WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 444-459. Research Article ISSN 2277– 7105

PREPARATION AND CHARACTERIZATION OF LUMEFANTRIN-PEG 4000 SOLID DISPERSIONS

[1]Mumuni A. Momoh, [2]Gwarzo S. Mahmud, [3]Musiliu O. Adedokun, [4]Mba C. Chukwuemeka and [5]A R. Oyi

[1]Drug Delivery Research Unit, Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Usmanu Danfodiyo Sokoto, Sokoto State, Nigeria.
[3]Department of Pharmaceutics and Pharmaceutical Technology, University of Uyo, Akwa- Ibom State, Nigeria.
[4]Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Enugu State, Nigeria.
[5]Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Article Received on
09 Oct 2015,

Revised on 31 Oct 2015,
Accepted on 22 Nov 2015,

***Correspondence for Author**
Mumuni A. Momoh
Drug Delivery Research Unit, Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Nigeria.

ABSTRACT

The objective of the present investigation was to explore the potential of solid dispersions (SDs) for the oral delivery of lumefantrin, a poorly water-soluble antimalarial agent. The SDs containing LMFT were formulated by employing melt fusion technique. Five different ratios of PEG-4000 and LMFT (1:1, 1:3, 3:1, 0:1 and 1:0) were prepared. The SDs was evaluated for particle size, encapsulation efficiency, *in vitro* drug release. The antimalarial activity of SDs formulated was evaluated in Plasmodium berghei infected mice. Thermograms of the SDs showed modifications in the peaks. Particle sizes of SDs were depend on the ratio of the PEG and drug. The SDs had high drug content and showed sustained release capability as compared to marketed sample. *In vivo* pharmacodynamic studies showed that LMFT-SDs exhibited significantly higher antimalarial activity ($P < 0.05$) as compared to the marketed formulation. LMFT-SDs is comparatively better with respect to antimalarial effect, sustained release and survival rate of the animal than marketed sample.

KEYWORDS: lumefantrin, solid dispersion, anti-malaria.

INTRODUCTION

Malaria threatens almost half the world population and is one of the most important infectious diseases worldwide.[1] The annual number of malaria cases worldwide is estimated to be around 500 million and over 90 % of malaria cases and the great majority of malaria deaths occur in sub-Saharan Africa. The mortality, recently estimated at 1.5 million people every year, has risen in recent years, probably due to increasing resistance to the common classical antimalarial drugs. The burden of this enormous toll, and the concomitant morbidity, is borne by the world's poorest countries. In humans, malaria is caused by four distinct blood–borne Apicomplexan parasite species: Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale. Amongst these, the most severe malaria is caused by P. falciparum which is responsible for almost all malaria related deaths. In P. falciparum infection, resistance has been observed to almost all currently used antimalarials, like amodiaquine, chloroquine, mefloquine, quinine and sulfadoxine/pyrimethamine.[2]

Lumefantrin (LMFT) is a synthetic aryl amino alcohol similar to mefloquine and halofantrine. It is a potent and rapidly acting antimalarial agent which is enlisted in WHO List of Essential medicines for the treatment of severe multi-resistant malaria in combination with artimisinin derivative such as artesunate or artemetheter.[3,4] It is active against P. vivax as well as chloroquine-sensitive and chloroquine-resistant strains of P. falciparum. However, lumefantrin is hardly use alone in the management of malarial due to passive poor aqueous solubility which has significantly hampers its therapeutic efficacy. Commercially, LMFT is available as a yellow tablets or caplets for oral therapy as a single formulation or formulated as multiparticulate tablet with other agents. However, the therapeutic potential of drug is significantly hindered due to its low and inconstant oral bioavailability.[5] The low and inconstant oral bioavailability of LMFT curtails from its poor aqueous solubility and thus resulting in incomplete absorption after oral administration (< 45 %). This indicates that large fraction of the dose remaining undissolved for absorption upon reaching, the non absorbable site in the large intestine. In construal to this, the SD of LMFT is likely to have great potential in improving oral bioavailability, solubility, dissolution rate and in turn the therapeutic efficacy of LMFT. Improving the aqueous solubility of LMFT can significantly impact their absorption in the GI when delivered orally.[6] To this end, enhancing the solubility of this class of drug substances has become a major point of interest in the pharmaceutical industry.

In considerations of the above mentioned facts, the objective of the present study was to formulate and evaluate commercially feasible oral form of LMFT as solid dispersion (SDs)

using hydrophilic polymer based on melt-fusion technique. Studies have shown that polymers with hydrophilic functional group are well suited for use as carriers in which the drug substance is dissolved at the molecular level.[7,9] Investigation also proved that such polymer when interact with active pharmaceutical ingredient (API) functional group or groups is/are capable of not only kinetically stabilizing the amorphous form in the solid-state, but also inhibiting precipitation in the liquid state, effectively providing maintenance of super saturation.[10,11] The use of polyethylene glycol (PEGs)-based polymeric carriers in solid dispersion systems is highly welcome in the pharmaceutical industries and is common among the formulation scientists due to their effectiveness as solubilizing as well as precipitation inhibitors. Additionally, the rationale behind the selected melt method is that, it is economic, environmentally friendly and avoids thermal degradation of drug, usage of organic solvent and sophisticated equipment. Also SD powders which are obtained by this method and selected polymer is physico-chemically stable and can be easily formulated.[12]

METHOD AND MATERIALS

Chemicals Lumefantrin (LMFT) was kindly provided by Healthcare Pvt. Ltd. Mumbai, India. Polyethylene glycol (PEG-400), (Cary Roth, Karlsruhe, Germany), monobasic potassium phosphate, sodium hydroxide and concentrated hydrochloric acid (BDH, Poole, England). Dialysis bags (MW 100 kDa) were procured from Sigma Chemicals (NJ, USA), Distilled water (Lion Water, University of Nigeria, Nsukka, Nigeria). All the excipients and reagents were used as received. Double distilled water was prepared freshly whenever required.

Parasite

Plasmodium berghei berghei NK 65 strain was obtained from the Malarial Research Unit of Nigeria Institute for Medical Research Yaba Lagos, Nigeria (NIMR) was used for *in vivo* evaluation of antimalarial activity. The strain was found to be free of contamination with Eperythrozoon coccoides after examination. The strain is well characterized in our Veterinary Laboratory Research Unit, University of Nigeria Nsukka, and it is known to provide high mortality in mice, providing a good model to estimate survival and antimalarial efficacy. It is sensitive to all antimalarial agents that are used currently.

Formulation of LMFT-SDs

Five different ratios of SDs were prepared 1:1, 1:3, 3:1, 1:0 and 0:1 (LMFT: PEG) and coded L-1, L-2 and L-3, L-4 and PE, respectively, using the fusion method. The required amount of PEG 4000 was melted in a beaker on a water bath maintained at 65–75 ^{o}C. The appropriate

quantity of LMFT weighed and was dispersed into the molten PEG 4000 and mixed thoroughly with a glass rod for 5 min. The mixture was cooled rapidly by placing the beaker in an ice bath for 5 min to solidify, then powdered in a mortar, sieved through a 65-mesh screen, the resultant yellowish powder were stored in a screw-cap vial at room temperature for further study.

Characterization of LMFT- SDs

Solubility Studies

An excess of LMFT was added to screw-capped vials containing PEG-4000 solution (0.5% w/v), prepared in phosphate buffer, pH 6.8. Vials were shaken mechanically at 38 ± 0.5 °C for 24 h. At equilibrium after 48 h, aliquots were withdrawn, filtered (0.22 µm pore size, USA) and the drug concentration was analyzed spectrophotometrically (Jenway, 63405, UK) at 336 nm.

Percent yield

Percentage yield were calculated to know about percent yield or efficiency of any method, thus it helps in selection of appropriate method of production. Solid dispersions were collected and weighed to determine the practical yield of the formulation using equation (1).

$$\% \text{ yield} = \frac{A_1}{A_2 + A_3} \times 100 \qquad (1)$$

where A_1 is the weight of the solid dispersion formulated (g), A_2 the weight of the LMFT (g) and A_3 the weight of the polymers (g).

Drug content

The solid dispersion equivalent to 30 mg of model drug were taken and dissolved separately in 25 ml of methanol. The solutions were filtered and were further diluted such that the absorbance falls within the range of standard curve. The absorbances of solutions were determined at 336 nm by UV-visible spectrophotometer. The actual drug content was calculated using the following equation as follows:

$$\% \text{ Drug content} = \frac{ADC}{TDC} \times 100 \qquad (2)$$

Where ADC is the weight of the actual drug content (g) and TDC is the theoretical content.

Morphological and particle size study

The morphology and the particle size of the SDs was determined by computerized image analysis on a photomicroscope (Lieca, Germany). Each of the batches was mounted on a slide and observed under a light microscope. With the aid of the software in the microscope, the particle morphologies were observed and photomicrographs taken. All these were done within one week of the formulation.

Differential Scanning Calorimeter (DSC)

Differential scanning calorimetry study was performed using differential scanning calorimeter (Mettler Toledo, DSC 822). Samples were heated in an open aluminum pans at a rate of 5 °C per min−1 in a 30 to 330 °C temperature range under a nitrogen flow of 40 ml/min. The crystallinity index (CI) of the SDs was calculated from the enthalpy of fusion using the following formula:

$$\% \text{ CI} = \frac{\text{Enthalpy of SDs (J/g)}}{\text{Enthalpy of polymer (J/g}} \text{ x } 100 \quad (3)$$

***In vitro* release**

The USP XXII rotating paddle apparatus (Erweka, GmbH Germany) was employed for this release study. The drug release study was carried out in phosphate buffer of pH 7.2 and 0.1 N HCl of pH 1.2 with 1 % Benzalkonium chloride (BKC). The polycarbonate dialysis membrane used as a release barrier was pre-treated by soaking it in the dissolution medium for 24 h prior to the commencement of each release experiment. In each case, 0.1 g of SDs was placed in the dialysis membrane bags (molecular weight cut off: 800-1000 kDa) containing 2.5 mL of the dissolution medium, securely tied with a thermo-resistant thread at both ends and then immersed in the dissolution medium under agitation provided by the paddle at 100 rpm, and maintained at 37±1 ^{0}C by means of a thermostatic water bath. At predetermined time intervals, 5mL portions of the dissolution medium were withdrawn and replaced with equal volume of the medium to maintain a sink condition, filtered with a pore size of 0.22 mm (Millipore filter, Delhi, India) and analyzed spectrophotometrically (Jenway, UK) at 336 nm. The amount of drug released at each time interval was determined with reference to the standard Beer's plot for LMFT in phosphate buffer. This test was carried out in triplicate for all the batches.

Animals

In house bred male Swiss albino mice aged 2–5 weeks with an average body weight of 45 g were used for the study. The animals, held at a temperature of 22±3 ◦C and 65% relative humidity, were fed a standard mouse diet and provided with clean drinking water ad libitum throughout the experiments. The animal experimental procedures in this study were reviewed and approved by the Animal Ethical Committee of the Department of Pharmaceutics, University of Nigeria Nsukka and were in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC).

***In vivo* antimalarial evaluation**

The protocol adopted in this *in vivo* study was designed on the basis of "Peters four day suppressive test" described by Peters et al.[13] The mice were infected by intraperitoneal inoculation of donor mouse blood diluted in acid citrate dextrose (ACD) buffer containing approximately 10^6 P. berghei P. berghei infected RBCs on day '0'. The mice were randomly divided into five groups of ten mice per group (n = 10 per group) as depicted in Table 1. Starting from day '0' to day '3' post infection animals in group A, B and C were given 20 mg/kg of L-1, L-2, and L-3, respectively by oral gavage. Animals in group D and E were treated with of normal saline (2.5 ml/kg) and market sample (MS) and served as negative and positive control respectively. On fourth day, blood was withdrawn from tail vein and the blood smears were prepared. Blood smears were fixed with methanol and stained with Giemsa stain and the parasites were counted. Parasitemia is the quantitative content of parasites in the blood. Parasitemia was reported as percentage parasitemia after counting 250 RBCs from each slide. The percentage reduction in the parasitemia level and the activity of different LMFT formulations was calculated by the following formula suggested in the standard protocol.[14]

$$\text{Activity} = \frac{\text{Mean parasitemia in trested group}}{\text{Mean parasitemia in control group}} \times 100$$

Kinetic analysis of *in vitro* release profiles

In order to understand the mechanism and kinetics of release of LMFT from the SDs preparation, the *in vitro* release data were fitted into various kinetic equations like zero order (Cumulative percent drug released vs. Time), first order (Log cumulative percent drug retained vs. Time), Higuchi (cumulative percent released vs. √T), Peppas (Log of cumulative

percent drug released vs. log Time) and Hixson-Crowell's cube root model ((Percentage retained)$^{1/3}$ vs.Time). The kinetic model that best fits the dissolution data was evaluated according to an earlier work.[15]

Statistical analysis

The results were expressed as mean of SEM. Statistical analysis of the data was carried out using **SPSS Version 20 (SPSS Inc. Chicago, IL.USA)** and Student's t-test to determine where there were statistically significant differences ($p \leq 0.05$).

RESULTS AND DISCUSSION

Drug present with different nature i.e amorphous and crystalline state. The amorphous substances are physically unstable due to their high energy state and tend to recrystallize upon storage. In order to stabilize these systems, various polymer carriers have been used because they readily generate amorphous forms and may be able to retain the amorphous nature of the drug upon storage. In this study three varying drug/polymer combination were formulated based on solid dispersion and evaluated for the stability as well as the efficacy.

Solubility studies

The solubility of different formulation of LMFT-SDs containing PEG and drug in the following ratios (1:1, 1:3 and 3:1) shows that the SD prepared with PEG: LMFT (1:3) presented higher dissolution concentration as compared with other formulations 1:1, 3:1. Results indicate that as the concentration of PEG increased, the solubility also increased. Maximum solubility (25 mg/ml) was attained in 1:3 as compared with pure drug (1.2 mg /ml) Table 1. Numbers of theories have been proposed on how SDs enhances the dissolution of drugs yet, but the mechanism by which the dissolution rate is improved is not fully understood, because there are comparatively few papers available which elucidate the mechanism (or mechanisms) involved in solid dispersion. The currently accepted range of possible mechanisms of enhanced dissolution effectively includes the following: reduction in particle size, increased wettability, dispersibility, and decrease in crystallinity of the drug, ease of complex formation between the drug and polymer and/or presence of drug in the water-soluble carrier in molecular form.

Percentage drug yield

The percentage of the SDs yield or recovered from the formulations (Table 1) indicate that all the LMFT-loaded SDs (batches L-1 to L-3) had overall higher recovery percentages than the

unloaded SD (L-4). The effect of drug /polymer ratio on the yield was not significant. Pharmaceutically, the yield or recovery rate of formulation is a key factor to be considered in the choice of formulation parameters, because it has direct impact on the productivity of the formulator as well as a major index in evaluating the cost of the final product.

DSC characterization

The results of DSC thermographs and the corresponding peaks of PEG-4000, pure drug, SDs formulated L-1, L-2 and L-3 are shown in Figs.1 (a-f). Thermograms of pure drug (LMFT) showed a sharp endothermic peak corresponding to melting at 135.2 ^{o}C (Fig. 1d) while that of PEG shows 68.2 ^{o}C (Fig. 1e). When the PEG was employed to formulate SDs, the DSC traces of the formulations showed various peaks according to drug-polymer ration. It was observed that the at 1:3 (PEG: LMFT) batch L-3, the DSC trace gave two different peaks of 65.2 ^{o}C and 132 ^{o}C (Fig. 1c), indicating that the SDs formulated had some drug that are not completely encapsulated within the polymer core. However the DSC traces of L-1 and L-2 showed a single peaks at 65 (Fig.1a) and 62 ^{o}C (Fig. 1b) respectively. The values of the peaks obtained in the SDs are much lower than the pure drug or the polymer as shown in the various thermographs and the overlayed (Fig. 1f). This result indicates that there was a drastic change in the degree of crystallization when the polymer and the drug were used in the SDs formulation. The lower traces of the DSC peaks are a clear indication that the SDs formed is less crystalline than individual materials used in the formulation. The pharmaceutical implication of the lower degree of crystallinity is that the SDs could generate more capacity for drug incorporation. More so, the release of the drug in the biological system could also be regulated.

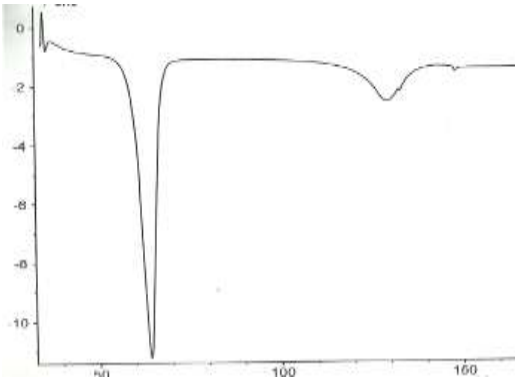


Fig. 1a. DSC thermogram of L-1.

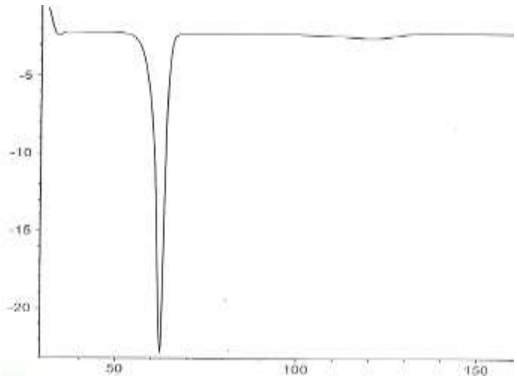


Fig. 1b. DSC thermogram of L-2.

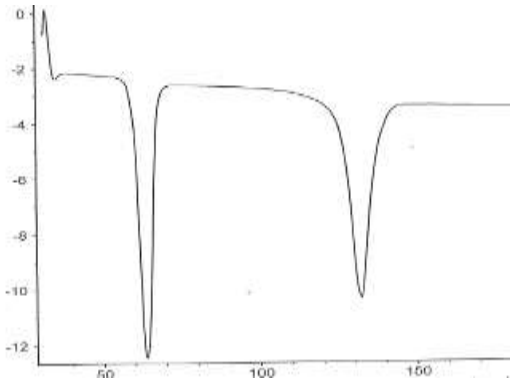


Fig. 1c. DSC thermogram of L-3.

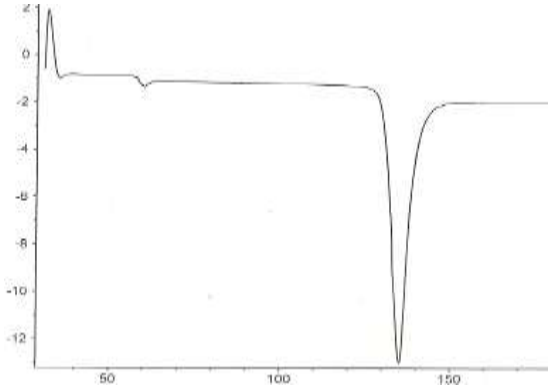


Fig. 1d. DSC thermogram of L-4.

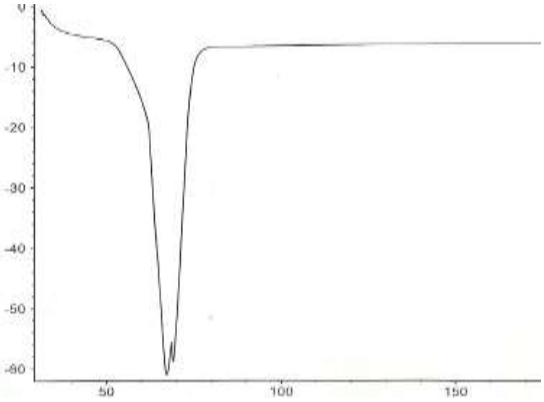


Fig. 1e. DSC thermogram of PEG alone

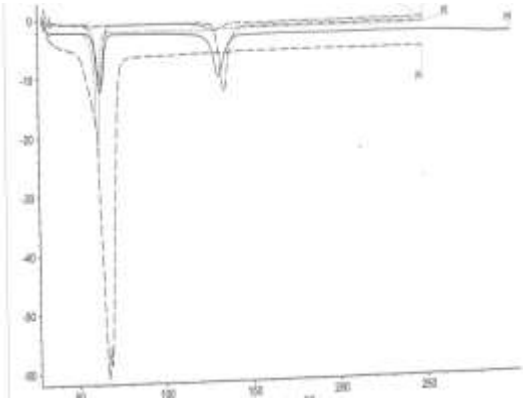


Fig.1f. DSC thermogram of the overlayed of the SDs

Drug content analysis

The drug content is an important variable for assessing the drug loading capacity of SDs and their drug release profiles, thus suggesting the amount of drug that would be available at the site of absorption. This parameter is dependent on the process of preparation, physicochemical properties of drug, and formulation variables.[16,17] It is also highly influenced by type of polymer, polymer concentration and solvent used to dissolve the drug and polymer.[18] The drug content was observed to be dependent on the proportion of polymer used in preparing the SDs. Table 1 shows that drug content generally increased with increasing amount of polymer in the formulation. Thus, SDs prepared with highest amount of polymer entrapped the highest amount of LMFT. However, in all case, the amounts of drug in all the SDs were very high indicating that the polymer ratios were adequately selected. Maximum (101.10 ± 0.21 %) and minimum (91.30 ± 0.21 %) drug content was found to be in L-1 and L-3 respectively.

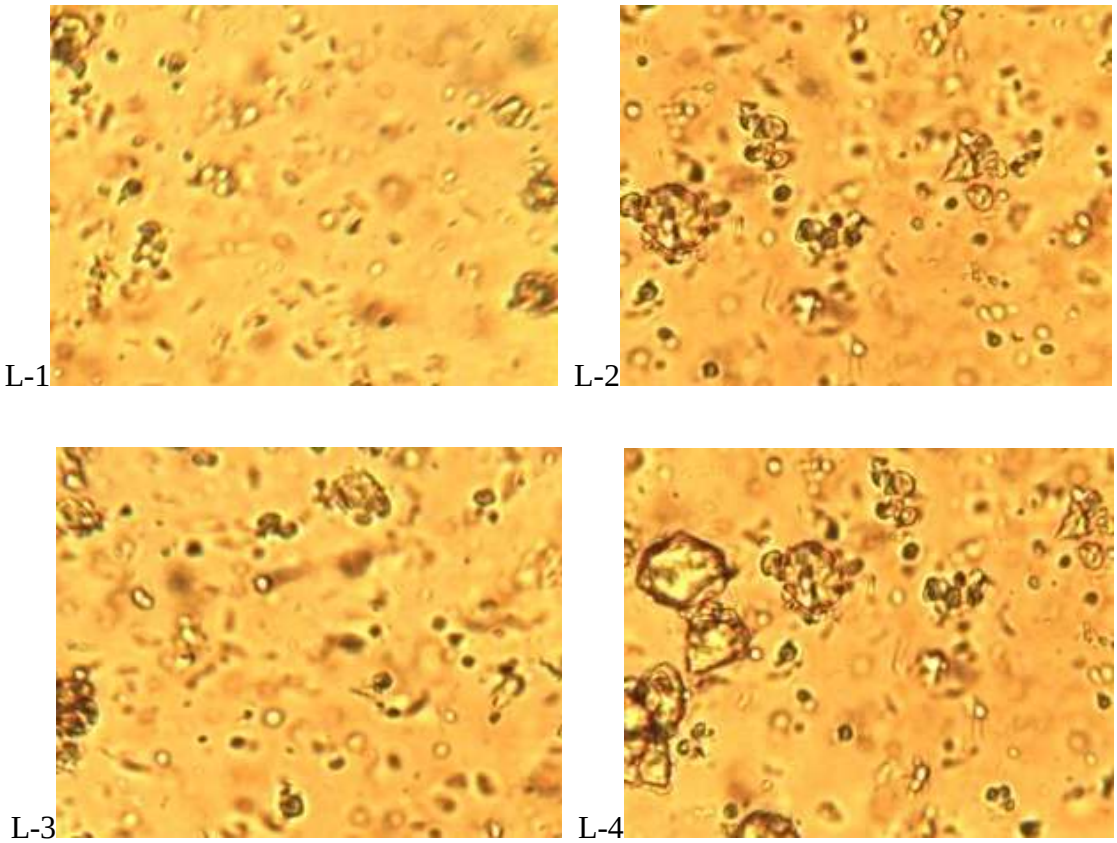
Table 1: Properties of LMFT-SDs formulations

Batches	PY (%)	PS (µm)	DC (%)	SE (µg/mL)
L-1	89.00 ± 0.21	128.00 ± 0.20	91.30 ± 0.21	126.20 ± 0.00
L-2	93.10± 0.10	102.00 ± 0.11	96.11 ± 0.11	191.10 ± 0.11
L-3	98.11± 1.10	84.00 ± 0.10	101.10 ± 0.21	211.10 ± 0.12
L-4	* * *	152.67± 0.13	* * *	56.3± 0.10

PY= percentage yield, PS= average particles size, DC= drug content and SE= solubility efficiency

Morphology and particle size analysis

The results of the morphological study Figs.2 (L-1 to L-4) of the SDs and the particle size of LMFT-SDs shown in Table 2 revealed that the SDs were within the acceptable range for microparticles meant for oral delivery. The maximum and minimum particle sizes were obtained for batch L-1 and L-3, respectively. Though, the particle size was affected by the ratio/combination of the drug and the polymer. From the results, particle size increased with increase in the amount of drug that is encapsulated in the SDs, this assertion is in agreement with the work done by some researchers.[19] The particle size however, varied significantly ($p < 0.05$) within the various batches of the formulation and across the batches. The particle size of SDs is important because it determines the site of administration of the drug formulations and also affects the bioavailability of formulated drug. Furthermore, particle size of SDs also determine the release ability of the formulation. In other words, Particles sizes in solid dispersions have been found to have a higher degree of porosity. The increase in porosity also depends on the carrier properties, for instance, solid dispersions containing linear polymers produce larger and more porous particles than those containing reticular polymers and, therefore, result in a higher dissolution rate. The increased porosity of solid dispersion particles also hastens the drug release profile.[20,21] Fortunately enough, all the LMFT-SDs formulations showed an acceptable range of particle size for oral administration formulation. Indicating that regardless of the ratio of drug to polymer ratio, the LMFT-SDs prepared were fit for oral administration based on particles size range.



Figs. 2 (L1 to L4). Photomicrographs of SDs formulated with varying ratio of PEG-4000 and LMFT.

***In vitro* release of LMFT for SDs**

The *in vitro* release profiles of LMFT-SDs formulation in phosphate buffer of pH 7.2 and pH 1.2 are shown in Figs. 3 and 4. In pH 7.2, batch L-3 of the formulation gave a maximum release of 75.1 ± 0.3 %, while L-2, L-1 and marketed sample (MS) gave maximum release of 68.9±11, 64.8 ±20 and 54.6 ±20 % respectively. Similarly, in pH 1.2, batch L-3 of the formulation gave maximum released of 82.30 ±13 %, while that of L-1, L-2 and reference sample gave maximum released of 75.12 ±20, 78.40 ± 0.2 and 67.11 ± 20 %, respectively. The results of the *in vitro* release of LMFT from the SDs showed that the formulation exhibited higher sustained release properties significantly different from the reference LMFT tablets ($p < 0.05$). The formulations gave a gradual and more sustained release of LMFT over the study period. The results showed that LMFT-SDs could be used once daily for the treatment of malaria at the recommended dose. However, the formulations showed more than 60 % release in all over the period of the study. Research have shown that The amorphous or metastable form will dissolve at the fastest rate because of its higher internal energy and greater molecular motion, which enhance the thermodynamic properties compared to

crystalline materials. Additionally, poorly water soluble crystalline drugs, when in the amorphous state tend to have higher solubility. From a thermodynamic point of view, amorphous solids, compared with corresponding crystalline solids, demonstrate excess in properties meaning they have higher free energy, entropy and specific volume. Amorphous solids exhibit higher transient solubility, dissolution rate, vapor pressure and molecular mobility. Normally, in order to dissolve a crystalline drug, energy is required to break up the crystalline lattice. This required energy is often considered as a barrier for the drug dissolution. In solid molecular dispersions, long-range crystalline structure is absent and the drug is dissolved or molecularly dispersed in a polymeric carrier. Here, the drug exists in an amorphous state which exhibits a higher kinetic solubility (up to a few orders of magnitude) and dissolution rate than that of the crystalline drug.[19,22]

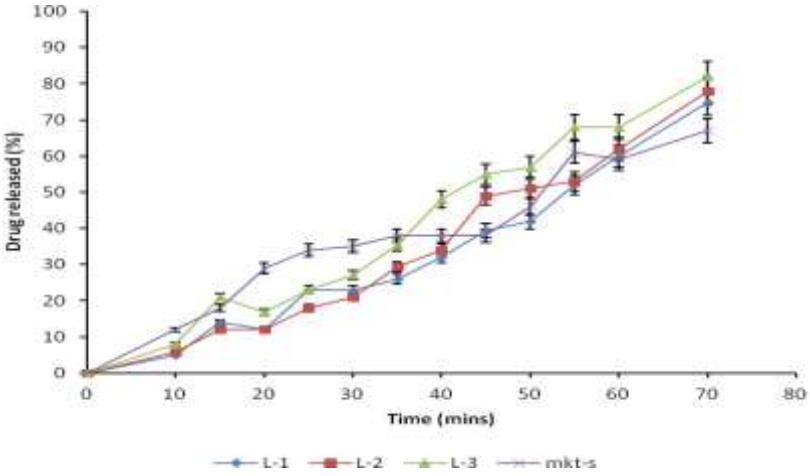


Fig. 3. Release profile of LMFT from solid dispersion in phosphate buffer pH 1.2 (n= 3). L-1–L-3 contain 1:1, 1:3 and 3:1 of LMFT: PEG respectively.

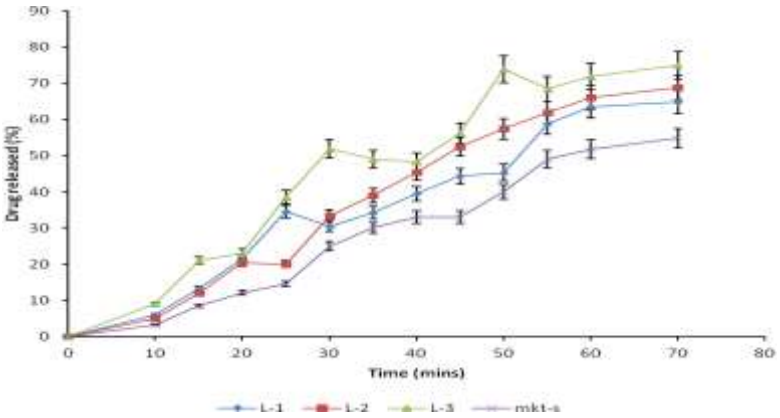


Fig. 4. Release profile of LMFT from solid dispersion in phopnate buffer at pH 7.2 (n= 3). L-1–L-3 contain 1:1, 1:3 and 3:1 of LMFT: PEG, respectively.

Drug release kinetics

The results of the *in vitro* release kinetics studied using four kinetic models showed that the zero-order plots were linear indicating that drug release followed zero-order kinetics model. The first-order plots of cumulative amount remaining versus time showed that drug release also followed first-order release kinetics. Therefore, the drug release followed mixed order of release including the zero and first-order release. Higuchi models gave high linearity, and n values that were above 0.5 for the formulated SDs, showing that drug release mechanism followed non-Fickian diffusion release[23] The release rate kinetics (k) for Higuchi model gave release rate constant significantly higher than that of the other models employed in the study confirming that the mechanism of drug release was predominantly by diffusion. The n values indicated that drug release followed non-Fickian or anomalous release. Therefore, drug release was by diffusion and erosion in both batches of SDs.[23]

Table 2: Kinetics of release of LMFT from the solid dispersions

Batches	Zero-order	First-order	Higuchi Square root	Hixson-Crowel
	(r^2)	(r^2)	(r^2)	(r^2)
L-1	0.845	0.952	0.978	0.982
L-2	0.917	0.779	0.987	0.974
L-3	0.888	0.899	0.972	0.984

***In vivo* anti-malarial actions of the LMFT-SDs formulations against P. berghei**

In vivo antimalarial efficacy of the various LMFT-SDs formulations were evaluated, though LUMFT is never use alone in the management, but it justification as combination therapy with other artemisinin derivatives is the motive behind this evaluation. The anti-malarial activity of the LUMFT-SDs formulation coded as L-1 to L-3 against P. berghei infected Swiss albino mice adapting the method of 4-day suppressive test as shown in Table 3. After four days of consecutive treatment with the various formulations in the P. berghei-infected mice, the changes in the parameters evaluated are shown (Table 2). Of the different batches of the formulation L-3 showed significant inhibition of parasitaemia compared higher to that of market sample that serve as positive. Mean parasitaemia (%) in the L-1, L-2 and L-3 are 4.10 ± 0.02, 4.99 ± 0.16 and 2.10 ± 0.10 respectively. In the control group the mean parasitaemia are 5.42 ± 0.12 and 7.20 ± 0.12 for positive and negative control respectively (Table 3). In all case, the batches of the LMFT-SDs (L-1 to L-3) prepared showed significant ($p < 0.05$) percent inhibitions as compared to control. In order words, the animals that received normal saline continued to have elevated parasitemia in their blood levels throughout the study period. This is because there was no LMFT in the NS. So the mice

remained infected all through the period and they all finally died. The LMFT-loaded SDs (L-1 to L-4) containing varying ratios of LMFT: PEG or dose-dependently lowered the blood parasitemia levels of the mice. Maximum blood parasitemia lowering level (75.01 ± 0.11 %) was encountered in the L-3 containing 3:1 of LMFT:PEG-4000 in the formulation, and this was quite comparable better than the value obtained (52.10 ± 0.91 %) encountered in the conventional or market tablet sample. Generally, the value obtained here is a function of the effective release of the LMFT from the SDs in the batches of the loaded formulation. Additionally, the solubilizing effect of the PEG-4000 was also proven here, because the SDs was able to solubilized the drug and effective release it out from it formulation. Interestingly, survivor rate of the animals after 14 days of the treatment was higher in the groups that were treated with LMFT-SDs formulations than the group treated with the market sample. Thus, LMFT-SD (L-3), showed higher survival rate as compared to that of L-1, L-2 and marketed formulation (Table 3), indicating that the high LMFT in L-3 may have resulted in higher clearance of the parasitemia thereby preventing the recurrent of the plasmodium with resultant increase in the survivor rate. Finally, the results of this Pharmacodynamic activity also suggest that with the help of solid dispersion approach, the therapeutic dose of LMFT can be significantly reduced which will be advantageous in reducing the dose related toxicity and dose related resistance issues associated with LMFT.

Table 3: *In vivo* anti-malarial activity of LMFT-SDs against P. berghei infected animal

Batch Code	Mean parasitemia (%)	Suppression of parasitemia (%)	Number of survivor after14 days (%)
A (L-1)	4.10 ± 0.02	61.11 ± 0.10	66.00
B (L-2)	4.99 ± 0.16	65.12 ± 0.23	87.00
C (L-3)	2.10 ± 0.10	75.01 ± 0.11	98.00
D (NS)	7.20 ± 0.12	00.00 ± 0.00	0.00
E (MS)	5.42 ± 0.12	52.10 ± 0.91	48.00

L-1 to L-3 are the various SD, NS= normal saline and MS= market sample

CONCLUSIONS

The solid dispersion (LMFT-SDs) significantly improved the solubility and therapeutic efficacy of LMFT as demonstrated in this novel drug delivery strategies against malaria parasite. *In vivo* studies clearly demonstrated that LMFT-SDs has significantly higher antimalarial activity as compared to the marketed formulation. Thus, the utility of SDs for enhancing the solubility and therapeutic efficacy of LMFT is established in this our laboratory animal based evaluation. LMFT-SDs can be a viable alternative to the existing

LMFT combination with other artemisinin derivatives for effective management of malarial parasites. Additionally, its ease of formulation and the cost effectiveness of the polymer suggest that LMFT-SDs could have a tremendous market potential. The acute and sub-acute toxicity studies and the general safety of the this novel formulation is ongoing in our research group.

Declaration of interest

The authors of this manuscript do not have a direct financial relation with the commercial identity mentioned in this manuscript that might lead to a conflict of interest. The authors do not have any conflict of interest in the preparation of this manuscript and they received no funding for this research work.

REFERENCES

1. Zurovac D, Ndhlovu M, Rowe AK, Hamer DH, Thea DM, Snow RW. Treatment of paediatric malaria during a period of drug transition to artemether-lumefantrine in Zambia: cross sectional study. BMJ, 2005; 331: 706-7.
2. Chadha R, Gupta S, Pathak N. Artesunate-loaded chitosan/lecithin nanoparticles: preparation, characterization, and *in vivo* studies. Drug Dev Ind Pharm, 2012; 38: 1538–46.
3. Dikasso D, Makonnen E, Debella A. *In vivo* anti-malaria activity of hydroalcoholic extract from Asparaginus africanus in mice infected with Plasmodium berghei. Ethiop J Health Dev, 2006; 20: 112–18.
4. Li Q, Weina P. Artesunate: The best drug in the treatment of severe and complicated malaria. Pharmaceuticals, 2010; 3: 2322–32.
5. Repka MA, Prodduturi S, Stodghill SP. Production and characterization of hot-melt extruded films containing clotrimazole. Drug Dev Ind Pharm, 2003; 29: 757–765.
6. Hancock BC, Zografi G Characteristics and significance of the amorphous state in pharmaceutical systems. J Pharm Sci, 1997; 86: 1–12.
7. McGinity JW, Zhang F, Repka MA, Koleng JJ. Hot-melt extrusion as a pharmaceutical process. Am Pharm Rev, 2001; 4(2): 25–36
8. WHO. (2009). World Malaria Report. World Health Organization: Geneva, Switzerland. 21 February 2010. Available online: http://www.who.int/malaria/publications/atoz/9789241563901/ en/index.html/ [last accessed 21 Jul 2010].

9. Osama AA, David SJ. Understanding the performance of melt extruded poly(ethylene oxide)-bicalutamide solid dispersions: characterisation of microstructural properties using thermal, spectroscopic and drug release methods. J Pharm Sci, 2012; 101: 200–213.
10. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Disc Today, 2007; 12: 1068–1075.
11. Rasenack N, Muller B. Development of novel ibuprofen loaded solid dispersion with improved bioavailability using aqueous solution. Arch Pharm Res, 2002; 19: 1894–1903.
12. Kim T, Shin J. Enhanced dissolution and bioavailability of poorly water soluble artemether using solid dispersion system. AAPS Annual Meeting, 2001; 21: □25.
13. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria. XXII. The value of drug-resistant strains of P. berghei in screening of blood schizonticidal activity. Ann. Trop. Med. Parasitol, 1975; 69: 155–171.
14. Ford JL, Rubinstein MH. Ageing of indomethacin-Polyethylene glycol 6000 solid dispersion, Pharm. Acta Helv, 1979; 54: 353-358.
15. Ofokansi KC, Adikwu MU, Okore V. Preparation and evaluation of mucin-gelatin mucoadhesive microspheres for rectal delivery of ceftriaxone sodium, Drug Dev Ind Pharm, 2007; 33: 691-700
16. Jain D, Panda AK, Majumdar DK. EudragitR S100 entrapped insulin microspheres for oral delivery. AAPS PharmSciTech, 2005; 6(1): 1-27.
17. Ichikawa H, Fukumori Y. Design of nanohydrogel-incorporated microcapsules for appropriate controlled-release of peptide drugs. Yakugaku Zasshi, 2007; 127(5): 813-823.
18. Yu L., Amorphous pharmaceutical solids:preparation, characterization and stabilization, Adv Drug Del Rev, 2001; 48: 27-42.
19. Zahedi P, Lee PI., Solid molecular dispersions of poorly water-soluble drugs in poly (2-hydroxyethyl methacrylate) hydrogels, Eur J Pharm Biopharm, 2007; 65: 320-328.
20. Vasconcelos T. and Costa P., Development of a rapid dissolving ibuprofen Solid dispersion, In PSWC-Pharmaceutical Sciences World Conference, 2007.
21. Serajuddin A.T.M., Solid dispersions of poorly water soluble drugs: early promises, subsequent problems and recent breakthroughs, J. Pharm. Sci, 1999; 88: 1058–1066.
22. Higuchi T. Mechanism of sustained action medication. Theoretical analysis of rate of solid drugs dispersed in solid matrices. J Pharm Sci, 1963; 52: 1145–9.
23. Ritger P.L., Peppas N.A.-A simple equation for description of solute release. I. Fickian and non-Fickian release from nonswellable devices in the form of slabs, spheres, cylinders or discs.-J. Cont. Rel., 1987; 5: 23-36.