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Formulation and Characterization of Solid Lipid Nanoparticles Containing Artemether and Lumefantrine for Treatment of *P. falciparum*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SH designed the study, wrote the protocol and author VC wrote the first draft of the manuscript. Author VP managed the literature searches, analyses of the study performed the spectroscopy analysis and author VJ managed the experimental process and final approval was done by author RKK. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To investigate sustained release of solid lipid nanoparticles containing Artemether and Lumefantrine against the *P. falciparum* by the combination of drugs that eventually will decrease the chance of drug resistance development.
Methods: Artemether and Lumefantrine come under BCS class II (poor aqueous solubility and high permeability) and these drug molecules possess low oral bioavailability due to improper dissolution and incomplete absorption. Novel formulation of Artemether and Lumefantrine may eliminate all of shortcomings and may lead to enhance oral bioavailability due to increase in solubility of these drugs. Liquid crystalline nanoparticles (cubosomes or hexosomes) containing

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Artemether and Lumefantrine were formulated by Hydrotropic dilution method. In this method ethanolic solution of GMO with drug and aqueous solution of Poloxamer 407 were prepared by vortexing. Water phase containing the Poloxamer (10% w/v) added to the ethanolic phase drop wise with continuously vortexing resulting in the precipitation of the GMO. A milky suspension is formed which indicate the formation of liquid crystallineas

Results: The average particle size of Artemether and Lumefantrine loaded SLNs decreased with increasing concentration of surfactant. SLNs of 193.5-194 nm with a Polydispersity index of (0.600 ± 0.10) were obtained at higher concentration of lipid and surfactant. Entrapment efficiency of Artemether and Lumefantrine were found 85% and 95.5% insolid lipid nanoparticles. Furthermore the stability of SLNs indicated with negligible drug leakage after 3 weeks in stability studies as per ICH guidelines. Physical and chemical stability study revealed no major change in particle size and entrapment efficiency of liquid crystalline nanoparticles.

Conclusion: The result concluded that Artemether and Lumefantrine were loaded in solid lipid nanoparticles that exhibited sustained release from the designed dosage form against the *P. falciparum* and decrease the chances of drug resistance development during the treatment.

Keywords: Solid lipid nanoparticles; artemether; lumefantrine; P. falciparum.

1. INTRODUCTION

Malaria is an infectious disease caused by protozoan parasites belonging to the genus *Plasmodium* and the parasite is transmitted to humans through the bite of female anopheles mosquitos. Clinically, malaria may be divided into '*complicated*' and '*uncomplicated*' disease, and these terms are applicable to both adults and children [1]. The drugs currently available for the treatment of malaria are sulfadoxine-pyrimethamine (SP) (Fansidar), chloroquine, quinine with or without tetracycline/ doxycycline or clindamycin, artesunate-amodiaquine (Coarsucam or ASAQ), artesunate-mefloquine (Artequin or ASMQ), artemether-lumefantrine (Coartem, Riamet, Faverid, Amatem, Lonart or AL), artesunate-sulfadoxine/ pyrimethamine (Ariplus or Amalar plus), dihydroartemisinin-piperaquine (Duo-Cotecxin or Artekin), artemisinin/ piperaquine/primaquine (Fast Elimination of Malaria through Source Eradication (FEMSE)), artesunate-pyronaridine (Pyramax) and atovaquone-proguanil.

The major drawback with antimalarial drugs is, the development of resistance during the treatment period, especially for two of the four species of the malarial parasites that naturally infect human beings, *P. falciparum* and *P. vivax* [2].

Hence simultaneous use of two antimalarials in combination, especially antimalarials that have two different mechanisms of action, has the efficacy to inhibit the development of resistance to either of the compounds [3]. Artemisinin-based Combination Therapy (ACT) is widely accepted to control the rapid emergence of resistance to the antimalarial drugs and slow down the spread of resistance to antimalarials and to improve the useful therapeutic life (UTL) of antimalarial agents [3].

2. MATERIALS AND METHODS

2.1 Materials

Artemether was a gift sample received from IPCA Pvt. Ltd Mumbai, India. Lumefantrine was a gift sample Mylan Laboratories, Hyderabad, India. Poloxamer 407 was procured from BASF Corp. India. Oleic Acid was procured from Avarice Laboratory Pvt. Ltd. India. All the Chemical reagents are of analytical grade.

2.2 Methods

2.2.1 Preformulation study

The color, odor and taste of the drugs were assessed as per the United States Pharmacopoeia (USP) 2009. Melting point of Artemether and Lumefantrine were determined by capillary rise method. Solubility of Artemether and Lumefantrine was determined in distilled water, methanol, ethanol, ethyl acetate, and chloroform. Particle size and shape of the drugs were analyzed using Research Microscope (Motic BA310) at magnification 10X and 40X. Partition coefficient of Artemether and Lumefantrine was determined by shake flask method. Artemether and Lumefantrine were characterized by Furiour transform infra red

spectroscopy (FTIR). λ_{max} of Artemether was determined in distilled water [4], buffer pH 1.2, pH 6.8, 0.1 N HCl [5] and methanol [6]. The calibration curves of Artemether in water, buffer pH 1.2, pH 6.8 and for Lumefantrine in 0.1 N HCl and methanol were prepared for further evaluation.

2.2.2 Formulation and development

Liquid crystalline (LC) nanoparticles (Cubosomes or Hexosomes) containing Artemether and Lumefantrine were formulated by Hydrotropic dilution method. In this method ethanolic solution of glycerol mono oleate (GMO) with drug and aqueous solution of poloxamer 407 were prepared by vortexing. Ethanol was used to dissolve monoolein, oleic acid, Artemether, Lumefantrine and aqueous phase was used to dissolve poloxamer 407. Water phase containing the poloxamer (10% w/v) was added to the ethanolic phase drop wise with continuous vortexing resulting in the precipitation of the GMO as described in Table 1. A milky suspension was formed which indicated the formation of liquid crystallineas described in Fig. 1. [1].

2.2.3 Characterization of artemether & lumefantrine liquid crystalline dispersion

2.2.3.1 Particle size analysis

The mean particle size and Polydispersity index were measured using laser diffraction on a Malvern Zetasizer Ver. 6.01r (Serial Number: MAL1027952, Malvern instruments Ltd.) at 25°C considering a viscosity of pure water 0.8872. The particle size was analysed by diluting the prepared formulations with distilled water [7].

2.2.3.2 Optical microscopic examination [8]

All the trial batches were examined for uniformity of dispersion (LCs), drug crystal and presence or absence of oil drops using the Research microscope (Motic Instrument BA310) at magnification of 40X. Prepared formulations were assessed at the interval of 2 days and 7 days. Sample slides were prepared and examined under microscope.

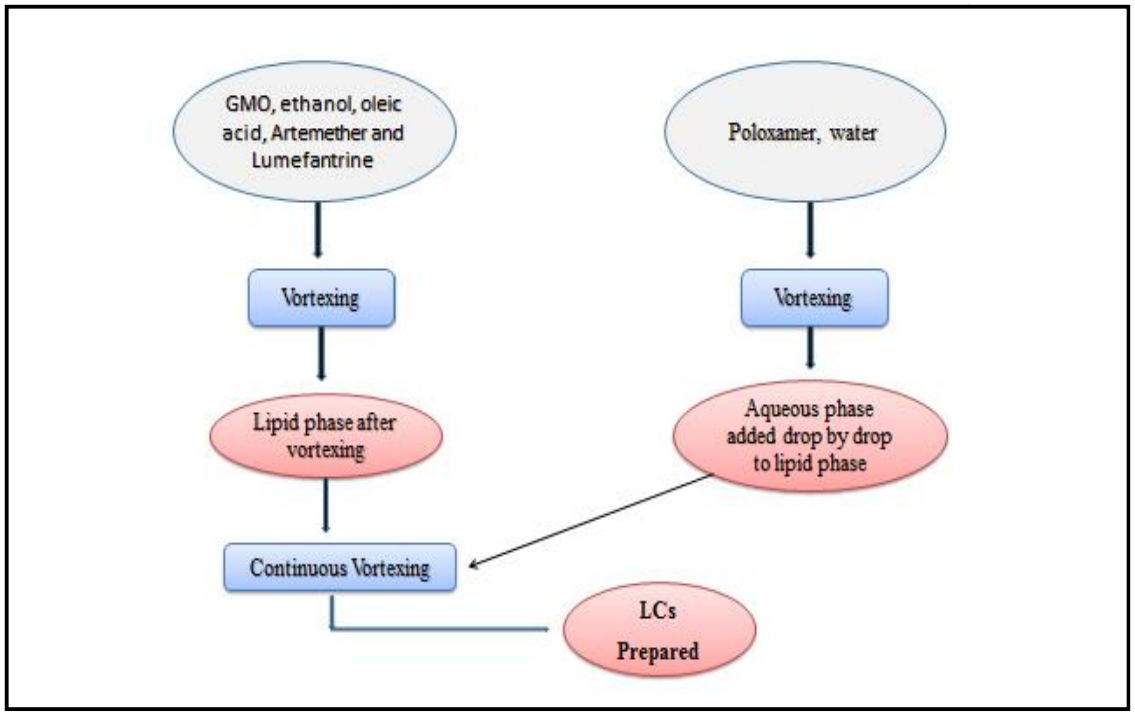


Fig. 1. Formulation chart for LCs of artemether and lumefantrine

Formulation and Development

Table 1. Composition of combination of artemether and lumefantrine liquid crystalline nanoparticles as per box-behnken design containing oleic acid*

Formulation code	Artemether (mg)	Lumefantrine (mg)	Oleic acid (mg)	Glyceryl monooleate (mg)	Poloxamer 407 (mg)	Ethanol (ml)	Water (ml)
F1	20	100	125	250	50	0.5	4.5
F2	15	75	125	250	50	0.5	4.5
F3	15	75	125	250	50	0.5	4.5
F4	15	75	125	250	50	0.5	4.5
F5	20	50	125	250	50	0.5	4.5
F6	10	75	150	250	50	0.5	4.5
F7	15	50	150	250	50	0.5	4.5
F8	15	50	100	250	50	0.5	4.5
F9	10	50	125	250	50	0.5	4.5
F10	15	100	150	250	50	0.5	4.5
F11	20	75	100	250	50	0.5	4.5
F12	10	100	125	250	50	0.5	4.5
F13	10	100	125	250	50	0.5	4.5
F14	15	75	125	250	50	0.5	4.5
F15	15	75	125	250	50	0.5	4.5
F16	15	100	100	250	50	0.5	4.5
F17	20	75	150	250	50	0.5	4.5

**Every formulation contain 5 ml formulation*

2.2.3.3 Entrapment efficiency (EE) [7]

Entrapment efficiency of Artemether and Lumefantrine was determined using the Nanosep device. The liquid crystalline dispersion of Artemether and Lumefantrine were centrifuged in cooling centrifuge (Remi Scientific Instruments) using Nanosep device (MWCO: 2-3 KD, Spectra Labs). 0.5 ml of prepared formulation was taken in Nanosep device and then placed in cooling centrifuge (Remi Scientific Instruments). The sample was then centrifuged at 13000 rpm for 30 min at 10°C. The aqueous phase was collected, and clear solution was analyzed for Artemether and Lumefantrine at 256 nm and 342 nm by UV spectroscopy. The preliminary trials were performed for the optimization of centrifugation speed and time. Time and speed 13000 rpm for 30 min was optimum to separate untrapped drug from the LCs as described Table 4. The encapsulation efficiency (E.E) was determined using the following equation:

EE(%)= (Total mass of ARTM or LMF-mass of ARTM or LMF in aqueous phase / Total equivalent mass of ARTM or LMF) x 100

2.2.4 Optimization and formulation

In development of formulation, Box-Behnken design was employed to study the effect of independent variables over the dependent variables. Independent variables were Artemether (X_1), Lumefantrine (X_2) and Oleic acid (X_3). Particle size (Y_1), entrapment efficiency of Artemether (Y_2) and entrapment efficiency of Lumefantrine (Y_3) were considered as dependent variables.

X_1 and X_2 are the polynomial terms which were included to investigate the non-linearity. From the Box-Behnken outcomes (responses) it was found that all the dependent variables were strictly dependent over the selected independent variables as they showed a wide variation among the 17 batches (F1-F17). The polynomial equation was used to draw a conclusion after considering the magnitude of coefficients and the sign carries i.e. positive or negative. The high value of correlation coefficient for the dependent variables showed a good fit. These equations may be used to estimate the response because small errors of variance were observed in the replicates.

The ANOVA of each response was carried out and the F statistics was applied to check whether the non-significant terms can be eliminated or not for the model. The Optimized formula was formulated for further evaluation.

2.2.5 Evaluation of optimized formulation

The optimized formulation was identified based on constraints using design expert software

(version 9.1.0, state ease Inc., Minneapolis, MN). The optimized formulation was formulated according to method given in Fig. 1 and evaluated for particle size, entrapment efficiency, *in-vitro* release, physical stability and chemical stability.

2.2.6 Physiochemical characterisation

2.2.6.1 Physical stability

Observed responses of OF1 were compared with predicted responses obtained by DoE. The closeness between the responses was the basis for the optimization of final formulation.

2.2.6.2 Visual assessment of phase separation

The initial stability of dispersions was evaluated visually in the eppendorf tube after sonication 10 min.

2.2.7 Creaming

Optimized formulation (OF1) was analyzed for creaming. Creaming involved the separation of dispersed phase from the liquid crystalline dispersion on storage under normal condition at room temperature. The dispersion type OF1 of LCN was oil in water. LCN were assessed for creaming by visual assessment conducted for three months. Optimized formulation was visually inspected on weekly basis up to three months.

2.2.8 Discoloration

Discoloration was assessed during storage period at 25-37°C. Visual inspection was done to assess the discoloration. Optimized formulation was observed for change in color after 24 hrs, and continued on weekly basis for next three months.

2.2.9 Chemical stability of optimized formulation

Chemical stability of Artemether and Lumefantrine entrapped in the liquid crystalline nanoparticles formulation (optimized formulation) was evaluated at 25-37°C for three months. The entrapment efficiency and particle size were considered as measuring parameters. These two parameters were evaluated during the storage period on weekly basis for three months.

2.2.10 *In-vitro* drug release study [5,7]

In-vitro drug release study of Artemether and Lumefantrine was performed in simulated gastric fluid pH 1.2 containing 1% w/v BKC and phosphate buffer of pH 6.8 containing 0.5% w/v SLS by using dialysis bag method. A dialysis membrane having pore size 2.4 nm and a molecular weight cut off 12000-14000 Dalton (Dialysis membrane-150, HiMedia, Mumbai, India) was used. The dialysis bag retains the nanoparticles and releases the free drug into the dissolution media. The dialysis membrane was pre-treated with sodium bicarbonate and EDTA solution and kept in diluted EDTA solution prior to use. The bag was washed with distilled water prior to use. 2 ml formulation of Artemether and Lumefantrine was placed in dialysis bag. Separate dialysis bags containing the formulation were immersed in 200 ml simulated gastric fluid for Lumefantrine and intestinal fluid (Artemether) maintained at 37±0.5°C and stirred at 100 rpm. Aliquots of the dissolution medium were withdrawn at definite intervals and the fresh dissolution medium was added to maintain a sink condition. Samples withdrawn from the dissolution medium were analyzed for the drug content by UV-spectroscopy. The drug content of each sample was calculated using following equation:

% Release of ARTM or LMF= (Mass of ARTM or LMF in releasing media / Total equivalent Mass of ARTM or LMF) x 100

3. RESULTS

3.1 Preformulation

The color, odor and taste evaluations of Artemether and Lumefantrine were complied with the given literature values. Melting point of the pure drugs were found 85±2°C for Artemether and 130±2°C for Lumefantrine. The solubility study of Artemether and Lumefantrine revealed that the procured drugs were Artemether and Lumefantrine. Particles of Artemether and Lumefantrine were found irregular shape and crystalline in nature, most of the particles of Artemether and Lumefantrine were found between 0-47 μm and 0-94 μm respectively. Partition coefficient of Artemether and Lumefantrine showed that drugs were highly lipophilic in nature. On FTIR analysis, the spectra of test samples of the drugs confirmed the reference spectra given in validation report of USP 2009. The absorption maxima (λ_{max}) of Artemether and Lumefantrine were found at 256 nm and 342 nm, respectively. The calibration equation for straight line was observed to

calculate further evaluation test of nanostructured formulation.

3.2 Evaluation of Artemether and Lumefantrine LCN

3.2.1 Particle size analysis

The mean particle size and Polydispersity index for all formulations were determined using laser diffraction on a Malvern Zetasizer Ver. 6.01r (Serial Number: MAL1027952, Malvern instruments Ltd.) at 25°C considering a viscosity of pure water 0.8872. Particle size analysis of the formed formulation was conducted with samples diluted in water. The results shown in Table 2.

3.3 Optical Microscopic Examination

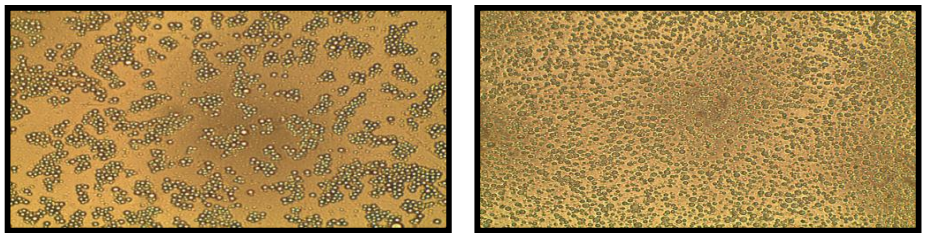
The trials which showed PDI values lower than 0.3 were examined for uniformity of dispersion using Research microscope (Motic BA310) at magnification of 40X. Prepared formulations were assessed at the interval of 2 days and 7 days. Sample slides were prepared and examined under microscope as shown in Figs. 2, 3 and 4.

3.4 Entrapment Efficiency

Entrapment efficiency of Artemether and Lumefantrine in liquid crystalline dispersion was determined using the Nanosep device for all formulations designed by Box-Behnken design. The liquid solution of Artemether and Lumefantrine (0.5 ml) was centrifuged in cooling centrifuge using Nanosep device (MWCO: 2-3 KD, Spectra Labs) at 13000 rpm and 10°C for 30 min at. Then aqueous phase was collected and analyzed for Artemether and Lumefantrine content at 256 nm and 342 nm respectively by UV spectroscopy.

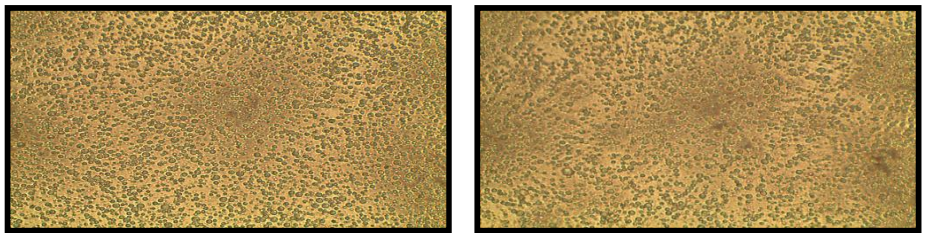
Table 2. Compositions and particle size of liquid crystalline nanoparticles of artemether and lumefantrine

kl	0Trial code	Composition			Particle size analysis	
		ARTM (mg)	LMF (mg)	Oleic acid (mg)	Z-average (d.nm)	PdI
1	F1	20	100	125	158	0.60
2	F2	15	75	125	176	0.50
3	F3	15	75	125	184	0.30
4	F4	15	75	125	162	0.40
5	F5	20	50	125	195	0.20
6	F6	10	75	150	165	0.22
7	F7	15	50	150	187	0.58
8	F8	15	50	100	200	0.48
9	F9	10	50	125	193.5	0.10
10	F10	15	100	150	180	0.45
11	F11	20	75	100	192	0.38
12	F12	10	100	125	164	0.41
13	F13	10	100	125	157	0.25
14	F14	15	75	125	182	0.35
15	F15	15	75	125	190	0.22
16	F16	15	100	100	156	0.38
17	F17	20	75	150	189	0.43



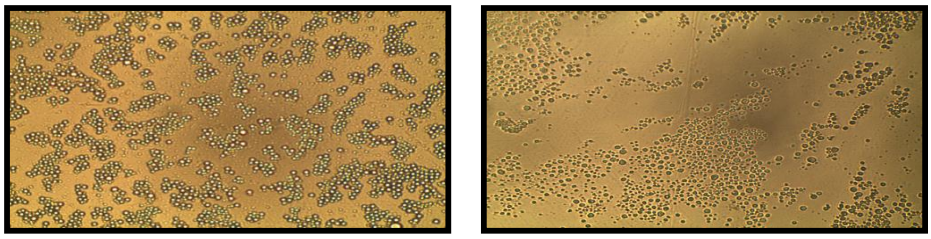
F1 dispersion after 2 days **F1 dispersion after 7 days**

Fig. 2. Microscopic examination of F1 (LCs dispersion, PDI 0.6) at 40X



F2 dispersion after 2 days **F2 dispersion after 7 days**

Fig. 3. Microscopic examination of F9 (LCs dispersion, PDI 0.1) at 40X



F3 dispersion after 2 days **F3 dispersion after 7 days**

Fig. 4. Microscopic examination of F9 (LCs dispersion, PDI 0.1) at 40X

Table 3. Microscopic examination of formed LCs of artemether and lumefantrine

S. no	Microscopic examination		
	Formulation code	Uniformity of dispersion	Oil drops
1	F1	++	—
2	F2	++	—
3	F3	++	—
4	F4	++	—
5	F5	+++	—
6	F6	+++	—
7	F7	+++	+
8	F8	++	+
9	F9	+++	—
10	F10	+++	+
11	F11	++	—
12	F12	++	—
13	F13	++	—
14	F14	++	—
15	F15	++	—
16	F16	++	—
17	F17	++	—

+++= Uniformity of dispersion; ++= Less Uniform; + = Oil drops present; —= Oil drops not present

The entrapment efficiency of Artemether and LMF was found in between 69.16±0.76% to 85.33±1.52% and 45±1% to 98.5±0.5%, respectively (Table 4).

3.5 Optimization and Validation of Prepared Liquid Crystalline Nanoparticles of Artemether and Lumefantrine

Optimization was performed using design expert software (version 9.1.0, state ease Inc., Minneapolis, MN). The model was evaluated in terms of statistically significant coefficient, standardized main effects, and ANOVA and R^2 values. The optimized formulation (OF1) was identified based on constraints. The optimized formulation as shown in Table 5 was formulated according to method given in Fig. 1 and evaluated for particle size, entrapment efficiency, *in-vitro* release, physical stability and chemical stability.

3.6 Stability Study of Optimized Formulation

3.6.1 Physical stability

Optimized formulation was evaluated for physical stability which included the study of phase separation, creaming and discoloration of product. Visual assessment of optimized formulation was conducted weekly for 3 months at storage condition 25-37°C and results are shown in Table 6.

Table 4. Entrapment efficiency of the liquid crystalline nanoparticles formulations of artemether and lumefantrine

S. no.	Formulation code	Entrapment efficiency (%)	
		Artemether	Lumefantrine
1	F1	70.5±1.32	45±1
2	F2	70.33±1.52	60.5±1.32
3	F3	70.67±1.154	62.67±1.52
4	F4	85.5±0.5	76.83±0.288
5	F5	69.16±0.76	84.33±2.08
6	F6	74±1	67±1
7	F7	75.26±0.83	84±0.5
8	F8	72.5±0.5	60±1
9	F9	85±0.5	98.5±0.5
10	F10	72.16±1.04	58±0.5
11	F11	75±1	68±1
12	F12	74.2±1.31	58.16±0.76
13	F13	74±0.5	57.5±1.32
14	F14	72±0.6	50±1
15	F15	85.33±1.52	55±0.5
16	F16	70.16±0.76	68±1
17	F17	65±0.5	74.16±1.04

Table 5. Composition of liquid crystalline nanocarriers of optimized formulation of1

Ingredients	Quantity per formulation
Artemether	10 mg
Lumefantrine	50 mg
Oleic acid	125 mg
Glyceryl monooleate	250 mg
Poloxamer 407	50 mg
Ethanol	0.5 ml
Water	4.5 ml

Evaluation of optimized formulation "OF1"

3.6.2 Chemical stability

Chemical stability of Artemether and Lumefantrine entrapped in the liquid crystalline nanoparticles formulation (optimized formulation) was evaluated at 25-37°C for three months. During this study entrapment efficiency and particle size were evaluated during the storage period on weekly basis for three months (Table 7).

3.6.3 *In-vitro* release study of optimized formulation (OF1)

In-vitro dissolution study for formulated liquid crystalline dispersion of Artemether and Lumefantrine was conducted separately in simulated gastric fluid (Lumefantrine) and simulated intestinal fluid (for Artemether) because the formulation was meant to be administered via oral cavity and two different media were selected based on dissolution medium stated in USP. *In-vitro* dissolution study for Artemether and Lumefantrine were conducted for 72 hrs.

In-vitro drug release study of optimized formulation by using the dialysis bag technique initially showed 20% drug release within 4 hrs, which can be explained by the fact that about 20% drug remained in aqueous phase and the rest of the drug was encapsulated within the liquid crystalline shells. About 60.0% of drug release was achieved at the end of 24 hrs and complete release occurred at the end of 72 hrs. This indicated that drug carrier can retain active drug moiety up to 72 hrs which indicates that drug can be completely leached out at slower rate as shown in Figs. 5 and 6.

4. DISCUSSION

Lipid based Liquid crystalline formulation of Artemether and Lumefantrine were developed and evaluated. The main objective was to formulate a lipid-based delivery system that may increase the bioavailability of BCS class II drugs. Such types of lipid-based drug carriers may keep the drug in dissolved state until the drug is completely absorbed and avoid gastrointestinal degradation of drugs. The present work was mainly focused to formulate liquid crystalline nanoparticles in combination that would improve the solubility of BCS Calss II drugs and could probably improve absorption of ARTM and LMF drugs and circumvent the drawback of poor bioavailability.

Table 6. Result of physical stability study

S. no	Parameters	1st month				2nd month		3rd month	
		Weeks				Weeks		Weeks	
		1st	2nd	3rd	4th	2nd	4th	2nd	4th
1	Phase separation	+++	+++	+++	+++	+++	+++	+++	+++
2	Creaming	—	—	—	—	—	—	—	—
3	Discoloration	—	—	—	—	—	—	—	—

Where: Phase separation: +++ = homogeneous milky dispersion: ++ = cloudy dispersion + = translucent dispersion, Creaming: + = creaming present: - = creaming absent, Discoloration: + = color changed: - = color not changed

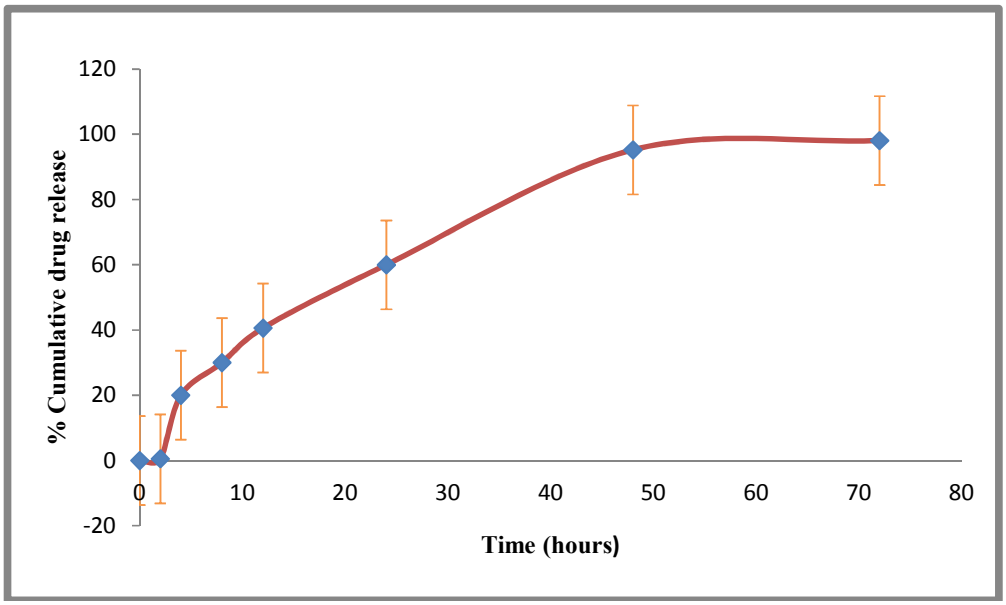


Fig. 5. *In-vitro* dissolution profile of artemether from optimized formulation

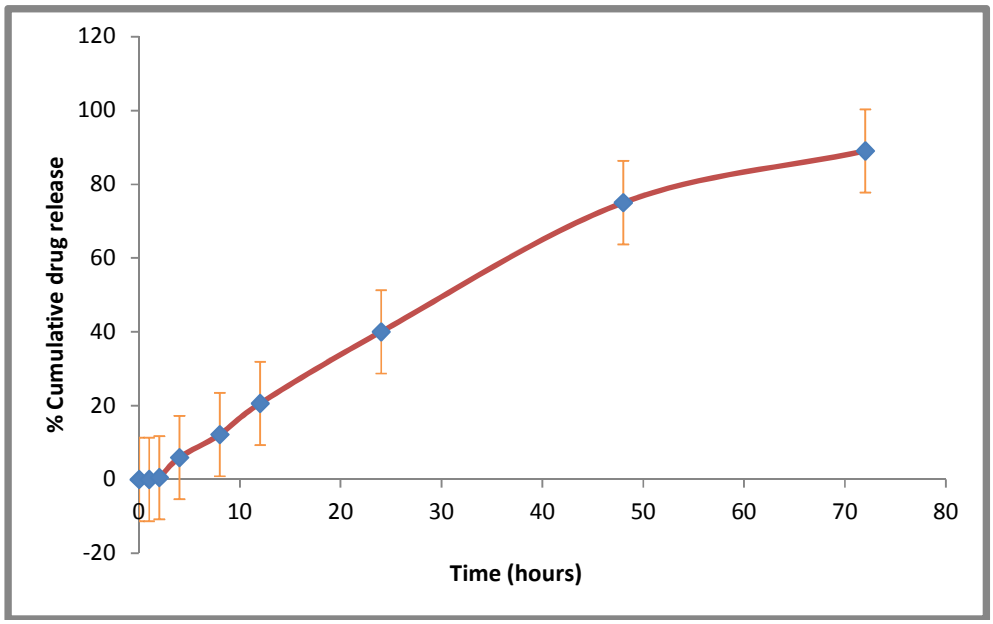


Fig. 6. *In-vitro* dissolution profile of lumefantrine from optimized formulation

Table 7. Chemical stability study at 25-37°C

S. no.	Parameters	Formulation code	At 25-30°C				
			1st month			2nd month	3rd month
			ARTM LMF	Initial	Day 10	Day 30	Day 60	Day 90
1	Particle size (nm)	OF1 (F9)		193.5±0.5	198.4±0.6	205±1	210±0.5	215±0.35
2	EE (%)	OF1 (F9)		85±0.5	83.33±1.52	81.6±0.4	78.4±0.6	75.0±0.5
				98.5±0.5	97.93±0.81	95.5±0.5	90.2±0.8	88.5±0.6

Each value represents mean ± standard deviation (n=3). EE = Entrapment efficiency

All the drugs and polymers were screened and optimized on the basis of Preformulation studies. Particle size analysis indicated that liquid crystalline dispersion contained a wide range of particle sizes, ranging from 158 nm to 200 nm and majority of particles lies within this range. The particle size variability depends upon drug concentration with respect to lipid availability (9). Polydispersity index indicated the width of particles and its distribution ranges from 0.1 to 0.6, which indicated that the dispersions of all the batches were not uniform because PDI value should be in the range from 0-1 and the value closer towards zero, possesses more homogeneity.

Based on the particle size analysis it has been found that on increasing the concentration of Artemether and Lumefantrine, particle size was decreased but on increasing the concentration of oleic acid as a lipid carrier above the 125 mg, particle size uniformity of dispersion was altered. Such an observation occurred due to decrease in hydrophilic domain of liquid crystalline structures. High particle size uniformity of dispersion indicated high PDI values. Based on particle size analysis, formulations were not excluded but kept for further research work and evaluations like microscopic examination and entrapment efficiency.

Average entrapment efficiency of Artemether was lies between 69-85%, which showed that Artemether possess good entrapment efficiency. It was due to the fact that Artemether is highly soluble in Glycerol monooleate (GMO) and is not affected by other factors. However, Lumefantrine showed variable entrapment efficiency due to the fact that Lumefantrine showed low solubility in GMO and high solubility in oleic acid (liquid lipid). The formulation which contained adequate quantity of oleic acid (≥125 mg) showed the highest entrapment.

The formulations were designed by Box-Behnken Design. The formulations that showed Polydispersity index values lower than 0.4 were uniformly dispersed in dispersion medium and the formulation with PDI greater than 0.4 was less uniform in dispersion medium. Such an observation occurred due to excess amount of oleic acid which was a critical factor and considered as an important factor for complete solubilisation of Lumefantrine and Artemether. Formulation containing oleic acid greater than 125 mg showed less uniform dispersion. It is important to choose a formulation that contains uniform dispersion as well as free from oil drops because oil drops might increase the chances of phase separation. Formulation F9 (LCs dispersion) did not contain any oil drop and possessed uniformity in dispersion throughout the study.

Based on the evaluation parameters including particle size analysis, microscopic examination and entrapment efficiency of prepared liquid crystalline nanoparticles of Artemether and Lumefantrine, it was found that trial F9 could be the optimized formulation (OF1).

From the BBD it was shown that all the parameters using for the evaluation of LCs dispersion were independent.

The entrapment efficiency for Artemether and Lumefantrine was recorded from highest to lowest amount of drugs with the variable concentration of oleic acid. It was shown that all the parameters used for the determination of entrapment effeciency were independent. The optimum formulation was selected based on the criteria of a particle size in the range of 150-200 nm, entrapment efficiency for Artemether 70-85% and entrapment efficiency of LMF in the range of 90-98% at the minimum level of both drugs and medium level of oleic acid. The optimized formulation of liquid crystalline nanoparticles of combination drugs, namely Artemether and Lumefantrine, containing oleic acid as solubilizing agent is give in Table 5.

Optimized formula was evaluated during the assessment of physical stability of optimized LCs dispersion of Artemether and Lumefantrine, 3 months stability studies at storage conditions in between 25-37°C. Formulation F9 (optimized formulation) did not show any sign of phase separation, creaming and discoloration which indicated that the optimized formulation is physically stable.

Table 7 shows that there was no significant change in particle size of the nanoparticles of Artemether and Lumefantrine. Polydispersity index of the liquid crystalline nanoparticles for optimized formulation during the storage period was found to be 0.1 to 0.2, which indicated the monodispersity of the liquid crystalline nanoparticles. The stability data provided the evidence of liquid crystalline nanoparticles being stable in terms of entrapment efficiency and particle size evaluation during the storage period.

In-vitro drug release study of optimized formulation by using the dialysis bag technique initially showed 20% release within 4 hrs. This can be explained by the fact that about 20% of drug remains in aqueous phase and the rest of drug remains encapsulated within the liquid crystalline particles. The initial release of drug may be due to unencapsulated drug. About 60.0% of release was achieved at the end of 24 hrs, and complete release occurred at the end of 72 hrs. Drug carrier can retain drug up to 72 hrs, which indicates that drug can be completely leached out at a slower rate.

In vitro release study of Lumefantrine using dialysis bag technique did not show any initial release within two hours. This might be due to the fact that about 98.5% drug was entrapped inside the formed liquid crystalline dispersion. After 2 hrs drug was slowly released and gradually increased. About 40% drug was released after 24 hrs. After 72 hrs the cumulative drug release was about 89±2%.

5. CONCLUSION

The study showed that Artemether and Lumefantrine loaded solid lipid nanoparticles may exhibit a sustained effect against *P. falciparum* and decrease the chance of drug resistance development during treatment.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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