

Formulation and evaluation of liquid crystalline nanoparticles of combination drugs of antimalarials: Preformulation part 1

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ABSTRACT

NovaMin is the trade name of a particulate bioactive glass containing the active ingredient calcium sodium phosphosilicate (CSPS) (chemical formula: CaNaO_6PSi). CSPS materials were originally developed as bone regenerative materials. Considering the antibacterial and mineralizing effects of bioactive glasses, they have been used in dental products also. NovaMin has been used in various dental products for treating tooth sensitivity. Recent studies have proved that it also contains the potential to prevent demineralization or aid in remineralization of tooth surfaces, reduction in gingival bleeding and gingivitis, whitening of teeth and erosion. The present manuscript discusses the mechanism of action of NovaMin, reviews its potential uses and also critically analyses it by enumerating its merits and demerits.

Keywords: Bioactive glass, calcium sodium phosphosilicate, dentinal hypersensitivity, NovaMin, remineralization

Introduction

Malaria is a complicated and sometimes acts as a fatal disease caused by parasitic mosquito which feeds on humans. According to WHO, in 2015, 214 million clinical cases of malaria occurred, and 43800 people died of malaria in total world population. As a drug resistance develops to existing drugs and treatments. Simultaneous use of two antimalarials is recommended especially when the antimalarial have two mechanisms of actions and has the efficacy to inhibit the development of resistance to either of the compounds.^[1]

In this study, a combination of drug, i.e., artemether (ARTM) and lumefantrine (LMF) was used. ARTM is a sesquiterpene lactone and methyl ether derivative of dihydroartemisinin, derived from artemisinin (qinghaosu) shows most potent and rapid onset of action against all *Plasmodium falciparum* strains. LMF belongs to the class of

arylamino alcohol and highly lipophilic and practically water-insoluble drug possess oral antimalarial activity.

ARTM and LMF have been included in the WHO list of essential medicine for the treatment of severe multidrug-resistant malaria. It was the first artemisinin-based combination therapy (ACT) approved in the USA for the treatment of malaria because of their high potency (at least 10 times more potent than other antimalarials), rapid onset of action, and favorable safety profile.

Rationale of ACT based combination therapy: Produce rapid clinical and parasitological cure, reduce the gametocyte carrier rate, ARTM, and LMF showed synergistic effect against the *P. falciparum* and oral combination of drug provides an initial rapid reduction in parasite biomass decrease the chance of drug resistance development.

Materials and Methods

Materials

LMF was a gift sample obtained from Mylan Laboratories, Hyderabad, India; ARTM was a gift sample received from IPCA Pvt. Ltd., Mumbai, India; poloxamer 407 as a polymer was procured from BASF Corp., India; glyceryl monooleate was procured from Danisco Cultor, India; oleic acid was purchased from Avarice Laboratory Pvt. Ltd., India; ethanol and methanol were received from Jiangsu Huaxi International, China; Conc. Orthophosphoric acid, hydrochloric acid, sodium bicarbonates, and n-octanol were purchased from Ranken RFCL Limited, New Delhi, India; potassium

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dihydrogen phosphate and sodium hydroxide were received from Titan Biochem. Ltd., Bhiwadi, India; ethylene diamine tetra acetic acid, disodium hydrogen orthophosphate were procured from Qualigens fine chemicals, Mumbai, India; Tween-80 was received from SD Fine-Chem Ltd., Mumbai, India. All the Chemical reagents were of analytical grade.

Methods

Preformulation study

Organoleptic evaluation

The color, odor, and taste of the drugs were assessed as per the USP 2009.

Melting point determination

The melting point of ARTM and LMF was determined by capillary rise method using digital melting point apparatus. Practically determined melting points were compared with the literature values.^[2]

Solubility studies

Solubility of ARTM and LMF was determined in distilled water. The observed results of the solubility of ARTM and LMF in different solvents were compared with the values given in literature values.

Microscopic examination

Particle size and shape of the drugs were analyzed using Research Microscope (Motic BA310) at magnification $\times 10$ and $\times 40$.

Partition coefficient^[3]

The partition coefficient of ARTM and LMF was determined by shake flask method. The samples were analyzed spectrophotometrically at 256 nm for ARTM and 244 nm for LMF.

Infra-red analysis of drugs

ARTM and LMF were characterized by Fourier transform infra-red (FTIR) spectroscopy at ARBRO analytical laboratory, New Delhi, India. K Br pellets of drugs were prepared and analyzed by FTIR. The obtained spectra of drugs were compared with reference spectra of ARTM and LMF.

Ultra-violet spectrophotometric analysis

Determination of λ_{\max} of ARTM in distilled water buffer pH 1.2 and buffer pH 6.8^[4]

About 10 mg of ARTM accurately weighed and transferred to 100 ml volumetric flask. Add 25 ml of 1 N HCl and heated on water bath for 20 min at $80^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The solution was then allowed to cool at room temperature, and the volume was made up with distilled water, buffer pH 1.2, and buffer pH 6.8 to get conc. of 100 $\mu\text{g}/\text{ml}$ of concentration and used as a stock solution. The stock solution was further diluted with distilled water to get a concentration of 20 $\mu\text{g}/\text{ml}$. The solution was scanned between 200 and 400 nm; distilled water was used as a blank. Observed results were compared with reference results.

Determination of λ_{\max} of LMF in 0.1 N HCl and methanol^[5,6]

About 10 mg of LMF accurately weighed and transferred to 100 ml volumetric flask. It was dissolved in 20 ml of 0.1 N HCl and methanol solutions containing 1% (w/v) benzalkonium chloride (BKC) as a co-surfactant. The final volume was made up with 0.1 N HCl. Pipetted out 2 ml from this solution into 10 ml volumetric flask diluted up to the mark with 0.1 N HCl to get 20 $\mu\text{g}/\text{ml}$ solution. The solution was scanned between 200 and 400 nm and obtained results were compared with the stated results.

Preparation of calibration curve of ARTM

Preparation of calibration curve of ARTM in water, buffer pH 1.2 and buffer pH 6.8

Accurately weighed 10 mg of ART was transferred into a 100 ml of volumetric flask. Add 25 ml of 1 N HCl and this solution was heated on the water bath for 20 min at $80^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The solution was allowed to cool at room temperature and made the volume with distilled water, buffer pH 1.2 and buffer pH 6.8 to get concentration of 100 $\mu\text{g}/\text{ml}$ and used as a stock solution. Suitable aliquots of the stock solution of ART (0.5-4 ml) were taken in 10 ml volumetric flasks and volume was made up to the mark with distilled water to prepare a series of standard solutions containing concentration range of 5-40 $\mu\text{g}/\text{ml}$. Absorbance of the samples were measured at 256 nm against blank. Blank was prepared by heating 2.5 ml 1 N HCl in the same condition as the stock solution was prepared and diluting up to the 10 ml with distilled water. The calibration curve was plotted for ART in the concentration range of 5-40 $\mu\text{g}/\text{ml}$ at 256 nm.

Preparation of calibration curve of LMF in water^[7]

Preparation of stock solution for calibration curve

Approximately, 10 mg of LMF was weighed and dissolved in 0.1 N HCl (1 ml)/methanol (9 ml) mixture and made up the volume 10 ml in standard volumetric flask. 1 ml of the resulting solution was pipetted out and dilute up to 10 ml in volumetric flask to get the concentration of 100 $\mu\text{g}/\text{ml}$ and used as a stock solution. The stock solution was further diluted into the series of solution to get the concentration range of 2.0-16 $\mu\text{g}/\text{ml}$; the absorption was taken at 342 nm.

Preparation of calibration curve of LMF 0.1 N HCl and methanol

About 10 mg of LMF accurately weighed and transferred to 100 ml volumetric flask. It was dissolved in 20 ml of 0.1 N HCl solution and methanol containing 1% w/w BKC as a cosolvent. After complete mixing of the drug, the volume was made up to 100 ml with 0.1 N HCl and used as a stock solution. The stock solution was further diluted into a series of solutions to get the concentration range of 10-60 $\mu\text{g}/\text{ml}$. The absorbance was taken at 342 nm.

Results and Discussion

The drugs ARTM and LMF were selected for the present research work, and drugs were characterized by different techniques. All the

observed results complied with literature value that showed procured drug was ARTM and LMF.

Organoleptic evaluation

The organoleptic properties of both drugs were complied with the given literature values as shown in Tables 1 and 2.

Melting point

The melting point of the pure drugs was found $85^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for ARTM and $130^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for LMF which complied with the range of literature values for ARTM and LMF as shown in Table 3.

Solubility studies

The solubility study of ARTM and LMF revealed that ARTM was soluble in ethanol and LMF was slightly soluble in ethanol, which complied with the literature values. This indicates that the procured drugs were ARTM and LMF as showed in Tables 4 and 5.

Microscopic analysis

Particle shape and size of ARTM and LMF were evaluated by microscopic analysis by research microscope (Figure 1).

Particles of ARTM were found irregular shaped crystalline in nature and most of the particles of ARTM were found between 0 and $47\text{ }\mu\text{m}$ as shown in Table 6. Similarly, LMF powder was also found irregular in shape and yellowish crystalline structure and particles lies between 0 and $94\text{ }\mu\text{m}$ as shown in Table 6. Practically examined data matched with literature values.

Partition coefficient

The partition coefficient of ARTM and LMF were determined by shake flask method and it was found that the experimentally determined partition coefficients of ARTM and LMF were complied with the reference values as shown in Table 7.

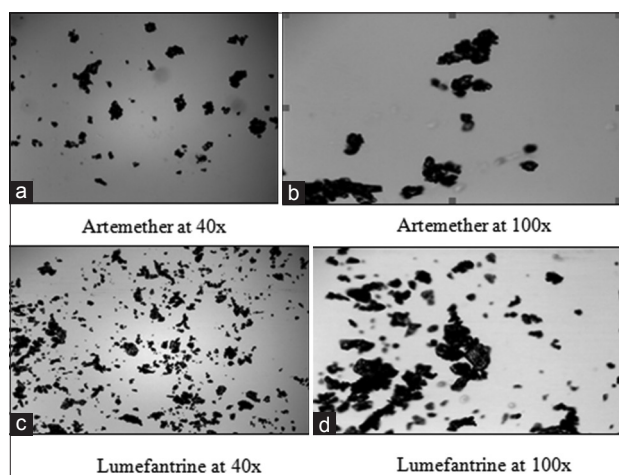


Figure 1: (a) Artemether at $\times 40$. (b) Artemether at $\times 100$. (c) Lumefantrine at $\times 40$. (d) Lumefantrine at $\times 100$

Table 1: Results of organoleptic evaluation of artemether

Evaluation parameters	Observed results	Reference results
Color	White	White
Odor	Odorless	Odorless

Table 2: Results of organoleptic evaluation of lumefantrine

Evaluation parameters	Observed results	Reference results
Color	Yellowish	Yellowish
Odor	Almond type odor	Almond type odor

Table 3: Melting point of artemether and lumefantrine

Parameter	Drug	Observed value	Reference value
Melting point	Artemether	$85 \pm 2^{\circ}\text{C}$	$86-88^{\circ}\text{C}$
	Lumefantrine	$130 \pm 2^{\circ}\text{C}$	$128-132^{\circ}\text{C}$

Table 4: Artemether solubility in different solvents

Solvent	Observed value	Reference value
Ethanol	++	Sparingly soluble
DMSO	++	Sparingly soluble
DMF	++	Sparingly soluble
Distilled water	-	Practically insoluble

++: Sparingly soluble, -: Practically insoluble, DMF: Dimethylformamide, DMSO: Dimethyl sulfoxide

Table 5: Lumefantrine solubility in different solvents

Solvent	Observed value	Reference value
Ethanol	++	Sparingly soluble
DMSO	++	Sparingly soluble
DMF	++	Sparingly soluble
Distilled water	-	Practically insoluble

+++ : Freely soluble, ++: Soluble, +: Slightly soluble, -: Practically insoluble, DMF: Dimethylformamide, DMSO: Dimethyl sulfoxide

Table 6: Particle size of procured drugs

Drugs	Particle size (μm)
Artemether	0-47
Lumefantrine	0-94

Table 7: Partition coefficient

Parameter	Drug	Observed value	Reference value
LogP o/w	Artemether	3.26	3.06-3.53
LogP o/w	Lumefantrine	2.9	2.29-3.52

Infra-red analysis

FTIR analysis of ARTM and LMF were conducted as follow:

Artemether

On FTIR analysis, obtained spectra of test sample of drug confirmed the reference spectra given in validation report of USP 2009. The peaks obtained in FTIR spectrum of test sample were examined and found in accordance with the functional groups present in chemical structure of ARTM. From this study, it was confirmed that procured drug was ARTM as shown in Figures 2 and 3, Table 8.

LMF

On FTIR analysis, obtained spectra of test sample of drug were similar to the reference spectra given in validation report of USP 2009. The peaks obtained in FTIR spectrum of test sample were examined and found in accordance with functional groups present in chemical structure of LMF. From this study, it was confirmed that procured drug was LMF as shown in Figures 4 and 5, Table 9.

Determination of λ_{\max} of ARTM and LMF

Determination of λ_{\max} of ARTM in water

λ_{\max} of ARTM in water after treating with 1 N HCl was found 256 nm which complied to the reference spectra of UV spectroscopy given in literature 256 nm. From obtained λ_{\max} , it was found that obtained drug was ARTM as shown in Figure 6.

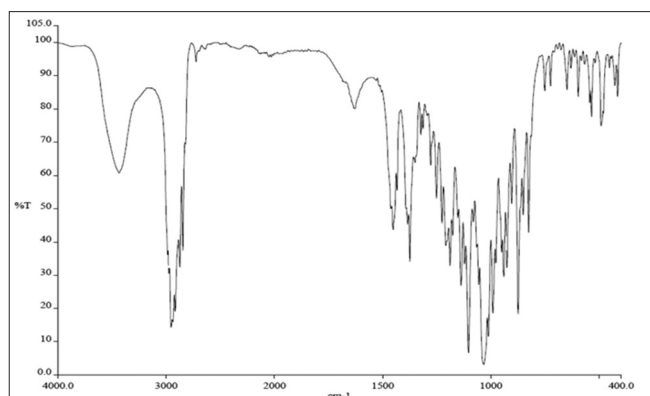


Figure 2: Reference Fourier transform infra-red spectra of artemether

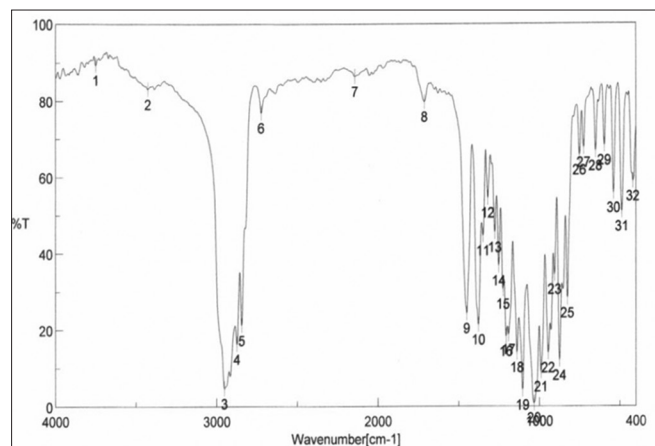


Figure 3: Fourier transform infra-red spectra of test sample of artemether

Determination of λ_{\max} of ARTM in buffer 1.2 pH

λ_{\max} of ARTM in buffer after treating with 1 N HCl was found 256 nm and which complied to the literature values as shown in Figure 7.

Table 8: Different peaks of artemether

Functional groups	Range of functional groups (cm ⁻¹)	Wave number (cm ⁻¹)
Aliphatic C-H	3500-3300	3425.92
C-O	1150-1050	1137.80
=C-O	1350-1150	1319.07
O=CH	2760-2700	2723.96

Table 9: Different peaks of lumefantrine

Functional groups	Range of functional groups (cm ⁻¹)	Wave number (cm ⁻¹)
O-H	3500-3000	3399.89
Aliphatic C-H	3500-3300	3425.92
C=C	1650-1550	1586.16
C-N	1350-1000	1307.5

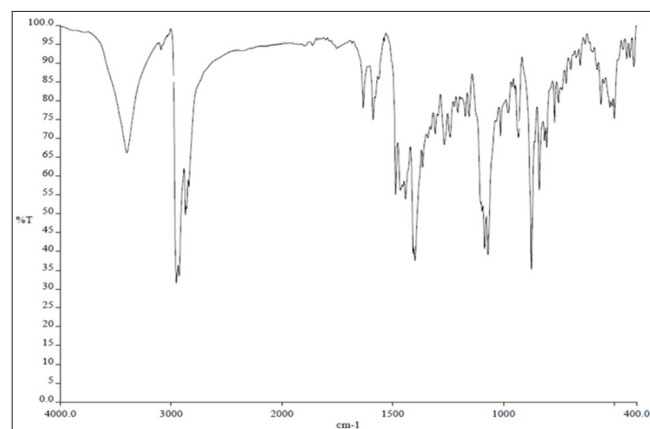


Figure 4: Reference Fourier transform infra-red spectra of lumefantrine

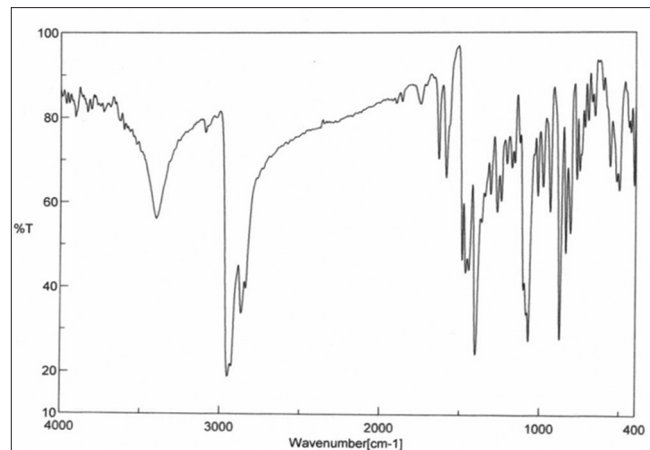


Figure 5: Fourier transform infra-red spectra of test sample of lumefantrine

Determination of λ_{\max} of ARTM in phosphate buffer pH 6.8

λ_{\max} of ARTM in phosphate buffer pH 6.8 after treating with 1 N HCl was found 256 nm and which compiled to the literature values as shown in Figure 8.

Determination of λ_{\max} of LMF in 0.1 N HCl

λ_{\max} of LMF in 0.1 N HCl was found 342 nm and which compiled to the given literature value as shown in Figure 9.

Determination of λ_{\max} of LMF in methanol

λ_{\max} of LMF in methanol was found 342 nm and considered for further analytical purpose as shown in Figure 10.

Preparation of calibration curve of ARTM in water

The calibration equation for straight line was observed to be $Y=0.01X-0.0343$ with correlation coefficient (R^2) of 0.9992 which was used to calculate the concentration of samples during analytical purposes as shown in Figure 11 and Table 10.

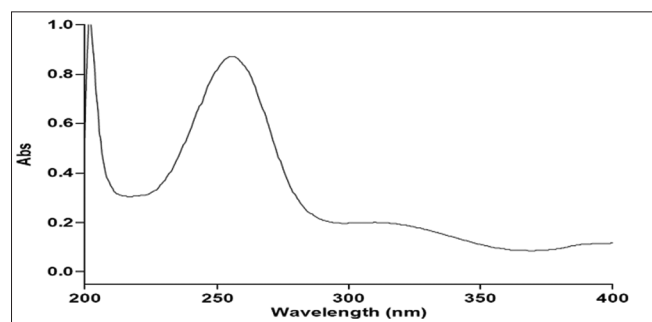


Figure 6: Absorption maxima of artemether in water

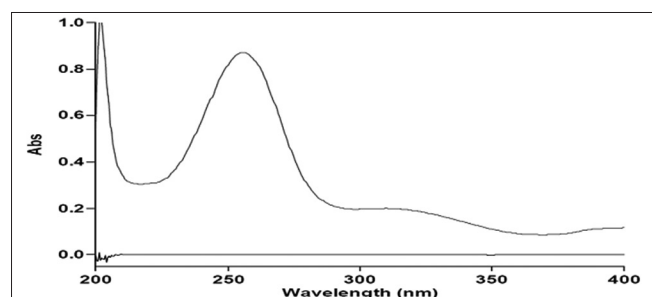


Figure 7: Absorption maxima of artemether in buffer pH 1.2

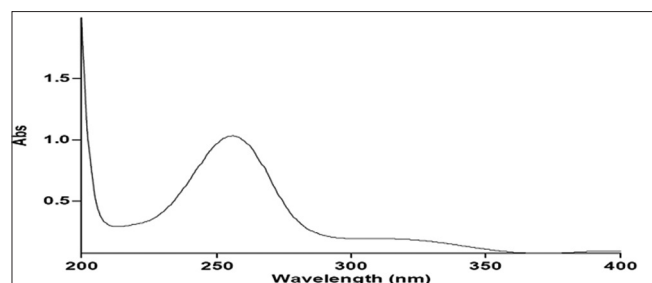


Figure 8: Absorption maxima of artemether in phosphate buffer pH 6.8

Preparation of calibration curve of ARTM in buffer pH 1.2

The calibration equation for straight line was observed to be $Y=0.021X-0.0044$ correlation coefficient (R^2) of 0.9997 which was used to calculate the concentration of samples during the dissolution study and other analytical purposes as shown in Figure 12 and Table 11.

Preparation of calibration curve of ARTM in phosphate buffer pH 6.8

The calibration equation for straight line was observed to be $Y=0.0254X-0.0085$ with correlation coefficient (R^2) 0.9984 which

Table 10: Preparation of calibration curve of artemether in water

Concentration ($\mu\text{g/ml}$)	Absorbance (nm)
5	0.0158 \pm 0.00055
10	0.0651 \pm 0.00051
15	0.1135 \pm 0.0017
20	0.1706 \pm 0.0017
25	0.2173 \pm 0.00021
30	0.2637 \pm 0.00010

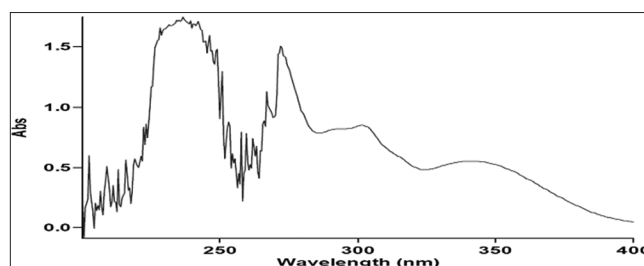


Figure 9: Absorption maxima of lumefantrine in 0.1 N HCl

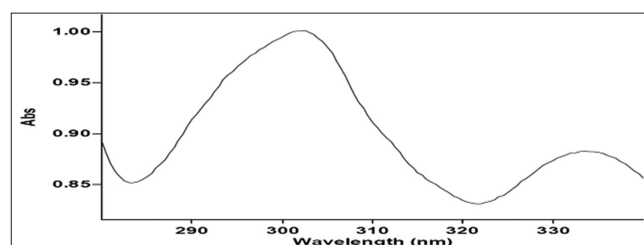


Figure 10: Absorption maxima of lumefantrine in methanol

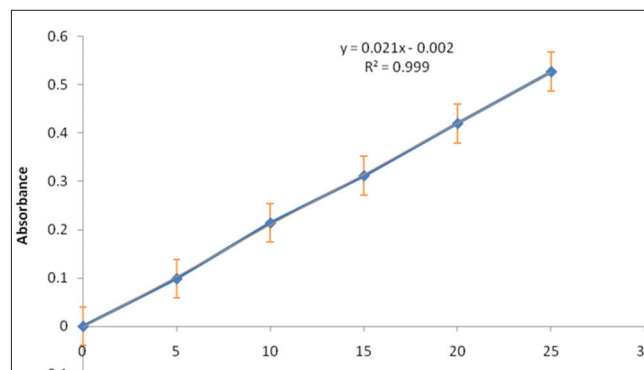


Figure 11: Calibration curve of artemether in water

was used to calculate the concentration of samples during dissolution study and other analytical purposes as shown in Figure 13 and Table 12.

Preparation of calibration curve of LMF in water

The calibration equation for straight line was observed to be $Y=0.0529X+0.0021$ with a correlation coefficient (R^2) 0.9982 which was used to analyze the concentration of drugs in various samples as shown in Figure 14 and Table 13.

Preparation of calibration curve of LMF 0.1 N HCl

The calibration equation for straight line was observed to be $Y=0.017X+0.0067$ with correlation coefficient (R^2) 0.9993 which was used to calculate the concentration of drug in samples

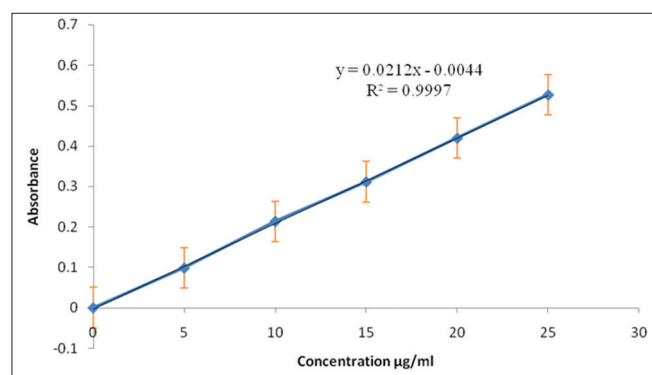


Figure 12: Calibration curve of artemether in buffer pH 1.2

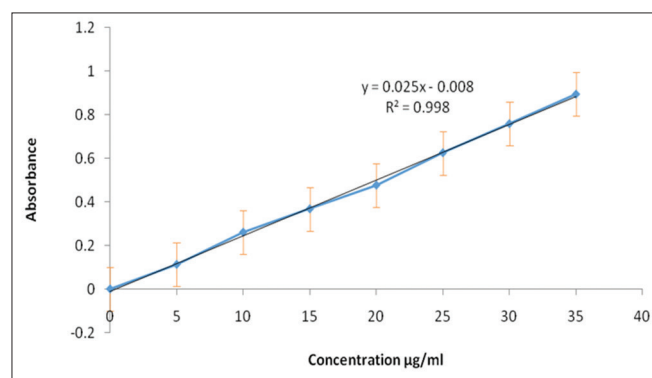


Figure 13: Calibration curve of artemether in phosphate buffer pH 6.8

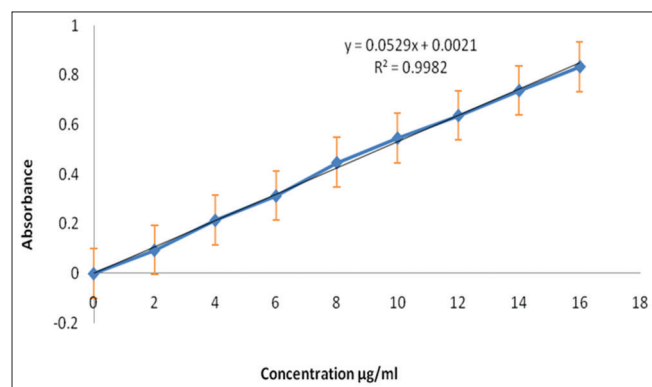


Figure 14: Calibration curve of lumefantrine in water

during dissolution study and other analytical purposes as shown in Figure 15 and Table 14.

Preparation of calibration curve of LMF in methanol

The calibration equation for straight line was observed to be $Y=0.0256X+0.0177$ with correlation coefficient (R^2) 0.9976 which was used to calculate entrapment efficiency and loading efficiency of nanostructured formulation as shown in Figure 16 and Table 15.

Discussion

The main aim behind the research work was to formulate a lipid based delivery system that may be useful for the increase in bioavailability of BCS class II drugs. Since such a lipid based drug carriers may keep the drug in dissolved state until the drug is completely absorbed and may avoid gastrointestinal degradation of drugs such as ARTM and LMF. The present work mainly focused to perform the preformulation studies to formulate the liquid crystalline nanoparticles in combination

Table 11: Preparation of calibration curve of artemether in buffer pH 1.2

Concentration (µg/ml)	Absorbance (nm)
5	0.0985±0.010
10	0.2139±0.006
15	0.3116±0.010
20	0.4197±0.025
25	0.5268±0.012
30	0.6323±0.038

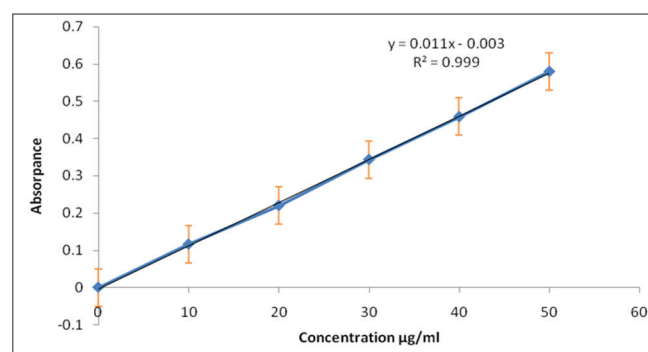


Figure 15: Calibration curve of lumefantrine in 0.1 N HCl

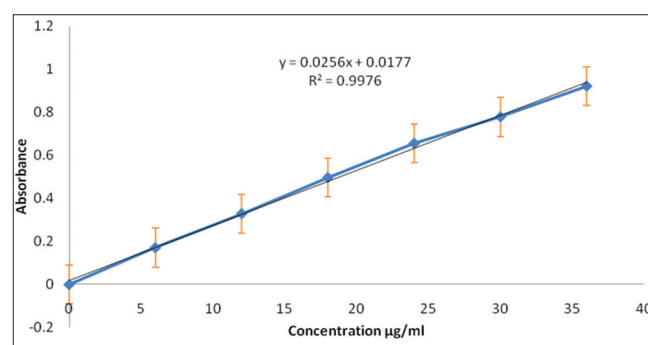


Figure 16: Calibration curve of lumefantrine in methanol

Table 12: Preparation of calibration curve of artemether in phosphate buffer pH 6.8

Concentration ($\mu\text{g/ml}$)	Absorbance (nm)
5	0.1126 \pm 0.010
10	0.26 \pm 0.006
15	0.3673 \pm 0.010
20	0.4759 \pm 0.025
25	0.6244 \pm 0.0012
30	0.7577 \pm 0.030
35	0.8926 \pm 0.034

Table 13: Preparation of calibration curve of lumefantrine in water

Concentration ($\mu\text{g/ml}$)	Absorbance (nm)
2	0.0943 \pm 0.0011
4	0.215 \pm 0.009
6	0.3134 \pm 0.0045
8	0.4476 \pm 0.0045
10	0.5465 \pm 0.0023
12	0.6374 \pm 0.0045
14	0.738 \pm 0.0010
16	0.8342 \pm 0.0018

of ARTM and LMF drugs and may circumvent the drawback of poor bioavailability.

The drugs ARTM and LMF were selected for the present research work, and drugs were characterized by different techniques such as melting point determination, solubility study, partition coefficient determination and FTIR analysis, determination of maximum wavelengths of drugs by UV spectroscopy. All the observed results complied with literature value that showed procured drug was ARTM and LMF.

Glyceryl monooleate and Poloxamer 407 along with Oleic acid were considered as novel excipients to formulate liquid crystalline dispersions of ARTM and LMF. Glyceryl monooleate is a lyotropic lipid and Poloxamer 407 used as a stabilizer to stabilize the formed liquid crystalline dispersion.

Conclusion

An attempt was made to perform the preformulation studies for the enhancement of solubility studies of ARTM and LMF. Pure sample of ARTM and LMF were supplied and used for further studies and identified successfully. ARTM and LMF were practically insoluble in water but soluble in ethanol, DMSO, and DMF. The partition

Table 14: Preparation of calibration curve of lumefantrine in 0.1 N HCl

Concentration ($\mu\text{g/ml}$)	Absorbance (nm)
0	0 \pm 0
10	0.1158 \pm 0.06
20	0.2197 \pm 0.013
30	0.3435 \pm 0.06
40	0.4585 \pm 0.05
50	0.58 \pm 0.02

Table 15: Preparation of calibration curve of lumefantrine in methanol

Concentration ($\mu\text{g/ml}$)	Absorbance (nm)
0	0 \pm 0
6	0.1709 \pm 0.06
12	0.3289 \pm 0.21
18	0.4966 \pm 0.10
24	0.6546 \pm 0.06
30	0.7777 \pm 0.38
36	0.9201 \pm 0.89

coefficient for ARTM and LMF were found 3.26 and 2.9. λ_{max} (256 nm, 342 nm) of ARTM and LMF were determined by UV-visible spectroscopy and IR Spectroscopy method.

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