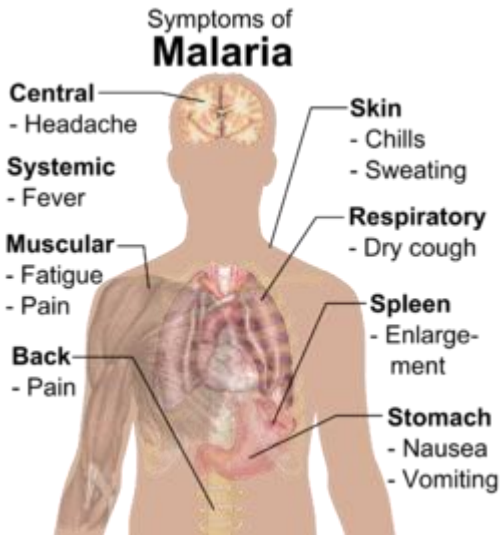
- a sensation of cold with shivering

- fever, headaches, and vomiting
- seizures sometimes occur in younger people with the disease
- sweats, followed by a return to normal temperature, with tiredness

In areas where malaria is common, many people recognize the symptoms as malaria and treat themselves without visiting a doctor.

Severe Malaria:

In severe malaria, clinical or laboratory evidence shows signs of vital organ dysfunction[47].



Symptoms of severe Malaria include:

- fever and chills
- impaired consciousness
- prostration, or adopting a prone position
- multiple convulsions
- deep breathing and respiratory distress

- abnormal bleeding and signs of anemia
- clinical jaundice and evidence of vital organ dysfunction

Severe malaria can be fatal without treatment.

COMPLICATIONS:

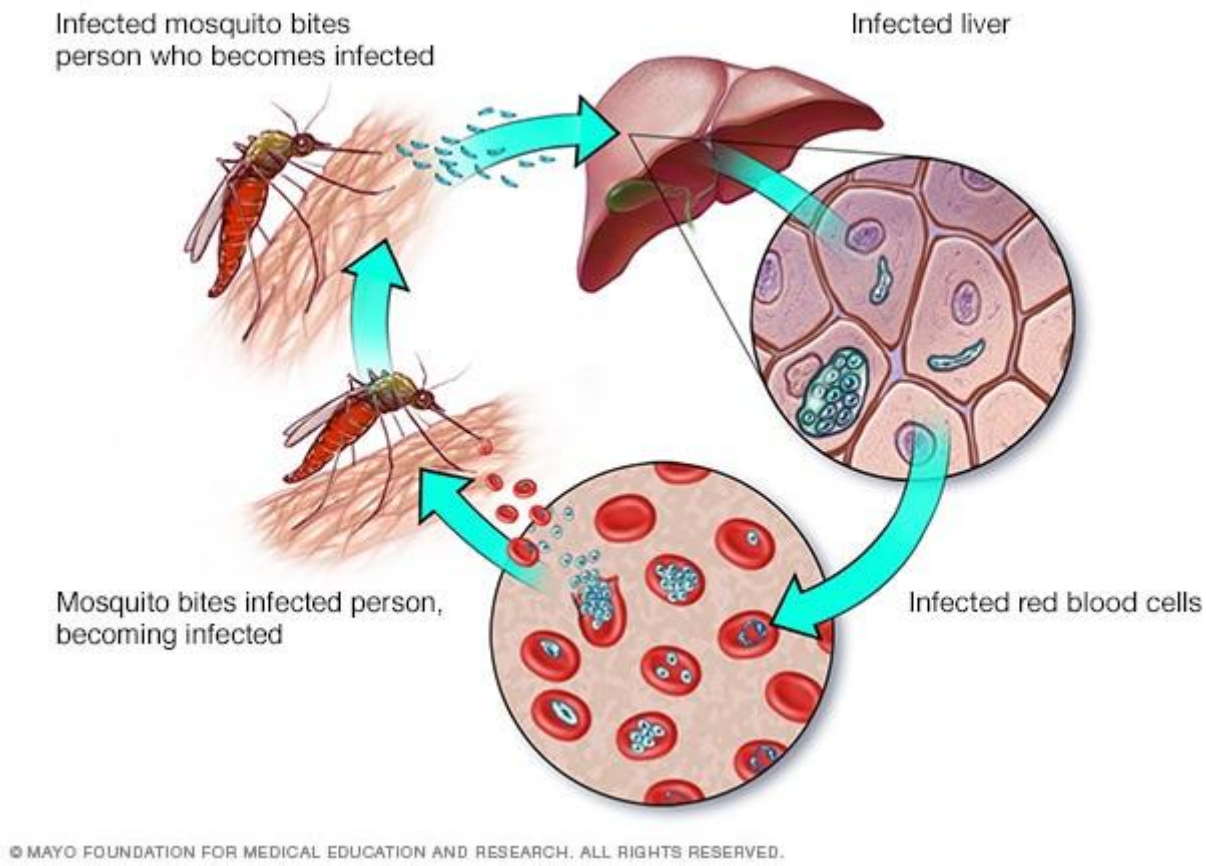
Malaria has several serious complications. Among these is the development of respiratory distress, which occurs in up to 25% of adults and 40% of children with severe *P. falciparum* malaria. Although rare in young children with severe malaria, acute respiratory distress syndrome occurs in 5–25% of adults and up to 29% of pregnant women[48]. Coinfection of HIV with malaria increases mortality. Renal failure is a feature of blackwater fever, where hemoglobin from lysed red blood cells leaks into the urine[49].

In most cases, malaria deaths are related to one or more serious complications, including[50]:

- **Cerebral malaria:** If parasite-filled blood cells block small blood vessels to your brain (cerebral malaria), swelling of your brain or brain damage may occur. Cerebral malaria may cause seizures and coma.
- **Breathing problems:** Accumulated fluid in your lungs (pulmonary edema) can make it difficult to breathe.
- **Organ failure:** Malaria can cause your kidneys or liver to fail, or your spleen to rupture. Any of these conditions can be life-threatening.
- **Anemia:** Malaria damages red blood cells, which can result in anemia.

- **Low blood sugar:** Severe forms of malaria itself can cause low blood sugar (hypoglycemia), as can quinine — one of the most common medications used to combat malaria. Very low blood sugar can result in coma or death[50].

CAUSES



Malaria transmission cycle:

Malaria is caused by a type of microscopic parasite. The parasite is transmitted to humans most commonly through mosquito bites. Malaria happens when a bite from the female *Anopheles* mosquito infects the body with Plasmodium[51]. Only the *Anopheles* mosquito can transmit malaria.

The successful development of the parasite within the mosquito depends on several factors, the most important being humidity and ambient temperatures.

Mosquito transmission cycle:

- **Uninfected mosquito:** A mosquito becomes infected by feeding on a person who has malaria.
- **Transmission of parasite:** If this mosquito bites you in the future, it can transmit malaria parasites to you.
- **In the liver:** Once the parasites enter your body, they travel to your liver — where some types can lie dormant for as long as a year[52].
- **Into the bloodstream:** When the parasites mature, they leave the liver and infect your red blood cells. This is when people typically develop malaria symptoms.
- **On to the next person:** If an uninfected mosquito bites you at this point in the cycle, it will become infected with your malaria parasites and can spread them to the other people it bites.

Other modes of transmission:

Because the parasites that cause malaria affect red blood cells, people can also catch malaria from exposure to infected blood, including[53]:

- From mother to unborn child
- Through blood transfusions

- By sharing needles used to inject drugs

RECURRENT MALARIA:

Symptoms of malaria can recur after varying symptom-free periods. Depending upon the cause, recurrence can be classified as either recrudescence, relapse, or reinfection. Recrudescence is when symptoms return after a symptom-free period. It is caused by parasites surviving in the blood as a result of inadequate or ineffective treatment. Relapse is when symptoms reappear after the parasites have been eliminated from blood but persist as dormant hypnozoites in liver cells[54]. Relapse commonly occurs between 8–24 weeks and is often seen in *P. vivax* and *P. ovale* infections.

However, relapse-like *P. vivax* recurrences are probably being over-attributed to hypnozoite activation. Some of them might have an extra-vascular merozoite origin, making these recurrences recrudescences, not relapses. One newly recognized, non-hypnozoite, possible contributing source to recurrent peripheral *P. vivax* parasitemia is erythrocytic forms in bone marrow. *P. vivax* malaria cases in temperate areas often involve overwintering by hypnozoites, with relapses beginning the year after the mosquito bite. Reinfection means the parasite that caused the past infection was eliminated from the body but a new parasite was introduced[55]. Reinfection cannot readily be distinguished from recrudescence, although recurrence of infection within two weeks of treatment for the initial infection is typically attributed to treatment failure. People may develop some immunity when exposed to frequent infections.

GENETIC RESISTANCE:

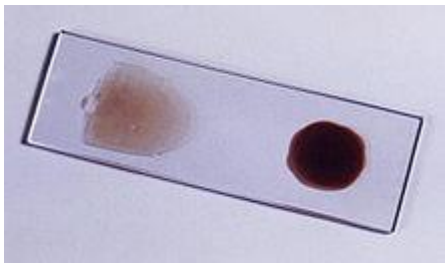
According to a 2005 review, due to the high levels of mortality and morbidity caused by malaria—especially the *P. falciparum* species—it has placed the greatest selective pressure on the human genome in recent history[56]. Several genetic factors provide some resistance to it including sickle cell trait, thalassaemia traits, glucose-6-phosphate dehydrogenase deficiency, and the absence of Duffy antigens on red blood cells.

The impact of sickle cell trait on malaria immunity illustrates some evolutionary trade-offs that have occurred because of endemic malaria. Sickle cell trait causes a change in the hemoglobin molecule in the blood. Normally, red blood cells have a very flexible, biconcave shape that allows them to move through narrow capillaries; however, when the modified hemoglobin S molecules are exposed to low amounts of oxygen, or crowd together due to dehydration, they can stick together forming strands that cause the cell to sickle or distort into a curved shape. In these strands the molecule is not as effective in taking or releasing oxygen, and the cell is not flexible enough to circulate freely. In the early stages of malaria, the parasite can cause infected red cells to sickle, and so they are removed from circulation sooner[55, 56]. This reduces the frequency with which malaria parasites complete their life cycle in the cell. Individuals who are homozygous (with two copies of the abnormal hemoglobin beta allele) have sickle-cell anaemia, while those who are heterozygous (with one abnormal allele and one normal allele) experience resistance to malaria without severe anemia. Although the shorter life expectancy for those with the homozygous condition would tend to disfavor the trait's survival, the trait is preserved in malaria-prone regions because of the benefits provided by the heterozygous form.

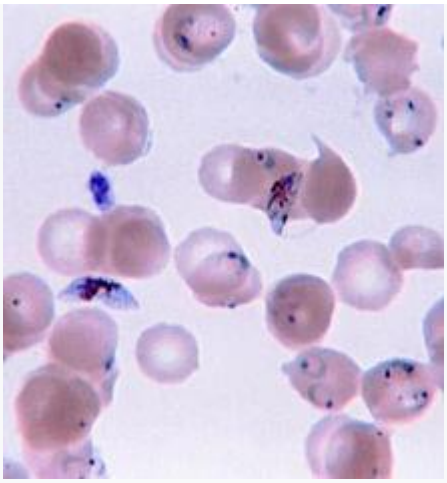
Liver dysfunction:

Liver dysfunction as a result of malaria is uncommon and usually only occurs in those with another liver condition such as viral hepatitis or chronic liver disease. The syndrome is sometimes called *malarial hepatitis*. While it has been considered a rare occurrence, malarial hepatopathy has seen an increase, particularly in Southeast Asia and India[57]. Liver compromise in people with malaria correlates with a greater likelihood of complications and death.

DIAGNOSIS:



The blood film is the gold standard for malaria diagnosis.



Ring-forms and gametocytes of *Plasmodium falciparum* in human blood

Owing to the non-specific nature of the presentation of symptoms, diagnosis of malaria in non-endemic areas requires a high degree of suspicion, which might be elicited by any of the following: recent travel history, enlarged spleen, fever, low number of platelets in the blood, and higher-than-normal levels of bilirubin in the blood combined with a normal level of white blood cells[56]. Reports in 2016 and 2017 from countries where malaria is common suggest high levels of over diagnosis due to insufficient or inaccurate laboratory testing.

Malaria is usually confirmed by the microscopic examination of blood films or by antigen-based rapid diagnostic tests (RDT). In some areas, RDTs must be able to distinguish whether the malaria symptoms are caused by *Plasmodium falciparum* or by other species of parasites since treatment strategies could differ for non-*P. falciparum* infections. Microscopy is the most commonly used method to detect the malarial parasite—about 165 million blood films were examined for malaria in 2010. Despite its widespread usage, diagnosis by microscopy suffers from two main drawbacks[57]: many settings (especially rural) are not equipped to perform the test, and the accuracy of the results depends on both the skill of the person examining the blood film and the levels of the parasite in the blood. The sensitivity of blood films ranges from 75–90% in optimum conditions, to as low as 50%. Commercially available RDTs are often more accurate than blood films at predicting the presence of malaria parasites, but they are widely variable in diagnostic sensitivity and specificity depending on manufacturer, and are unable to tell how many parasites are present[58].

In regions where laboratory tests are readily available, malaria should be suspected, and tested for, in any unwell person who has been in an area where malaria is endemic. In areas that cannot afford laboratory diagnostic tests, it has become common to use only a history of fever as the indication to treat for malaria—thus the common teaching "fever equals malaria

unless proven otherwise". A drawback of this practice is overdiagnosis of malaria and mismanagement of non-malarial fever, which wastes limited resources, erodes confidence in the health care system, and contributes to drug resistance. Although polymerase chain reaction-based tests have been developed, they are not widely used in areas where malaria is common as of 2012, due to their complexity.

CLASSIFICATION:

Malaria is classified into either "severe" or "uncomplicated" by the World Health Organization (WHO). It is deemed severe when *any* of the following criteria are present, otherwise it is considered uncomplicated[59].

- Decreased consciousness
- Significant weakness such that the person is unable to walk
- Inability to feed
- Two or more convulsions
- Low blood pressure (less than 70 mmHg in adults and 50 mmHg in children)
- Breathing problems
- Circulatory shock
- Kidney failure or hemoglobin in the urine
- Bleeding problems, or hemoglobin less than 50 g/L (5 g/dL)
- Pulmonary oedema
- Blood glucose less than 2. 2 mmol/L (40 mg/dL)
- Acidosis or lactate levels of greater than 5 mmol/L
- A parasite level in the blood of greater than 100, 000 per microlitre (µL) in low-intensity transmission areas, or 250, 000 per µL in high-intensity transmission areas

Cerebral malaria is defined as a severe *P. falciparum*-malaria presenting with neurological symptoms, including coma (with a Glasgow coma scale less than 11, or a Blantyre coma scale less than 3), or with a coma that lasts longer than 30 minutes after a seizure[60].

Various types of malaria have been called by the names below:

Name	**Pathogen**	**Notes**
algid malaria	*Plasmodium falciparum*	severe malaria affecting the cardiovascular system and causing chills and circulatory shock
bilious malaria	*Plasmodium falciparum*	severe malaria affecting the liver and causing vomiting and jaundice
cerebral malaria	*Plasmodium falciparum*	severe malaria affecting the cerebrum
congenital malaria	various plasmodia	plasmodium introduced from the mother via the fetal circulation
falciparum malaria, *Plasmodium*	*Plasmodium falciparum*	

Name	Pathogen	Notes
falciparum malaria, pernicious malaria		
ovale malaria, *Plasmodium ovale* malaria	*Plasmodium ovale*	
quartan malaria, malariae malaria, *Plasmodium malariae* malaria	*Plasmodium malariae*	paroxysms every fourth day (quartan), counting the day of occurrence as the first day
quotidian malaria	*Plasmodium falciparum*, *Plasmodium vivax*	paroxysms daily (quotidian)
tertian malaria	*Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*	paroxysms every third day (tertian), counting the day of occurrence as the first

Name	Pathogen	Notes
transfusion malaria	various plasmodia	plasmodium introduced by blood transfusion, needle sharing, or needlestick injury
vivax malaria, *Plasmodium vivax* malaria	*Plasmodium vivax*	

PREVENTION:

If you live in or are traveling to an area where malaria is common, take steps to avoid mosquito bites. Mosquitoes are most active between dusk and dawn. To protect yourself from mosquito bites, you should[61]:

- **Cover your skin.** Wear pants and long-sleeved shirts.
- **Apply insect repellant to skin and clothing.** Sprays containing DEET can be used on skin and sprays containing permethrin are safe to apply to clothing.
- **Sleep under a net.** Bed nets, particularly those treated with insecticide, help prevent mosquito bites while you are sleeping.

Methods used to prevent malaria include medications, mosquito elimination and the prevention of bites. There is no vaccine for malaria. The presence of malaria in an area requires a combination of high human population density, high anopheles mosquito population density and high rates of transmission from humans to mosquitoes and from mosquitoes to humans. However, unless the parasite is eliminated from the whole world, it could re-establish if conditions revert to a combination that favors the parasite's reproduction. Furthermore, the cost per person of eliminating anopheles mosquitoes rises with decreasing population density, making it economically unfeasible in some areas[62].

Prevention of malaria may be more cost-effective than treatment of the disease in the long run, but the initial costs required are out of reach of many of the world's poorest people[63]. There is a wide difference in the costs of control (i. e. maintenance of low endemicity) and elimination programs between countries.

In areas where malaria is common, children under five years old often have anemia, which is sometimes due to malaria. Giving children with anemia in these areas preventive antimalarial medication improves red blood cell levels slightly but does not affect the risk of death or need for hospitalization.

MEDICATION:

There are a number of medications that can help prevent or interrupt malaria in travelers to places where infection is common. Many of these medications are also used in treatment. In places where *Plasmodium* is resistant to one or more medications, three medications—mefloquine, doxycycline, or the combination of atovaquone/proguanil (*Malarone*)—are frequently used for prevention. Doxycycline and

the atovaquone/proguanil are better tolerated while mefloquine is taken once a week[64]. Areas of the world with chloroquine sensitive malaria are uncommon.

The protective effect does not begin immediately, and people visiting areas where malaria exists usually start taking the drugs one to two weeks before they arrive, and continue taking them for four weeks after leaving (except for atovaquone/proguanil, which only needs to be started two days before and continued for seven days afterward). The use of preventative drugs is often not practical for those who live in areas where malaria exists, and their use is usually only in pregnant women and short-term visitors. This is due to the cost of the drugs, side effects from long-term use, and the difficulty in obtaining anti-malarial drugs outside of wealthy nations. During pregnancy, medication to prevent malaria has been found to improve the weight of the baby at birth and decrease the risk of anemia in the mother[65]. The use of preventative drugs where malaria-bearing mosquitoes are present may encourage the development of partial resistance.

TREATMENT:

Malaria is treated with antimalarial medications; the ones used depends on the type and severity of the disease. While medications against fever are commonly used, their effects on outcomes are not clear.

Simple or uncomplicated malaria may be treated with oral medications[60, 65].

- **Artemisinin-based combination therapies (ACTs).** The most effective treatment for *P. falciparum* infection is the use of artemisinins in combination with other antimalarials (known as artemisinin-combination therapy, or ACT), which decreases resistance to any single drug component. These additional antimalarials include: amodiaquine,

lumefantrine, mefloquine or sulfadoxine/pyrimethamine. [94 Another recommended combination is dihydroartemisinin and piperaquine. ACT is about 90% effective when used to treat uncomplicated malaria. To treat malaria during pregnancy, the WHO recommends the use of quinine plus clindamycin early in the pregnancy (1st trimester), and ACT in later stages (2nd and 3rd trimesters).

- **Chloroquine phosphate.** Chloroquine is the preferred treatment for any parasite that is sensitive to the drug. But in many parts of the world, the parasites that cause malaria are resistant to chloroquine, and the drug is no longer an effective treatment.

- Treatment of *P. vivax* requires both treatment of blood stages (with chloroquine or ACT) and clearance of liver forms with primaquine. Treatment with tafenoquine prevents relapses after confirmed *P. vivax* malaria.

Severe and complicated malaria are almost always caused by infection with *P. falciparum*. The other species usually cause only febrile disease. Severe and complicated malaria are medical emergencies since mortality rates are high (10% to 50%). Cerebral malaria is the form of severe and complicated malaria with the worst neurological symptoms. Recommended treatment for severe malaria is the intravenous use of antimalarial drugs. For severe malaria, parenteral artesunate was superior to quinine in both children and adults[66]. In another systematic review, artemisinin derivatives (artemether and arteether) were as efficacious as quinine in the treatment of cerebral malaria in children. Treatment of severe malaria involves supportive measures that are best done in a critical care unit. This includes the management of high fevers and the seizures that may result from it. It also includes monitoring for poor breathing effort, low blood sugar, and low blood potassium.

RESISTANCE:

Drug resistance poses a growing problem in 21st-century malaria treatment. Resistance is now common against all classes of antimalarial drugs apart from artemisinins. Treatment of resistant strains became increasingly dependent on this class of drugs. The cost of artemisinins limits their use in the developing world. Malaria strains found on the Cambodia–Thailand border are resistant to combination therapies that include artemisinins, and may, therefore, be untreatable[67]. Exposure of the parasite population to artemisinin monotherapies in subtherapeutic doses for over 30 years and the availability of substandard artemisinins likely drove the selection of the resistant phenotype. Resistance to artemisinin has been detected in Cambodia, Myanmar, Thailand, and Vietnam, and there has been emerging resistance in Laos. Resistance to the combination of artemisinin and piperaquine was detected in 2013 in Cambodia, and has since spread through Laos, Thailand and Vietnam (with up to 80 percent of malaria parasites resistant in some regions).

PROGNOSIS:

When properly treated, people with malaria can usually expect a complete recovery. However, severe malaria can progress extremely rapidly and cause death within hours or days. In the most severe cases of the disease, fatality rates can reach 20%, even with intensive care and treatment. Over the longer term, developmental impairments have been documented in children who have suffered episodes of severe malaria. Chronic infection without severe disease can occur in an immune-deficiency syndrome associated with a decreased responsiveness to *Salmonella* bacteria and the Epstein–Barr virus.

During childhood, malaria causes anemia during a period of rapid brain development, and also direct brain damage resulting from cerebral malaria[68]. Some survivors of cerebral malaria have an increased risk of neurological and cognitive deficits, behavioural disorders, and epilepsy. Malaria prophylaxis was shown to improve cognitive function and school performance in clinical trials when compared to placebo groups.

NO VACCINE YET:

Scientists around the world are trying to develop a safe and effective vaccine for malaria. As of yet, however, there is still no malaria vaccine approved for human use

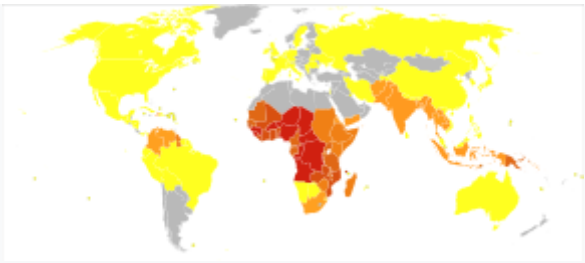
EPIDEMIOLOGY:

Distribution of malaria in the world:[120] ♦ Elevated occurrence of chloroquine- or multi-resistant malaria

♦ Occurrence of chloroquine-resistant malaria

♦ No *Plasmodium falciparum* or chloroquine-resistance

♦ No malaria



Deaths due to malaria per million persons in 2012

0–0

1–2

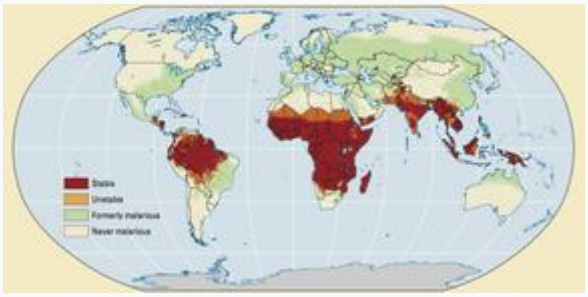
3–54

55–325

326–679

680–949

950–1, 358



Past and current malaria prevalence in 2009

The WHO estimates that in 2015 there were 214 million new cases of malaria resulting in 438, 000 deaths. Others have estimated the number of cases at between 350 and 550 million for falciparum malaria The majority of cases (65%) occur in children under 15 years old. About 125 million pregnant women are at risk of infection each year; in Sub-Saharan Africa, maternal malaria is associated with up to 200, 000 estimated infant deaths yearly. There are about 10, 000 malaria cases per year in Western Europe, and 1300–1500 in the United States. About 900 people died from the disease in Europe between 1993 and 2003. [65] Both the global incidence of disease and resulting mortality have declined in recent years. According to the WHO and UNICEF, deaths attributable to malaria in 2015 were reduced by 60% from a 2000 estimate of 985, 000, largely due to the widespread use of insecticide-treated nets and

artemisinin-based combination therapies. In 2012, there were 207 million cases of malaria. That year, the disease is estimated to have killed between 473, 000 and 789, 000 people, many of whom were children in Africa. Efforts at decreasing the disease in Africa since the turn of millennium have been partially effective, with rates of the disease dropping by an estimated forty percent on the continent[69].

Malaria is presently endemic in a broad band around the equator, in areas of the Americas, many parts of Asia, and much of Africa; in Sub-Saharan Africa, 85–90% of malaria fatalities occur. An estimate for 2009 reported that countries with the highest death rate per 100, 000 of population were Ivory Coast (86. 15), Angola (56. 93) and Burkina Faso (50. 66). A 2010 estimate indicated the deadliest countries per population were Burkina Faso, Mozambique and Mali. The Malaria Atlas Project aims to map global levels of malaria, providing a way to determine the global spatial limits of the disease and to assess disease burden. This effort led to the publication of a map of *P. falciparum* endemicity in 2010 and an update in 2019. As of 2010, about 100 countries have endemic malaria. Every year, 125 million international travellers visit these countries, and more than 30, 000 contract the disease. The geographic distribution of malaria within large regions is complex, and malaria-afflicted and malaria-free areas are often found close to each other. Malaria is prevalent in tropical and subtropical regions because of rainfall, consistent high temperatures and high humidity, along with stagnant waters where mosquito larvae readily mature, providing them with the environment they need for continuous breeding[70]. In drier areas, outbreaks of malaria have been predicted with reasonable accuracy by mapping rainfall Malaria is more common in rural areas than in cities. For example, several cities in the Greater Mekong Subregion of Southeast Asia are essentially malaria-free, but the disease is prevalent in many rural regions, including along international borders and forest fringes. In contrast, malaria in Africa is present in both rural and urban areas, though the risk is lower in the larger cities.

SOCIETY AND CULTURE:

Economic impact:

Malaria is not just a disease commonly associated with poverty: some evidence suggests that it is also a cause of poverty and a major hindrance to economic development. Although tropical regions are most affected, malaria's furthest influence reaches into some temperate zones that have extreme seasonal changes. The disease has been associated with major negative economic effects on regions where it is widespread. During the late 19th and early 20th centuries, it was a major factor in the slow economic development of the American southern states[69].

A comparison of average per capita GDP in 1995, adjusted for parity of purchasing power, between countries with malaria and countries without malaria gives a fivefold difference (US$1, 526 versus US$8, 268). In the period 1965 to 1990, countries where malaria was common had an average per capita GDP that increased only 0. 4% per year, compared to 2. 4% per year in other countries.

Poverty can increase the risk of malaria since those in poverty do not have the financial capacities to prevent or treat the disease. In its entirety, the economic impact of malaria has been estimated to cost Africa US$12 billion every year. The economic impact includes costs

of health care, working days lost due to sickness, days lost in education, decreased productivity due to brain damage from cerebral malaria, and loss of investment and tourism. The disease has a heavy burden in some countries, where it may be responsible for 30–50% of hospital admissions, up to 50% of outpatient visits, and up to 40% of public health spending.

Cerebral malaria is one of the leading causes of neurological disabilities in African children. Studies comparing cognitive functions before and after treatment for severe malarial illness continued to show significantly impaired school performance and cognitive abilities even after recovery. Consequently, severe and cerebral malaria have far-reaching socioeconomic consequences that extend beyond the immediate effects of the disease.

COUNTERFEIT AND SUBSTANDARD DRUGS:

Sophisticated counterfeits have been found in several Asian countries such as Cambodia, China, Indonesia, Laos, Thailand, and Vietnam, and are an important cause of avoidable death in those countries. The WHO said that studies indicate that up to 40% of artesunate-based malaria medications are counterfeit, especially in the Greater Mekong region. They have established a rapid alert system to rapidly report information about counterfeit drugs to relevant authorities in participating countries[71]. There is no reliable way for doctors or lay people to detect counterfeit drugs without help from a laboratory. Companies are attempting to combat the persistence of counterfeit drugs by using new technology to provide security from source to distribution.

Another clinical and public health concern is the proliferation of substandard antimalarial medicines resulting from inappropriate concentration of ingredients, contamination with other drugs or toxic impurities, poor quality ingredients, poor stability and inadequate packaging. A 2012 study demonstrated that roughly one-third of antimalarial

medications in Southeast Asia and Sub-Saharan Africa failed chemical analysis, packaging analysis, or were falsified.

ERADICATION EFFORTS:

Members of the Malaria Commission of the League of Nations collecting larvae on the Danube delta, 1929

Several notable attempts are being made to eliminate the parasite from sections of the world, or to eradicate it worldwide. In 2006, the organization Malaria No More set a public goal of eliminating malaria from Africa by 2015, and the organization claimed they planned to dissolve if that goal was accomplished. As of 2018 they are still functioning. Several malaria vaccines are in clinical trials, which are intended to provide protection for children in endemic areas and reduce the speed of transmission of the disease.

As of 2012, The Global Fund to Fight AIDS, Tuberculosis and Malaria has distributed 230 million insecticide-treated nets intended to stop mosquito-borne transmission of malaria[71]. The U. S. -based Clinton Foundation has worked to manage demand and stabilize prices in the artemisinin market. Other efforts, such as the Malaria Atlas Project, focus on analysing climate and weather information required to accurately predict the spread of malaria based on the availability of habitat of malaria-carrying parasites. The Malaria Policy Advisory Committee (MPAC) of the World Health Organization (WHO) was formed in 2012, "to provide strategic advice and technical input to WHO on all aspects of malaria control and elimination". In November 2013, WHO and the malaria vaccine funders group set a goal to

develop vaccines designed to interrupt malaria transmission with the long-term goal of malaria eradication.

Malaria has been successfully eliminated or greatly reduced in certain areas. Malaria was once common in the United States and southern Europe, but vector control programs, in conjunction with the monitoring and treatment of infected humans, eliminated it from those regions. Several factors contributed, such as the draining of wetland breeding grounds for agriculture and other changes in water management practices, and advances in sanitation, including greater use of glass windows and screens in dwellings. Malaria was eliminated from most parts of the United States in the early 20th century by such methods, and the use of the pesticide DDT and other means eliminated it from the remaining pockets in the South in the 1950s as part of the National Malaria Eradication Program.

In 2015 the WHO targeted a 90% reduction in deaths from malaria by 2030 and Bill Gates said in 2016 that he thought global eradication would be possible by 2040.

In 2018, WHO announced that Paraguay was free of malaria, after an eradication effort that began in 1950.

As of 2019, the eradication process is ongoing, but with the current approaches and tools, it is will be very hard to achieve a world free of malaria. Approaches may require investing more in research and greater universal health care[72]. Continuing surveillance will also be important to prevent return of malaria in countries where the disease has been eliminated.

RESEARCH:

The Malaria Eradication Research Agenda (malERA) initiative was a consultative process to identify which areas of research and development (R&D) must be addressed for worldwide eradication of malaria.

Vaccine:

A vaccine against malaria called RTS, S, was approved by European regulators in 2015. It is undergoing pilot trials in select countries in 2016.

Immunity (or, more accurately, tolerance) to *P. falciparum* malaria does occur naturally, but only in response to years of repeated infection. An individual can be protected from a *P. falciparum* infection if they receive about a thousand bites from mosquitoes that carry a version of the parasite rendered non-infective by a dose of X-ray irradiation. The highly polymorphic nature of many *P. falciparum* proteins results in significant challenges to vaccine design. Vaccine candidates that target antigens on gametes, zygotes, or ookinetes in the mosquito midgut aim to block the transmission of malaria. These transmission-blocking vaccines induce antibodies in the human blood; when a mosquito takes a blood meal from a protected individual, these antibodies prevent the parasite from completing its development in the mosquito[73]. Other vaccine candidates, targeting the blood-stage of the parasite's life cycle, have been inadequate on their own.] For example, SPf66 was tested extensively in areas where the disease is common in the 1990s, but trials showed it to be insufficiently effective.

MEDICATION:

Malaria parasites contain apicoplasts, organelles usually found in plants, complete with their own genomes. These apicoplasts are thought to have originated through the endosymbiosis of algae and play a crucial role in various aspects of parasite metabolism, such as fatty acid biosynthesis. Over 400 proteins have been found to be produced by apicoplasts and these are now being investigated as possible targets for novel anti-malarial drugs.

With the onset of drug-resistant *Plasmodium* parasites, new strategies are being developed to combat the widespread disease[74]. One such approach lies in the introduction of synthetic pyridoxal-amino acid adducts, which are taken up by the parasite and ultimately interfere with its ability to create several essential B vitamins. Antimalarial drugs using synthetic metal-based complexes are attracting research interest.

- (+)-SJ733: Part of a wider class of experimental drugs called spiroindolone. It inhibits the ATP4 protein of infected red blood cells that cause the cells to shrink and become rigid like the aging cells. This triggers the immune system to eliminate the infected cells from the system as demonstrated in a mouse model. As of 2014, a Phase 1 clinical trial to assess the safety profile in human is planned by the Howard Hughes Medical Institute.
- NITD246 and NITD609: Also belonged to the class of spiroindolone and target the ATP4 protein.

OTHER:

A non-chemical vector control strategy involves genetic manipulation of malaria mosquitoes. Advances in genetic engineering technologies make it possible to introduce foreign DNA into the mosquito genome and either decrease the lifespan of the mosquito, or make it more resistant to the malaria parasite[75]. Sterile insect technique is a genetic control method whereby large numbers of sterile male mosquitoes are reared and released. Mating with wild females reduces the wild population in the subsequent generation; repeated releases eventually eliminate the target population.

Genomics is central to malaria research. With the sequencing of *P. falciparum*, one of its vectors *Anopheles gambiae*, and the human genome, the genetics of all three organisms in the malaria lifecycle can be studied. Another new application of genetic technology is the ability

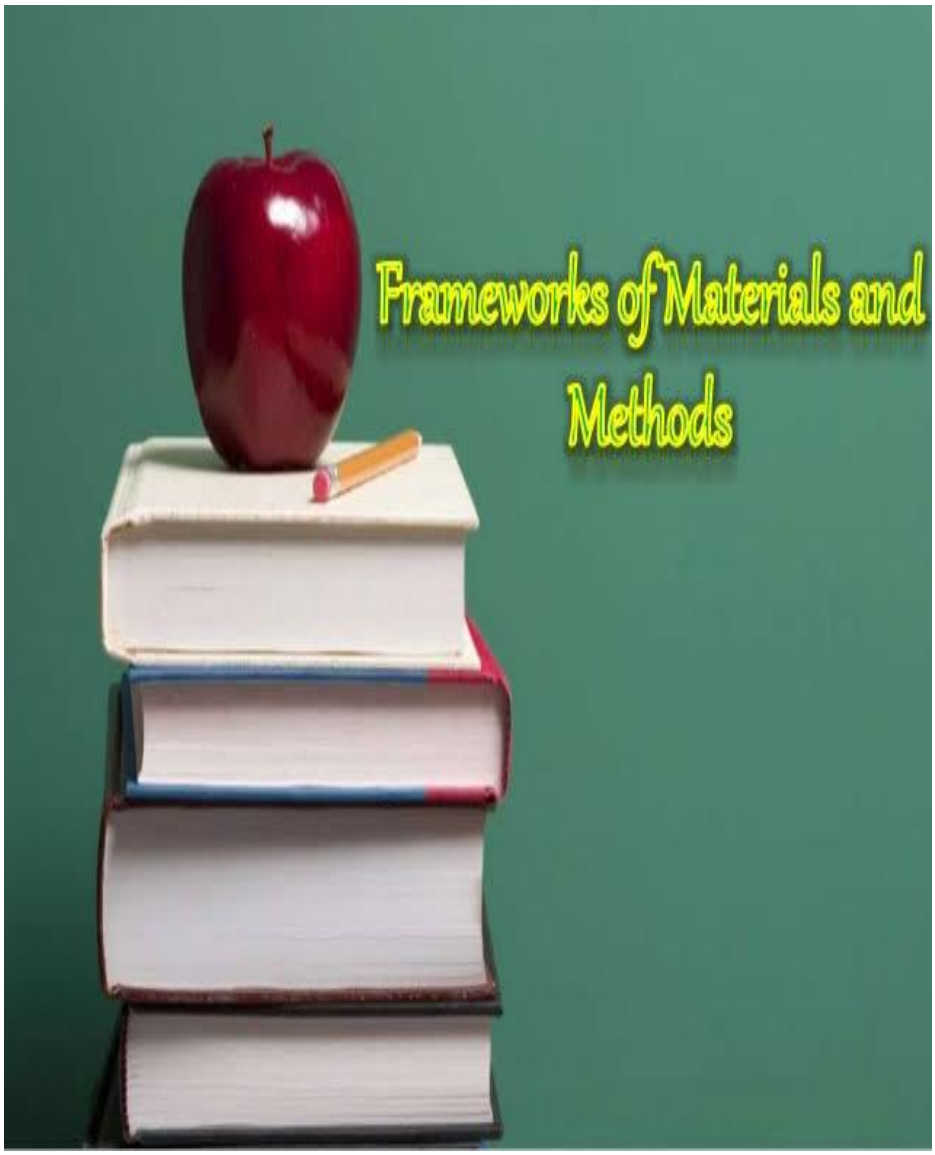
to produce genetically modified mosquitoes that do not transmit malaria, potentially allowing biological control of malaria transmission.

In one study, a genetically-modified strain of *Anopheles stephensi* was created that no longer supported malaria transmission, and this resistance was passed down to mosquito offspring[76].

Gene drive is a technique for changing wild populations, for instance to combat or eliminate insects so they cannot transmit diseases (in particular mosquitoes in the cases of malaria, zika, dengue and yellow fever).

OTHER ANIMALS:

Nearly 200 parasitic *Plasmodium* species have been identified that infect birds, reptiles, and other mammals, and about 30 species naturally infect non-human primates. Some malaria parasites that affect non-human primates (NHP) serve as model organisms for human malarial parasites, such as *P. coatneyi* (a model for *P. falciparum*) and *P. cynomolgi* (*P. vivax*[75, 76]). Diagnostic techniques used to detect parasites in NHP are similar to those employed for humans. Malaria parasites that infect rodents are widely used as models in research, such as *P. berghei*. Avian malaria primarily affects species of the order Passeriformes, and poses a substantial threat to birds of Hawaii, the Galapagos, and other archipelagoes[77]. The parasite *P. relictum* is known to play a role in limiting the distribution and abundance of endemic Hawaiian birds. Global warming is expected to increase the prevalence and global distribution of avian malaria, as elevated temperatures provide optimal conditions for parasite reproduction[78].


Frameworks of Materials and Methods

7. MATERIALS AND METHODS

List of Materials

Table 6. 1. Materials used

MATERIALS	SUPPLIER
Artemisinin	Sigma aldrich pvt. ltd
Egg Phosphatidyl choline	Sigma aldrich pvt. ltd
Cholesterol	Sigma aldrich pvt. ltd
Chloroform	M/S SD Fine Chemicals, Mumbai, India
Methanol	M/S SD Fine Chemicals, Mumbai, India
Potassium di hydrogen phosphate	M/S SD Fine Chemicals, Mumbai, India
Ortho phosphoric acid	M/S SD Fine Chemicals, Mumbai, India

Table 6. 2. Equipment list

S.NO	EQUIPMENTS	MODEL
1.	Electronic balance	Metler Toledo AG 135.
2.	Ultra centrifuge	Remi instruments, Mumbai.
3.	Mechanical stirrer	Remi instrument.
4.	DSC	Schimadzu DSC-60.
5.	Particle size analyser	Malveran master sizer.
6.	UV spectrophotometer	Schimadzu 1710, Mumbai.
7.	USP dissolution apparatus	Lab india, DS8000.

METHODS

PREFORMULATION STUDIES:

Preparation of calibration graph for Artemisinin:

Preparation of calibration curve in pH 1. 2, pH 7. 4 and pH 6. 8 buffer solutions:

An accurately weighed amount of Artemisinin 100mg was dissolved in small volume of buffer solutions in each of three 100 ml volumetric flask and the volume was adjusted to 100 ml with 1. 2 pH buffer in first volumetric flask, 7. 4 pH buffer in second volumetric flask and the third one was adjusted to 100 ml with 6. 8 pH buffer. A series of standard solution containing in the concentration range from 10 to 50 µg/ml of Artemisinin were prepared for 1. 2 pH buffer solution, 7. 4 pH buffer solution and 6. 8 pH buffer solution separately, absorbance was measured at 195 nm and calibration graph was plotted using concentration versus absorbance.

Drug-excipient compatibility study by DSC:

Differential scanning calorimetry (DSC):

Samples of individual components as well as each drug-excipient were weighed (Mettler Electronic balance) directly in pierced aluminum crucible pans (5-10 mg) and scanned in the 50-300°C temperature range under static air, with heating rate of 10 ºC /min, using shimadzu DSC-60 equipment.

METHOD OF PREPARATION[32-37]

Table 6. 3. Formula used for the preparation of Artemisinin Liposomes:

S. NO	FORMULATION	DRUG (mg)	Phosphatidyl Choline (mg)	Cholesterol (mg)
1.	**AL-1**	100	50	100
2.	**AL-2**	100	75	100
3.	**AL-3**	100	100	100
4.	**AL-4**	100	125	100
5.	**AL-5**	100	150	100

METHOD:

PREPARATION OF LIPOSOMES:

- Liposomes of Artemisinin were prepared by evaporation of solvent followed by hydration. Briefly, the selected lipids, drug and cholesterol were dissolved in a mixture of chloroform and methanol (ratio 2:1 v/v) in a 250ml round bottom flask.
- The solvent was evaporated in the rotary flash evaporator. The thin dry lipid film thus formed was hydrated using aqueous hydrating medium distilled water at 65°C.

- The procedure was repeated for the preparation of five batches of Artemisinin liposomes using various concentrations of phospholipids **(AL-1 to AL-5).**
- The formed liposomal dispersion was sonicated in probe sonicator using ice bath to prevent temperature induced distortion of liposomes.

CHARACTERIZATION STUDIES:

- Particle size and Zeta potential
- Encapsulation efficiency
- Drug content
- *In vitro* drug release

Particle size and Surface charge:

Surface charge is important in adhesion and interaction of particle with cells. The zeta-potential is used to measure the cell surface charge density. It can be measured using Malvern-Zeta sizer. The prepared liposomes were evaluated for their particle size and surface charge by photon correlation spectroscopy (PCS) using zeta sizer. The formulations were diluted to 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second (kcps). Analysis was carried out at 25°C with an angle of detection of 90°. In this experiment six replicates were taken for the measurement. The results were given in results and discussion section.

Drug content:

1gm of Artemisinin liposomes were accurately weighed and transferred into a 25ml volumetric standard flask. The sample was dissolved with methanol. 1ml of this solution was diluted to 25ml with the purified water. The standard Artemisinin was dissolved and diluted with same methanol and water respectively.

Then the standard and sample absorbance was measured at 195 nm using UV-Visible spectrophotometer. The percentage of drug content was calculated.

The results were given in results and discussion section.

Entrapment efficiency:

The drug loaded liposomes in buffer solutions were subjected to centrifugation at 15000 rpm for 30 min. The supernatant liquid was separated and 1ml of this solution was diluted with buffer solution and the absorbance was measured at 195 nm. The amount of Artemisinin unentrapped in the supernatant was calculated. The amount of Artemisinin entrapped was determined by subtracting amount of free unentrapped Artemisinin from the total amount of Artemisinin taken for the preparation.

The results were given in results and discussion section.

***In vitro* release:**

In vitro release studies were performed for 24 h using dialysis membrane by using the Franz diffusion cell. The prepared Artemisinin liposomes formulations were placed inside a dialysis membrane and immersed in buffer pH 6. 8. At predetermined time intervals the sample was withdrawn and the amount of Artemisinin released was determined by measuring the absorbance at 195 nm using a UV-Visible spectrophotometer. From the absorbance values the

cumulative percentage drug release was calculated. The results were given in results and discussion section.


Results

8. RESULTS AND DISCUSSION

Preformulation studies:

Preparation of calibration graph for Artemisinin

Standard calibration data of Artemisinin in pH 1. 2, 7. 4 and 6. 8 buffers at 195 nm

Table 7. 1. Absorbance of Artemisinin in buffer solutions

S. no	Concentration (µg/ml)	Absorbance		
		pH 1. 2	pH 7. 4	pH 6. 8
1	10	0. 045	0. 051	0. 065
2	20	0. 091	0. 103	0. 131
3	30	0. 137	0. 154	0. 196
4	40	0. 182	0. 205	0. 263
5	50	0. 274	0. 257	0. 327

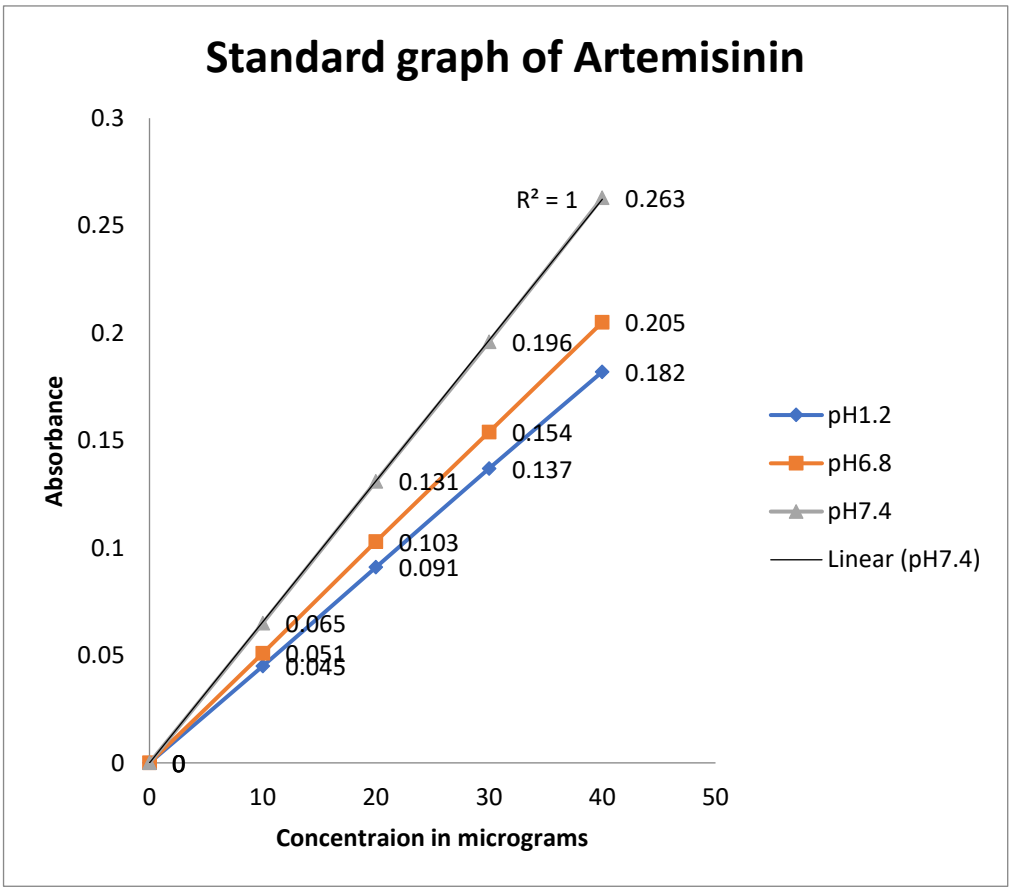


Fig. 7. 1. Calibration curve of Artemisinin in pH 1. 2, 7. 4 and 6. 8 buffers

Standard calibration curve of Artemisinin was carried out in 1. 2 pH, 7. 4 pH and 6. 8 pH buffer at 195 nm. The r^2 value in the entire medium shows nearly 1, which signifies linearity.

DSC analysis:

DSC of Artemisinin showed a sharp endothermic peak at about 206. 26^0C (melting point). The physical mixture of Artemisinin with other excipients also showed the same thermal behavior (206. 28^0C) as the individual component. DSC results also revealed that the physical mixture of Artemisinin with excipients showed superimposition of the thermogram. There was no significant change observed in melting endotherm of physical mixture of Artemisinin and excipients.

Hence from the DSC study, it was found that there was no interaction between Artemisinin and other excipients used in the formulation.

The DSC thermogram were given in the **Fig. 7. 2 and 7. 3**

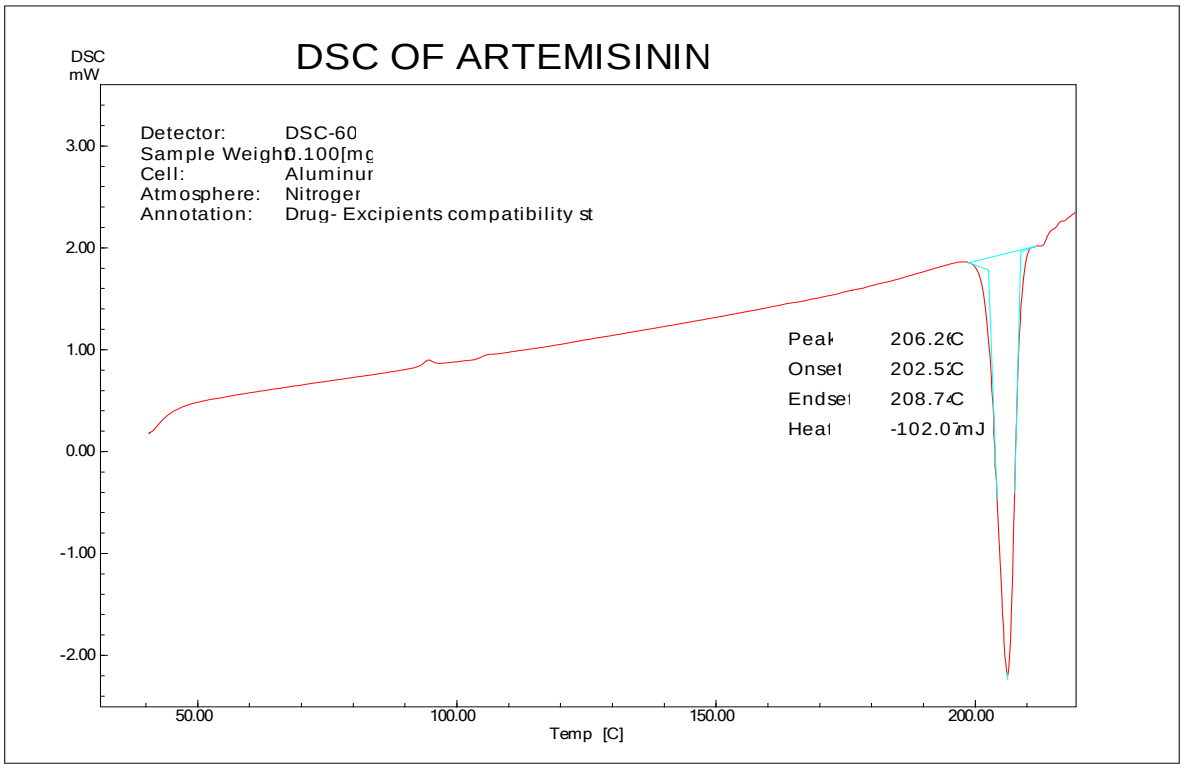


Fig. 7. 2

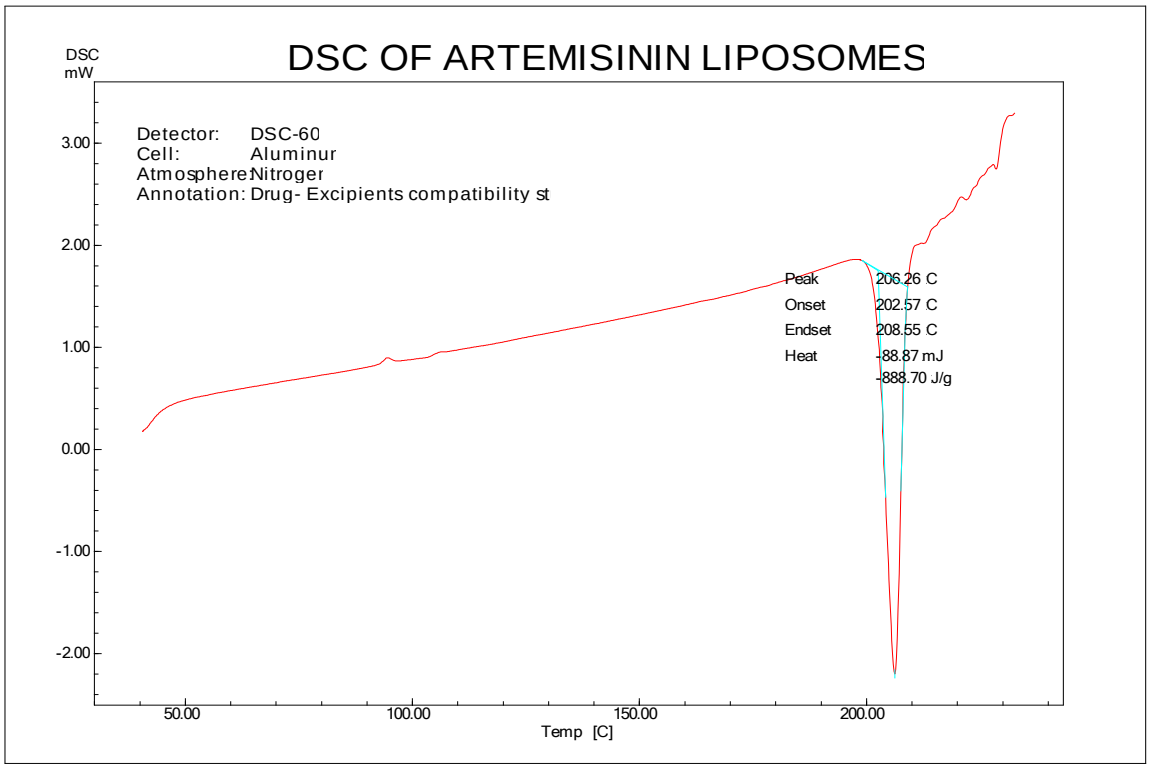


Fig. 7. 3

Fig. 7. 2 and 7. 3. DSC Thermogram of Artemisinin and Artemisinin Liposomes

Drug –Excipients accelerated compatibility study - Physical observation and assay

Upon analysis of the drug excipient mixture for their physical characteristics no colour change was observed. Based on the chemical evaluation it was found that there was no significant change observed indicating that the drug is compatible with the added ingredients. The results of this study were given in Table 7. 2

Table 7. 2. Physical characteristics of Artemisinin

S. No	Physical parameters	Results
1	Description	Off white powder
2	Melting point	196°C
3	Loss on drying	0. 35%
4	Assay	98. 58%

Table 7. 3 Physical characteristics of individual drug and excipients

S. No	Sample ID	Initial description	Final description
1.	Artemesinin	Off white powder	No change
2.	Phosphatidyl choline	Yellowish brown semisolid mass	No change

Table 7. 4 Physical characteristics of Drug-Excipient mixture

S. No	Sample ID	Initial description	Final description
1	Artemesinin	Off white powder	No change
2	Artemesinin + Phosphatidyl choline	Yellowish powder	No change

Table 7. 5 Chemical characteristics of Drug-Excipient mixture

S. No	Sample ID	Initial assay (%)	Final assay (%)
1.	Artemesinin	98. 52%±0. 24	98. 51%±0. 23
2.	Artemesinin+Phosphatidylcholine	98. 53%±0. 18	98. 53%±0. 11

n = 3; Mean ± S. E. M.

Table. 7. 6 Drug content and Entrapment efficiency Particle size and Zeta potential of Artemisinin Liposomes.

Trials	Zeta potential (mV)	Particle size (nm)	Entrapment Efficiency (%)	Drug Content (%)
AL-1	-17. 6	236. 8	37. 84	98. 37
AL-2	-18. 3	239. 5	56. 79	98. 45
AL-3	-19. 2	243. 7	61. 53	98. 49
AL-4	-20. 7	248. 8	76. 47	98. 50
AL-5	**-23. 4**	**252. 4**	**88. 76**	**98. 53**

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 252.4	Peak 1:	170.5	55.1	103.7
PdI: 0.789	Peak 2:	1528	44.9	1325
Intercept: 0.938	Peak 3:	0.000	0.0	0.000

Result quality : Good

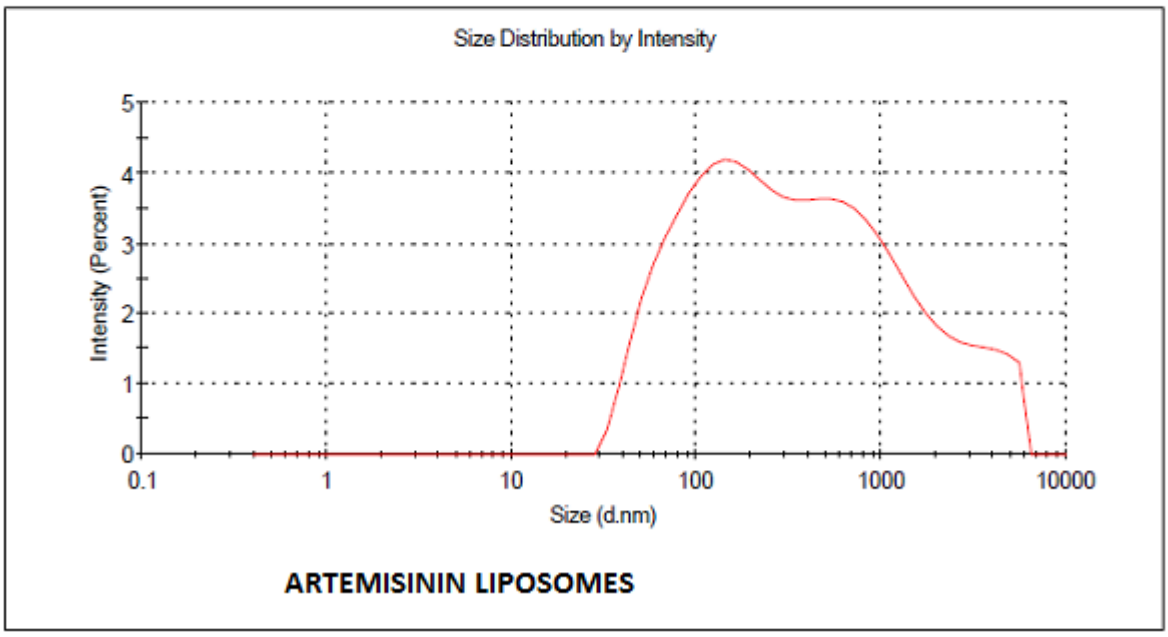


Fig. 7. 3 Particle size of optimized Artemisinin liposomes (AL-5)

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -23.4	Peak 1:	6.33	100.0	6.40
Zeta Deviation (mV): 6.40	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0714	Peak 3:	0.00	0.0	0.00
Result quality : Good				

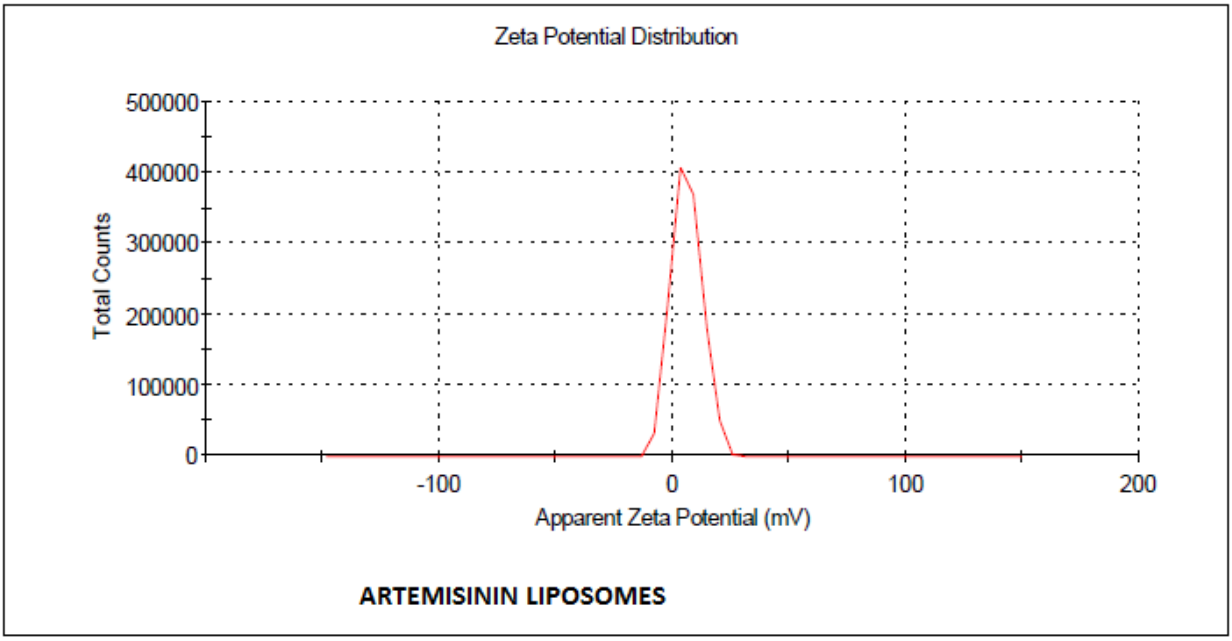


Fig. 7. 4 Zeta potential of optimized Artemisinin liposomes (AL-5)

- Particle size and entrapment efficiency of the **Artemisinin Liposomes (AL-1-AL-5)** were increased with increasing **Phosphatidyl Choline** concentration.
- This may be due to high amount of availability of Phospolipid to encapsulate the drug, upon increasing the **Phosphatidyl Choline** concentration, number of layers coated the drug was increased, this resulted in increased particle size and entrapment efficiency.
- Further increase in the **Phosphatidyl Choline** concentration **(AL-1-AL-5)**, there is no much increase in the entrapment efficiency due to the availability of the drug to be incorporated is low which is not enough for further encapsulation of drug by **Phosphatidyl Choline**.
- Based on the results of Particle size and entrapment efficiency of the **Artemisinin Liposomes** (**AL1**- **AL-5**), the trial **AL-5** which contains **150mg of Phosphatidyl Choline** concentration was selected as the best formulation

***In- vitro* drug release:**

Table 7. 7 *In vitro* release studies of Artemisinin Liposomes

S. NO	Time (Hrs)	%CUMULATIVE DRUG RELEASE				
		AL-1	AL-2	AL-3	AL-4	AL-5
1	0. 5	48. 67	40. 57	29. 87	20. 77	15. 84
2	1	76. 92	68. 64	57. 94	45. 86	32. 75
3	6	**98. 52**	90. 63	79. 37	67. 93	53. 27
4	12	98. 51	**98. 51**	87. 58	76. 65	65. 39
5	16	98. 50	98. 48	**98. 55**	84. 39	74. 67
6	20	98. 48	98. 47	98. 54	**98. 53**	87. 29
7	24	98. 46	98. 48	98. 52	98. 49	**98. 54**

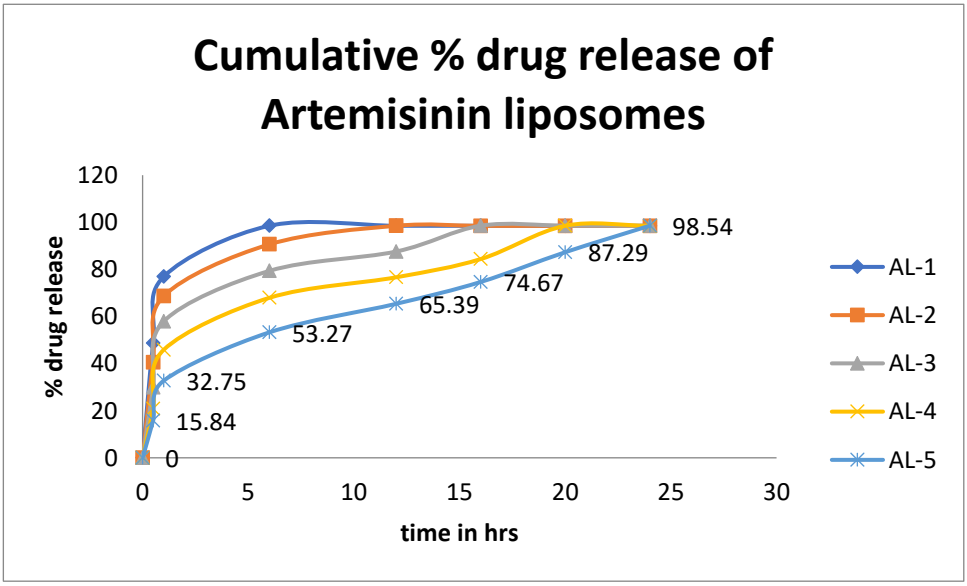


FIG. 7. 6

- From the *in vitro* drug release study results, the maximum percentage drug release **98. 54** at the end of 24hwas observed with trial **AL-5** which contains **150mg of Phosphatidyl choline**

- Below **150mg of Phosphatidyl choline** concentration as in the case of trials **AL-1 to AL-4,** the maximum percentage drug release **98. 52 %, 98. 51 % , 98. 55** and **98. 53 %** were obtained at the end of 6h. 12h, 16h and 20h respectively which was not desirable.

- Above **150mg of Phosphatidyl choline** concentration, reduction in drug release was observed for all the trials **(AL-1 to AL-5)**. The maximum percentage drug release for **AL-5** was found to be **98. 54%** at the end of 24h was obtained.

- From the *in vitro* drug release data for **AL-1 to AL-5,** it was observed that increase in **Phosphatidyl choline** concentration delays the drug release due to increased particle size and reduced surface area of the prepared liposomes.

- From all the formulations, **AL-5** was selected as best formulation due to its ideal particle size (**252. 4 nm**), Zeta Potential **(-23. 4)** , high entrapment efficiency (**88. 76%**) and desirable drug release **98. 54 %** at the end of 24 h.

9. SUMMARY AND CONCLUSIONS

The active pharmaceutical ingredient **Artemisinin** was evaluated for its Organoleptic properties and solubility. The results obtained were satisfactory.

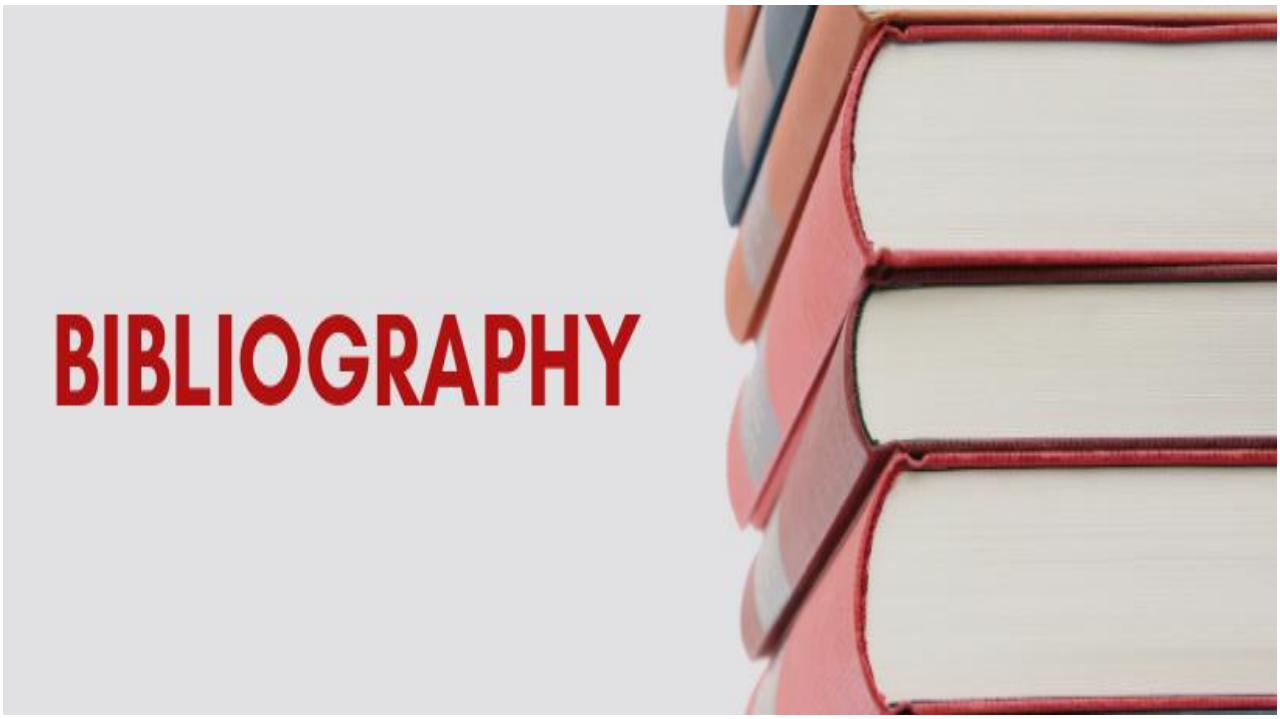
Artemisinin liposomes were prepared by solvent evaporation, followed by hydration with water and the **Phosphatidyl choline** concentrations were optimized by various trials

In the present study liposomes containing **Artemisinin** was prepared. The effect of increase in **Phosphatidyl choline** concentration in various parameters like **particle size, zeta potential and *in vitro*** release profile were studied.

The **Artemisinin** liposomes were formulated and evaluated for its drug content, entrapment efficiency, particle size analysis, zeta potential and *invitro* drug release profile.

Based on the results of **Artemisinin** liposomes formulations **(AL-1- AL-5)** formulation **AL-5** was selected as the best formulation in which the particle size was **252. 4 nm** and the entrapment was **88. 76%**.

The *in vitro* % drug release of **AL-5** formulation was **98. 54 %** and it was found to be suitable formulation for the treatment of Malaria. Hence it can be concluded that the newly formulated controlled release liposomal drug delivery systems of **Artemisinin** may be ideal and effective in the treatment of Malaria by allowing the drug to release continuously for 24 hrs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