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Development of SMEDDS using natural lipophile: Application to β-Artemether delivery

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ABSTRACT

The objective of the present investigation was to formulate self-microemulsifying drug delivery systems (SMEDDS) using a novel, indigenous natural lipophile (N-LCT) as an oily phase. SMEDDS based on N-LCT and commercially available modified oil (Capryol 90) were formulated and their application in improving the delivery of a lipophilic anti-malarial drug, β-Artemether (BAM) was also evaluated. BAM-loaded SMEDDS were characterized with respect to mean globule size and *in vitro* drug release profile in comparison to the marketed formulation (Larither®). Comparative *in vivo* anti-malarial performance of the developed SMEDDS was evaluated against the (Larither®) in Swiss male mice infected with lethal ANKA strain of *Plasmodium berghei*. The parameters studied were percent parasitemia, activity against time and animal survival period. Both the BAM–SMEDDS showed excellent self-microemulsification efficiency and released >98% of the drug in just 15 min whereas (Larither®) showed only 46% drug release at the end of 1 h. The mean globule size for optimized BAM–SMEDDS was <100 nm. The anti-malarial studies revealed that BAM–SMEDDS resulted in significant improvement in the anti-malarial activity ($P < 0.05$) as compared to that of (Larither®) and BAM solubilized in the oily phases and surfactant. The developed SMEDDS highlight safety for use and potential applications of indigenous natural lipophile in the development of novel colloidal drug carriers.

1. Introduction

The oral delivery of lipophilic drugs presents a major challenge because of the low aqueous solubility. Lipid-based formulations have been shown to enhance the bioavailability of drugs administered orally (Hou et al., 2003; Sarkar, 2002; Gao et al., 2004; You et al., 2005). Widening availability of lipidic excipients with specific characteristics offer flexibility of application with respect to improving the bioavailability of poorly water-soluble drugs and manipulating their release profiles (Attama and Nkemnele, 2005). Lipids may have considerable clinical impact. Ingested food containing lipids can significantly alter postprandial drug absorption and its bioavailability (Charman et al., 1997; Fleischer et al., 1999). Gursoy and Benita (2004) and Kang et al. (2004) formulated self-emulsifying drug delivery systems for lipophilic drugs and showed that same can be used for the design of formulations in order to improve the oral absorption and bioavailability of highly lipophilic compounds. Similarly, SMEDDS is an isotropic, anhydrous mixture of drug, lipophile and surfactant/s that form fine oil-in-water microemulsion (globule size <100 nm) when introduced into aqueous phase under conditions of gentle agitation. SMEDDS are regarded as an attractive approach because of high drug solubilizing capacity and improvement in both rate and extent of absorption by the lymphatic uptake. Ideally, these novel formulations allow the drug to remain in dissolved state throughout the transit through the gastrointestinal tract thereby enhancing the bioavailability of poorly water-soluble therapeutic agents with reproducible plasma profiles (Constantinides, 1995; Constantinides and Scalart, 1997). Such formulations can be encapsulated into various types of capsules. The finished product is then administered to the patient as a solid dosage form (Yamahira et al., 1979). There are different categories of vehicles which can be selected in order to prepare a lipidic carrier. Khoo et al. (1998) demonstrated enhanced drug absorption when using long chain triglycerides (LCT) compared with medium chain triglycerides (MCT) in the SMEDDS formulations. These findings were attributed to maximal stimulation of lymphatic transport by the LCT. Hence, the objective of the present investigation was to formulate self-microemulsifying drug delivery systems (SMEDDS) using a novel, indigenous natural lipophile (N-LCT) as an oily phase. N-LCT is a refined, vegetable oil obtained from pressed fruit seed kernel. It is an edible oil having 1:2.37:1.36 ratio of saturated fatty acid (SFA):mono-unsaturated fatty acid (MUFA):poly-unsaturated

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fatty acid (PUFA) and contains triglycerides of C16–C18 fatty acids. Studies indicated that the rate of intestinal absorption of N-LCT was similar to that of the other Pharmacopoeial vegetable oils such as, sunflower, sesame and groundnut oil (Bhattacharya, 1987); suggesting that the N-LCT is acceptable for human consumption and pharmaceutical applications. The N-LCT offers many other advantages such as, easy availability in large quantities from natural source, toxicologically safe, completely biocompatible and cost-effective replacement for commercial triglycerides and modified oils.

In the present investigation, SMEDDS based on N-LCT and commercially available modified oil were formulated and their application in improving the delivery of a lipophilic anti-malarial drug, β-Artemether (BAM) was also evaluated. The emergence of drug-resistant parasite strains indicates an urgent need for discovering new and effective anti-malarial therapeutics as well as effective utilization of existing drugs through the concept of novel drug delivery systems (Fidock et al., 2004). BAM is a potent and rapidly acting anti-malarial drug which is listed by WHO as an Essential Drug for the treatment of severe multi-resistant malaria. This potent schizonticide drug is practically insoluble in water, belongs to BCS class II and has oral bioavailability of ∼45%. The generally recommended oral and parenteral administration, once a day for at least 5 days seems reasonable in view of clinical efficacy. The marketed dosage forms available for BAM are tablet, powder filled capsule and intramuscular injection. The oral formulations of BAM are rapidly but incompletely absorbed, limiting its use in malaria (Karbwang et al., 1997) whereas parenteral oily formulation leads to pain on injection and poor patient compliance. Pharmacokinetics of the BAM suggests its clinical efficacy is dependent on the formulation (Bunnag et al., 1991). There is need to develop a formulation which offers quick dissolution of BAM in order to yield improvement in bioavailability and therapeutic efficacy. In view of this, SMEEDS appeared to be an attractive approach for the delivery of BAM. In the present investigation, BAM–SMEDDS were developed and were evaluated for *in vitro* and *in vivo* performance.

2. Materials and methods

2.1. Materials

BAM was kindly provided as a gift sample by Ipca Laboratories Ltd. (Mumbai, India). Refined N-LCT was a gift sample from Charbhuja Trading Agencies and Pvt. Ltd., India. Caproyl 90 (Propylene glycol monocaprylate), Gelucire 44/14 (Lauroyl macrogol glycerides) and Plurol Oleique CC 497 (Polyglyceryl 6-dioleate) were obtained as a gift sample from Gattefosse', France through Colorcon Asia Ltd. (Goa, India). Cremophor EL (PEG-35-castor oil) was kindly provided by BASF India Ltd. (Mumbai, India). Transparent empty hard gelatine capsules were obtained as a gift sample from Associated Capsules Ltd. (Mumbai, India). All other chemicals and solvents were purchased from Merck International and s. d. Fine Chemicals Ltd., Mumbai, India.

2.2. Acute oral toxicity studies

Acute oral toxicity studies were conducted for a newly explored N-LCT in the development of novel drug delivery system. The acute oral toxicity was determined in Swiss albino mice ($n = 24$) to estimate lethal dose (LD_{50}) as per the OECD guidelines 420, 423 and 425 (OECD, 2001). The study protocol was approved by the Institutional Animal Ethical Committee (Approval No. UICT/PH/IAEC/0405/8).

2.3. Screening of components (solubility studies)

The saturation solubility of BAM in N-LCT, various commercial modified oils, surfactants and co-surfactants was determined. Briefly, excess amount of BAM was added to each screw-capped test tube containing 1 ml of lipophile or surfactant or co-surfactant. After sealing, the test tubes were shaken in an isothermal shaker ($37.0 \pm 1.0\,^{\circ}C$) for 72 h. Each tube was centrifuged at 5000 rpm for 15 min and the amount of BAM present in the supernatant was determined by HPLC method. The components were selected for further studies depending on the maximum drug solubility in oil phase, surfactant and co-surfactant.

2.4. HPLC analysis of BAM

The quantity of BAM solubilized in various vehicles was determined by using HPLC method reported by Chimanuka et al. (2002). The parameters were Jasco PU-2080 Plus Intelligent HPLC pump (Jasco, Japan) equipped with a Jasco UV 2075 Intelligent UV/VIS detector (Jasco, Japan), Rheodyne 7725 injector (Rheodyne, U.S.A.), Jasco Borwin Chromatography Software (Version 1.50) integrator software, Hi Q Sil C_{18} (4.6 mm × 250 mm and 10 μm particle size) column, mobile phase: acetonitrile:water (75:25) at flow rate 1 ml/min, detection at 214 nm with retention time at 8.2 min

2.4.1. Construction of pseudo-ternary phase diagrams

The pseudo-ternary phase diagrams were constructed by titration of homogenous liquid mixtures of oil, surfactant and co-surfactant with water at room temperature (Djordjevic et al., 2004). N-LCT or Capryol 90 (Gattefossé, France) with Plurol Oleique CC 497 (1:1) was the oil phase, Cremophor EL or Tween 80 was the surfactant and Gelucire 44/14 was the co-surfactant. At K_m values 1.5 and 1 (surfactant:co-surfactant ratio), oily mixtures of oil, surfactant and co-surfactant were prepared varied from 9:1 to 1:9 and weighed in the same screw-cap glass tubes and were vortexed. Each mixture was then slowly titrated with aliquots of distilled water and stirred at room temperature to attain equilibrium. The mixture was visually examined for transparency. After equilibrium was reached, the mixtures were further titrated with aliquots of distilled water until they showed the turbidity. Clear and isotropic samples were deemed to be within the microemulsion region. No attempts were made to completely identify the other regions of the phase diagrams. Based on the results, appropriate percentage of oil, surfactant and co-surfactant was selected, correlated in the phase diagram and were used for preparation of SMEDDS containing BAM.

2.5. Measurement of mean globule size

Each unit dose of SMEDDS containing 40 mg of BAM was added in 900 ml of the distilled water filtered through 0.45 μm membrane filters (Pall Life Sciences, India). The globule size and polydispersity index (P.I.) of the resultant microemulsion were determined by photon correlation spectroscopy (PCS) on N4 Plus Submicron Particle Size Analyzer (Beckman Coulter, USA) at a scattering angle of 90°. All measurements were performed in triplicate at a temperature of $20.0 \pm 2.0\,^{\circ}C$.

2.6. In vitro dissolution studies

The optimized SMEDDS formulations were filled volumetrically in transparent hard gelatin capsules (size zero). Each capsule contained 40 mg BAM. *In vitro* drug release profile of the BAM from the SMEDDS was evaluated using USP XIII Dissolution Testing Apparatus I at 100 rpm, with dissolution medium pH 1.2 buffer (900 ml), temperature $37.0 \pm 1.0\,^{\circ}C$ and sampling intervals 0, 5,

10, 15, 30, 45 and 60 min. The samples were analyzed by HPLC at 214 nm with abovementioned parameters. The release profile of developed SMEDDS of BAM was comparatively evaluated with the commercial (Larither®, IPCA Labs., India) capsule dosage form.

2.7. In vivo efficacy of BAM–SMEDDS

In vivo anti-malarial activity and efficacy of the developed SMEDDS of BAM was comparatively evaluated against marketed (Larither®, IPCA Labs., India). The study protocol was approved by the Institutional Ethical Committee of the Tata Institute of Fundamental Research (TIFR) and the work was carried out in the same premises. The "Peter's four day suppressive test" (Peters et al., 1975) was utilized for the study. The lethal ANKA strain of *Plasmodium berghei* was used in the experiment. In-house bred Swiss male mice (weighing around 25–30 g, each) free of mycoplasma were infected by intra peritoneal inoculation of donor mouse blood diluted in ACD (acid citrate buffer) containing approximately 10^6 infected RBCs on day '0'. The animals were divided into nine groups ($n = 6$) as per the treatment mentioned, Group I (Positive Control, no drug treatment), Group II (Blank SMEDDS, 4 mg/kg of blank SMEDDS of N-LCT), Group III (Blank SMEDDS, 4 mg/kg of blank SMEDDS of Capryol 90), Group IV (BAM SMEDDS, 4 mg/kg of BAM SMEDDS of N-LCT), Group V (BAM SMEDDS, 4 mg/kg of BAM SMEDDS of Capryol 90), Group VI (Marketed Formulation, 4 mg/kg of Larither®, IPCA Labs. India), Group VII (BAM in oil vehicle, 4 mg/kg of BAM in N-LCT), Group VIII (BAM in oil vehicle, 4 mg/kg of BAM in Capryol 90), Group IX (BAM in Surfactant, 4 mg/kg of BAM in selected surfactant). The animals were held at a temperature of 22 °C and 65% relative humidity and were fed a standard mouse diet with clean drinking water *ad libitum*. Starting from day '0' to day '3' of post-infection, the different groups of mice were given respective treatment by oral gavage. The dose was suitably diluted with the distilled water, shaken-well and orally administered. The parameters under investigation were mean percentage parasitemia against time (in days), percent activity against time (in days) and the animal survival period. The parasite counts were made on day 4, 8, 10, 15 and 20 from thin blood smears of tail blood, fixed with methanol and stained with Giemsa's stain. Parasitemia was reported as mean percentage parasitemia after counting 1000 RBCs from each slide. Percent anti-malarial activity was also calculated as suggested in the standard protocol (Fidock et al., 2004) and the animals were also observed for their survival till death.

The mean percentage parasitemia was expressed as mean ± S.D. and assessed by two-tailed paired '*t*' test or ANOVA followed by Tukey's multiple comparison test using Graphpad Instat Demo version. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Acute oral toxicity studies

Although reports suggest that the N-LCT is biocompatible and would have good applications in oral delivery, it was essential to validate the claims in those reports by carrying out acute oral toxicity studies. The study revealed biocompatibility and safety of the N-LCT for oral delivery. No sign of mortality was seen at different doses of 5, 50, 300 and 2000 mg/kg when administered orally. All the physiological functions and animal behavior were normal at higher dose levels. Hence, it could be concluded that the estimated LD_{50} of N-LCT is above 2000 mg/kg body weight when given orally.

3.2. Screening of components (solubility studies)

BAM showed high solubility in commercial modified oil Capryol 90 (281.74 ± 3.48 mg/ml) and also in N-LCT (271.27 ± 4.16 mg/ml). In the surfactants, Cremophor EL and Tween 80 showed maximum drug solubilization 293.40 ± 3.69 mg/ml and 262.58 ± 3.12 mg/ml, respectively. The solubility of BAM in the other components such as Plurol Oleique CC 497 (oil) and Gelucire 44/14 and Tween 20 (surfactants) was comparatively less as compared to the aforementioned components. For further studies, Cremophore EL was selected as a surfactant and Gelucire 44/14 was selected as a cosurfactant. Gelucire 44/14 is a known bioavailability enhancer and also plays role in reduction of mean particle size (Aungst et al., 1997). It was observed that N-LCT showed complete miscibility and no phase separation with other components when blended with Plurol Oleique CC 497 at 1:1 ratio. Therefore, a mixture of Plurol Oleique CC 497 and N-LCT (at 1:1 ratio) was used as an oily phase for the further studies.

3.3. Construction of pseudo-ternary phase diagrams

Phase diagrams were constructed to obtain the proportion of components that can result in maximum microemulsion existence area. The area of microemulsion existence is depicted in Fig. 1a and b with shaded color. The maximum area of microemulsion existence at for N-LCT obtained at $K_m = 1.5$ while for Capryol 90 it was at $K_m = 1$, irrespective of Cremophor EL or Tween 80 as a surfactant. The optimized compositions of the developed SMEDDS are shown in Table 1.

3.4. Measurement of mean globule size

The results are shown in Table 2. It is evident from the table that the SMEDDS of BAM containing Gelucire 44/14 yielded smaller globule size (<100 nm) as compared to the SMEDDS which are devoid of Gelucire 44/14 (>300 nm), irrespective of Cremophor EL or Tween 80 as a surfactant.

3.5. In vitro dissolution studies

The *in vitro* dissolution studies are performed in order to ensure the quick release of the drug the dissolution medium and they also act as an important quality control tool for the dosage forms. Furthermore, *in vitro* dissolution studies also give an idea about the self-microemulsification efficiency of the developed system. The *in vitro* dissolution profile of S1, S2, S3, S4 and Larither® was evaluated in simulated gastric fluid ($n = 3$). It was observed that all the SMEDDS formulations S1, S2, S3 and S4 released more than 98% of BAM within 15 min. All the formulations dispersed almost instantaneously after dissolution of the capsule shell indicating the high self-microemulsion efficiency of the developed formulations. The graphs of the dissolution profile are not shown as figure

Table 1
Composition of various SMEDDS formulation used in the study

Components	Composition			
	S1	S2	S3	S4
BAM	40 mg	40 mg	40 mg	40 mg
N-LCT	25	25	–	–
Capryol 90	–	–	25	25
Cremophor EL	25	–	20	–
Tween 80	–	25	–	20
Gelucire 44/14	17	17	20	20
Plurol Oleique CC 97	25	25	25	25

Table 2
Globule size and polydispersity index various SMEDDS ($n = 3$)

System	Globule size (nm)		Polydispersity index	
	With Gelucire 44/14	Without Gelucire 44/14	With Gelucire 44/14	Without Gelucire 44/14
S1	61.9 ± 25.3	286 ± 35.1	0.684	1.139
S2	73.1 ± 22.7	337 ± 51.4	1.105	1.66
S3	31.7 ± 7.8	195 ± 13.6	0.720	0.851
S4	52.2 ± 14.3	307 ± 31.7	0.782	1.117

because they are almost overlapping. The marketed formulation (Larither®) showed only 46% drug release at the end of 60 min. This clearly demonstrates the superior performance of the developed SMEDDS as compared to Larither®. The SMEDDS are expected to quickly present BAM in solubilized form in gastric fluids after ingestion and would provide large interfacial area for BAM absorption.

3.6. *In vivo efficacy of BAM–SMEDDS*

For the *in vivo* anti-malarial studies, SMEDDS containing Tween 80 were selected due to the greater safety profile of Tween 80 as compared to Cremophore EL on oral administration (Rowe et al., 2003). The results of anti-malarial studies are summarized in Table 3. At end of the treatment (day 4), when compared to Control (29.58 ± 4.95%), SMEDDS treatment Group V (13.70 ± 1.05%) and Group IV (14.31 ± 1.66%) showed significantly less ($P < 0.001$) mean percentage parasitemia. The results of the marketed formulation, Group VI (18.39 ± 2.40%) were also statistically significant ($P < 0.001$) against the Control on the same day. Interestingly, both the BAM–SMEDDS formulations demonstrated significantly less parasitemia ($P < 0.05$) as compared to that of marketed formulation of BAM (Larither®). This clearly demonstrates the advantage and superiority of SMEDDS approach over the marketed formulation. It is also noteworthy that the BAM solubilized in individual SMEDDS components did not show significantly different anti-malarial activity as compared to that of Larither®. Hence, it can be inferred that the higher efficacy of the SMEDDS formulation is a result of the combination of all the SMEDDS components and the nanostructure of the microemulsion. On 20th day, all the groups except blank showed significantly lower parasitemia ($P < 0.05$) as compared to that of Control group. Interestingly, one of the SMEDDS still showed significantly lower parasitemia ($P < 0.05$) as compared to that of Larither® whereas all the other formulations did not show statistically different anti-malarial activity. The results of survival studies are shown in Fig. 2 and Table 3. It is clearly evident that groups treated with SMEDDS showed highest number of survivors (83%) whereas group treated with Larither® showed 100% mortality at the end of 30 days.

The results of *in vivo* studies revealed that anti-malarial efficacy by BAM–SMEDDS prepared with N-LCT were analogous with Capryol 90. The *in vivo* findings further discovered the fact that solubilized form of drug in an oil vehicle could not be as effective as its SMEDDS. High drug solubilization capacity and self-microemulsification efficiency by the SMEDDS significantly contributed to the absorption. Readily uptake of oil globules (with solubilized drug) from the resultant microemulsion at the absorption site resulted in faster onset and thereby the anti-malarial efficacy. Surfactant vehicle group had shown anti-malarial activity initially but eventually it reduced. This could be because of drug precipitation that may lead to incomplete absorption. The *in vivo* performance from the marketed formulation could be attributed to the poor wettability of the formulation and drug release.

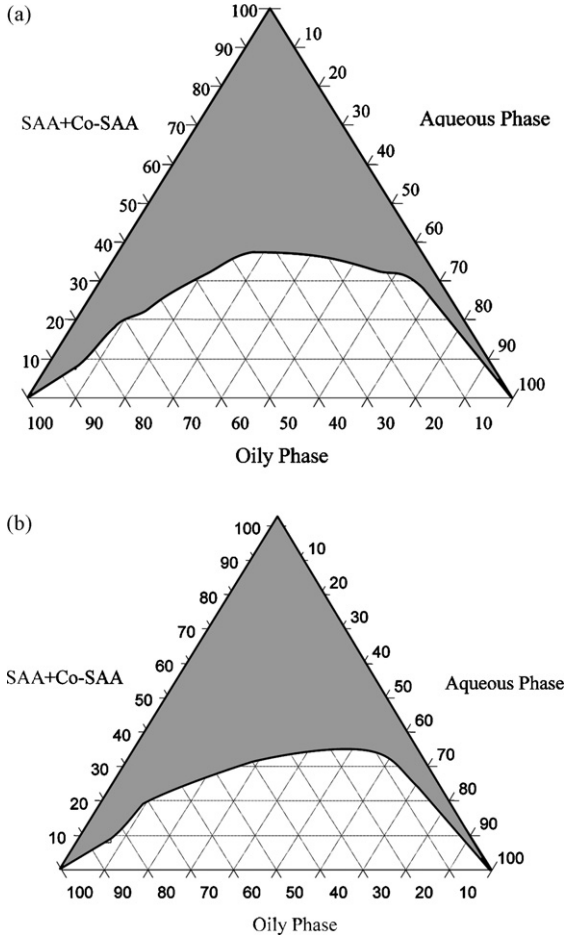

Fig. 1. (a) Ternary phase diagram at K_m 1.5. Oily phase = N-LCT and Plurol Oleique CC 497 (1:1), SAA (surfactant) + Co-SAA (co-surfactant) = Cremophor EL/Tween 80 + Gelucire 44/14 at K_m 1.5 and aqueous phase = distilled water. The shaded color region indicates microemulsion existence area. (b) Ternary phase diagram at K_m 1. Oily phase = Capryol 90 and Plurol Oleique CC 497 (1:1), SAA (surfactant) + Co-SAA (co-surfactant) = Cremophor EL/Tween 80 + Gelucire 44/14 at K_m 1 and aqueous phase = distilled water. The shaded color region indicates microemulsion existence area.

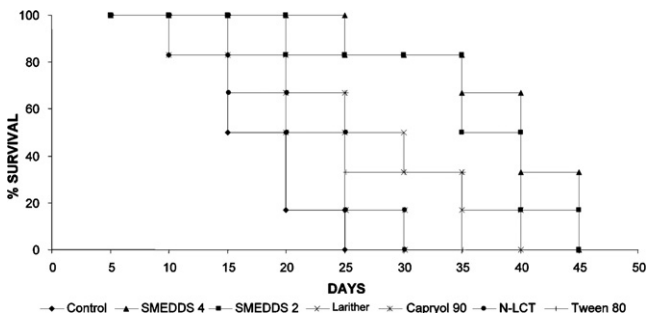

Fig. 2. Survival plot for the various treatment groups ($n = 6$ in each group).

Table 3
In vivo anti-malarial activity of various formulations in *P. berghei* infected mice ($n = 6$)

Sr. No.	Treatment group	Average% parasitemia ± S.D.		% activity		No. of survivals ($n = 6$, $t = 30$ days)
		4th day	20th day	4th day	20th day	
I	Control	29.58 ± 4.95	54.02 ± 0.00	–	–	0
II	Blank 2	28.29 ± 5.11	53.23 ± 0.73	4	1	0
III	Blank 4	28.71 ± 3.77	52.95 ± 0.00	3	2	0
IV	S2	14.31 ± 1.66[a]	38.33 ± 3.81	52	29	5
V	S4	13.70 ± 1.05[a]	35.11 ± 4.16[a]	54	35	5
VI	Larither®	18.39 ± 2.40	42.35 ± 4.18	38	22	2
VII	N-LCT	20.93 ± 3.92	41.00 ± 5.70	29	24	0
VIII	Capryol 90	21.02 ± 2.32	40.43 ± 3.03	29	25	0
IX	Tween 80	19.17 ± 3.98	40.30 ± 8.76	35	25	2

All the other groups except blank showed significantly lower parasitemia as compared to the Control ($P < 0.001$).
[a] Significantly less as compared to the Larither® ($P < 0.05$).

4. Conclusion

The present research work could be summarized as successful development of SMEDDS for lipophilic anti-malarial drug, BAM using an indigenous natural lipophile as a novel excipient. The experimental results with utilization of newly explored lipophile were satisfactory and comparable to that of commercially available modified triglyceride. Thus, use of indigenous natural lipophile would be a safe and promising alternative in the field of novel drug delivery systems. Significant improvement in drug solubility and thus overcome dissolution rate-limited absorption of BAM may be realized with *in vitro* drug release studies. SMEDDS of BAM as oral immediate release, capsule dosage form deemed to be the efficacious and patient compliant delivery system in the anti-malarial therapeutics.

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