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Research Article

Pharmaceutics for effective drug dosage

Preparation and Characterization of Advanced Nano medicine For Malaria: Box Behnken Design Approach

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Abstract: In tropical countries, malaria is a larger cause of sickness and death in adults and children. Drug resistance with antimalarial therapy has now become a serious problem worldwide and it is the basic reason for the need of combination therapy. Artemisinin-based combination therapy (ACT) is widely used nowadays. The drugs selected for the study from ACT were an Artesunate (AST) and Lumefantrine (LUM). In this study, the optimized Solid-Self nanoemulsifying drug delivery system (S-SNEDDS) formulation of AST and LUM was developed using Box Behnken design (BBD) which showed greater *in-vitro* drug release when compared to marketed formulation. Both the drugs AST and LUM show high solubility in Anise oil, Tween 80 (surfactant), and PEG 400 (co-surfactant). Ternary phase diagrams were used to select nanoemulsifying regions. It was found to have a maximum nanoemulsion region with a 2:1 surfactant to co-surfactant ratio. The effect of formulation variables was studied by BBD. Thirteen formulations were prepared and were further characterized based on globule size, PDI, zeta potential, self-emulsification time, cloud point, % transmittance and in vitro dissolution profiles. The optimized Liquid-SNEDDS (L-SNEDDS) with RHLB 13.76 gives globule size (82.8 nm), % T (96.7 %), *in vitro* release of AST (98.4 %) and LUM (97.07%) at 45 min. was converted into S-SNEDDS by using neusilin US2 as an adsorbent. The S-SNEDDS was characterized by SEM, globule size (98.24 nm), % T (95.02 %), *in vitro* release of ART (95.18 %) and LUM (96.4%) at 45 min. DSC and FTIR study for S-SNEDDS assure that there was an absence of any chemical interaction within the drug and carrier. SEM, X-ray diffraction studies confirmed that drugs exist in amorphous nature. The present research successfully developed S-SNEDDS for bioavailability enhancement of AST and LUM in fix dose combination

Keywords: Artesunate and Lumefantrine, Solid self-emulsifying drug delivery system, Box-Behnken design, Antimalarial formulation

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1. INTRODUCTION

The oral drug delivery of poorly water soluble or lipophilic drugs is frequently associated with low bioavailability, high intra and inter subject variability. In order to overcome these problems of poor solubility, many techniques are adopted these days. Some of them include solid dispersion technique, inclusion complexation, liquid solid compaction, self-emulsifying drug delivery systems etc.[1] In the present context, the failure of the conventional delivery system due to various factors like problems associated with absorption, altered metabolism, poor drug solubility, variability in plasma drug concentration and the effect of food, has given rise to the search for newer methods in case of delivery of a drug through oral route. For improving the bioavailability and solubility of such oral drug delivery systems, it is required to formulate suitable formulations.[2] The main challenge for the formulation scientist has been the formulation and development of poorly water-soluble moieties. The lipid-based formulation methodology has seen a wide range of interest in improving the oral bioavailability and the drug solubilization in the Gastrointestinal Tract (GIT) of BCS class II and IV drugs. Current investigations support the usage of lipid-based formulations to tackle the formulation challenges of poorly soluble drugs. Common pharmaceutical excipients used in self-nano emulsifying drug delivery systems (SNEDDS) containing bio enhancers like cremophor, tween 80, PEG 400 are reported to facilitate absorption by inhibiting glycoprotein efflux hence enhancing the bioavailability.[3,4] Malaria is an acute infectious disease caused by the bite of female Anopheles mosquitoes belonging to genus *Plasmodium* which flies high in humid and swampy areas. Being the most insidious species, *Plasmodium falciparum* is a rapid fulminating disease, the symptoms of which are persistent high fever, orthostatic hypotension, and massive erythrocytosis. Plasmodium falciparum infection can lead to capillary occlusion thereby causing death if treatment is not initiated promptly. Plasmodium vivax causes a mild form of malaria, *Plasmodium malaria* is most common in tropical regions and *Plasmodium ovale* is often encountered.[5] The resistance acquired by the parasite to drugs, abstained from the development of new therapeutic challenges, particularly in the controlling of resistance caused by P. falciparum. The efficacy of a drug treatment particularly to plasmodium species and each stage of its life cycle is being targeted.[6,7] In tropical countries Malaria is a larger cause of sickness and death in adults and children. Drug resistance with antimalarial drugs has now become a serious worldwide question and it is the basic reason for the decrease in antimalarial drug efficacy and limits the choice in various parts of the world.[8] Artemisinin based antimalarial drugs is the most essential class of antimalarial presently available, due to their effectiveness against among all the available classes of antimalarial drugs to which parasites are resistant.[9]The aim of the present study was to contribute to the understanding of the physicochemical principles, key factors in predicting the performance and applicability of Self-nanoemulsifying drug delivery system (SNEDDS) for the improvement in dissolution performance of poorly water soluble/lipophilic drugs.

2. MATERIALS AND METHODS

2.1 Materials

Artesunate and LUM was obtained as a gift sample from IPCA Lab Ltd., Mumbai, India. Anise oil was obtained from Merck chemicals, Mumbai, PEG 400 and Tween 80 from Loba chemicals and Neusilin US II was obtained from FUJI Chemical industries, Japan.

2.2 Methods

2.2.1 *Solubility studies*

Solubility studies were conducted by placing an excess amount of the drug in a 2.5 ml in stoppered tubes containing 1 ml of vehicle (oil, surfactant or co-surfactant). Then the mixture was vortexed using cyclone mixer (REMI CM 101DX, REMI Equipment's, Mumbai, India) and kept at 25°C in orbital shaker (REMI motors, RIS-24BL, INDIA) for 48 h to facilitate the solubilisation. The samples were centrifuged at 5000 rpm for 10 min to remove undissolved drugs. The supernatant was taken and diluted with methanol for quantification of drugs by UV-Vis double beam spectrometer, (UV-1800 Shimadzu, JAPAN) in particular wavelengths of both drugs.[10]

2.2.2. *Selection of oil, surfactant and Co-surfactants*

Selection of oil was based on the solubility of the drug in the oil. Solubility of AST and LUM drugs in different oils (Anise oil, Oleic acid, Arachis oil, Lemon oil, Capryol 90) was estimated. Based on emulsification efficiency, selection of surfactant surfactant from Tween® 20, Tween® 80, Labroglycol 90 and Span® 80 and co-surfactant from PEG-200, PEG-400, Labrosol was done.Emulsification efficiency was determined based on number of inversions of volumetric flask to form a uniform emulsion and % transmittance of the emulsion was measured after 2 h at 638.2 nm using double beam UV-Vis spectrophotometer (UV-1800 Shimadzu, JAPAN) by taking distilled water as blank.[10]

2.2.3. *Construction of ternary phase diagram*

Self-emulsifying drug delivery system (SEDDS) containing AST and LUM was formulated by pseudo ternary phase diagram using a water titration method. An isotropic mixture of oil, surfactant, co-surfactant, and drugs was formed. The ternary phase diagram was constructed using selected oil, surfactant, co-surfactant. Surfactant mixture (Smix) ratios were chosen in increasing concentration of surfactant with respect to co-surfactant (1:1, 2:1,2:1, 3:1). The oil phase and specific Smix ratios were mixed thoroughly in different weight ratios ranging from 1:9 to 9:1 in separate glass vials. 0.1 ml from each ratio is titrated with distilled water separately and stirred using a magnetic stirrer at 37°C. Percentage transmission of all the ratios is then measured using a UV-visible double beam spectrophotometer (UV-1800 Shimadzu, JAPAN) at 638.2 nm. The ratios with > 80 transmissions are treated as good emulsions and were used for constructing ternary phase diagrams using CHEMIX SCHOOL Version 7.0 (Arne Stadnes, MN, USA) software.[11,12]

2.2.4. Optimization of L-SNEDDS formulation

From the pseudo ternary phase diagram, the nanoemulsion region was selected. The results revealed that Anise oil, Tween 80, and PEG 400 were used in varying ratios of exhibited the largest nanoemulsion area. Moreover, it was also observed that an increasing the amount of Anise oil above 40% caused an increase in droplet size as well as polydispersity index (PDI), whereas, increase in surfactant

and co-surfactant percentage above 60% revealed in a decrease in droplet size and PDI. Based on these results, the study has been carried forward towards the creation of DoE to the investigate effect of formulation variables (oil, surfactant and co-surfactant) on various responses like mean globule size (Y1), polydispersity index (Y2), % transmittance (Y3), % drug release of AST (Y4) and % drug release of LUM (Y5), (please see Results and discussions). Box-Behnken design (BBD) was used for designing various batches of L-SNEDDS of AST and LUM using Design-Expert version 11.0.0 software. All these variables were operated at three levels (+1, 0 and −1). In order to allow the estimation of pure error, a total of 13 experiments were designed by the software. Experiments were run in random order to increase the predictability of the model. The amount of AST and LUM added to formulations was 240 mg and 40 mg and it was kept constant, moreover, type of oil (Anise oil), type of surfactant (Tween 80) and co-surfactant (PEG 400) were also kept constant for all the experiments.[12,14,15] However, concentration of Anise oil (X1), Tween 80 (X2) and PEG 400 (X3) was varied. The design was used to statistically optimize the independent variables: concentration of oil, concentration of surfactant and concentration of co-surfactant and the significant response factors used to assess the quality of the SNEDDS formulation, including droplet size (Y1), poly dispersibility index (Y2), Percentage transmittance (Y3), Percentage of Drug released of AST in 30min (Y4) and Percentage of drug released of LUM in 30 min (Y5), were determined.(Table 1) The results obtained for each response were fitted to a quadratic polynomial model explained by a nonlinear Eq. 1:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_2 X_3 + \beta_6 X_1 X_3 + \beta_7 X^2_{\ 1} + \beta_8 X_2^{\ 2} + \beta_9 X_3^2 \ldots\ldots\ldots\ldots (1)$$

where y is the measured response, β0–β9 are regression coefficients and X1, X2 and X3 are independent factors. The models were validated by analysis of variance (ANOVA), lack of fit, and multiple correlation coefficient (R2) tests.

Table 1. ***Variables used in the Box–Behnken Design***

Independent variables	Levels, Actual (Coded)		
	Low (−1)	Medium (0)	High (+1)
X1: Amount of Anise oil added (mg)	170	170	170
X2: Amount of Tween 80 added (mg)	1260	1260	1260
X3: Amount of PEG 400 added (mg)	222	1356	716
Dependent variables			
Y1: Droplet size (nm)		Minimized	
Y2: Poly dispersibility index		Minimized	
Y3: Percentage transmittance (%)		Maximized	
Y4: Percentage drug release of AST in 30 min		Maximized	
Y5: Percentage drug release of LUM in 30 min		Maximized	

2.2.5. *Preparation of L-SNEDDS*

Formulations of AST and LUM loaded L-SNEDDS were prepared using anise oil and Smix in the ratios of 2:1 along with surfactant and co-surfactant in the ratios of 1:1, 2:1, 3:1. These were prepared by adding 240mg of AST and 40mg LUM loaded oil into Smix at 37°C by thorough mixing. The mixture was equilibrated for 24 h and observed for any signs of turbidity or phase separation. Compositions of developed formulations were shown in Table 4.[16]

2.2.6. *Preparation of S-SNEDDS from L-SNEDDS:*

The S-SNEDDS was prepared from L- SNEDDS using Neusilin US2 as a solid adsorbent. The adsorbent was added in increments to a fixed aliquot of L-SNEDDS and mixed vigorously until a free-flowing powder blend was obtained. Then powder blend formulates into different dosage forms i.e. hard gelatine capsule and tablet.[17]

2.2.7. *Characterization of optimized S- SNEDDS*

2.2.7.1. *Powder flow properties*

The S-SNEDDS powders were further subjected to micromeritics characterization for true, bulk, and tapped density, flow rate, angle of repose, Carr's compressibility index. [18]

2.2.7.2. *Globule size determination*

The reconstitution property of S-SNEDDS was checked by determining globule size and polydispersity index by using nano particle analyzer (HORIBA scientific & SZ-100) [19]

2.2.7.3. *Zeta Potential*

The particle is one of the factors determining the physical stability of emulsion and suspension. The particles are equally charged, the higher is the electrostatic repulsion between particles higher is the physical stability. Typically, particle charge is quantified as called zeta potential. Zeta potential of the sample was determined using Zetasizer (HORIBA scientific & SZ-100) [19]

2.2.7.4. *Scanning electron microscopy (SEM)*

The microphotographs of Neusilin US2 and optimized S-SNEDDS were taken for their morphological characteristics using SEM (S-4100, Hitachi, Shiga, Japan). The samples were placed on a brass stub with adhesive tape and it was made electrically conductive by coating in a vacuum (6 pas) along with platinum using an ion. [19]

2.2.7.5. *X-Ray powder diffraction (X-RPD)*

X-RPD patterns of Neusilin US2 and S-SNEDDS were recorded by X-ray diffractometer (Shimadzu, Maxima, X-RPD-7000, Tokyo, Japan). They were recorded at room temperature using monochromatic Cu-Ká radiation at 30 mA

and 40 kV over 10-80 p range at a step size of 30 per min and an intensity range of 0-1500 counts. [19]

2.2.7.6. *Differential Scanning Calorimetry (DSC) study*

The DSC thermograms were recorded using a differential scanning calorimeter. DSC of drug samples was performed using Mettler Toledo, at a heating rate of 10^0C approximately 2-5 mg of each sample heated in a pierced aluminium pan. Thermal data analysis of the DSC thermograms was conducted using STARe software (version 12.10) from 300°C to 3000°C. [19]

2.2.7.7. *In- vitro release study*

AST and LUM loaded S-SNEDDS formulation was filled in (size 0) capsule and 12mm Tablet. The quantitative in vitro release test was performed in 900 ml 0.1N HCL as a dissolution medium maintained at 37±0.5^0 C using USP type II dissolution apparatus. The paddle was rotated at 50 rpm. 5 ml aliquots were collected periodically (5, 10, 15, 30, 45, 60, 120 min) and replaced with a fresh dissolution medium. Aliquots after filtration through Whatmann filter paper and diluted with methanol. Analysis was carried out using a UV spectrophotometer at 400-200 nm. Results were compared with Marketed tablet, prepared tablet and capsule of AST and LUM formulation. The dissolution experiments were carried out in triplicate, and data were expressed as mean ±S.D. The drug release data were further analyzed to investigate release kinetic from S-SNEDDS by different mathematical models.[2,17,18] Several theories and kinetic models describe the dissolution of drugs from immediate release and modified release dosage forms. The quantitative elucidation of the values obtained in the dissolution assay is made easy by the usage of a generic equation that mathematically translates the dissolution curve function of some parameters related to the pharmaceutical dosage forms. Drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t or Q (t).[19,20]

2.2.8. *Accelerated stability studies*

The optimized solid-SNEDDS formulation was subjected to accelerated stability studies which is carried out at 40°C / 75 % ± 5 % RH as per ICH guidelines, in the sealed amber glass vials. They were assayed for particle size, PDI, and % T periodically for 3 months.[13,20]

3. STATISTICAL ANALYSIS

Statistical significance was evaluated using the Student's t-test, all data are represented as mean ± standard deviation (SD) with $p < 0.05$ considered statistically significant. A Box–Behnken statistical design applied using Design-Expert version 11.0.0 software (State- Ease Inc. Mineapolis, USA) to prepare L-SNEDDS to evaluated main and interaction effects of independent variables on the formulation.[21]

4. RESULTS AND DISCUSSION

The solubility of AST and LUM in oils, surfactants and co-surfactants is examined to choose the components for SNEDDS formulation. The solubility determines the drug loading capacity in the oil. Among all the oils examined (Anise oil, Oleic acid, Arachis oil, Lemon oil, Capryol 90) AST and LUM is highly soluble in Anise oil with solubility of 1350mg/ml in AST and 1876.2mg/ml in LUM which is shown in Table 2.

Table 2. ***Solubility of AST and LUM in different oils, surfactants, co-surfactants***

Solubility of AST and LUM	mg/ml for AST (mean, n=3)	mg/ml for LUM (mean, n=3)
OIL		
Anise oil	**1350.0**	**1876.2**
Arachis oil	445.0	82.36
Capryol-90	131.67	51.31
Labrafac-PG	39.33	94.21
Lemon oil	615.2	78.947
Oleic acid	1.265	342.1
Isopropyl myristate	18.5	40.0
SURFACTANTS		
Span 80	31.25	0.203
Tween 80	**3910.0**	**3342.0**
Labroglycol 90	92.0	0.5631
CO-SURFACTANT		
PEG-400	**810.0**	**195.23**
Labrosol	23.5	94.4

Among all the surfactants (Tween® 20, Tween® 80, Labroglycol 90 and Span® 80), Tween 80 was selected as the surfactant for the study as it has shown the highest solubility and percentage transmission (86.06) which indicates formation of clear nano-emulsion (Table 2). Co-surfactants further lower the interfacial tension improving the stability of the nanoemulsion. It also improves the dispersibility and drug absorption from the formulation. Selection of co-surfactant was also based on solubility and emulsification efficiency. Among all the co-surfactants (PEG 400, PEG 200 and Labrasol), PEG 400 was found to show highest percentage transmittance (91.73%) when used with Tween® 80 as surfactant.

3.1. *Ternary phase diagram*

To identify nano-emulsifying regions and to optimize the concentration of selected oil, surfactant and co-surfactant, ternary phase diagrams were plotted.[23, 24] Based on the monophasic region obtained in ternary phase diagram, the optimum concentrations of oil, surfactant, and co-surfactant were established for SNEDDS formulation. Fig. 1 shows the

ternary phase diagram with selected oil, surfactant, and co-surfactant and the shaded area indicates nanoemulsion region. Systems containing more than 25 % oil phase were found to be out of nano emulsification region. Surfactant concentration less than 34 % resulted in turbid emulsions, signifying the importance of surfactant concentration. A high concentration of Smix was required to produce stable nano-emulsions of desired globule size[23]

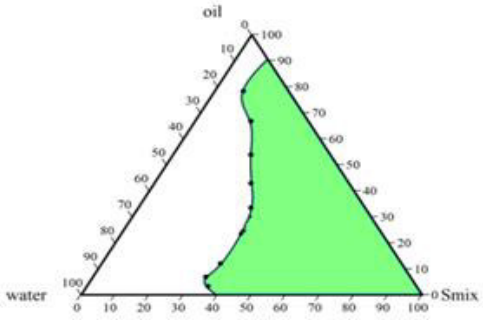


Fig1: ***Ternary phase oil: Smix (2:1) [Anise oil: Tween 80-PEG 400 (2:1)]***

3.2. *Optimization of L-SNEDDS formulation*

In our work, The experiments were designed using Design-Expert software version 11.0.0 software. A total of 13 experiments were carried out to study the formulation factors that affect the particle size, PDI , % Transmission, % DR of AST and % DR LUM. Response data for all experimental runs of Box Behnken design are presented in Table 3. The responses were fitted into Quadratic models. The obtained models were validated using an ANOVA. The coefficient of determination (R2) closest to unity indicated a good model. p Values lower than 0.05 indicated that the regression equations were statistically significant.[21]

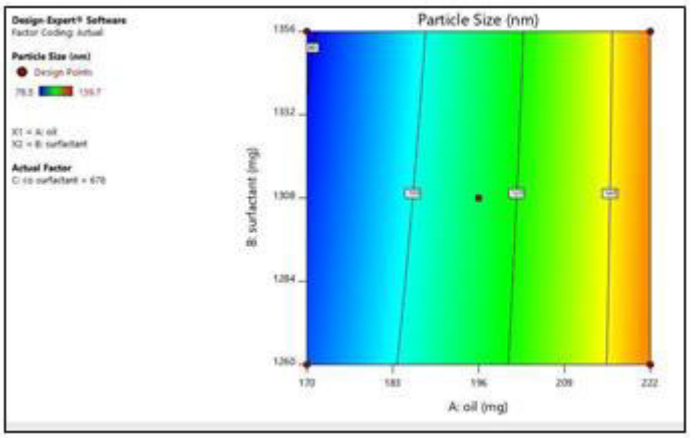
Table 3. ***Formulation optimization Design of L-SNEDDS***

Run	Amt. of oil (mg) X1	Amt. of surfactant (mg) X2	Amt of Co-surfactant (mg) X3	Globule size (nm) Y1	PDI Y2	%T (%) Y3	% AST (%) Y4	% LUM (%) Y5
AL1	222	1308	716	150	0.27	85	83	86
AL2	170	1308	716	78	0.37	81	86	84
AL3	196	1260	716	127	0.31	83	81	86
AL4	222	1308	640	159	0.34	86	89	91
AL5	170	1308	640	86	0.42	88	92	88
AL6	196	1356	678	112	0.21	87	95	95
AL7	196	1356	716	102	0.36	86	89	85
AL8	222	1260	678	147	0.24	90	91	93
AL9	222	1356	678	145	0.32	92	93	92
AL10	196	1260	640	105	0.41	89	92	94
AL11	170	1356	678	80	0.29	94	98	89
AL12	196	1356	640	126	0.31	92	94	91
AL13	170	1260	678	89	0.41	91	93	89

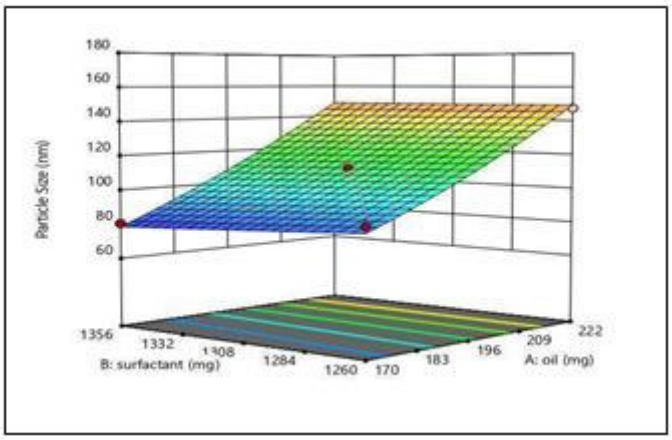
(mean, n=3)

3.3. *Effects of independent variables on the responses in experimental design*

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. Globule size = $+112.40 + 78.01X_1 - 1.91X_2 - 2.26X_3 + 1.69X_1X_2 - 0.350X_1X_3 - 11.32X_2X_3 + 3.27X_1^2 + 0.0925X_2^2 + 3.08X_3^2$ The Model F-value of 32.07 implies the model is significant. There is only a 0.79% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case X1, X2, X3 are significant model terms. The interaction reports showed that as oil concentration increases it also increases the globule size where as Smix concentration increases it decreases the globule size. Thus, oil concentration has negative effect on globules size. The Smix concentration plays a major role in reduction of globule size. The globule size interaction is shown in Fig.. 2(A).



(A)



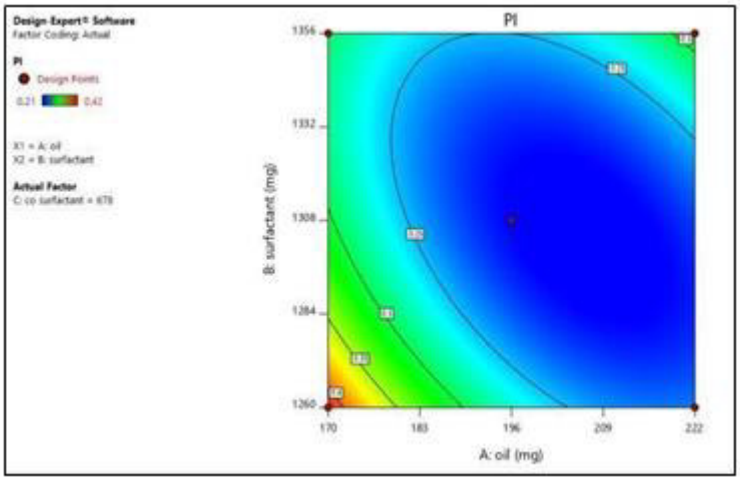
(B)

Fig. 2. Interaction Report of Globule Size

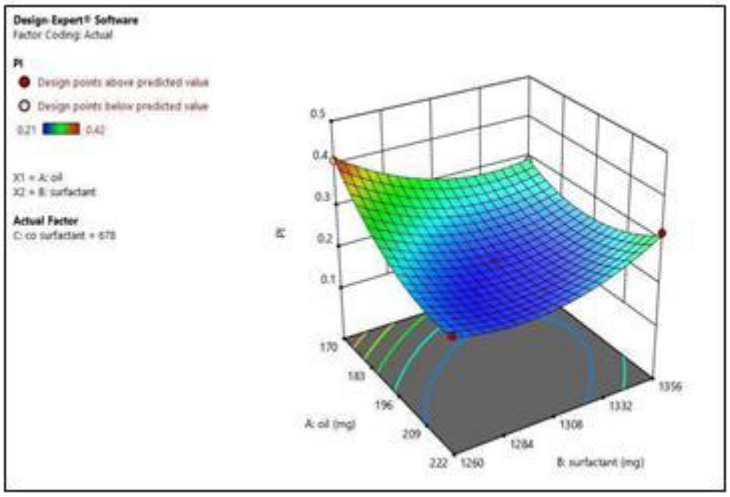
Three Dimensional plots Fig.. 2 (B) showed that as oil concentration increases it also increases the globule size whereas the Smix concentration increases it also decreases the Globule size. The least globule size is observed in formulation with mean globule size of 82.4 nm which has 10% oil and 90% Smix which could be due to higher concentration of Smix as compare to oil. This could be due to decreased surface tension by the presence of PEG. The analysis of globule size reveals that it significantly increases due to addition of Tween 80. Globule size of the formulation with combination of surfactant was less. Effective size reduction after addition of drug was observed for all the drug loaded formulations. The size reduction for the combination of surfactant can be because of the synergistic effect of the combination of the surfactant.

$$PDI = +0.2100 - 0.0400X1 - 0.0113X2 - 0.0213X3 + 0.0500X1X2 - 0.0050X1X3 + 0.0375X2X3 + 0.0538X1^2 + 0.0512X2^2 + 0.0862$$

The Model F-value of 20.69 implies the model is significant. There is only a 1.50% chance that an F-value this large could occur due to noise. P-values less than 0.05 indicate model terms are significant. In this case X1, X3, X1X2, X2X3, $X1^2$, $X2^2$, $X3^2$ are significant model terms. The interaction reports showed that as oil concentration increases it also increases the PDI whereas Smix concentration increases it decreases the PDI. The Smix concentration plays a major role in decreasing the PDI. The interaction is shown in Fig. 3A and 3B



(A)



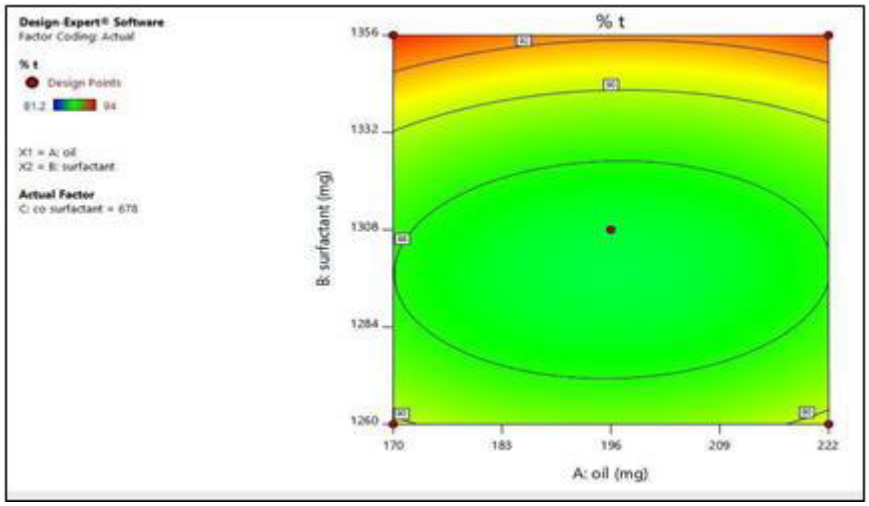
(B)

Fig 3: *(A) Response surface plots showing influence of Amount of Oil and Amount of Surfactant on PDI. (B) Contour plot showing relationship between various levels of Oil and Surfactant to attain minimum PDI*

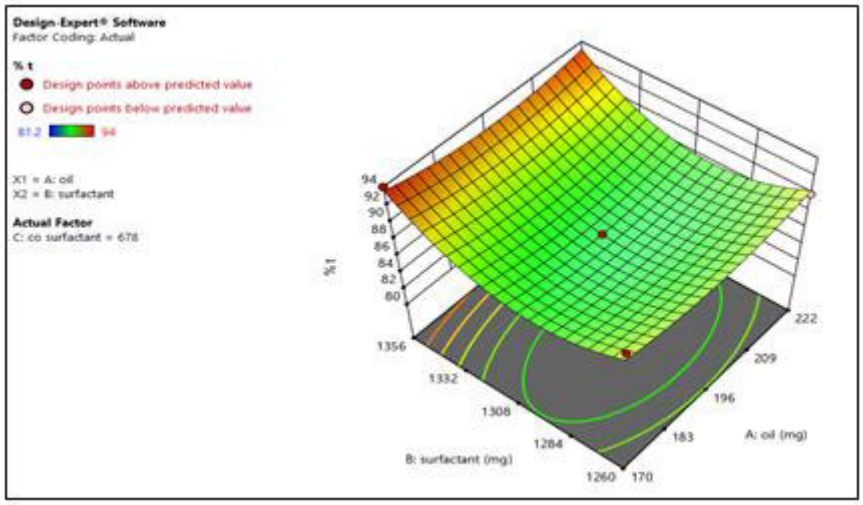
The clarity of micro emulsion was checked by transparency measured in term of transmittance. SNEDDS form o/w emulsion since water in external phase. Formulation showed 87% transmittance. These indicate the high clarity of nano emulsion

$$\%T = +87.00–0.0500X1+1.52X2–2.53X3–0.1500X1X2+1.35X1X3+0.000X2X3+1.15*X1^2 + 3.70*X2^2 -3.00*X3^2$$

The Model F-value of 11.49 implies the model is significant. There is only a 3.46% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case X2, X3, $X2^2$, $X3^2$ are significant model terms. The % T depends on oil concentration and Smix concentration. The result show increases the concentration oil the % T decrease where as Smix concentration increases it increase the % T. Thus, oil concentration has shown the effect on % T. 3D Plots showed that as oil concentration increases it also decreases the % transmittance whereas the Smix concentration increases it also increases the % T Fig 4A and 4B.



(A)



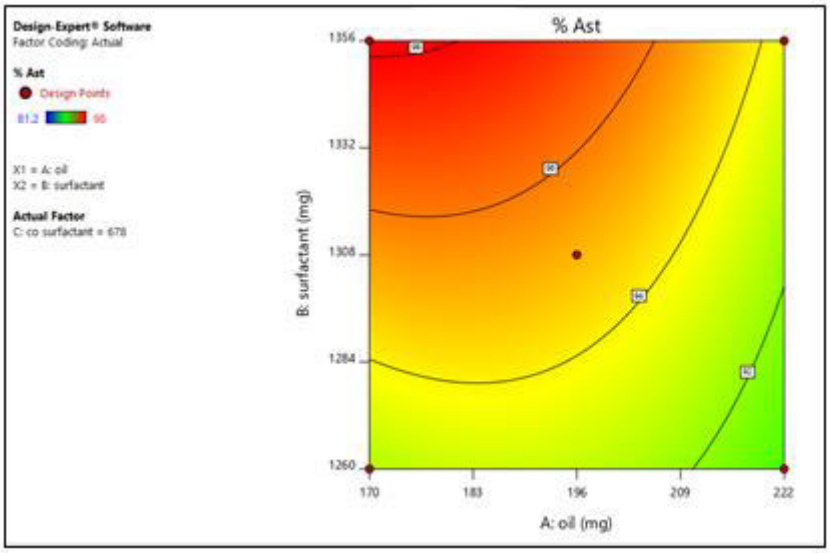
(B)

Fig. 4: (A) Response surface plots showing influence of Amount of Oil and Amount of Surfactant on % Transmittance (B) Contour plot showing relationship between various levels of Oil and Surfactant to attain minimum % Transmittance

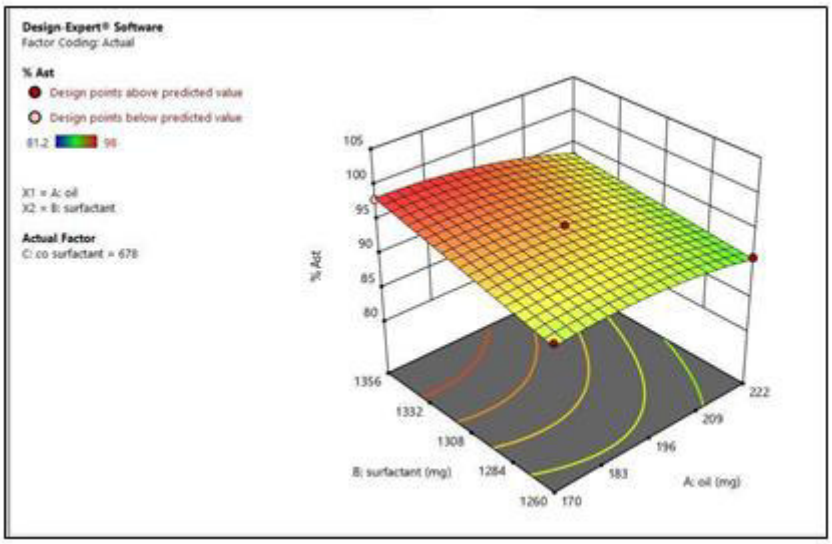
Percent drug release of AST in formulation prepared showed good release almost above 95% in 30 minutes. The formulation prepared shows that as oil concentration increases it retards the release of AST. The increase in concentration of surfactant: co-surfactant ratio causes increase in the drug releases. Almost all formulations showed good release in 30 Min. indicating that it is immediate release formulation

$$\%DR \text{ of AST} = +95.00-1.60X1+2.10X2-3.52X3-0.7500X1X2+0.0500X1X3+1.45X2X3-1.20X1^2-0500X2^2-5.90X3^2$$

The Model F-value of 33.74 implies the model is significant. There is only a 0.74% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case X1, X2, X3, $X3^2$ are significant model terms. The results showed that as there is increase in the S-Mix concentration increases there is increase in the release of the drug whereas oil concentration shows retard release. About 70% of Smix and about 30% of oil resulted in 95% of drug release Fig. 6. Drug release shows that it depends upon the two factors i.e. concentration of oil and Smix. The 3D plot shows characteristic interaction which is shown in Fig.5A and 5B



(A)



(B)

Fig 5: ***(A) Response surface plots showing influence of Amount of Oil and Amount of Surfactant on % Drug release of AST (B) Contour plot showing relationship between various levels of Oil and Surfactant to attain minimum % Drug release of AST***

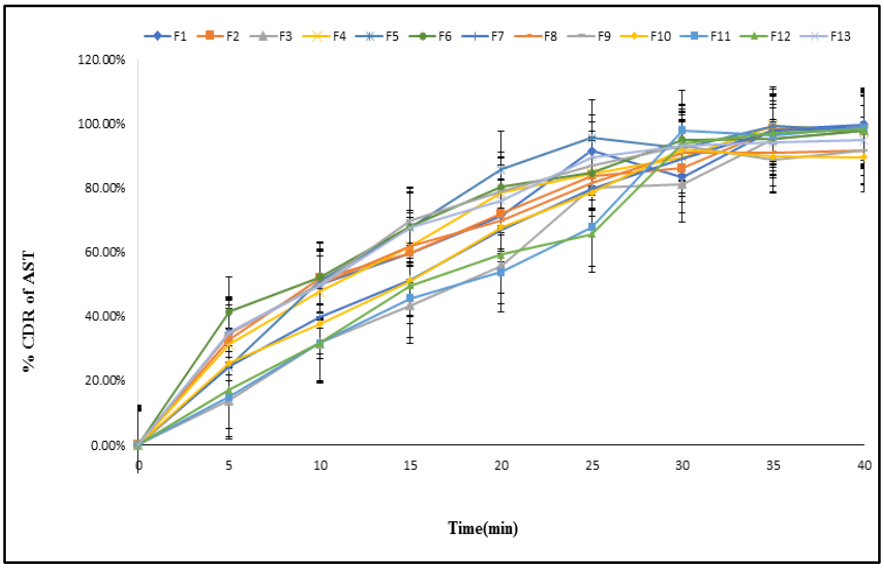
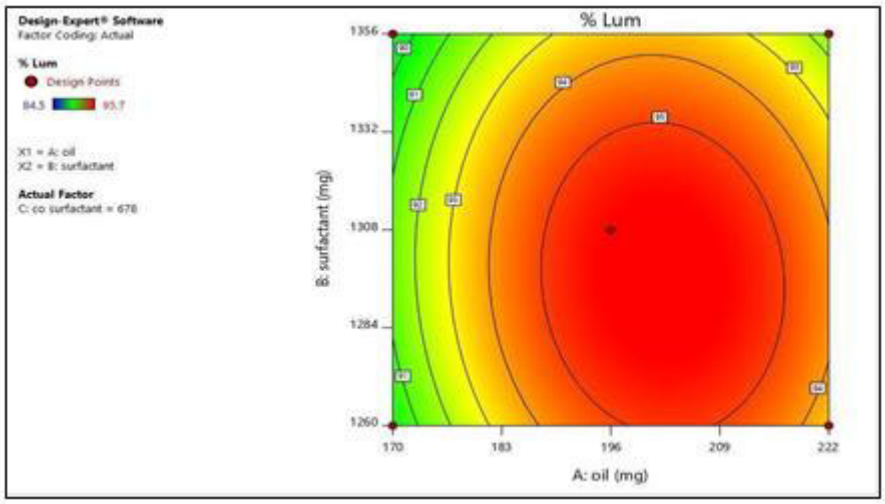


Fig 6: % drug release of AST

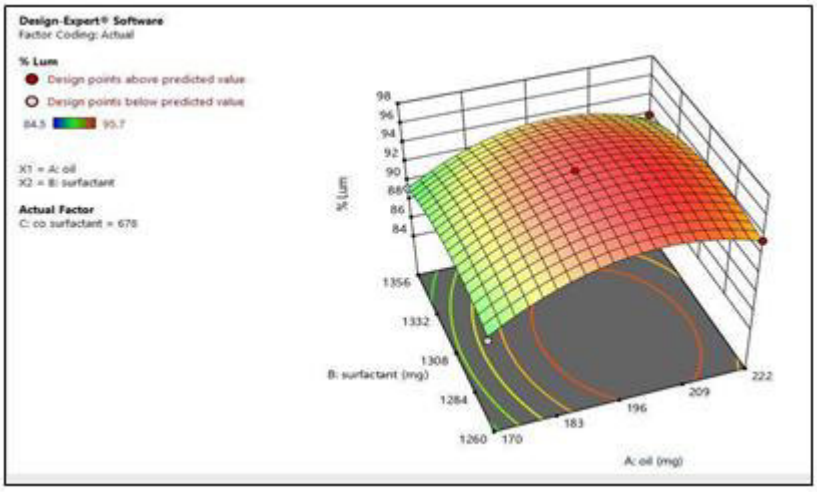
Percent Drug Release of LUM in the prepared formulation showed good release almost above 91% in 30 minutes. The formulation prepared showed clearly that as oil concentration increase it tends retards the release of LUM. The increase in concentration of surfactant: co-surfactant ratio causes increase in the drug releases. Almost all formulations showed good release in 30 Min. indicating that it is immediate release formulation

% DR of LUM =+95.70 + 1.40X1 - 0.6875X2 - 2.89X3 - 0.2750X1X2 - 0.2250X1X3 + 0.400X2X3 -3.07$X1^2$-1.50$X2^2$-4.85$X3^2$

The Model F-value of 14.78 implies the model is significant. There is only a 2.43% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case X1, X2, $X1^2$, $X3^2$ are significant model terms. The results showed that as there is increase in the Smix concentration there is increase in the release of the drug whereas oil concentration increases show decreased drug release. About 70% of Smix and about 30% of oil resulted in 91% of drug release Fig. 8. Drug release shows that it depends upon the two factors i.e. concentration of oil and Smix. The 3D plot shows characteristic interaction which is shown in Fig. 7A and 7B.



A



B

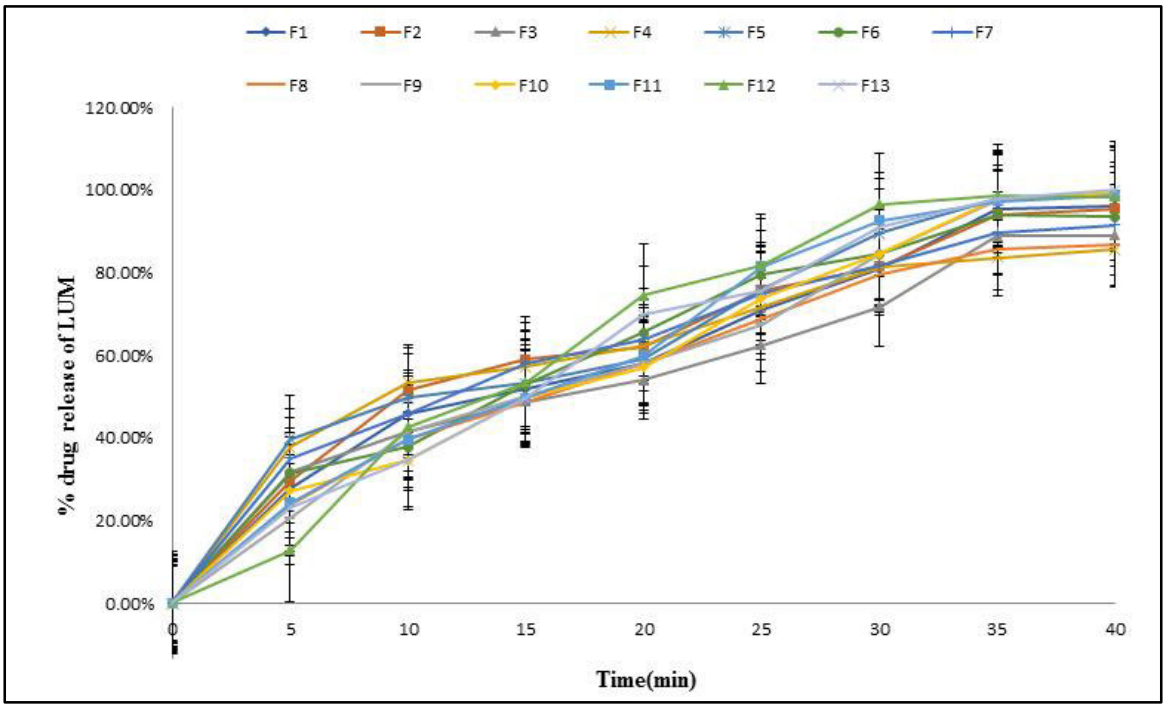


Fig 8: % drug release of LUM

Table 4. *Composition of optimized L-SNEDDS formulation*

Sr.no.	Content	Quantity
1	Oil (Anise Oil)	170mg
2	Surfactant (Tween 80)	1294.31mg
3	Co-surfactant (PEG 400)	662.61mg
4	Artesunate (AST) drug	240mg
5	Lumefantrine (LUM) drug	40mg

3.4. Formulation and Evaluation of S-SNEDDS

The S-SNEDDS was prepared by a mixture of L-SNEDDS containing AST and LUM drugs with different inert solid carriers in various proportions as shown in table 5. The Neusilin US2 shows good adsorption properties and flow properties after the formulation of S-SNEDDS, which was then evaluated for drug content, powder flow properties, and characterized by DSC, XRD, Zeta potential, and SEM for physical state analysis.

Table 5. *Selection of different adsorbents.*

Sr.no.	Adsorbent	Quantity taken (gm)	Specific surface area(m^2/g)
1	Areosil 200	1.977	130
2	**Neuslin US2**	**1.322**	**300**
3	Silica Oxide	2.043	180
4	Mg carbonate	3.933	800
5	Talc	2.900	110
6	MCC	3.560	427
7	Ca carbonate	2.400	30

3.5. *Characterization of S- SNEDDS Blend.*

3.5.1. *Powder flow properties:*

The results evaluation of powder S-SNEDDS for flow Characteristics is presented in table 6.

Table 6. *Flow properties of S-SNEDDS*

Properties	S- SNEDDS Formulation
Bulk Density (gm/ml)	0.678gm/mL ±0.048
Tapped density (gm/mL)	0.08196gm/mL ±0.0 56
Carr's Index %	8.88 ±0.5
Hasuner ratio	1.098 ±0.7
Angle repose(°)	25.11 ±0.3
Flow property	Excellent

The solid formulation had shown excellent flow properties. The flow properties of the batch were shown in table 6. The observed value of angle of repose, bulk density, and tapped density was 25.11°, 0.678gm/mL, and 0.08196gm/mL respectively. The flow properties of the batch showed good flow properties.[18] It can easily fill into the hard gelatine capsule and can also use to formulate conventional dosage forms like tablet SNEDDS formulation.

3.5.2. *Globule Size & Polydispersity index determination*

Globule size was determined by the Horiba particle size analyser. The smaller the droplet size, the larger the interfacial surface area will be provided for drug absorption. Polydispersity is the ratio of standard deviation to means droplet size, so it indicates the uniformity of globule size within formulation. The higher the polydispersity, the lower the uniformity of the droplet size in the formulation. globule size for S-SNEDDS was found to be 102 ±0.024nm with polydispersity index 0.449.The fine particle size of S-SNEDDS helps to improve the solubility of poor water-soluble drug and ultimately help to improve the oral bioavailability of such drugs.[21]

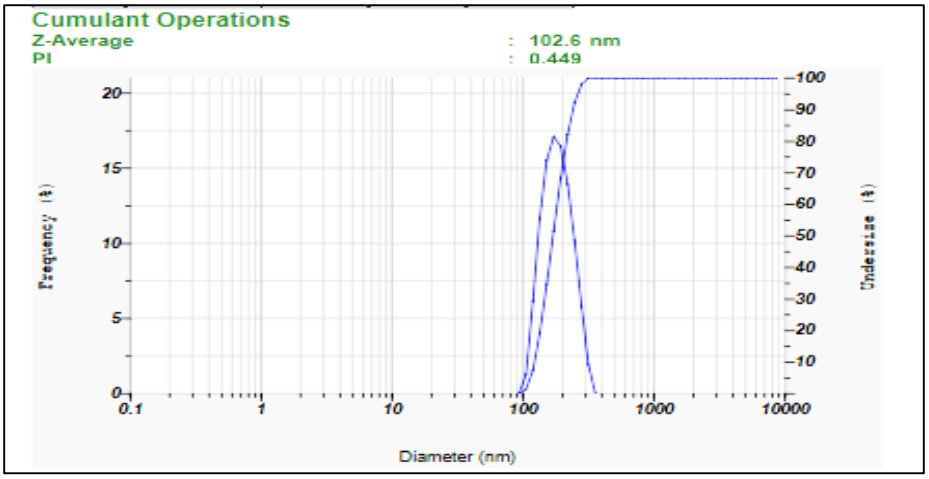


Fig 9 : *Globule size of S-SNEDDS*

3.5.3. *Zeta Potential*

The particles with zeta potentials more positive than +30mV are normally considered stable.[21] The particle with zeta potential more negative than -30mV are normally considered stable. The results of the zeta potential study of formulation were determined by Horiba Zetasizer SZ100. The zeta potential of formulation increased with an increase in surfactant concentration value. Zeta potential of S-SNEDDS of the formulation was observed -17.0 mV.

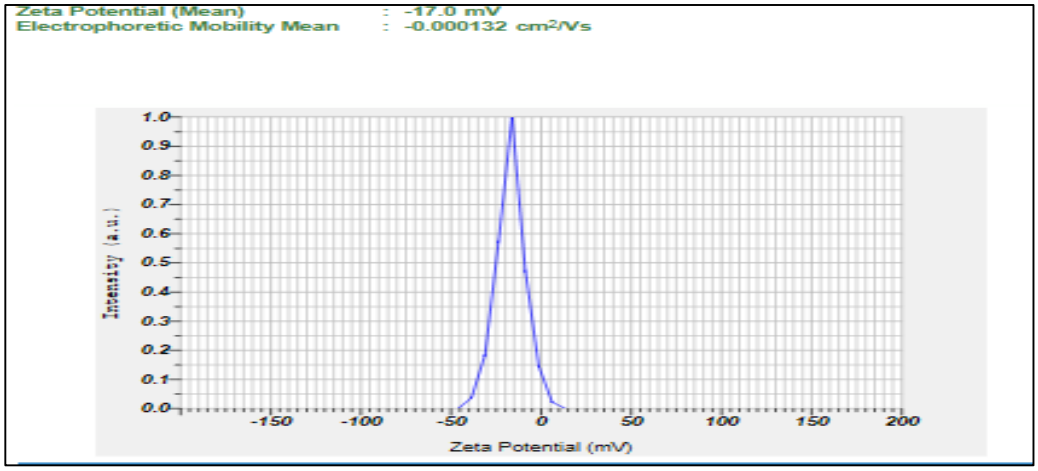


Fig10: *Zeta potential of S- SNEDDS*

3.5.4. % *Drug content:*

Drug loading in the SNEDDS formulation is mostly dependent on its solubility in a particular types of lipids, surfactants , and co-surfactant. The results of % drug content of optimized formulation by adsorption method were found to be 79.8±0.772%. The SNEDDS formulation shows a high drug loading capacity and is suitable to improve the solubility of poorly water-soluble drugs.[19]

3.5.5. *X-RPD analysis*

X-ray diffraction study is a potential tool for the evaluation of stability during storage and use. The crystalline nature of pure drugs AST and LUM was further recognized by its diffraction pattern, which concluded that the drug is present in Crystalline form as sharp peaks are observed. [19, 23] The X-ray diffraction pattern of S-SNEDDS supported the presence of AST and LUM in the crystalline state due to sharp peak intense peak not observed. The formulation was not indicating significant crystalline peaks, which confirmed the molecular dispersed state of AST and LUM in the formulation

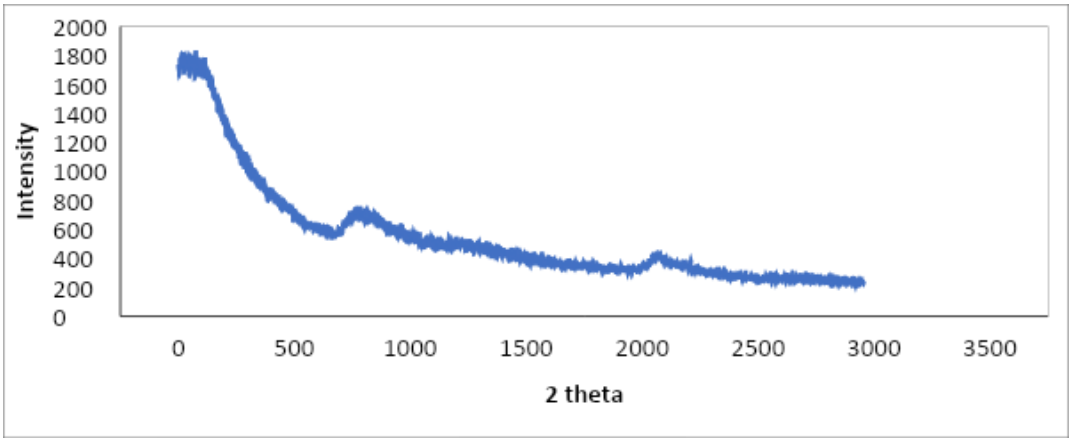


Fig 11: *X-RPD of Formulated S-SNEDDS*

3.5.6. *Differential scanning calorimetry (DSC)*

The DSC curves of pure drugs AST and LUM, Neusilin US2, and S-SNEDDS formulations are shown in Fig.. 17. Pure drugs showed sharp endothermic peaks at about, corresponding to their melting points and indicating their crystalline nature. Neusilin US2 showed a flat line with no melting endotherm, owing to its amorphous nature. It is important to note that the endothermic peaks of the drugs were absent in the S-SNEDDS formulations prepared with Neusilin US2 as a carrier. This showed that the SAT and LUM drugs have got dissolved completely in the formulation. Moreover, adsorption of AST and LUM loaded L-SNEDDS on amorphous Neusilin US2 through adsorption would have further resulted in the creation of a complete amorphous state of the formulation. In order to have better insight, the DSC results were correlated with XRD studies. [19,23]

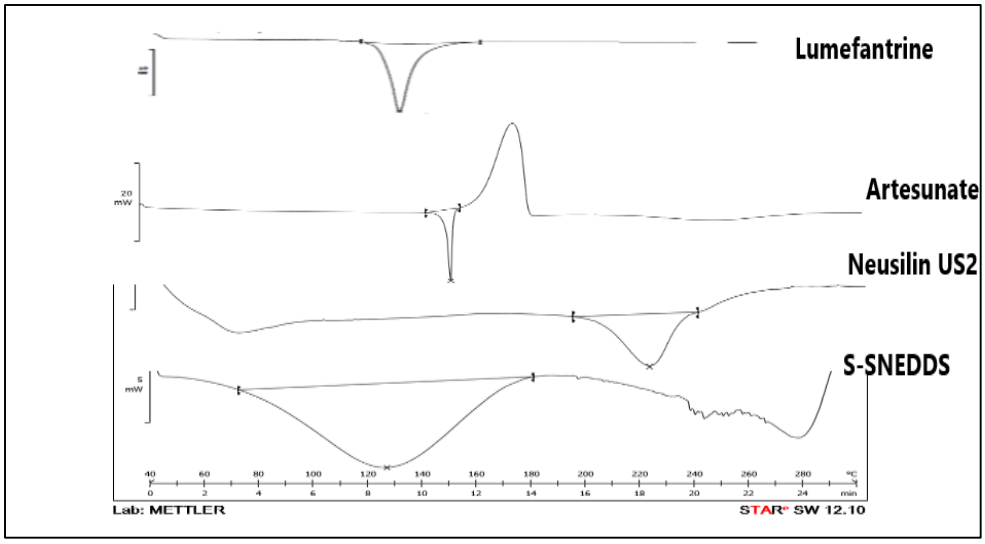


Fig12: *DSC analysis of S- SNEDDS.*

3.5.7. *Scanning Electron Microscopy*

The SEM studies revealed the formulation of spherical smooth surface particles of solid AST and LUM formulation. The SEM images of pure Neusilin US2 and S-SNEDDS were shown in Fig... Neusilin US2 appeared as smooth-surfaced porous particles. AST and LUM are crystalline in shape. The SEM studies revealed formulation of spherical smooth-surfaced particles of solid AST and LUM formulation.[19, 23]

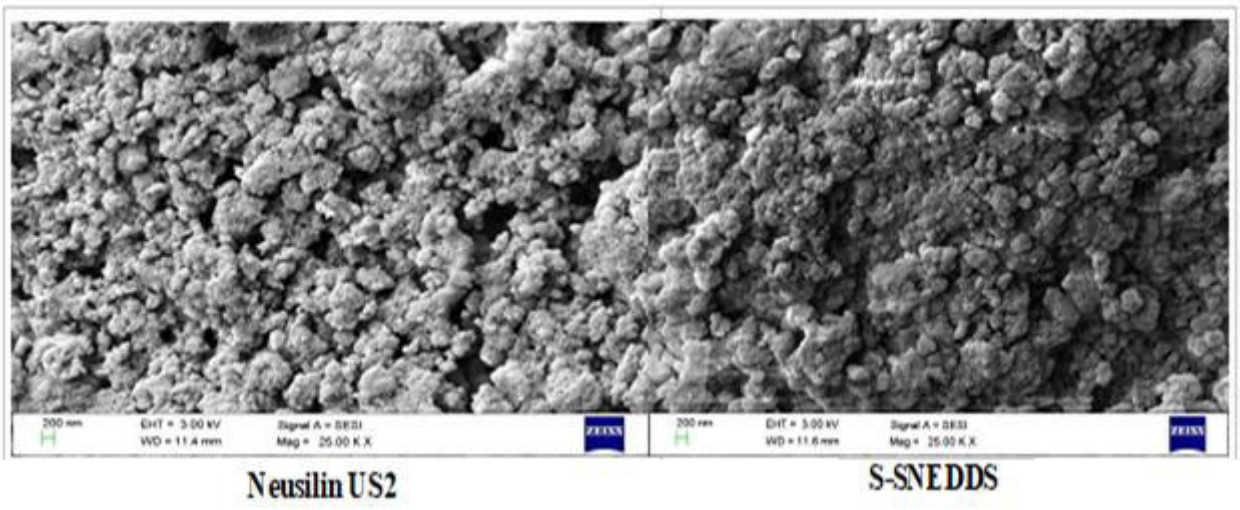
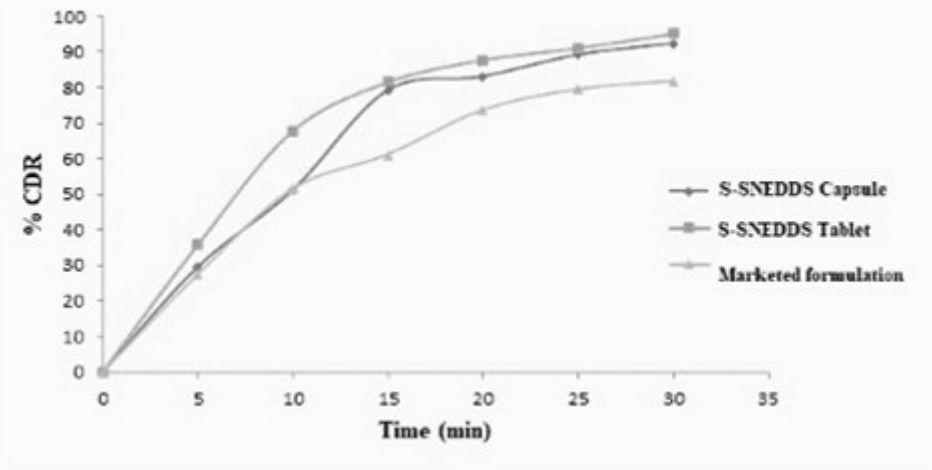


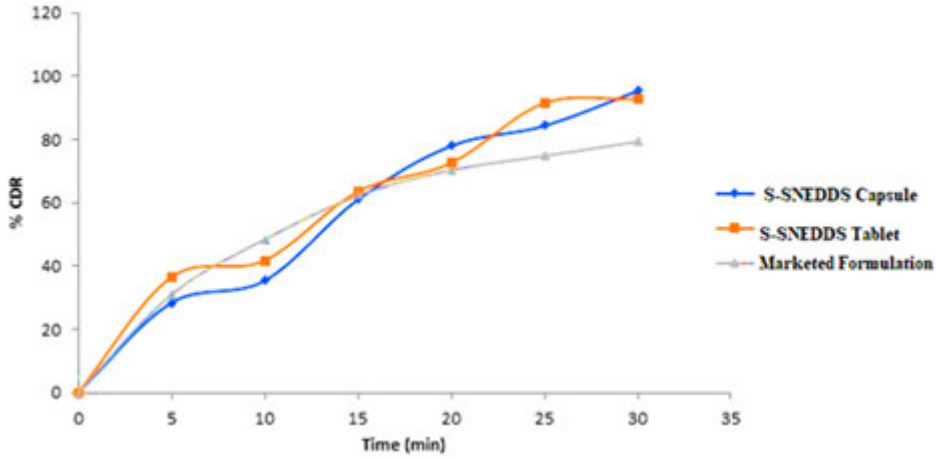
Fig 13: *Morphology of Neusilin US2 and S-SNEDDS using SEM analysis*

3.5.8. *In vitro dissolution studies*

S-SNEDDS formulation showed significantly higher drug release as compared to marketed AST and LUM tablet (Azunate L) (Table 7, Fig.. 19, Fig.. 20) (Results are expressed as mean ± SD). Formulated Capsules and Tablets showed more than 90% of AST and LUM drugs release in 30 minutes while marketed tablets showed 86% in AST and 90% in LUM drug release in 30, respectively. Spontaneous formation of nanoemulsion of SNEDDS formulation could be the reason for the faster rate of drug release into the Dissolution medium. The dramatic increase in the rate of release of AST and LUM from S- SNEDDS compared to the marketed formulation can be attributed to its quick dispersibility and ability to keep the drug in the solubilized state. Thus, this greater availability of dissolved drugs from the SNEDDS formulation could lead to higher absorption and higher oral bioavailability.[19, 23]



(A)



(B)

Fig. 14: In vitro diffusion studies of AST(A) and LUM (B)drug from 1. S-SNEDDS Capsule, 2. S-SNEDDS Tablet, 3. Marketed formulation

3.5.9. Mathematical models to predict the drug release

The data obtained after the dissolution testing was subjected to various kinetic models. The highest value of R in capsule showed R-value is 0.9979 in AST and 0.9985 in LUM, Tablet Showed R value is 0.9973 in AST and 0.9979 in LUM and Marketed Tablets show R-value is 0.9995 in AST and 0.9978 in LUM its confirmed for Korsmeyer –Peppas model (best fit model) for AST and LUM SNEDDS formulation.[23]

Table 8. *Regression coefficient of kinetic model*

Batch	Best fit model	R		n	
		AST	LUM	AST	LUM
SNEDDS Capsule	Korsmeyer- peppas	0.9979	0.9985	1.264	1.2498
SNEDDS Tablets	Korsmeyer- peppas	0.9973	0.9979	1.103	1.066
Marketed tablet (Azunte L)	Korsmeyer- peppas	0.9995	0.9978	1.294	1.461
L-SNEDDS	Korsmeyer-peppas	0.9986	0.9971	1.314	1.544

3.6. *Accelerated Stability Study*

The stability study was in compliance with ICH guidelines. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity for the drug substance. The effect of the accelerated testing of the formulation under storage i.e. S-SNEDDS formulation for three months was studied. Results of the stability study were shown in Table 9 for drug content, globule size, and %Transparency under specified storage condition. Results of the stability study show that there was no significant difference found between initial globule size, drug content, and % Transparency for formulation and no moisture uptake and no sealing integrity of capsule was changed after storage for three months. This study concluded that formulation retained physical stability during three months.[21, 22]

Table 9. ***Accelerated stability study data***

Month	Globule size	% Transmittance	Drug Content
0 month	118.04±0.78	88.54±0.06	75.85±0.472
1 month	120.78±0.59	91.77±0.70	79.8±0.124
2 months	118.31±0.24	88.9±0.25	78.57±0.142
3 months	115.78±0.99	91.85±0.30	77.47±0.450

(mean ±SD, n=3)

5. CONCLUSION

In this study, the optimized solid-SNEDDS formulation using Box Behnken design of AST and LUM was developed which showed greater in-vitro drug release when compared to marketed formulation. The developed S-SNEDDS formulation formed fine oil in water nanoemulsion when contacted with water with a narrow distribution size. Studies like SEM, XRD, DSC showed the presence of AST and LUM in amorphous or molecular dispersion form in the final formulation. Thus we may conclude that improvement in solubility, dissolution rate thereby oral bioavailability was achieved by preparing SNEDDS for AST and LUM.

6. ACKNOWLEDGEMENT

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7. AUTHORS CONTRIBUTION STATEMENT

Anuradha G. More, Parag R. Potbhare, Padmaja S. Kore, P. D. Chaudhari, Pratiksha U. Kshirsagar, Radhika R. Baheti, Surbhi C. Gupta contributed to the design and implementation of the research, to the analysis of the results and writing of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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