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Artesunate Loaded Self Nanoemulsified Drug Delivery System: A Preliminary Study for Improved Efficacy in the Treatment of Malaria: Formulation, Characterization and Bio-Distribution Study

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Abstract

The current study focused on formulation and evaluation of Artesunate loaded Self Nanoemulsified Drug Delivery System (SNEDDS). The research oriented towards the establishment of the pharmacokinetic parameters and biodistribution of Artesunate for SNEDDS. Artesunate nanoemulsion were developed using lipid, surfactant and Co Surfactant respectively (Capryol 90, Cremophor EL and Ethanol) by Spontaneous emulsification method. The investigation includes various characterization studies viz., particle size distribution, poly dispersibility index, zeta potential, viscosity, refractive index, % transmission and conductivity. The results were found to be 110.4 nm, 0.386, -36.6 mV, 19.54 cps, 1.287, 100, 367.2 µS/cm respectively with the optimized formulation 5. The *in vitro* drug release of Artesunate from SNEDDS formulation for was extremely significant when compared to pure drug suspension and the marketed formulation. The maximum release of drug from SNEDDS, marketed formulation and pure drug suspension was 98.78%, 62.78% and 20.88% respectively. The pharmacokinetic parameters of C_{max}, $AUC_{(0-2\ h)}$, $AUC_{(0-\infty)}$, Kel, T_{max} and MRT were found to be 2467 ± 11.98 ng/ml, 1278 ± 0.18 h.ng/ml, 3278 ± 0.78 h.ng/ml, 1.04 ± 0.07 h-1, 1.0 h and 1.87 ± 0.01 h, respectively. From biodistribution studies the concentration of dihydroartemisinin (metabolite of Artesunate) was found to be maximum in the order of liver>lung>kidney>spleen>brain>heart. The highest concentration of 1951.8 ng/g was found to be in the liver.

Keywords: Artesunate; Dihydroartemisinin; Self-Nanoemulsifying drug delivery; Bio-distribution

Introduction

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 [1]. For improving the bioavailability and solubility of such oral drug delivery system, it is required to formulate suitable formulations. The main challenge for the formulation scientist has been the formulation and development of poorly water soluble moieties [2-5]. The lipid based formulation methodology has seen wide range of interest in improving the oral bioavailability and the drug solubilisation in the Gastrointestinal Tract (GIT) of BCS class II and IV drugs [6-9]. Current investigations support the usage of lipid based formulations to tackle the formulation challenges of poorly soluble drugs. There has been a considerable growth in the past 15 years on Lipid Based Drug Delivery System (LBDDS) as novel drug delivery system to deals with the problem associated with low solubility and high permeability (BCS Class II) [10] in case of wide range of new chemical moieties. Common pharmaceutical excipients used in self-nano emulsifying drug delivery system (SNEDDS) containing bio enhancers like cremophor, tween 80 are reported to facilitate absorption by inhibiting glycoprotein efflux hence enhancing the bioavailability [11]. Malaria is an acute infectious disease caused by the bite of female Anopheles mosquitoes belongs to genus Plasmodium which flies high in humid and swampy areas. Being the most insidious species, *Plasmodium falciparum* is 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 malariae* is most common in tropical regions and *Plasmodium ovale* is often encountered. The resistance acquired by the by the parasite to drugs, abstained for the development of new therapeutic challenges, particularly in the controlling of resistance caused by *P. falciparum* [12]. The efficacy of a drug treatment particularly to plasmodium species and each stage of its life cycle is being targeted. The main objectives of anti-malarias are to prevent and treat patient suffering from malaria and also complete eradication of the parasite from the body [13]. The main aim of the research was to find out the enhancement of bioavailability by loading artesunate with poor bioavailability in nano-emulsion (Nanodroplets) formulated with various components like lipid, Surfactant and Co Surfactant (SCoS), using spontaneous emulsification method (Aqueous titration method).

Materials and Method

Materials

Artesunate (complementary sample) obtained from Mylan laboratories, Ltd., (Hyderabad, India).

Capryol 90 was purchased from gatteffosse India Pvt. Ltd. (Mumbai,

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India). Cremophore EL brought from sigma Aldrich Corporation, (Bangalore). Ethanol purchased from Merck chemicals India Pvt. Ltd. (Mumbai). The chemical used in the research was analytically graded.

Solubility studies

Solubility of artesunate was found in different oils through (Shake flask method) [14] through the addition of an excess quantity of drug to 2 ml of oil in volumetric flasks. The temperature of flasks was maintained at 25 ± 0.5°C using an isothermal shaker (IKA® KS 4000i, Germany) for time duration of 72 h for reaching equilibrium. The equilibrated sample remained detached from shaker and was centrifuged at 4000 rpm for 15 min and filtration of supernatant was done by using 0.45 µm membrane filter. Artesunate concentration in the supernatant was determined by using UV spectrophotometer.

Screening of surfactant and Co Surfactant (SCoS)

Screening of the surfactant and co surfactants was established by the formation of emulsion in the respective oil phase. The ability to emulsify was determined by mixing of oil phase, surfactant and aqueous phase to form a uniform emulsion [15].

Purity and melting point of the drug

A small quantity of sample was placed into a capillary fusion tube and sealed from one end with the help of the burner. The capillary tube was placed in the melting point determining apparatus (Capillary tube melting point apparatus) containing liquid paraffin. The temperature of the liquid paraffin was gradually increased and the temperature at which sample started to melt was observed and noted.

Drug interaction studies by FTIR

Compatibility of drug, lipid, Surfactant, Co Surfactant and formulation were studied using FTIR spectrophotometer (Shimadzu, Japan). A physical mixture of drug, lipid and surfactants (either alone or in combination) was prepared using anhydrous potassium bromide (KBr) in 1:4 ratios. About 100 mg of this mixture was ground into fine powder followed by compression using a hydraulic press at 15 tons, to form a thin transparent KBr pellet. Each KBr pellet was scanned at a resolution of 2 cm at 4 mm/s of a wave number region from 4000 to 400 cm^{-1}. The result of physical mixture was compared with pure drug, lipid, surfactant and matching of the IR peak was done to detect any appearance or disappearance of peaks [16].

Formulation of artesunatenano emulsion (NE)

Nanoemulsions were formulated by the spontaneous emulsifying method (Aqueous titration method). They were formulated basically by mixing of oil, water and surfactant Co Surfactant (SCoS), in correct ratio, followed by mild agitation. Nanoemulsion regions alone were constructed using Pseudo ternary phase diagrams [17].

Construction of pseudo ternary phase diagrams

The study was done to know the formation of oil in water (o/w) nanoemulsion with 4 components viz. Oil, surfactant, co-surfactant, and aqueous phase. Oil ratio was kept constant and different proportions of SCoS from 1:1 to 1:3 ratios in each group were mixed. Nine combinations of oil and SCoS, 1:1 to 1:9 were made to cover maximum ratios which represent phases outer boundaries formed in the phase diagram. For every weight ratio of oil and SCoS titration of water was performed. Transparency and flow ability of the NEs were observed visually. In the phase diagram, only the NE regions were plotted [18].

Evaluation of nanoemulsion

Thermodynamic stability studies: The formulations were exposed to the subsequent stability studies [19]. A. Heating and cooling cycle: Cooling cycle was done in refrigerator at 4°Cand heating cycle was done in hot air oven at 45°C for 48 h. The formulations in which no physical and chemical changes were observed and chosen for the centrifugation test. B. Centrifugation: Centrifugation study for the selected formulations was do neat 3500 rpm for 30 min. Formulations were subjected for the freeze thaw cycle in which no phase separation occurred. C. Freeze thaw cycle: Freeze thaw cycles were carried out in deep freezer where the formulation was stored at temperature of -2°C and +25°C around 48 h. The formulations that passed the thermodynamic stability tests were studied further.

Particle size, zeta potential and polydispersity index (PDI): The determination of particle size and zeta potential of nanoemulsion were done by photon correlation spectroscopy with a Malvern Zetasizer Nano ZS90 at 25°C. A proper dilution with samples was dispersed in preparation medium and placed in polystyrene cells for PDI measurement and disposable plain folded capillary zeta cells for zeta potential measurement [20].

Refractive index, % transmittance, viscosity and conductivity: To determine the drug loaded formulations an Abbe-type refractometer (Macro Scientific Works, Delhi) was been used. The percentage transmittance of NE formulations was measured by using Shimadzu UV-Visible spectrophotometer. DVE viscometer (Brookfield Engineering Laboratories, Inc.,) was used to determine the viscosity of the formulation. Around 0.5 g of sample was reserved for analysis without dilution and the sample was analyzed by using spindle no. 63 at different rpm at 25 ± 0.5°C. Electro-conductivity of the resultant system was measured by an electro-conductometer and examined NEs were arranged with 0.01 N aqueous solution of sodium chloride instead of doubled distilled water. The measurements were done in triplicate at 25 ± 1°C [21,22].

In vitro release studies

Drug loading was done based on the dose equivalent in 1 ml of optimized nano emulsion. The *in vitro* drug release test was done in 250 ml pH 6.5 biorelevant media using USP dissolution apparatus Type I at 50 rpm at 37 ± 5°C for quantitative analysis. The optimized Self-Nano Emulsifying Drug Delivery System (SNEDDS) (Preconcentrate) containing single dose of Artesunate in oil and SCoS was filled in capsule size 2. At each interval of time 0, 0.5, 1, 1.5, and 2 h samples were withdrawn with the substitution of equal amount of dissolution media. Samples were filtered and analyzed in UV-Spectrophotometer at 210 nm. The release of drug from SNEDDS formulation was also compared with the conventional tablet and pure drug suspension [23].

In vivo pharmacokinetic study

The experiments were carried out after getting the approval of the CPCSEA and IAEC, from JSS College of Pharmacy, Ooty. Reg. No: JSSCP/IAEC/M.PHARM/PH.CEUTICS/02/2014-15. CPCSEA and their guidelines were followed throughout the experiment.

HPLC Method

The HPLC system consist of a mobile phase delivery pump (LC-20 AD; Shimadzu, Japan), a photodiode array (PDA) detector (SPDM20 A; Shimadzu, Japan) and a 20 µL loop (Rheodyne). A C18 reverse-phase column (Phenomenex Gemini C18, 250 × 4.6 mm i.d., 5 µ) was used for drug separation. The estimation of drug content was analyzed

using a mobile phase acetonitrile: 50 mM potassium dihydrogen orthophosphate pH 4.8 (50:50, v/v), at the 1.0 ml/min and UV wavelength at 210 nm [24].

Estimation of drug in rat plasma

Healthy Male Sprague-Dawley rats, fasted overnight, with a weight of about 250-300 g were used for in vivo experiment. The animals were provided with water and libitum in fasting and during the experimentation. Early in the morning zero periods fasting blood samples were withdrawn. The animals were then categorized into 4 groups, every group having three animals 1.25 mg/kg dose administered based on the surface area ratio of rat and man through oral gavage. Group 1 was given conventional marketed formulation, group 2 was given SNEDDS formulation, group 3 was given pure drug suspension and group 4 were used as control which was used for plasma spiking. 0.5 ml of blood samples was withdrawn from the Retro orbital puncture with a Capillary tube at 0, 15, 30, 45, 60, 90, 120 min. The blood samples were collected in a RIA vial containing anti-coagulant (0.4 ml of 2.5% sodium citrate), centrifuged at 4000 rpm for 5 min and the plasma samples were separated and stored at -20°C. Deprotonation of the plasma samples were carried out and the drug was extracted by protein precipitation method using methanol and then analyzed by HPLC. One way ANOVA method was used for predicting the significant difference using Graphpad Prism software [25,26].

Pharmacokinetic data analysis

Non-compartmental analysis of the individual concentration-time data using PhoenixWinNonlin® v6.3 software (Pharsight Corporation, Mountain view, CA, USA) was performed for the calculation of pharmacokinetic parameters. The pharmacokinetic parameters viz., maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the plasma concentration-time curve. The elimination rate constant (Kel) was obtained from the least-squares fitted terminal log-linear portion of the plasma concentration-time profile, and area under the plasma concentration time curve from 0 to 2 h ($AUC_{0-2\ h}$) was obtained by the linear trapezoidal rule, and area under the curve from 0 h extrapolated to infinity ($AUC_{0-\infty}$) was calculatedas$AUC_{0-2\ h}$+CT/Kel where CT signifies the plasma concentration at the last measurable sampling time [27].

Biodistribution studies

Same animals were used after a wash out period of 2 months. The animals were taken care of according to CPCSEA guidelines. The animals were provided using food and water ad libitum and fasted for 12 h prior to the commencement of the experiment. The tissue distribution studies were performed, immediately after cervical dislocation; the organs (heart, liver, spleen, lung, kidney and brain) were collected. The isolated tissues were placed in normal saline solution to remove blood content and blotted dry with tissue paper. The isolated organs were further crushed individually with the help of triple blade stirrer and centrifuged at 10000 rpm for 10 min. The supernatant solutions were collected and stored at -70 ± 2°C for drug content analysis [28].

Results and Discussions

Solubility studies of drug in various oils

Solubility is an important parameter in the formulation of SNEDDS, where drug remains in liquid which solubilized in the oil phase. For solubility study, the oil phase in which the drug showed highest concentration was selected. The evidence from Figure 1 showed

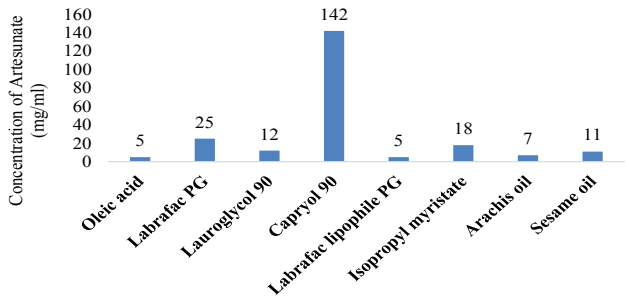

Figure 1: Solubility studies.

that capryol 90 has maximum solubility of artesunate 142 ± 0.54 mg/ml. Hence for the formulation of SNEDDS, capryol 90 was selected. Due to the more affinity of drug towards the respective oil solubility of the drug has been increased.

Screening of surfactants and co-surfactants

From the screening studies, the SCoS mixture of cremophor EL and ethanol for capryol 90 were found to produce clear and uniform o/w emulsion. Hence, cremophor EL and ethanol were selected for the formulation. The selected SCoS mixture was found to produce a clear and uniform emulsion with capryol 90.

Purity and melting point of the drug

The Merck Index states that Artesunate melts at about 135-137°C. The melting point of Artesunate observed was 136.33°C, which states that the sample complies to be pure with no impurities.

Drug interaction studies by FTIR

From the obtained FTIR studies it was seen that there were no drug interactions with the excipients. The prominent peaks in artesunate spectra as follows 2883 cm^{-1} to CH stretching vibration, 3000-3500 cm^{-1} to OH stretching vibration, 1755 cm^{-1} to C=O stretching vibration and 1212 cm^{-1} to C-O stretching vibration. The functional groups along with corresponding peaks of pure artesunate and in physical mixture of artesunate were no interactions between artesunate and selected Oil+SCos. Hence, pure dug and physical mixture of excipients are compatible with each other.

Pseudo ternary phase diagram study

By constructing phase diagrams, phase behavioural studies were performed which represents the boundaries of dissimilar phases, arrangements and to examine the structural organization of the emulsions formed. To prevent the coalescence of the designed NE by the concentration of surfactant and co-surfactant this is responsible for forming the barrier at the interface. To improve the thermodynamic stability of the NE formulation, the SCoS should be adsorbed at the crossing point, thus decreasing the energy required for NE formation. Among all ratios from 1:1 to 1:3 in SCoS ratio 1:1 produced the clear solution of Oil and SCoS of 1:5, 1:6, 1:7, 1:8 and 1:9 these formulations covered the most of the nano-emulsion area and those formulations were selected for the further studies. The optimized formulations ternary phase diagram shown in Figure 2.

Formulation selection of artesunate NE Oil phase Capryol 90, SCoS: Cremophor EL and Ethanol

Different concentration of oil, which solubilize single dose of drug

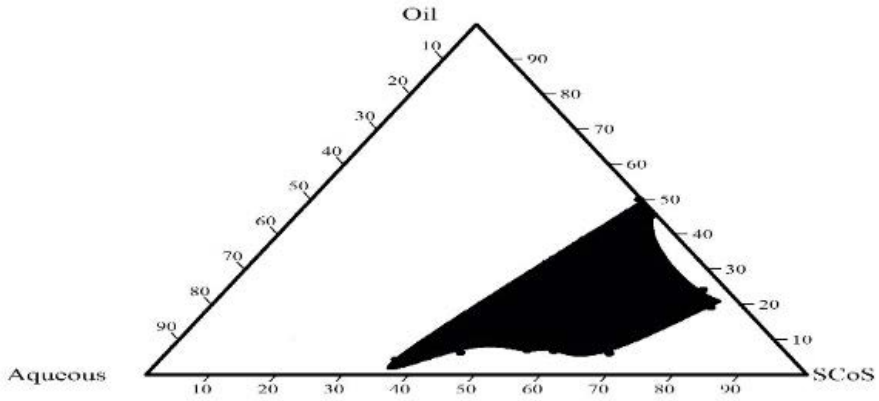

Figure 2: Ternary phase diagram of optimized formulation 5.

were selected at 5% intervals (10%, 15%, 20%, 25% and 30%)from each phase diagram, so that, largest number of formulations could be selected which covers the nanoemulsion area of the phase diagram. Only individual formulations were selected having least concentration of SCoS for forming nanoemulsion. The results were shown in Table 1. The optimization was done and the drug was dissolved in the respective oil and mixed with SCoS to form self-emulsifying concentrate. Since the solubility of the drug was well above to the dose, the total amount of drug was assumed to be present in the oil phase.

SCoS	Oil: SCoS								
1:1	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
	E	E	E	E	NE	NE	NE	NE	NE
1:2	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
	M	E	E	EG	E	M	E	**NE**	**NE**
1:3	1:1	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
	E	E	M	EG	EG	E	EG	E	M

NE: Nanoemulsion; E: Emulsion; EG: Emulsion gel; M: Milky

Table 1: Visual observation during aqueous phase titration for formulation selection by using SCoS ratio 1:1 to 1:3.

Evaluation of NE

Thermodynamic stability studies: The formulations which are been selected from the pseudo ternary phase diagram with ratio of SCoS 1:5, 1:6, 1:7, 1:8 and 1:9 were subjected to various thermodynamic stability studies. The nanoemulsion that are kinetically stable and undergo phase-separation that was differentiated by thermodynamic stability studies. This indicates the formulations comprise adequate quantity of SCoS concentration which is needed for NE formulation, which declines the energy necessary for NE formation. Hence, the stability of NE is due to decrease in energy. The entering of NE through the GI tract, it undergoes infinite dilution leading to phase separation because of poor dispersibility. By dispersing in the aqueous milieu of the GIT formulations which conceded the dispersibility studies can certain to remain as NE. The dilution of the oral NE through the GI fluids will result in the gradual desorption of the surfactant located at the globule interface. From the Table 2 all formulations which passed the thermodynamic stability test and dispersibility test were undergone for the investigation of globule size, zeta potential, % transmission, viscosity, refractive index and PDI analysis. Formulations, which are clear and prevented from turbidity are considered as stable, whereas others are considered to be unstable.

Characterization studies: The increase in concentration of oil in the formulation globule size increases and decreases with increase in the SCoS concentration. Among the formulations which passed the thermodynamic stability tests, formulation 5 was found to have a mean globule size of 110.4 nm with a PDI 0.386, and zeta potential -36.6 mV with 100% transmission was selected as the optimized formulation, subsequently other formulations mean droplet size and PDI were found to be larger than formulation5 and wider range of particle size distribution which is not desirable. The other parameters for all the formulations were found to be good. Based on least mean particle size the optimized formulation was selected and PDI. The additional parameters like viscosity, refractive index and conductivity were found to be acceptable for all the formulations. The results were mentioned in the Table 3. Since the formulations are translucent, % transmission is almost maximum which is also supported by refractive index. The PDI was also very minimum shows the droplets are uniformly distributed and zeta potential was found to be -36.6 mV, due to presence of free fatty acid which shows that the formulation is stable. From the Figures 3 and 4 shown, the formulation 5 was selected for drug loading and *in vivo* and *in vitro* studies.

Drug loading

The optimized formulation 5 was been selected for the drug content analysis. Since the solubility of the drug in oil was well above to the dose of the drug, capacity of oil phase used remained equivalent to the dose of the drug i.e., artesunate 50 mg, Oil and SCoS volume is 0.35 ml and 0.2 ml respectively.

In vitro drug release

In vitro dissolution studies were performed in pH 6.5 biorelevant media for formulation 5 and compared with marketed and pure drug suspension. pH 6.5 was selected based on the literature support that which says that very short residence time of lipid based drug delivery systems in gastric pH and maximum absorption takes place in small intestine whose pH is 6.5. The solubilisation of lipophilic molecule occurs at upper GI tract in which pancreatic fluids and biliary lipids are secreted which enhances solubilisation process. The residence time in upper GI is limited and the transit time in small intestine is 3.5 to 4.5 h. Hence the pH 6.5 is used as a dissolution media. To investigate the drug

Formulation	SCoS	% Oil	% CoS	% Water	Heating and Cooling Cycle	Centrifugation	Freeze thaw Cycle	Dispersibility
1	1:1	1.49	16.84	6.7	P	P	P	P
2	1:1	1.36	12.10	7.2	P	P	P	P
3	1:1	1.31	10.4	7.4	P	P	P	P
4	1:1	1.12	8.9	8.9	P	P	P	P
5	1:1	0.84	7.6	11.8	P	P	P	P

Note: P: Pass

Table 2: Thermodynamic stability studies (mean ± S.D, n=3).

Formulation	Mean Droplet Size (nm)	PDI	Zeta Potential (mV)	% Transmission	Viscosity (cps)	Refractive Index	Conductivity (µS/cm)
1	284.1	0.639	-25.96	89.1	23.14	1.754	415.2
2	219.25	0.964	-22.07	97.2	28.78	1.687	397.5
3	217.56	0.756	-20.04	97.9	25.14	1.517	378.4
4	227.39	0.807	-23.08	94.9	24.78	1.487	348.8
5	110.4	0.386	-36.6	100	19.54	1.287	367.2

Table 3: Physical characterization studies of nanoemulsion.

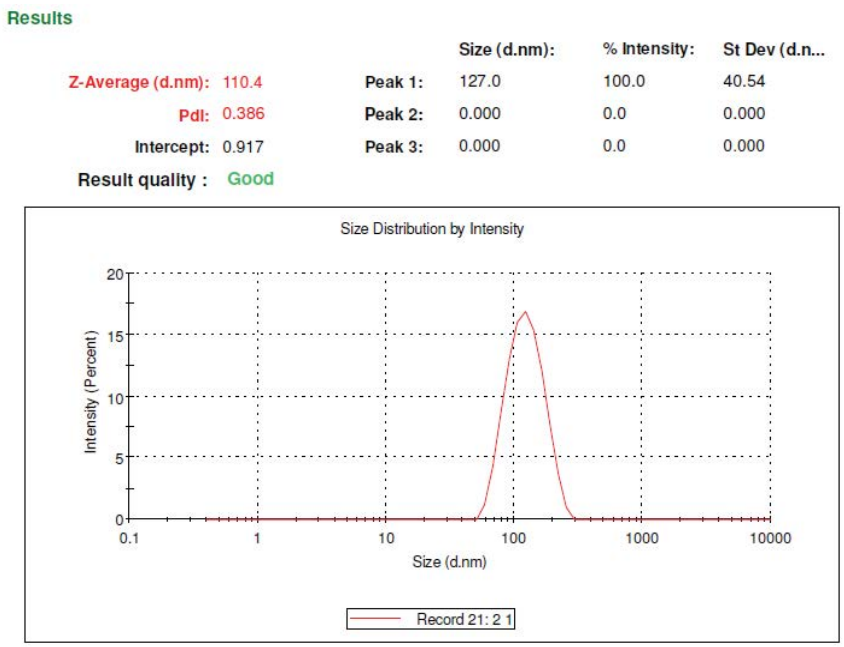

Figure 3: Size distribution report for optimized formulation 5.

dissolution from SNEDDS, marketed tablet formulation and pure drug suspension containing same quantity of drug, comparative dissolution studies were performed. *In vitro* dissolution studies indicated that Artesunate released an initial amount of 30.77% at 15 min for SNEDDS formulation, whereas the marketed formulation was 22.78% and for pure drug it was 10.78%. The release rates were significant compared to both pure drug suspension and for marketed formulation. The reason for this could be the smaller droplet size and PDI, which lead to the increased surface area permitting a faster release rate with a maximum release within 90 min. At the end of 120 min almost all the drug was found in solution. At the end of the study SNEDDS formulation did not show any precipitation or aggregation of the particles. The release profile shows that the SNEDDS preserved enhanced *in vitro* dissolution and which would eventually enhance dissolution of drug. Since the drug is low soluble only 20.88% of drug was released from pure drug suspension. Marketed formulation may contain some of the solubility enhancement formula hence artesunate of around 62.78% was found in solution, whereas the SNEEDS released around 98.78%. Since the drug is available in droplet form and the formation is colloidal which could able to keep the drug in solution along micelles & reverse micelles. The drug release from the SNEDDS formulation for Artesunate was extremely significant in comparison with the pure drug suspension and the marketed formulation. The results were shown in Figure 5.

In vivo pharmacokinetic studies

The *in vivo* release of artesunate in Sprague-Dawley rats for SNEDDS, marketed tablet and pure drug suspension were used to calculate the pharmacokinetic parameters. For the absorption of artesunate the rate limiting step was dissolution. The obtained result showed that the rate

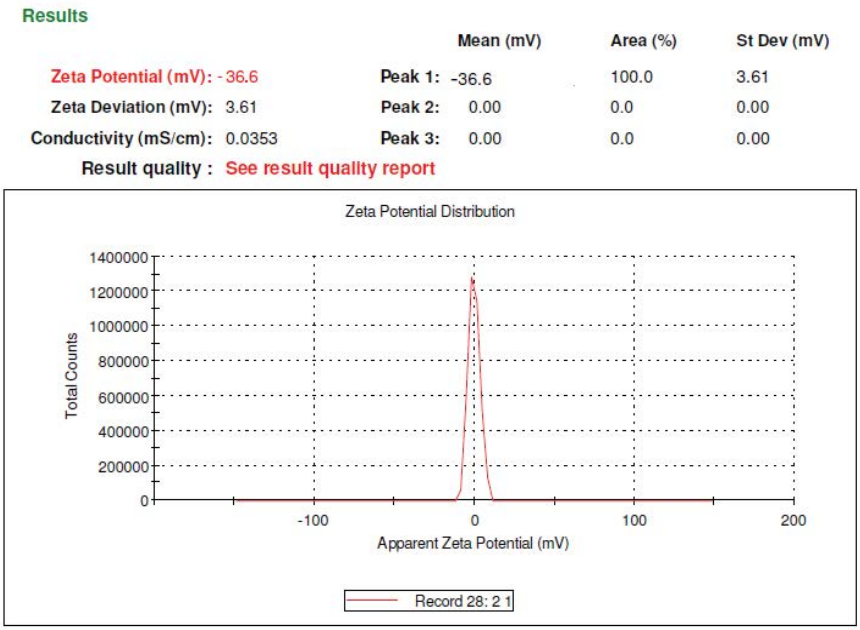

Figure 4: Zeta potential report for optimized formulation 5.

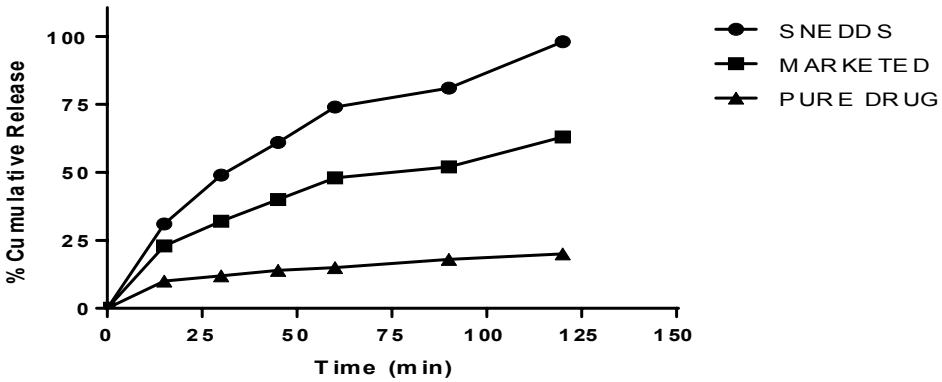

Figure 5: Comparison on % cumulative release of SNEEDS, marketed conventional formulation and pure drug suspension.

PK Parameters	Dihydroartemisinin (DHA)		
	SNEDDS	Marketed	Pure drug suspension
C_{max} (ng/ml)	2467 ± 11.98	1940 ± 15.87	640 ± 0.34
T_{max} (h)	2	3	5
K_{el} (h^{-1})	1.01 ± 0.07	1.28 ± 0.04	1.50 ± 0.14
$AUC_{(0-t)}$ (ng.h/ml)	6278 ± 0.18	3455 ± 10.47	1110 ± 0.01
$AUC_{(0-\infty)}$ (ng.h/ml)	8478 ± 0.78	4240 ± 0.55	1540 ± 0.27
MRT (h)	1.87 ± 0.01	1.46 ± 0.02	0.64 ± 0.04

Table 4: Pharmacokinetic parameters of DHA after oral administration (mean ± S.D., n=3) (*p<0.05).

limiting step in case of SNEDDS was dispersion of the drug into the aqueous gastrointestinal environment and plays an important role in absorption. We can conclude that, on subsequent oral administration, SNEDDS dissolve spontaneously to arrange as NE in the GI fluid where the active components are present in a solubilized form, and the small droplet size offers a large surface area for drug absorption. According to the literatures, the oil will afford rapid and wide-ranging of absorption due to ultra-fine dispersion. The increase in the permeability of the oil across the cell membrane, and lymphatic transport was due to high concentration of surfactant in SNEDDS, due to which the formulation

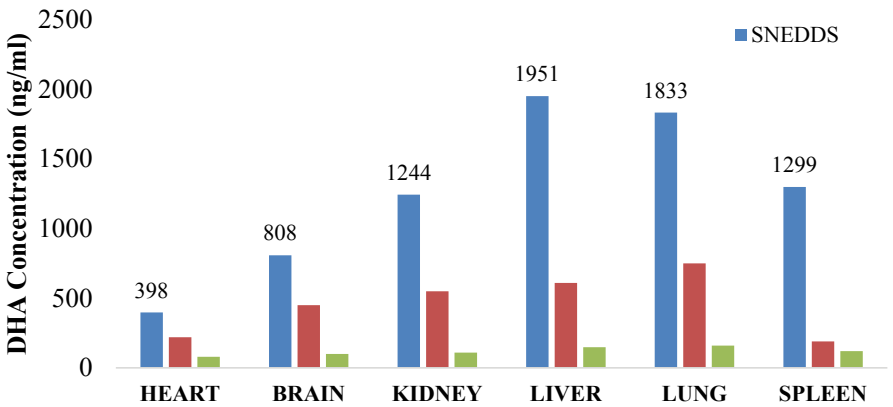

Figure 6: Comparison on biodistribution studies of SNEEDS, marketed conventional formulation and pure drug suspension.

takes around 2 h to achieve Tmax. Whereas the conventional marketed formulation did not show similar result, considering that it takes more time for disintegration and to go into the solution. Hence it took more than 2 h to achieve T_{max}, and moreover the extent of absorption which is evident fromAUC_{0-t} and $AUC_{0-\infty}$, the SNEDDS achieved maximum plasma concentration, which is statistically significant. From the study it is evident that SNEDDS containing artesunate was well absorbed with a C_{max} of 2467 ng/ml in 2 h (T_{max}), whereas the marketed and pure drug suspension resulted in 1940 ng/ml in 3 h and 640 ng/ml in 5 h respectively. SNEDDS formulation had an elimination rate constant which is 1.01, 1.28 and 1.40 for SNEDDS, marketed and pure drug suspension respectively (The pharmacokinetic parameters were calculated by non-compartmental analysis of individual concentration-time data using Phoenix WinNonlin® v 6.3 software (Pharsight corporation, Mountain view, CA, USA)). This may be due to the maximum concentration of drug enters the systemic circulation via lymphatics. The mechanism behind this may be the presence of fatty acids which triggered chylomicron synthesis in enterocytes in response to lipid digestion. The literature supports that Chylomicron consisting of triglycerides, surrounded by polar phospholipids and apoprotein, serves to solubilize the lipid in the aqueous environment of the blood that tends to drag lipids towards lymphatic system. Thus, enhances the bioavailability. This process also overcomes the pre-systemic metabolism. It is statistically evident since the statistical value of $P<0.05$ was achieved with a significant of 0.0106. This could be due to the fact that lipid droplets are taken by the chylomicrons. The results were mentioned in Table 4.

Biodistribution studies

Concentrations of dihydroartemisinin in DHA (metabolite of Artesunate) were been determined in various tissues like liver, spleen, heart, lung, brain and kidney of SD rats. The tissue distribution of Artesunate was analyzed by a non-compartment model and the results are showed in Figure 6. The concentration of DHA in all the collected tissues at 2 h were in the order of the maximum is, liver>lung>kidney>s pleen>brain>heart. The highest concentration of 1951.8 ng/g was found in the liver, which is more than 13 times that of pure drug and 3.19 times increase when compared with marketed formulation. Hence showed more significant on a P value of 0.0031 ($P<0.05$). This could be due to the fact lipid nanoemulsion is well taken by the reticuloendothelial system in association with the micelles and reverse micelles.

Conclusion

In conclusion, SNEDDS loaded Artesunate was been formulated for improving the pharmacokinetics of by the oral route. From the results it can be concluded that the SNEDDS formulation for the artesunate to contain oil phase Capryol 90 with Cremophor EL and ethanol as SCoS 1:1 mixture. The formulations exhibited nano droplet size with least PDI, & stable zeta potential for formulation 5. The study also suggests that the SNEDDS formulation showed high bioavailability and rate of drug release when compared to the conventional marketed formulation. SNEDDS also distributed higher towards the target site liver. Hence the formulation with Capryol 90 and Cremophor EL & Ethanol can allow the use of the potent antimalarial Artesunate effectively in case of high-risk malarial patients.

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