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Preparation, Optimization and Characterization of Solid Lipid Nanoparticles Containing Antimalarial Drugs

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ABSTRACT:

Objective: The research aims to prepare and characterize solid lipid nanoparticles (SLNPs) containing artemether.
Method: The solvent diffusion approach was employed to produce artemether-loaded SLNPs. Artemether is one of the most widely used antimalarial medications, and it is the only one that can cure the relapsing form of malaria. Artemisinin has low water solubility, low bioavailability, and a short half-life, necessitating continual dose to maintain acceptable therapeutic drug-plasma concentration; therefore, we solve this issue by creating solid lipid nanoparticles. Nano formulation of pharmaceuticals in an appropriate drug carrier system has been extensively explored and found to have the potential to improve bioavailability, hence increasing activity, reducing dose frequency, and, as a result, lowering toxicity.
Results: The produced SLNs had particle size, polydispersity index (PDI), and zeta potential (ZP) values ranging from 211.6 to 389.9 nm, 0.277 to 0.723, and -17.8 to -16.5 mV, respectively. SEM, XRD, PDI, Zeta potential, Entrapment efficacy, and percentage yield, as well as all other examinations, were completed successfully. The optimized F6 batch released medicine at a rate of 98.07%. Optimized batch involves research like Differential scanning calorimeter thermograms showed that the medication is more physically stable in manufactured formulations. Negligible changes in drug characteristic peaks in Fourier transform infrared spectra indicated that there was no interaction between the various components in the nanoparticle formulation.
Conclusion: Solid lipid nanoparticles containing artemether were successfully developed. The improved F6 batch released 98.07% of the medication within 6 hours. The optimized batch was stored for stability testing.

Keywords: Solid lipid nanoparticle, Artemether, Malaria, Nanoparticle, Bioavailability.

INTRODUCTION

Malaria, one of the world's worst diseases, is most widespread in tropical and subtropical climates (Paliwal et al 2023). The World Health Organization (WHO) announced in 2021 that over 620,000 people died from malaria in 2020, out of an expected 241 million cases. 95% of malaria cases were reported to occur in Africa, with children under the age of five accounting for almost 80% of these deaths (Akbari et al., 2022; Altammar, 2023). The female Anopheles mosquito is the principal vector of this potentially fatal parasitic disease, which spreads most

during the hot and humid seasons. These mosquitoes poison human bloodstreams with Plasmodium parasites (Phillips et al 2017).

Only five of the genus Plasmodium's over one hundred species have been shown to infect humans: *P. vivax, P. ovale, P. knowlesi, P. malariae,* and the most prevalent, P. falciparum (Duffy, P. E. et.al 2020) Artemisinin-based combination therapy (ACT) can be used to prevent, treat, and manage all of them, despite differences in immunology, morphology, medication response, and recurrence patterns (Miller et al., 2013, Cowman et al 2016). Malaria is prevented using two complimentary strategies: chemoprophylaxis and mosquito bite prevention. In malaria, the use of antimalarial drugs as "protection" against the disease is known as chemoprophylaxis or chemoprevention (Waters, 2016).

To avoid mosquito bites, people should apply insect repellents containing 50% diethyltoluamide (DEET) to their skin, cover their beds with mosquito nets, wear long sleeves and pants at all times, and treat clothing, tents, and other materials with permethrin (White, 2011, Zuckerman, 1977). Despite being preventable and curable, malaria has not been totally eradicated because the parasite has developed resistance to current combination therapy drugs containing artemisinin (Carmona-Fonseca et al., 2022).

Despite changes to prevention, diagnostic, and treatment operations during the outbreak, governments around the world have mainly held the line against further setbacks to malaria control, according to the report's 2022 edition. Malaria mortality worldwide are estimated to reach 619,000 in 2021, up from 625,000 in the first year of the pandemic. Prior to the pandemic, there were 568,000 fatalities in 2019 (Magwaza et al., 2023, (Ellen et al., 2023). Malaria cases increased in 2020 and 2021, albeit at a slower rate than between 2019 and 2020. Malaria occurrences rose to 247 million in 2021, up from 245 million in 2020 and 232 million in 2019 (Chaves et al 2022).

Artemether

Artemether is a lipid-soluble derivative of artemisinin. It comes in both oral and intramuscular forms. It can also be coupled with lumefantrine in a fixed-dose formulation. Artemether-lumefantrine is one of the WHO-recommended ACTs, and the majority of countries utilize it when they transition from less effective to ACT drugs (Deng et al., 2020; Duong et al., 2020). Several clinical experiments have investigated artemether's pharmacokinetics, either alone or in combination. Artemether is rapidly absorbed when administered orally, reaching its peak concentration within 2 hours and engaging significant first-pass metabolism. The primary active metabolite is DHAB (Esu et al., 2019). Artemether binds strongly (>90%) to α1-acid glycoprotein, albumin, and lipoproteins. The process of elimination has a half-life of two to four hours (Garud et al., 2012; Genç et al., n.d).

Artemether is an antimalarial medication used to treat uncomplicated acute malaria. It is used in conjunction with lumefantrine to increase efficacy (Ghasemiyeh & Mohammadi-Samani, 2018). This combination medicine is effective against Plasmodium spp. erythrocytic stages and can be used to treat infections caused by *P. falciparum* and unknown Plasmodium species, including those acquired in chloroquine-resistant areas.

Artemether is converted in the body to the active metabolite dihydroartemisinic. The medicine works against *P. falciparum* erythrocytic stages by blocking nucleic acid and protein synthesis (Gujjari et al., 2022; Kekani & Witika, 2023). Artemether is used in combination with lumefantrine to increase efficacy (Kojom Foko et al., 2019). Artemether has an immediate beginning of action and is quickly eliminated by the body. Artemether is intended to give rapid symptom relief by lowering the quantity of malaria parasites (Krishnatreyya et al., 2019; Mishra, 2021).

Solid lipid nanoparticle (SLN)

Solid lipid nanoparticles (SLN) were developed in the early 1990s as an alternative colloidal carrier system to emulsions, liposomes, and polymeric nanoparticles for controlled drug

delivery (Mukherjee et al., 2009). SLN is made up of a matrix of solid lipids that are held together in an aqueous dispersion by surfactants or polymers. The dispersion can be spray-dried or lyophilized to get a dry product (Nguyen & Duong, 2022). Solid lipid nanoparticles can provide particle form and physical integrity while also stabilizing sensitive components (Omari et al., 2006). SLN are nanostructures made up of solid lipids such as glyceryl behenate (Compritol), stearic triglyceride (tristearin), cetyl palmitate, and glycerol tripalmitate (tripalmitin), with diameters ranging from 50 to 1,000 nm (Peto et al., 2022; Qushawy & Nasr, 2019).

Lipids have been considered as an alternative carrier to overcome the limitations of polymeric nanoparticles, particularly for lipophilic medications. Such small lipid particles are known as solid lipid nanoparticles (SLNs), and they are becoming increasingly popular among formulators worldwide (Scioli Montoto et al., 2020; Sinha et al., 2010). SLNs are colloidal carriers created in the previous decade to replace traditional carriers such emulsions, liposomes, and polymeric nanoparticles (Viegas et al., 2023).

MATERIAL AND METHODS

Material

Artemether was procured from Niksan Pharmaceuticals, Glycerol Monostearate, Soya Lecithin Purchased from Research Pvt Ltd., Poloxamer 407, Pluronic F 127 from Loba Chemie Pvt Ltd., and solvents such as Methanol n-hexane Purchased from SD Fine Chemicals Pvt Ltd. All compounds were analytical reagent grade.

Method of preparation of solid lipid nanoparticles

SLNs containing artemether were produced utilizing the solvent diffusion method. The aqueous phase was created by dissolving the surfactant and co-surfactant in 50 mL of double distilled water. The organic solution was made by thoroughly dissolving the medication (artemether) and lipid (GMS) in 5 mL of water-miscible solvent (methanol) in a water bath at 70 0C. The resulting organic solution was injected into 50 ml of an aqueous phase containing the surfactant with continuous stirring at 1,000 rpm (Remi Instruments Ltd, Mumbai, India) at 61 0C for 1 hour (Aceto et al., 2023; Aljaeid & Hosny, 2016; Bhalekar et al., 2017; Bodke et al., 2024; Chinaeke et al., 2015).

The resulting nanosuspension was allowed to cool to ambient temperature. As the temperature drops, the lipid droplets solidify, resulting in SLNs. Optimizing the formulation was carried out with several variables such as concentration of lipid, surfactant, and co-surfactant.

EVALUATIONS OF SOLID LIPID NANOPARTICLES

Preparation of calibration curve in methanol

10 mg of artemether was accurately weighed and placed in a 100 mL volumetric flask, followed by 25 mL of 1 N HCl. The solution was heated on a water bath at 80±2 ºC for 30 minutes, then cooled at room temperature. The volume was increased to 100 ml with methanol to achieve a concentration of 100 µg/ml and used as a stock solution (Da Silva De Barros et al., 2021). Methanol was used to prepare further dilutions of this stock solution. The absorbance of these samples was measured using a Shimadzu -1700 UV/Visible spectrometer at 254 nm. (Figure 1)

Particle size

The physical stability of SLNs is determined by their particle size. The most effective methods for detecting particle size are photon correlation spectroscopy (PCS) and laser diffraction (LD) (Dandagi et al., 2014). The variability in intensity of diffused light is caused by particle movement. Photon correlation spectroscopy (PCS) detects particle sizes from 3 nm to 3 m, while laser diffraction detects particle sizes from 100 nm to 180 m. PCS is a good approach for characterizing nanoparticles, but it can also identify larger microparticles. The LD method is

based on the diffraction angle's relationship with particle size. Smaller particles scatter more strongly at high angles than larger particles (Dhome et al., 2018).

Zeta potential

To measure zeta potential, utilize a zeta potential analyzer or meter. Before measuring size and zeta potential, SLN dispersions are diluted 50-fold with their original dispersion preparation fluid (Ekambaram & Abdul Hasan Sathali, 2011). In the absence of any complicating factors, such as steric stabilizers or hydrophilic surface appendages, a greater zeta potential value may result in particle disassociation. Zeta potential measurements can help predict storage. Colloidal dispersion stability. The significance of the zeta potential is related to colloidal dispersion stability. In the dispersion phase, the Zeta potential indicates the degree of repulsion between neighboring charged particles. As the molecules and particles are small enough, a high zeta potential ensures stability, suggesting that the solution or dispersion will not aggregate (Fouad et al., 2015; Gathirwa et al., 2014).

Surface morphology

Electron microscopy confirmed the morphological characteristics of solid lipid nanoparticles. The surface morphology of solid lipid nanoparticles was investigated using scanning electron microscopy. Scanning electron microscopy (SEM) produces three-dimensional pictures of particles and their surface morphology. (Figure 2) TEM (transmission electron microscopy) can reveal the shape and size of nanoparticles, as well as their internal structure (Granja et al., 2023; Jain & Vyas, 2023).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) can assess the degree of crystalline structure in lipid particles (Kushwaha et al., 2013). It is a thermo-analytical technology that uses lipid enthalpy to quickly and accurately assess the degree of crystal structure of lipids. Powder X-ray diffractometry (PXRD) is another non-destructive technique used to describe crystalline materials and investigate the crystal structure of SLN (Liang et al., 2023; Maiti et al., 2023).

***In vitro* drug release**

Drug release investigations in vitro were carried out utilizing a modified Franz diffusion cell (Nair et al., 2012; Nemattalab et al., 2022, 2022; Odera et al., 2024; Parashar et al., 2016). A dialysis membrane with a pore size of 2.4 nm and a molecular weight cutoff of 12,000-14,000 was utilized. The dialysis membrane was steeped in double-distilled water for 12 hours before being mounted in a Franz diffusion cell (Parvez et al., 2020). The donor compartment held 1 ml of SLN formulation, whereas the receptor compartment had 12 ml of dialysis solution (phosphate buffer, pH 6.4). At a set time, 5 ml of the sample was extracted from the receiver compartment via the side tube. Fresh double distilled water was added to keep the volume consistent. (Figure 3)

Fourier Transform Infrared (FTIR) Study

Fourier transform infrared (FTIR-8400S, Shimadzu) testing confirmed the medication's interaction with lipids. The FTIR spectrum of Artemether was obtained using the conventional KBr Disc/Pellet method. The samples were created by combining anhydrous KBr powder and compressing into pellets. The FTIR spectra were collected at a range of 4000-400 cm−1, with a resolution of 4 cm−1 over 45 scans (Raina, 2013) (Figure 4).

Differential Scanning Calorimetry.

DSC is commonly used to assess the crystallinity and polymorphism properties of SLN components (Ramadan et al., 2023). It is important because the lipid matrix and the entrapped drug may undergo a polymorphic transition, resulting in unwanted drug ejection during storage (Ryan et al., 2023). A 3mg sample of Artemether, GMS, Artemether, and GMS was introduced one after the other into the Shimadzu 60 DSC. (Figure 5).

Entrapment efficiency

The drug's entrapment efficiency is calculated based on its concentration alone in the dispersion medium. Centrisart was employed for ultracentrifugation, which is a filter membrane (molecular weight cutoff 20,000 Da) located at the bottom of the sample recovery chamber (Shahab-Navaei & Asoodeh, 2023). The SLNs and encapsulated drug remain in the outer chamber, while the aqueous phase moves inside the sample-collecting chamber. To determine the amount of medication in the aqueous phase, use HPLC or a UV spectrophotometer.

$$\% \text{ Entrapment efficiency} = \frac{[(\text{Initial drug weight of free drug})}{[\text{ Weight of initial drug}]} \times 100\%$$

Drug content

The total amount of drug in the formulation was determined by dissolving 1 ml of the formulation in 10 ml of methanol. The amount of artemether in each sample was determined using a UV spectrophotometer (1700, Shimadzu, Japan) by measuring the absorbance at a λ max value of 240 nm (Singh et al., 2021).

Stability testing

Storage stability testing revealed that plain and coupled SLN formulations kept at 41℃ were more stable than those kept at room temperature (Wang et al., 2023). The average particle size of nanoparticles has been shown to grow during storage, which may be due to particle aggregation under different storage conditions. This effect was limited in formulations stored at 41°C, demonstrating that temperature can be used to control aggregation, with 41°C being the optimal storage temperature. The various SLN formulations were stored at 41℃ and room temperature, and the remaining drug content was estimated after 30, 60, and 90 days (Wu et al., 2021).

RESULTS AND DISCUSSION

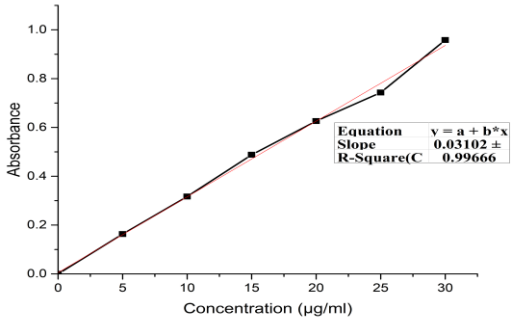


Figure 1. Calibration curve of artemether in methanol

Table 1. Optimization of lipid concentration

Batch code	Lipid concentration(mg)	Size(nm)	PDI	Zeta potential	%TDC	%E.E	% Yield
F-1	10	358.7	0.317	-24.3	49.32	72.39	42.6
F-2	20	307.8	0.329	-18.6	53.80	76.42	39.7
F-3	30	342.2	0.357	-20.3	57.4	79.3	41.4
F-4	40	261.1	0.382	-17.9	59.3	78.4	40.7
F-5	50	217.6	0.420	-28.2	61.2	80.73	44.20
F-6	60	264.3	0.398	-21.7	60.4	75.4	45.5
F-7	70	239.4	0.410	-19.0	62.4	74.6	39.2
F-8	80	252.9	0.4.9	-22.3	61.92	79.2	41.9
F-9	90	274.4	0.394	-23.2	59.2	78.6	46.6

F-10	100	281.9	0.378	-20.8	58.7	71.8	45.2

Table 2. Optimization of co-surfactant concentration (Poloxamer).

Batch code	Poloxamer Concentration (%)	Size (nm)	PDI	Zeta potential	%TDC	%E. E	% Yield
F-11	0.5	218.8	0.435	-28.7	58.1	79.78	45.3
F-12	0.75	246.3	0.512	-22.6	56.8	71.29	41.8
F-13	1.0	223.7	0.639	-27.2	59.2	65.83	36.2

Table 3. Optimization of surfactant concentration

Batch code	Soya lecithin (%)	Size (nm)	PDI	Zeta potential	%TDC	%E. E	% Yield
F-14	0.5	288.4	0.603	-20.9	60.20	61.8	39.2
F-15	0.75	282.9	0.317	-22.6	61.8	73.4	39.86
F-16	1.0	278.2	0.414	-24.2	58.4	76.3	42.69

Table 4. Optimization of stirring speed

Batch code	Stirring speed (rpm)	Size (nm)	PDI	Zeta potential	% TDC	% E. E	% Yield
F-17	700	389.9	0.277	-21.0	49.91	78.19	39.99
F-18	800	303.7	0.321	-23.1	46.16	73.46	36.00
F-19	900	289.2	0.551	-24.3	58.19	76.1	42.98
F-20	1000	215.6	0.492	-25.2	59.98	82.64	36.39
F-21	1100	250.4	0.472	-22.0	52.10	69.85	42.45
F-22	1200	282.5	0.522	-24.0	56.29	73.68	49.68

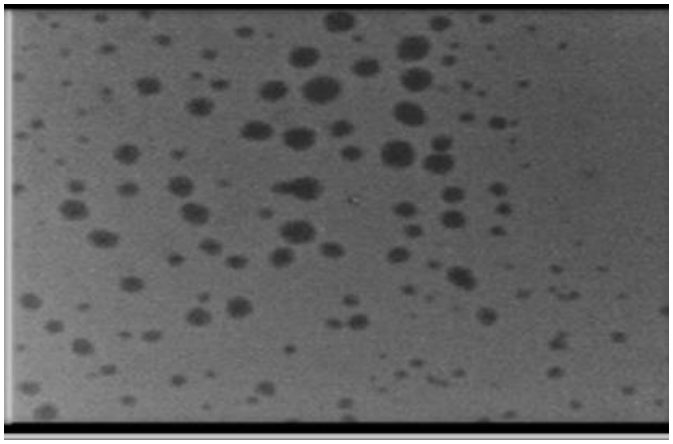


Figure 2. SEM of solid lipid nanoparticle of artemether

Table 5. Optimized formula for SLN

Sr. No.	Parameters	Quantity
1	Lipid concentration	60 mg
2	Surfactant concentration	1 %
3	Co-surfactant concentration	0.5%
4	Stirring speed	1000 rpm

Table 6. Cumulative % drug released from optimized formulation

Sr. No.	Time (hours)	% Cumulative drug release of (µg/ml)
1	0.5	58.3±0.781
2	1.0	69.1 ±0.628
3	2.0	70.2 ±0.529
4	3.0	76.9 ±1.058
5	4.0	84.8 ±0.591
6	5.0	91.3 ±0.853
7	6.0	98.02 ±1.521

data represents mean ± SD, (n=3).

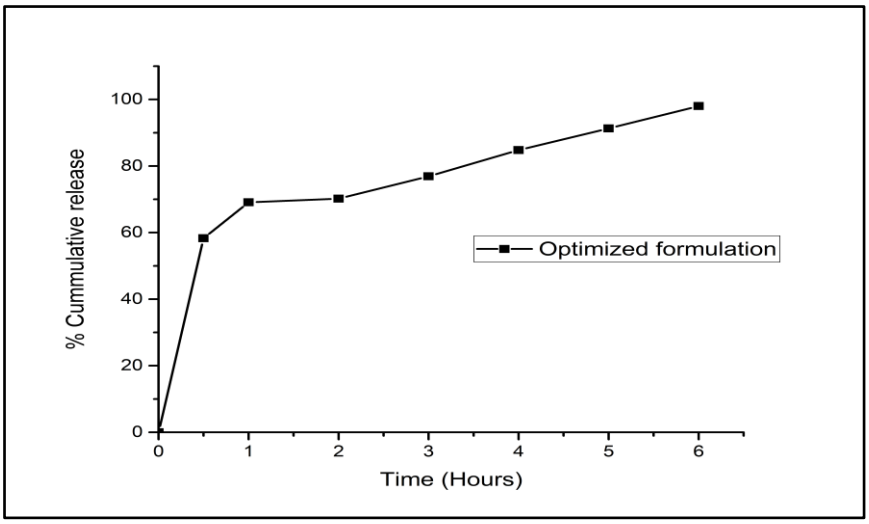


Figure 3. Cumulative % drug released of optimized formulation

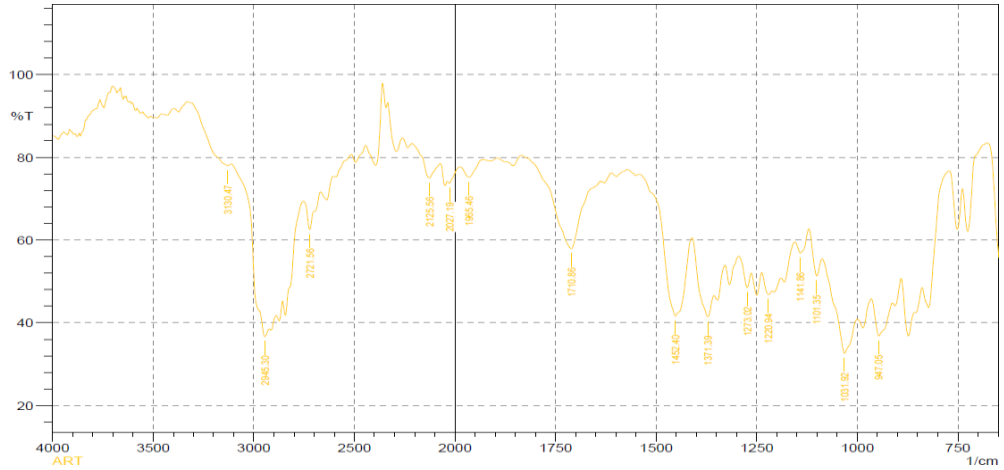


Figure 4. FTIR Spectra of artemether

Table 7. Characteristic IR absorption bands of artemether.

Sr. No	Wave Number (cm-1)	Characteristic Absorption
1	2945.30	C-H Stretching of CH3
2	2721.56	Aliphatic C-H stretching
3	1024	C-O stretching of C-O-C (ether)
4	2933.85	C-H stretching of saturated (cyclo-alkane)
5	2815	C-H stretching of CH3

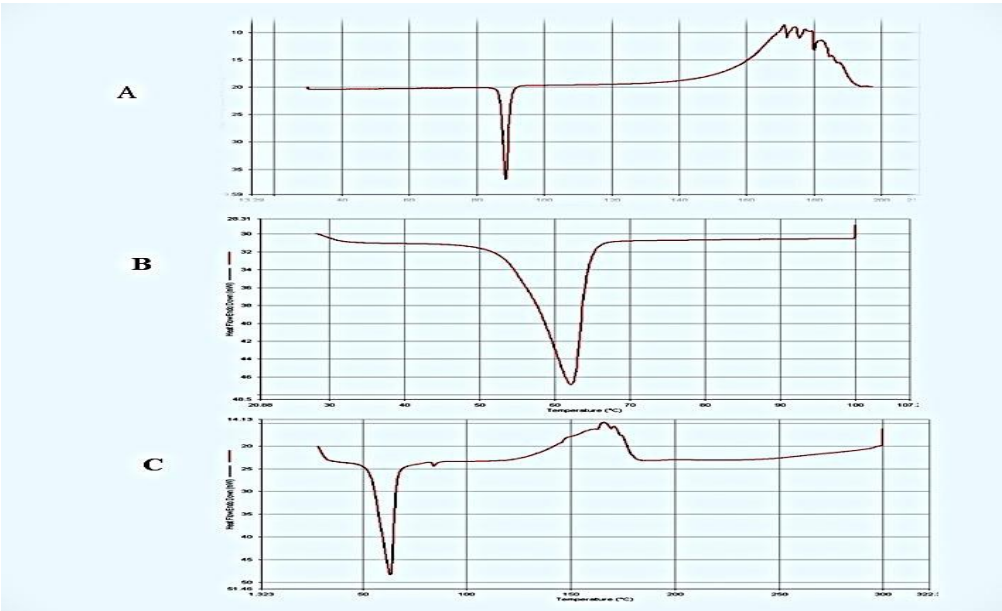


Figure 5. DSC of (A) Artemether, (B) GMS, (C) Artemether and GMS.

Optimization of formulation by Design of expert

The preparation of solid lipid nanoparticles involves various process variables but optimization was carried out by considering following factor**.** The three Factors Considered are Effect of lipid concentration, Effect of surfactant concentration, Effect

of co surfactant concentration. The Effect of the three Factors Considered was Studied on the Following Responses such as Percent yield of SLN, Percent entrapment efficiency of drug, Cumulative percent release of drug, Total drug content. (Figure 6,7,8)

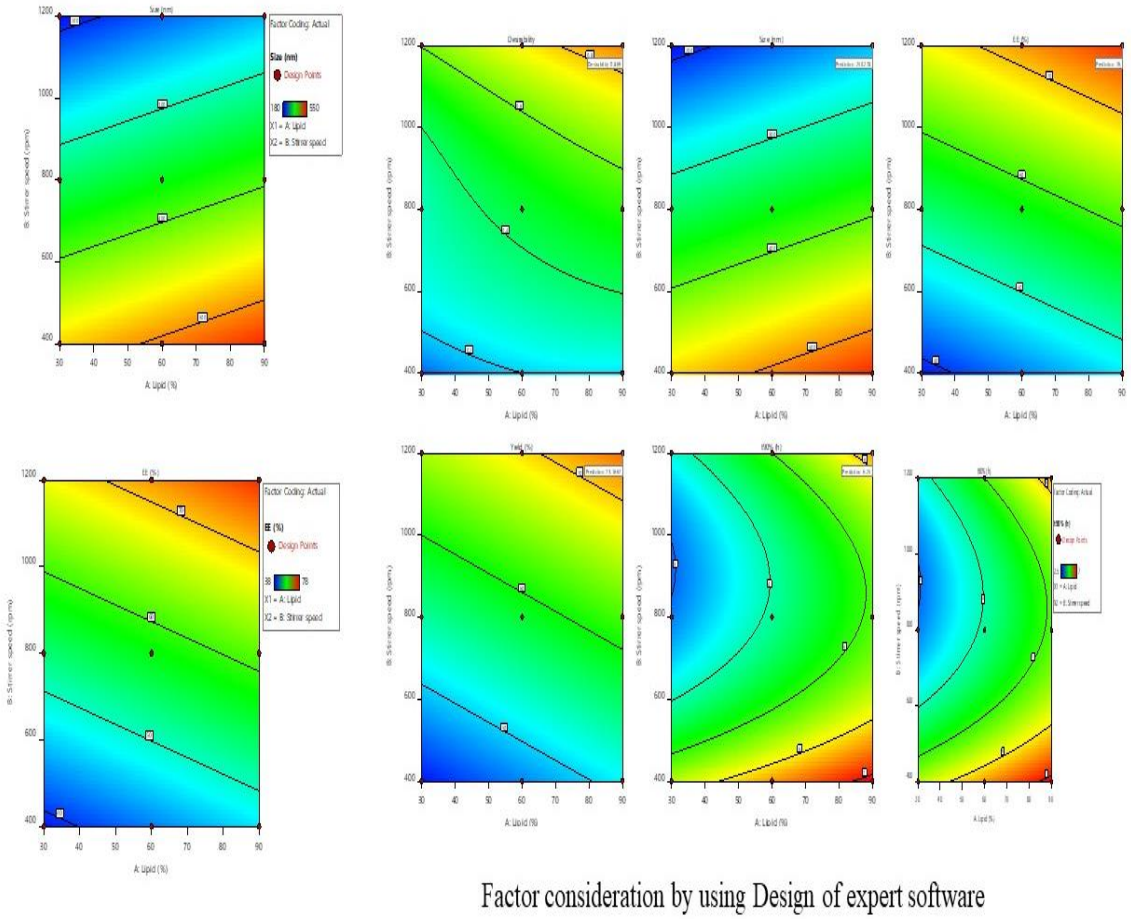
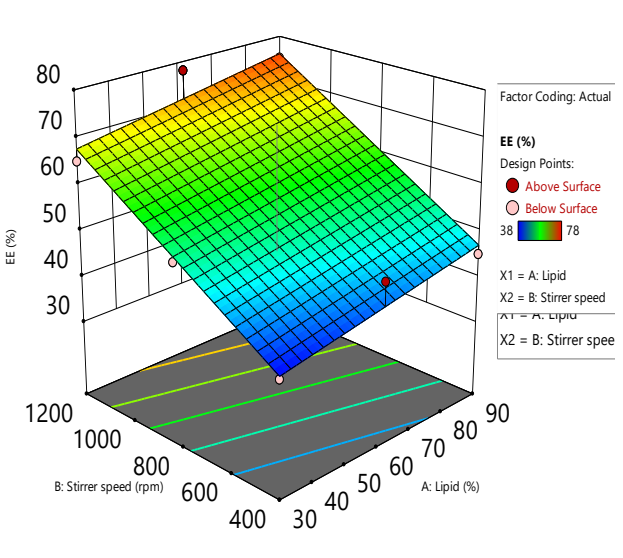
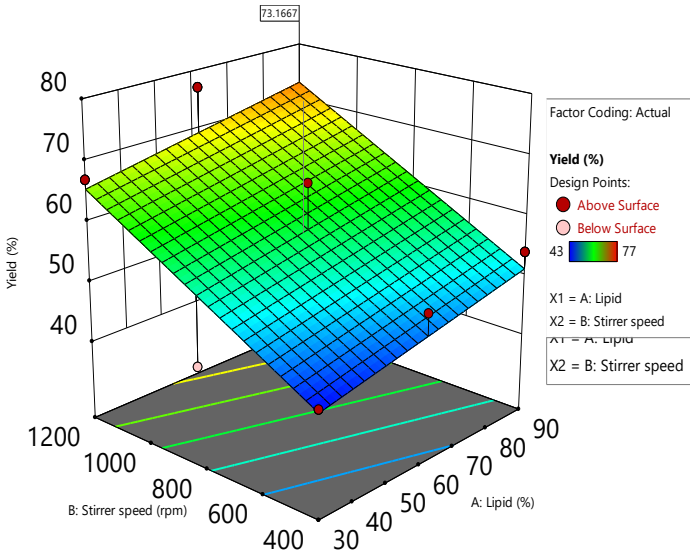


Figure 6. Factor consideration by using Design of expert software





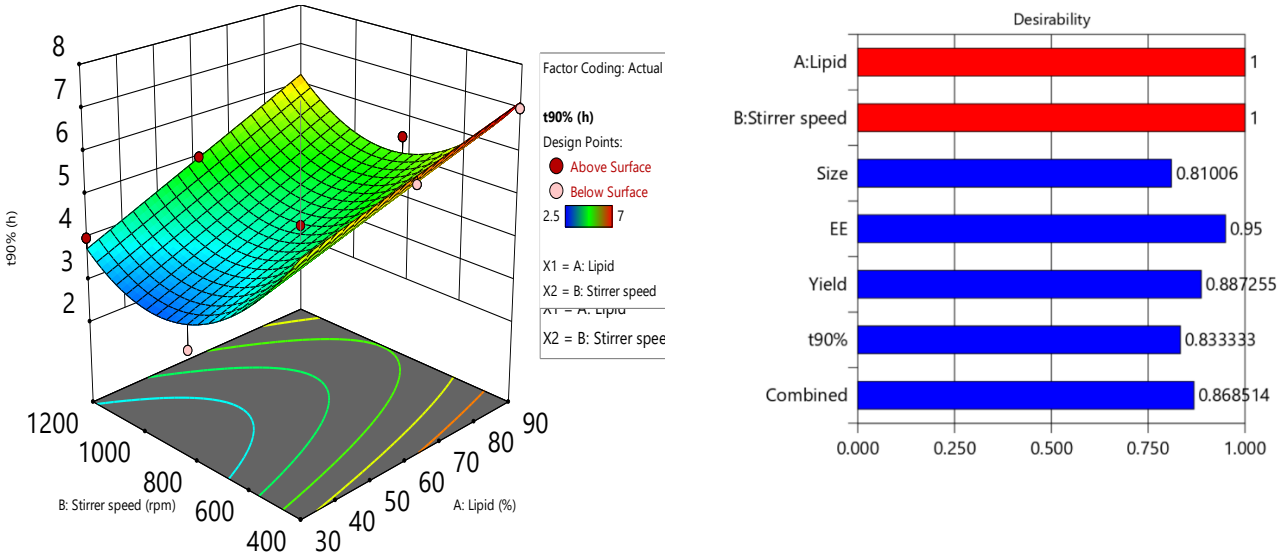


Figure 7. Graphical presentation of factor and its evaluated responses by using Design of expert software

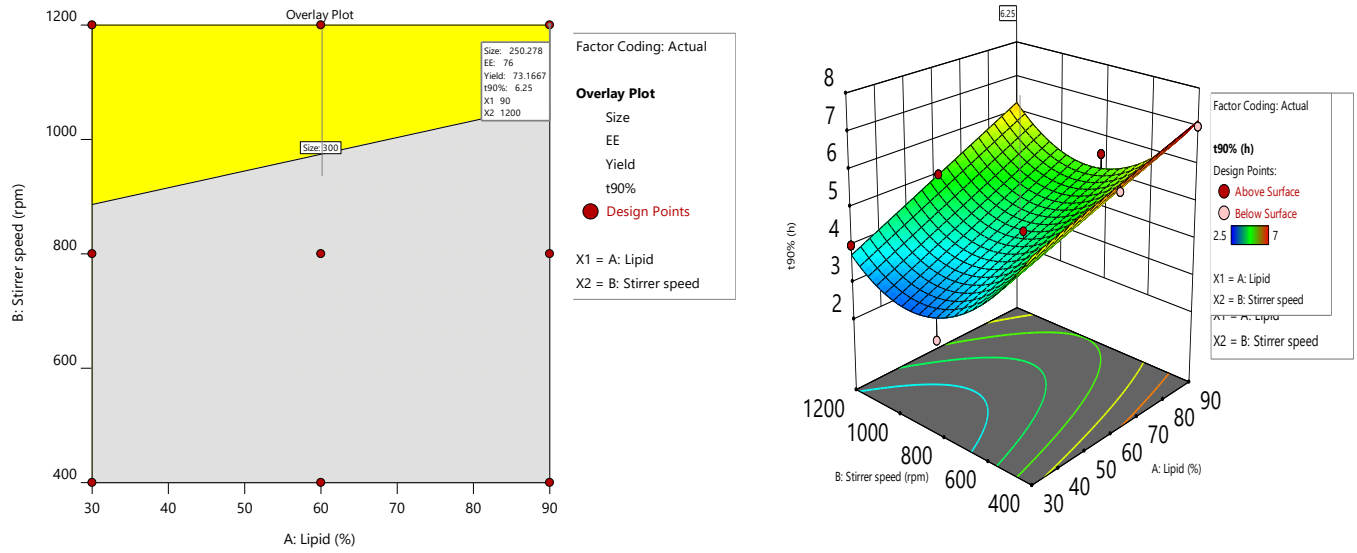


Figure 8. Optimization batch results using design of expert

CONCLUSION

A solid lipid nanoparticle containing Artemether was successfully synthesized. Artemisinin has limited water solubility, low bioavailability, and a short half-life, necessitating continual dose to maintain acceptable therapeutic drug-plasma concentration. We successfully addressed this issue by creating solid lipid nanoparticles. With their superior characteristics and benefits over other traditional forms of administration, SLNs have piqued the interest of many researchers, and other colloidal equivalents of SLN have proven to be a significant advancement in nanotechnology due to their efficacy and safety as a carrier in medicinal administration. Lipid nanoparticles can transport both hydrophilic and lipophilic medicines. In malaria treatment, SLN as a colloidal drug carrier combines the benefits of polymeric nanoparticles and liposomes, including better physical stability, the ability to include

lipophilic and hydrophilic drugs, cost-effectiveness, and ease of scaling up and production. SLNs have the potential to release medicines in specific places over time. SLNs are created using a variety of complicated procedures. SLNs are widely used in drug development, medicine distribution, and diagnostics, among other medical uses.

ABBREVIATIONS

SLN- Solid Lipid Nanoparticle

FUNDING

Nil.

AUTHORS CONTRIBUTIONS

The authors confirm contribution to the paper as follows:
Study concept and design by Dr. Reenu Yadav, Data interpretation by Dr. Bharat W. Tekade, Practical work and draft manuscript preparation by Mr. Swapnil Phalak, Data collection by Mr. Vishal Bodke. All authors reviewed the results and approved the final version of the manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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