Formulation and Evaluation of Nanoliposome of Artemether and Lumefantrine of Antimalarial Drugs

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Abstract

Malaria is the most life-threatening disease prevalent in tropical and subtropical countries causing about 1–2 million deaths every year worldwide. *Plasmodium vivax, P. falciparum, P. malariae* and *P. ovale* are four distinct species that are causative agent for malaria in human beings. There are only a limited number of clinically effective antimalarial agents in existing practice. Problems such as poor and erratic oral bioavailability, lack of dose proportionality and degradation in gastrointestinal tract are the main reasons for poor clinical effectiveness of existing antimalarial agents. Rapid drug resistance and wide spread presence are the major hurdle to combat malaria. In addition, development of new chemical entities (NCEs) and launching of effective formulation into the market takes a long time and requires lot of revenue. In the past, solid dispersions (Abdul-Fattah et al., 2002), liposomes, polymeric nanoparticle and dendrimers have been developed and explored to counter the malaria. To improve the efficacy of the antimalarial agents, different types of route of administration such as transdermal and rectal routes have been utilized. To enhance the therapeutic efficacy of currently existing drugs, combination therapy may be proved as a boon which involves the participation of two antimalarial drugs with distinct site and mode of action. From long, combination therapy is preferred over monotherapy for malarial treatment; and among various combination therapies, artemisinin-based combination therapy is most preferred.

1.0 INTRODUCTION:

1.1 MALARIA PROGRESSION AND PATHOGENESIS

Malaria is a life-threatening infectious disease caused by parasites of the genus *Plasmodium* that are transmitted by infected *Anopheles* mosquitoes. Malaria remains a major cause of morbidity and mortality in tropical and subtropical regions of the world (Figure 2.1) (Gething et al., 2011). According to WHO, there were nearly 300 million episodes of malaria in 2016 that resulted in about 8 lakhs deaths (WHO., 2016). Increased prevention, control measures and new therapeutic interventions have led to a reduction in malaria mortality rates by more than 20% globally since 2000. Early diagnosis and treatment of malaria reduce disease, incidence and transmission of the parasite, thus contributing to the reduced death rates.

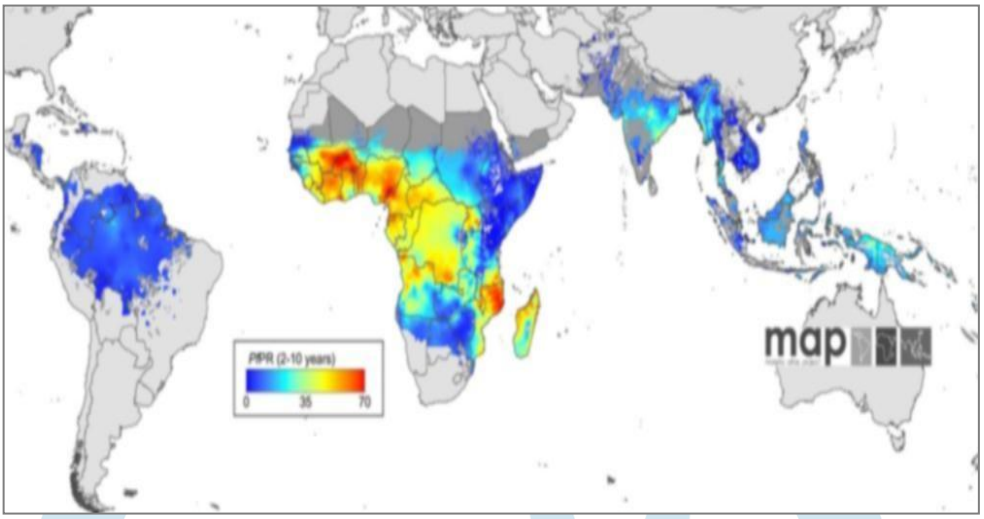


Fig.1.1: Geographical distribution of *P. falciparum* malaria

The five *Plasmodium* species affecting humans are: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. *P. falciparum* causes the most deadly, severe cases and affects naive adults, primigravid women and children. *P. vivax* produces less severe symptoms but it is more widespread and can cause a relapsing form of malaria due to the reactivation of hypnozoites in the liver. Development of drug resistance could result in an expansion of this debilitating and deadly infection (Baird et al., 2004; Price et al., 2009). The other species are found less frequently, representing only 5% of total malaria cases

Malaria infection (Figure 2.2) starts when a parasitized female *Anopheles*, during a blood meal, releases sporozoites into the circulation and then to the liver. Sporozoites invade hepatocytes in a few minutes and each parasite will develop and replicate into thousands of new merozoites (Prudencio et al., 2006).

These are released into the circulation to infect RBCs, where they undergo several cycles of asexual replication. Each asexual intraerythrocytic cycle lasts approximately 48 hours and is responsible for the clinical symptoms of malaria.

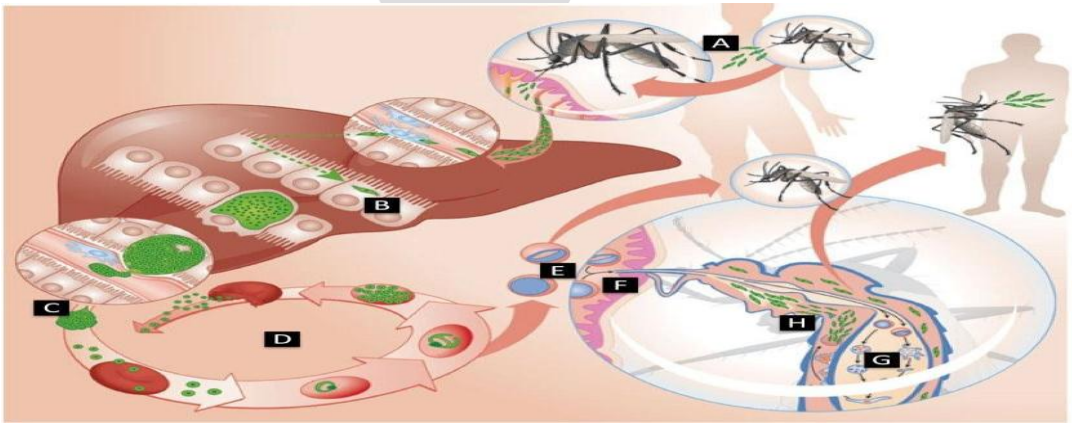


Fig. 1.2: *Plasmodium* life cycle. (A) Injection of sporozoites, (B) migration to the liver,

(C) release of merozoites into the bloodstream, (D) invasion of RBCs and asexual replication, (E) generation of gametocytes, (F) ingestion of gametocytes by the mosquito, (G) fertilization of gametes and (H) migration of sporozoites to the salivary glands and release during the next blood meal,

Inside RBCs, merozoites develop into ring, trophozoite and schizont stages, replicating to produce from 16 to 32 daughter merozoites that are released during egression. Free merozoites are then able to invade other erythrocytes to perpetuate the asexual blood-stage cycle, leading to an exponential increase in parasitemia (Prudencio et al., 2006).

Occasionally; some parasites differentiate into sexual erythrocytic stages, female or male gametocytes that, after a subsequent blood meal, reach the mosquito's midgut. Here, fertilization of gametes occurs forming ookinetes which transform into oocysts from which sporozoites are released and migrate to the mosquito salivary glands to restart the cycle at the next blood meal.

There are several important features of *P. falciparum* biology that make it a successful parasite and contribute to *Plasmodium* survival and transmission within an ever-changing host environment, such as its high asexual multiplication rate, an efficient evasion of host immunity through sequestration in the peripheral circulation, its elevated antigenic variation and the redundancy in erythrocyte invasion pathways. *P. falciparum* invasion involves the interaction of several parasite ligands with receptors that line the RBC surface (Mackinnon et al., 2010). The malaria parasite is capable not only of using a number of these receptors but also of varying the primary route used and adapting to variation in RBC surface receptors and still successfully infect the human host. Despite the substantial impact of this pathogen on human health, much of its basic biology is poorly understood (Callaway et al., 2007). Whilst each stage of the parasite lifecycle is the subject of intensive research, the centrality of blood stage infection to disease pathology has led to extensive effort towards understanding some of its core biological processes.

1.2 MALARIA TREATMENT

Accurate diagnosis of malaria is part of an effective disease management, and it is usually based on clinical suspicion and on the detection of parasites in the blood. Clinical suspicion of malaria should be confirmed with a parasitological diagnosis: microscopical observation and rapid diagnostic tests.

1.2.1 Antimalarial drugs

Effective malaria chemotherapy aims at treatment of the patient combined with blocking the infection of the parasite for the vector by exploiting the differences in metabolism between parasite and the host. The therapeutic efficacy of the drugs is limited by parasite resistance and unspecific drug toxicity (Salako et al., 1984). Current antimalarial drugs can be classified according to their biological activity (Figure 2.4) or their chemical structure (Suryanaryana **)**

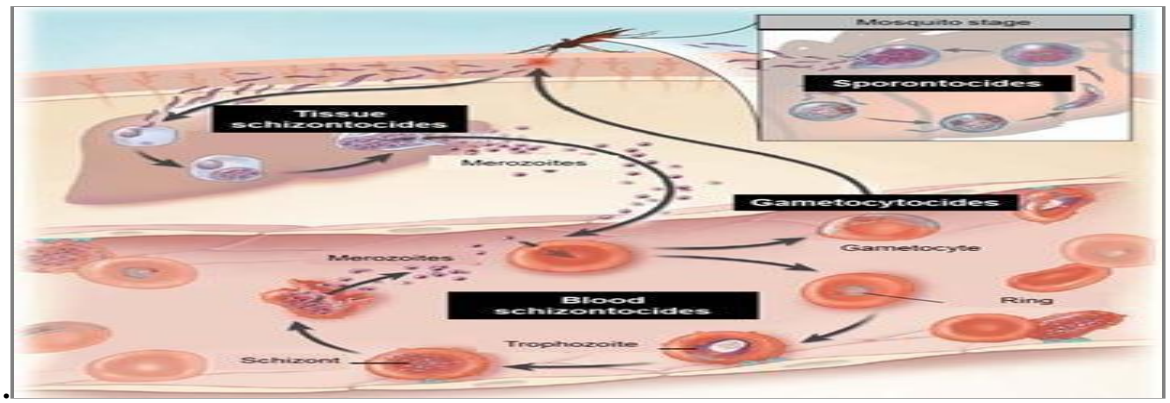


Fig. 2.4: Antimalarial drug activity in the life cycle of *Plasmodium*, showing tissue

schizontocides, blood-stage schizontocides, gametocytocides and sporontocides

(a) Blood schizontocides: Drugs acting on blood stages of the parasite, inhibiting the growth inside RBCs and terminating clinical attacks of malaria. Drugs belonging to this class can have a quick mode of action: chloroquine, quinine, mefloquine, halofantrine, artemisinin and its derivatives, or a slow mode of action: pyrimethamine, sulfadoxine, sulfones, tetracyclines and atovaquone (Davis et al., 2003).

(b) Tissue schizontocides: These drugs act on the primary tissue forms of Plasmodium in the liver. By blocking liver stages, further development of the infection can be prevented. However, since no symptoms are presented yet at this stage of the infection, this therapy is not used. Primaquine, pyrimethamine, proguanil and sulfonamides have activity against this stage.

(c) Hypnozoiticides: Drugs acting on hypnozoites from *P. vivax* and *P. ovale*, which remain latent in the liver. Primaquine is the only available drug for this stage.

(d) Gametocytocides: These drugs kill the sexual forms of the parasite in the blood, preventing transmission of the infection to the mosquito. Chloroquine and quinine have gametocytocidal activity against *P. vivax* and *P. malariae*, but not against *P. falciparum*. Primaquine and artemisinins have gametocytocidal activity against all human malarial parasite species.

(e) Sporontocides: Drugs that prevent the development of oocysts in the mosquito, blocking malaria transmission. Primaquine and chloroguanidine have activity against this stage.

Antimalarials can be also classified according to their chemical structure and mechanism of action in different groups: quinine-related drugs, artemisinin and its derivatives, antifolates and other antimalarials (Murambiwa et al., 2011).

2.0 DRUG PROFILE

Profile of Artemether

Artemether is an ether derivative of artemisinin used for the treatment of malaria particularly multi-drug resistant strains of *P.falciparum*. It is the most widely used artemisinin derivative with higher lipid solubility and better toleration.

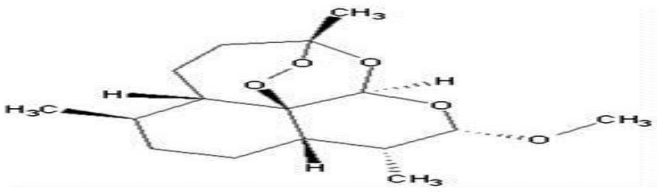


Fig. 2.1: Structure of artemether

Chemistry: It is available as white crystalline powder with melting point in the range of 86-90˚C. Its molecular mass is 298.4 and its chemical name is (3R,5aS,6R,8aS,9R,10S, 12R,12aR)-decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2- benzodioxepin.

It is practically insoluble in water; very soluble in chloroform, dichloro methane and acetone; very freely soluble in methanol, ethanol and ethyl acetate.

Pharmacokinetics: After oral administration, peak plasma concentrations occur around 2-3 hours. Following intramuscular injection, absorption is highly variable and peak plasma concentrations generally occur after around 6 hours. Absorption is slow and erratic and times to peak can be 18 hours or longer, especially in children with poor peripheral perfusion. Artemether is metabolized to DHA, the active metabolite. After oral administration DHA predominates, whereas after intramuscular injection artemether predominates. Biotransformation is mediated via the cytochrome P450enzyme CYP3A4. Artemether is 95% bound to plasma proteins. The elimination half-life is approximately 1 hour, but following intramuscular injection the elimination phase is prolonged because of continued absorption. No dose modifications are required in renal or hepatic impairment.

2.2 Profile of Lumefantrine

Lumefantrine, previously called benflumetol, has synthesized by the 1970s by the Academy of Military Medical Sciences in Beijing, China. It conforms structurally, physicochemically and in mechanism of action to the aryl amino alcohol group of antimalarial agents including quinine, halofantrine and mefloquine. The exact mechanism of action of lumefantrine on plasmodia is not fully understood. Most likely, it interacts with heme, a degradation product of the hemoglobin metabolism, with the active ingredients in the digestive vacuole of the malaria parasite. However, studies show that it may also interfere with the synthesis of nucleic acids and proteins.

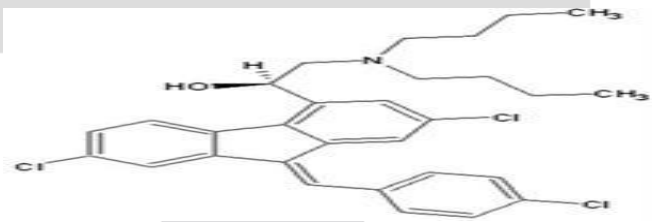


Fig. 2.2: Structure of lumefantrine

Chemistry*:* It is available as yellow crystalline powder with melting point in the range of 128 - 132˚C. Its molecular mass is 528.9 and its chemical name is 2-Dibutylamino-1-[2, 7- dichloro-9-(4-chlorobenzylidene)-9H-fluoren4-yl]-ethanol. It is practically insoluble in water; slightly soluble in ethanol and methanol; soluble in dichloromethane; and freely soluble in ethyl acetate. Lumefantrine can be assayed by non-aqueous titration and should contain not less than 98.0% and not more than 102.0% of $C_{30}H_{32}Cl_3NO$, calculated with reference to the dried substance.

Pharmacokinetics: The pharmacokinetic properties of lumefantrine are reminiscent of characteristics of halofantrine (White et al., 1999). Oral bioavailability is variable and is highly dependent on administration with fatty foods. With meal, absorption increases by 108% and is lower in patients with acute malaria than in convalescing patients. After oral administration, peak plasma levels occur

approximately after 10 h. The terminal

elimination half-life is around 2 to 3 days in healthy volunteers and 4 to 6 days in patients with clinically relevant *P. falciparum* infections.

3 EXPERIMENTAL WORK

3.1 MATERIALS

Artemether and lumefantrine were procured as a gift sample from IPCA Laboratories (Indore, India). oy lecithin was provided as a gift sample by Kamani Oil Industries Ltd (Mumbai, India). Cholesterol was purchased from SD Fine Chemicals (Mumbai, India). All other solvents used in the study were of analytical grade and were purchased from Merck Ltd (Mumbai, India).

3.2 PREFORMULATION STUDIES

3.2.1 Estimation by UV spectroscopy

UV visible spectroscopic method for analysis of artemether and lumefantrine was adopted in the present work. Separately, an accurately weighed quantity of drugs was dissolved in methanol to generate a stock solution having concentration of 2.0 mg/ml. Stock solution was further diluted to 100 mL to produce standard solution having concentration of 200 µg/ml. The standard solution was serially diluted with methanol to get working standard solutions having concentration of 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml. The wavelength of maximum absorbance was determined through double beam UV visible spectrophotometer against methanol as a blank. The standard plot was plotted by estimating absorbance wavelength of maximum absorbance for both the drugs and data was subjected to linear regression analysis in Microsoft Excel. Simultaneous estimation was done to observe interference of drugs with each other during analysis.

3.2.2Validation of method of estimation

The method was validated by determining linearity, range, accuracy and precision for both the drugs.

3.2.3 Solubility studies

The solubility of artemether and lumefantrine was determined in different solvents. An excess quantity of both the drugs was added in 5 mL of each solvent in screw capped glass test tubes and shaken for 12 hr at room temperature (Christensen et al., 2007). The solution was filtered, diluted and the solubility was determined by estimating drug by UV spectroscopy.

3.2.4 FTIR

Fourier transform infra-red (FT-IR) spectroscopy will be used to characterize the drugs. Spectra of artemether and lumefantrine were obtained using the potassium bromide disk method in absorbance mode in the spectral region of 4000-400 cm^{-1}.

3.2.5 DSC

Differential scanning calorimetry (DSC) scans of pure drugs were taken using DSC (Mettler Toledo, Germany) to characterize the drugs. The analysis was performed with a heating range of 10 °C to 250 °C and at a rate of 10 °C/min in nitrogen atmosphere. The sample weight was approximately 5 mg.

3.3 VESICLE PREPARATION

Liposomes were prepared by ether injection method under controlled condition. Briefly, soy lecithin and cholesterol at varied concentrations were first dissolved in ether (20 ml) in a glass beaker. Drugs, either individually or in combination, were separately dissolved in chloroform. Both the solution were mixed with each other to obtain an organic immiscible

phase. The organic phase was then slowly injected into 25 ml of aqueous phase containing pluronic F68. The solution was magnetically stirred and the temperature was maintained at 60°C to evaporate the organic phase. Traces of organic phase were then further removed by using rotary evaporator under reduced pressure. The formed dispersion was then sonicated using ultrasonicator (Hielscher Ultrasonics GmbH, Germany) to achieve size reduction. The nanoliposomes were then freeze dried in presence of sucrose as cryoprotectant. Suitability of sucrose as cryoprotectant at used concentration was checked by rehydration of lyophilized nanoliposomes with distilled water which shows ability to form aqueous suspension. All formulations were stored at 5°C until used. Formulations were prepared using different concentrations of artemether and lumefantrine alone and in combinations to optimize the final formulation (Liang et al., 2013).

3.4 VESICLE CHARACTERIZATION

3.4.3 Vesicle size

The mean vesicle size and polydispersity index (PI) of the samples were determined by dynamic light scattering using Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at 25°C. The vesicle aggregation was determined by calculating the change (%) in mean particle size of the vesicular dispersion after storage at room temperature for 15 days.

$$\text{Vesicle aggregation (\%)} = \frac{\text{Size after storage} - \text{Initial size}}{\text{Initial size}} \times 100$$

3.4.4 Vesicle morphology

Vesicle morphology was determined by transmission electron microscopy (TEM). Samples, stained with 1% uranyl acetate, were analyzed by TEM (Morgani 268D, Netherlands) at AIIMS, New Delhi. Data acquisition was done on the AMT image capture.

3.4.5 Drug entrapment and loading

To determine drug loading (DL) and entrapment efficiency (EE), the dried NLs were weighed and added to known volume of methanol and sonicated for 10 min. This causes lysis of vesicles and solubilization of drugs in methanol. The solution was then centrifuged at 2000 rpm for 10 min, supernatant liquid was removed and the amount of entrapped drugs, artemether and lumefantrine, were determined by UV spectrophotometry (UV 1700, Schimadzu, Japan) at absorbance of 260 nm and 286.2 nm, respectively according to developed UV method (Parashar et al., 2013). DL and EE were calculated based on the following equation:

$$\text{DL (\%)} = \frac{\text{Weight of drug in NLs}}{\text{Weight of NLs recovered}} \times 100$$

$$\text{EE (\%)} = \frac{\text{Total drug in NLs}}{\text{Total amount of drug}} \times 100$$

For drug leakage, the EE of the vesicles was determined after agitating the formulations on water bath shaker for 30 min.

3.5 IN VITRO EVALUATIONS

3.5.1 Drug release studies

Dialysis bag method was used for in vitro release study of nanoliposomes. Dialysis bag (molecular weight cut off: 12,000 g/mole; Sigma, USA) was filled with NLs equivalent to 20 and 120 mg of artemether and lumefantrine, respectively and then immersed in 50 ml phosphate buffer solution (pH 7.4) maintained at 37 ± 0.5 °C under mild agitation (50 rpm). 1 ml sample of the medium was taken out at pre-determined time intervals (0.5, 1, 2.5, 5, 10, 15, 20, 25 and 30 h), and replaced with the same amount of fresh medium. The withdrawn samples were assayed for drug content by measuring absorbance at 260 nm for artemether and at 286.2 nm for lumefantrine against the blank using UV

spectrophotometer (UV 1700, Schimadzu, Japan) (Parashar et al., 2013). The following equation was used to calculate % drug release.

$$\%\ \text{drug release} = \frac{\text{Amount of drug in releasing medium}}{\text{Total amount of drug}} \times 100$$

4.0 RESULTS AND DISCUSSION

4.1 DRUG IDENTIFICATION

4.1.1dentification of artemether

The melting point value of artemether is reported to be 89–90 ºC (Achhrish et al.,2012) and the DSC thermograms of artemether also depict as the endothermic peak within the range of 89–90 ºC . indicating that the obtained sample is of artemether.

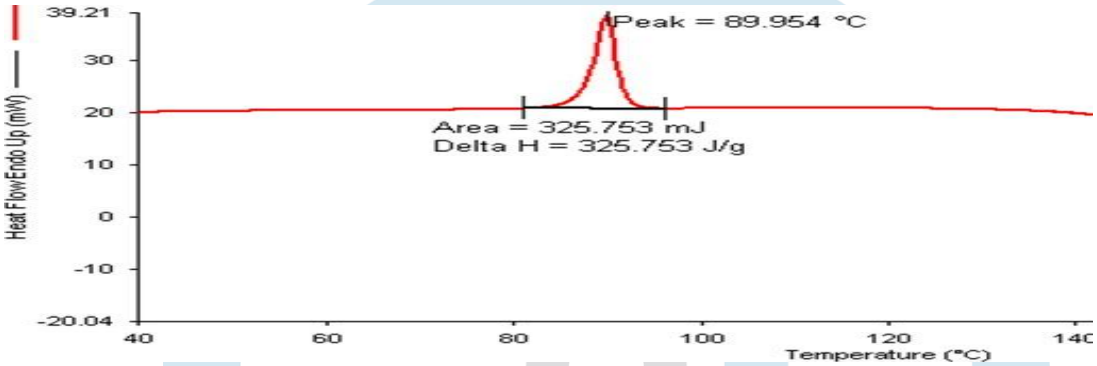


Fig.: DSC thermograms of artemether

Further confirmation was obtained by FTIR analysis wherein the spectra obtained matches the standard spectra of artemether . The results of the assay indicate that the obtained artemether has a purity of 99.95 %.

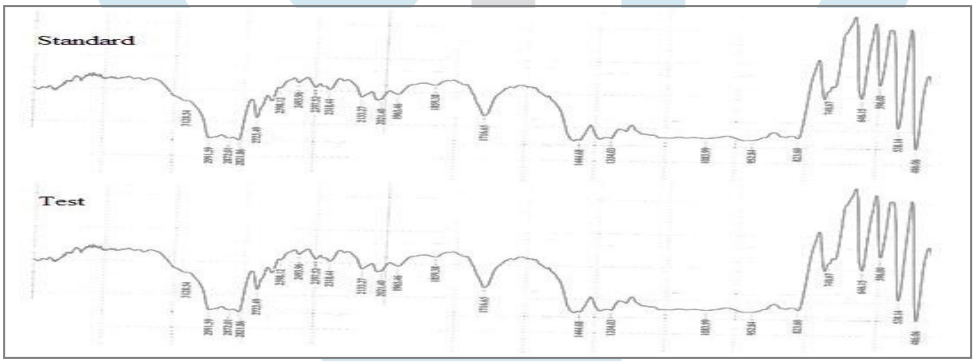


Fig.: FTIR spectra of artemether

4.1.2 Identification of lumefantrine

The DSC thermograms of lumefantrine showed endotherm at 133.6 ºC epresenting melting point of the drug which correlates well with reported value of 133- 135 ºC (Achhrish et al., 2012).
Fig.:DSC thermograms of lumefantrine

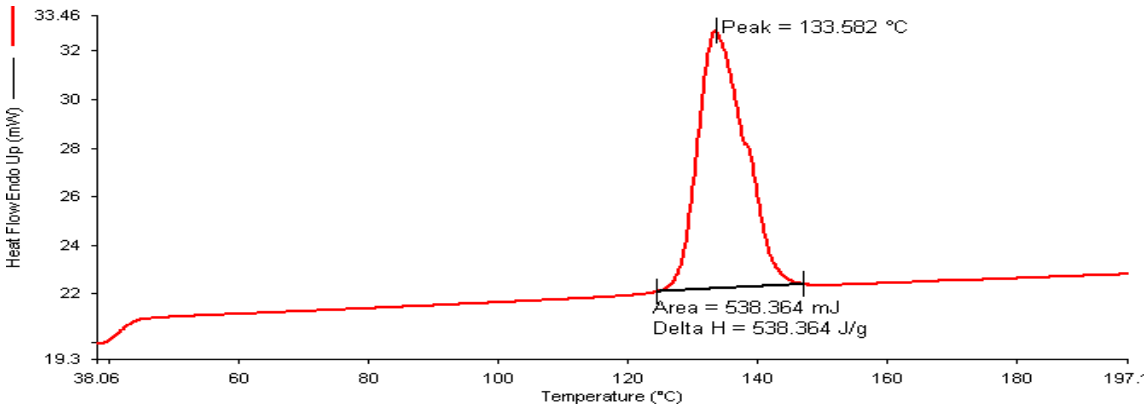
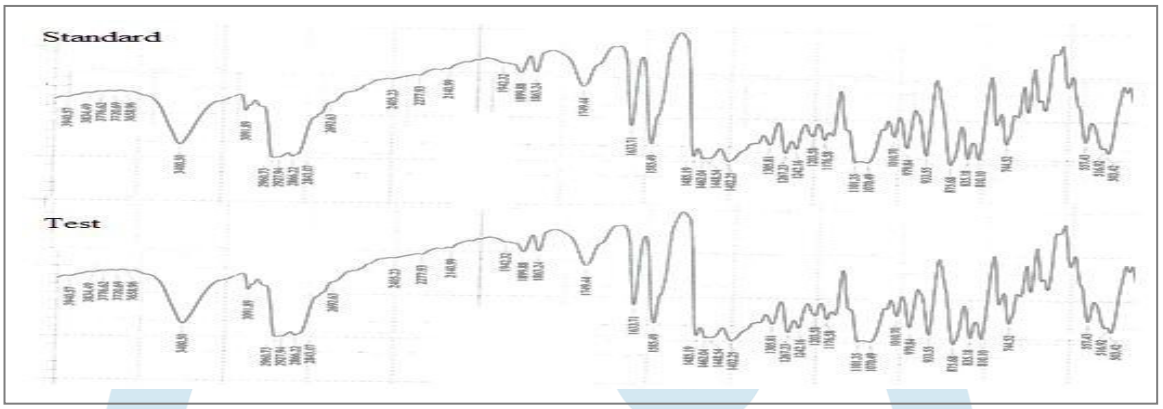


Fig. : FTIR spectra of lumefantrine

FTIR spectra of obtained lumefantrine exactly matched the standard FTIR spectra indicating that the obtained drug sample is of lumefantrine . Assay results indicated that the obtained sample of lumefantrine is 99.91% pure.



4.2 ANALYTICAL (UV) METHOD DEVELOPMENT

In the present work, UV spectroscopic method was developed for the simultaneous estimation of ART and LUM. For analysis through UV spectrophotometer, for both the drugs, calibration plot in the concentration range of 5-40 µg/ml was prepared in methanol. The absorbance values obtained are tabulated in Table and calibration plots are shown in Fig.

Table: Standard curve values for artemether and lumefantrine

S. No	Artemether		Lumefantrine	
	Conc. (µg/ml)	Abs. (268 nm)	Conc. (µg/ml)	Abs. (302 nm)
1	5	0.101	5	0.080
2	10	0.180	10	0.150
3	15	0.246	15	0.220
4	20	0.347	20	0.316
5	25	0.441	25	0.376
6	30	0.547	30	0.441
7	35	0.660	35	0.521
8	40	0.750	40	0.650

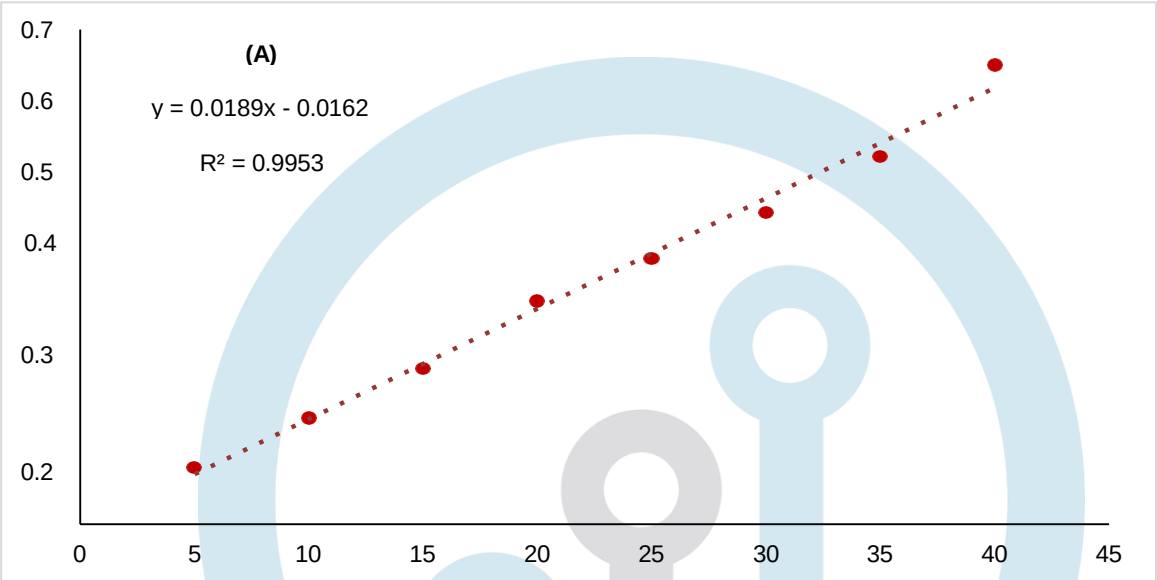
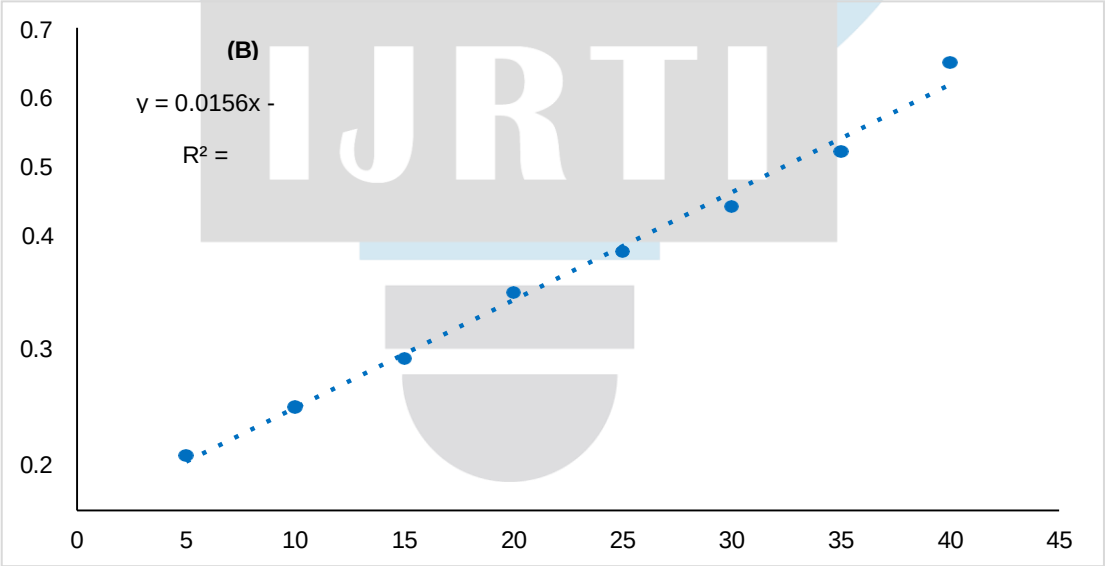


Fig.: Calibration plot of (A) artemether and (B) lumefantrine



Dilutions were prepared for both the drugs from their respective stock solutions and scanned in the spectrum mode from 400 nm to 200 nm. ART and LUM showed absorbance maxima at 268 nm and at 302 nm, respectively. The overlain spectra of both these drugs is shown in Fig.

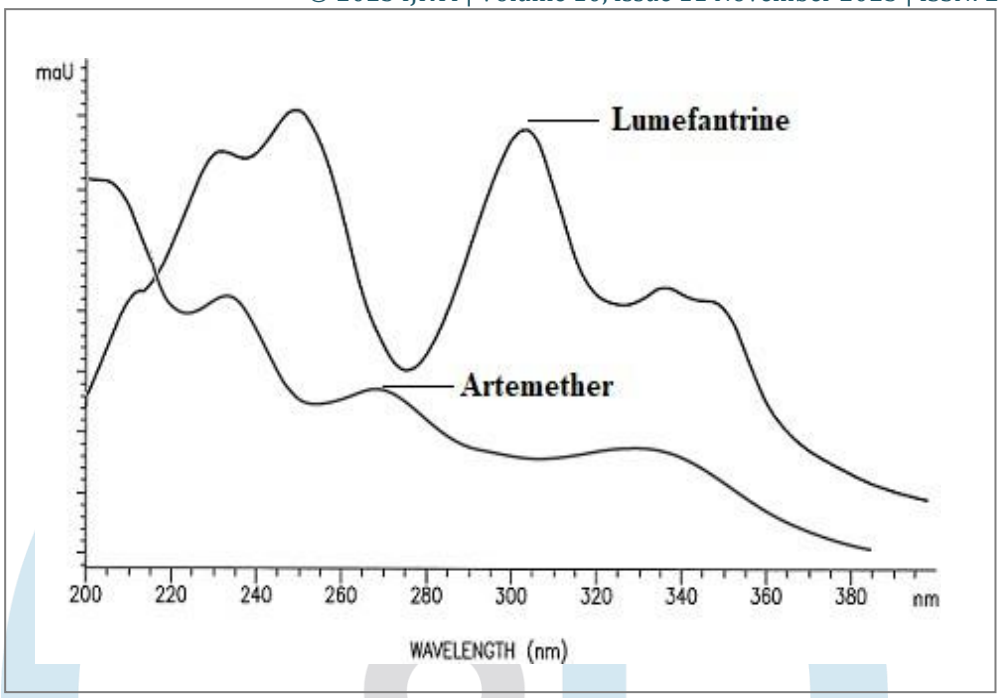


Fig.: Overlain spectra of artemether and lumefantrine

The method was checked by analyzing a solution containing known concentration of both drugs. The mixed standards in the Beer-Lamberts range for each drug containing 10, 25 and 40µg/ml of ART and 10, 25 and 40µg/ml of LUM, respectively, were prepared by diluting appropriate volumes of standard stock solutions. The scanning of mixed standard solutions was carried out in the range of 400 nm to 200 nm in spectrum mode with measurement at 268 nm and 302 nm. The results are shown in Table. Results clearly stated that the absorption of drugs does not interfere with each other.

Table: Absorbance of mixed standards containing artemether and lumefantrine

S. No	Mixed Standards Conc. (µg/ml)		Abs. (nm)	
	Artemether(ART)	Lumefantrine(LUM)	268 nm(ART)	302 nm(LUM)
1	10	10	0.181	0.149
2	25	25	0.439	0.372
3	40	40	0.753	0.652

The method was validated statistically for range, linearity, precision, accuracy, LOD, LOQ and repeatability.

Linearity and Range: The linearity for ART and LUM was determined at eight concentration levels, ranging from 5-40 µg/ml for both the drugs. Good coefficient of correlation value of 0.9953 and 0.9917 for artemether and lumefantrine, respectively indicates linearity of the proposed method of analysis.

Precision and Accuracy: Precision and accuracy of the method was proved by using stock solutions containing 5 µg/mL of ART and 30 µg/ml of LUM. Intraday precision and accuracy was done by repeating the assay three times of six replicate dilutions of the same concentration after every two hours on the same day whereas interday precision and accuracy of the assay was determined by repeating the intra-day assay on three different days.Precision was expressed as the percentage coefficient variation (CV, %) of measured concentrations for each calibration level whereas accuracy was expressed as percent recovery [(drug found / drug present) x 100].The results of precision and accuracy are shown in Table. The low CV (%) values and high accuracy values represent that the developed method is precise and accuracte over the concentration range analyzed.

4.3 VESICLE PREPARATION AND OPTIMIZATION

- **Optimization of liposome vesicles (LVs)**

Although several preparation methods are reported in literature for the preparation of liposomes but in the present study Ether Injection Method was selected because it is most common and scalable technique resulting in uniform liposomes. For parenteral administration size of nanoliposomes is the most important factor. Apart from size encapsulation efficiency of drugs in these vesicles is critical for development of successful formulation. Thus the optimization of liposomes is done based on size and encapsulation efficiency of drugs in liposomes. Preliminary studies suggest that amount of cholesterol, sonication energy, hydration temperature and freeze-drying plays a vital role in LV formation and drug encapsulation and thus these parameters were critically assessed for optimization of LVs (Liang et al., 2013)

- **Influence of cholesterol**

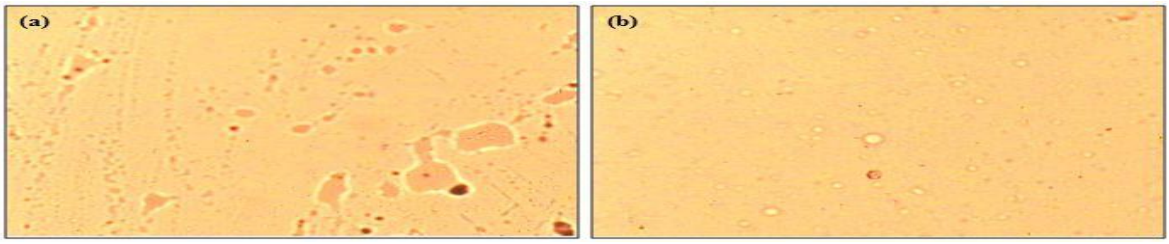


Fig. .Microscopic images of LVs (a) without cholesterol, (b) at optimum cholesterol

- **Influence of hydration temperature**

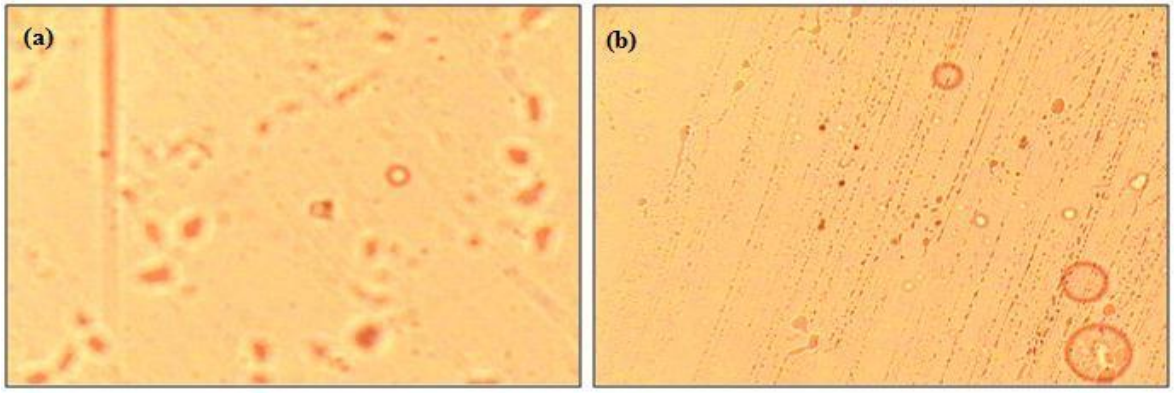


Fig.: Microscopic images of LVs formed at (a) 25 °C & (b) 45 °C

The final optimized process and formulation parameters are shown in box below:

Parameter	**Optimized Condition**
Lipid: cholesterol ratio	80:20
Hydration temperature	45°C
Sonication amplitude	80%
Sonication time	3 min
Volume of organic phase	20 ml of ether
Volume of hydration medium	25 ml of aqueous phase containing pluronic F68
Cryoprotectant	2% Sucrose

The particle size graph and TEM image of the optimized LVs are also shown in Fig. The diameter of particle observed by TEM was smaller than that from particle size analyzer. The reason may be the formation of hydration layer around the NLs dispersed in water to measure size in case of PCS method, while the NLs measured in TEM were taken after drying them on the copper grid, which are void of these hydration layer.

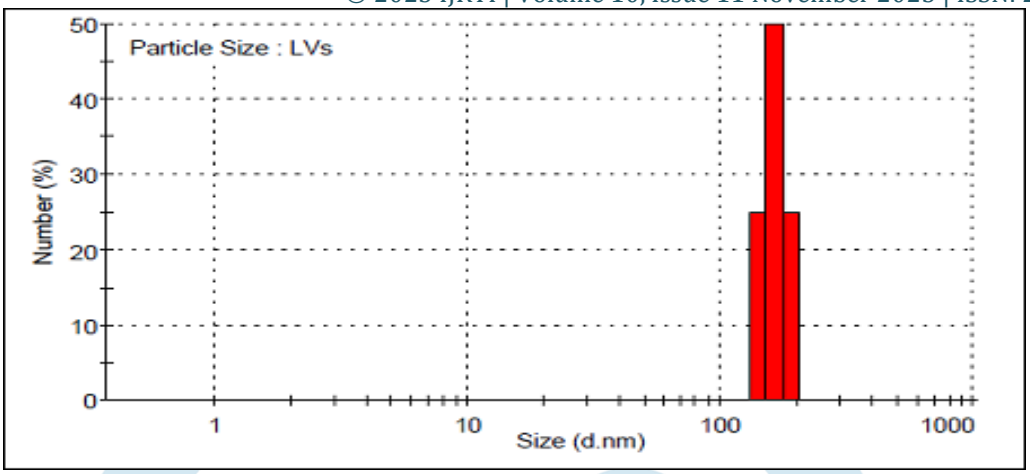


Fig. Particle size graph of the optimized LVs, particle size: 125.3 nm

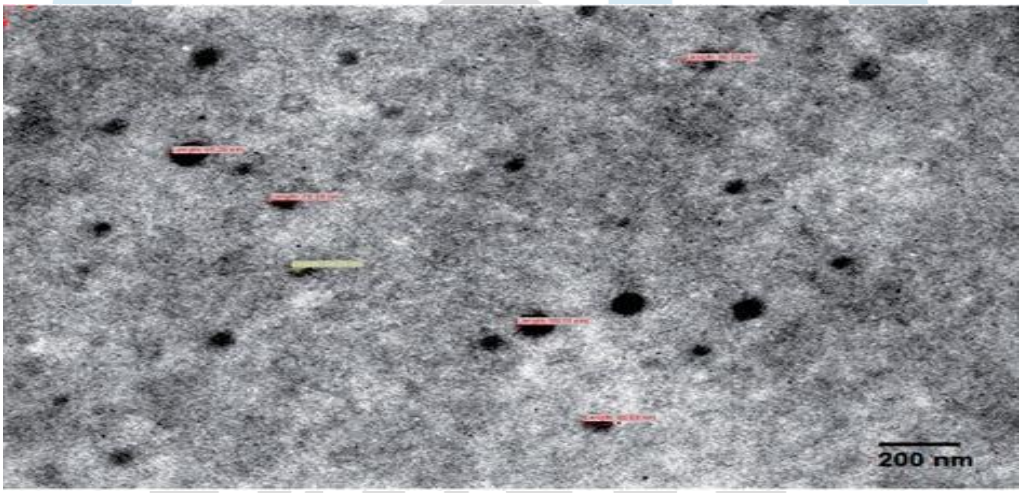


Fig.: TEM image of the optimized LVs

- **IN VITRO DRUG RELEASE**

The feasibility of using NLs to deliver both the drug in a manner to show the maximum cidal effect on the parasite was the key issue of the current investigation. Dialysis bag method was used to evaluate the release of artemether and lumefantrine from NLs and phosphate buffer (pH 7.4) as release medium and cumulative percentages of drug released were plotted as a function of time .

During preparation of the formulation, the drug remains distributed over the particle surface. In such cases, the initial release of drug from the dosage form becomes significant. In our study, it has been observed that both the drug molecules appeared to be released from NLs in a biphasic way, which characterized by an initial burst release or rapid release followed by a step of slower release or sustained release pattern.

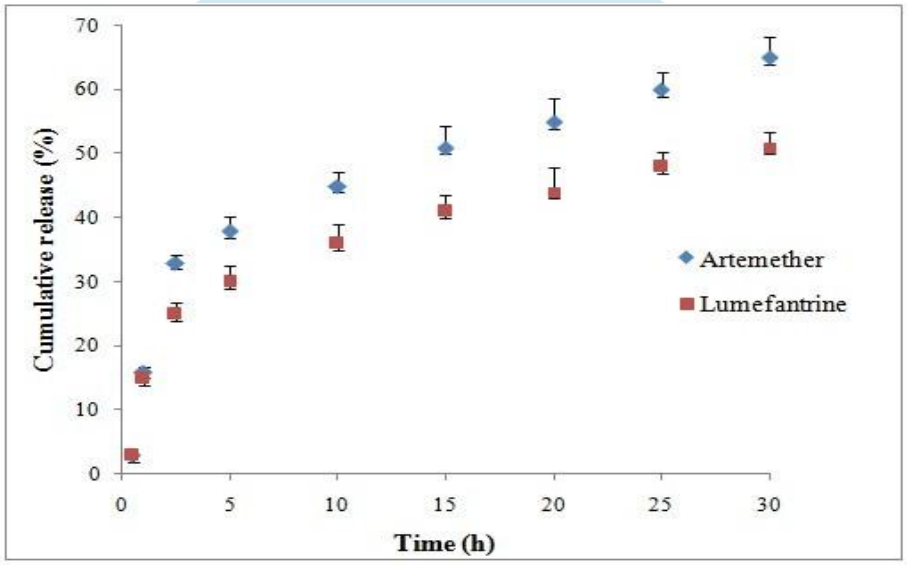


Fig.: ***In vitro*** **release profile of artemether and lumefantrine from nanoliposomes**

The burst effect was observed between 1 and 2.5 hour, in which ~15–35% of the initial drug was released from NLs, and the release rates were in an increasing order in a continuous way for up to 30 hour, reaching percentage of cumulative release close to 65% of artemether and 51% of lumefantrine. The reason behind burst effect may be due to presence of drug particles distributed on the surface whereas the sustained effect are due to the presence of the drug which is present in the core of the NLs, which takes time to diffuse out from the internal core region to outer releasing medium. Another important observation from the study observed was; rate and extent of artemether release from NLs was higher than that of lumefantrine.Reason may be probably due to high hydrophobicity of lumefantrine (log P=9.19) compared to artemether (logP=3.48), which shows the preference of lumefantrine to stay within the hydrophobic core of NLs rather than releasing into the hydrophilic media (Amin et al., 2013) or, due to the smaller molecular structure of artemether compared to lumefantrine,which might have diffused easily from the liposomal core into the releasing media faster than its counterpart. The release pattern strongly indicates that fast release of artemether will help to reduce the parasite burden initially, and lumefantrine will be available for the system for prolonged time period to clear the remaining parasite.

5.0 SUMMARY

Malaria is the most life-threatening disease prevalent in tropical and subtropical countries causing about 1–2 million deaths every year worldwide. *Plasmodium vivax, P. falciparum, P. malariae* and *P. ovale* are four distinct species that are causative agent for malaria in human beings. There are only a limited number of clinically effective antimalarial agents in existing practice. Problems such as poor and erratic oral bioavailability, lack of dose proportionality and degradation in gastrointestinal tract are the main reasons for poor clinical effectiveness of existing antimalarial agents. Rapid drug resistance and wide spread presence are the major hurdle to combat malaria. In addition, development of new chemical entities (NCEs) and launching of effective formulation into the market takes a long time and requires lot of revenue. In the past, solid dispersions (Abdul-Fattah et al., 2002), liposomes, polymeric nanoparticle and dendrimers have been developed and explored to counter the malaria. To improve the efficacy of the antimalarial agents, different types of route of administration such as transdermal and rectal routes have been utilized. To enhance the therapeutic efficacy of currently existing drugs, combination therapy may be proved as a boon which involves the participation of two antimalarial drugs with distinct site and mode of action. From long, combination therapy is preferred over monotherapy for malarial treatment; and among various combination therapies, artemisinin- based combination therapy is most preferred.

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