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FORMULATION AND *IN VITRO* EVALUATION OF PHOSPHOLIPON® 90 H-BASED MICROPARTICLES FOR ORAL DELIVERY OF ARTEMETHER-LUMEFANTRINE

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ABSTRACT

Lipid-based formulations enhance the delivery of poorly water-soluble drugs. In this study, Phospholipon® 90 H (P90H)-based microparticles co-formulation of artemether-lumefantrine (ATM-LUM) were prepared by emulsion-solvent evaporation method and evaluated for particle morphology, pH, yield, drug content, encapsulation efficiency (EE) and *in vitro* drug release. The *in vitro* drug release was studied over an 8 h period using a commercial tablet sample as reference. Microscopic examination of the formulations showed that spherical microparticles were formulated. The drug content and EE values were generally low for both drugs. This was attributed to probable poor dissolution of the drugs in the media used. The optimized formulation ($5M^m$) had the best pH stability over a one-month period. The products showed low and slow release profiles. However, the P90H-based microparticles formulation had significantly ($p< 0.05$) higher amounts of the drugs released relative to the reference, despite the generally low levels. The study showed that P90H could be used as a lipid carrier for formulation of ATM-LUM microparticles by emulsion-solvent evaporation technique. Further studies are recommended for the product development.

KEYWORDS: Microparticles; Phospholipids; Artemisinin-based combination therapies (ACTs); Artemether; Lumefantrine; Malaria.

INTRODUCTION

Malaria is an infectious disease caused majorly by the species of female anopheles mosquito known as *Plasmodium falciparum* and is characterized by vomiting, diarrhea, coughing, jaundice of the skin and eyes, chills, fever, sweats, muscle aches and headache [1]. A complicated form of malaria is associated with shock, bleeding, kidney and liver failure, central nervous system problems, coma and death. Other common plasmodium species include: *Plasmodium vivax, P. falciparum, P. ovale, P. maleriae*, and*P. knowlesi* [2]. *P. falciparum* is responsible for roughly 50 % of all malaria cases and has three major stages namely, the liver/exo-erythrocytic, blood/erythrocytic and sporogenic cycles [3, 4]. These cycles are due to the fact that *P. falciparium* does not have a fixed structure but normally undergoes continuous changes during the course of its life style. The common anti-malarial drugs are known to target different sites within these stages. For instance, the artemisinins are blood schizonticides. Artemisinin combination therapy (ACT) has been recommended by the World health organization (WHO) for the treatment of *P. falciparum* malaria [1, 5]. *P. falciparum* is known to

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be responsible for all severe human illnesses and deaths that are associated with complicated and severe malaria. Women are known to be susceptible to severe malaria during their first pregnancy [6], though susceptibility to severe malaria is drastically reduced in subsequent pregnancies as a result of increased antibody levels against variant surface antigens that normally appear on infected red blood cells [7]. ACTs are effective due to the fact that the artemisinin component kills the majority of parasites at the commencement of the treatment, while the more slowly-acting partner drug clears the remaining ones [1]. Artemisinins have short half-lives; they are not used for malaria prophylaxis, and have to be administered multiple times each day. The oral route of drug administration is normally limited by problems related to physiochemical properties of the drug such as, poor solubility, low permeability, instability and rapid metabolism. These challenges lead to decreased oral bioavailability [8].

Over the years, efforts have been made to overcome the challenges to bioavailability through new drug formulation designs. When a poorly water-soluble, lipophilic drug for oral delivery is co-administered with a fatty meal the absorption increases as a result of increased solubilization by the fatty milieu [9]. Therefore, there tends to be increased interest in the formulation of lipophilic drugs with lipid base as a means of improving the drug solubilization in the GIT. Lipid-based drug delivery system (LBDDS) is one of the emerging technologies designed to address such challenges. LBDDSs are known to enhance the bioavailability of poorly water-soluble drugs and are also used for targeted and controlled release [10]. LBDDSs are known to exhibit a greater carrier capacity for drugs and also have high stability. With the solubilization and encapsulation of lipophilic drugs in lipid excipients, there would be an increase in absorption, resulting in enhanced bioavailability [11, 12]. Advanced drug delivery systems are being developed using lipid carriers such as phospholipids in order to overcome some of the limitations that are associated with the conventional drug forms.

Phospholipids are esters of fatty acids with glycerol containing an esterified phosphoric acid and a nitrogen base [13, 14]. They are lipid materials naturally present in large amounts in nerve tissue, brain, liver, kidney, pancreas, and heart cell membranes. They increase the rate of fatty acid oxidation, act as carriers of inorganic ions across membranes, help blood-clotting and act as prosthetic group to certain enzymes. Phospolipids have been applied as excipients for various functionalities in drug delivery.

Artemether (ATM) is a white crystalline powder derived from artemisinin; it is freely soluble in acetone, methanol and ethanol but insoluble (or poorly soluble) in water (log*P* = 3.53; *pKa* = 3.48) [15, 16]. Lumefantrine (LUM) is a yellow crystalline material with a bitter almond odour, almost insoluble in water and aqueous acids, freely soluble in N,N-dimethylformamide, chloroform and ethyl acetate; soluble in dichloromethane; and slightly soluble in ethanol and methanol (log*P* = 9.19; *pKa* = 8.73 and 13.49) [15, 16]. Artemisinin derivatives and quinolone-related compounds are among the major classes of drugs currently used to treat malaria. ATM-LUM combination is indicated for the treatment of uncomplicated *P. falciparum* malaria. Due to the challenges associated with the conventional delivery forms [15, 16], the combination of ATM and LUM in a new improved and probably well tolerated oral form with less adverse effects and comparable bioavailabilities would be useful against uncomplicated multi-drug resistant *P. falciparum* malaria [17]. The ATM is converted to dihydroartemisinin, which rapidly disappears from plasma with a half-life of about 45 minutes. The LUM component is eliminated more slowly, having half-life of 3-6 days, thereby clearing the remaining parasites. Some lipid-based formulations had been prepared for improved delivery of ATM and LUM. Nnamani *et al*. prepared liquid-solid nanostructured lipid carriers by hot homogenization method for co-delivery of ATM and LUM [9]. Sustained release of ATM from a solid lipd microparticle formulation based on solidified reverse micellar solution (SRMS) has been reported [18]. The formulation of ATM microspheres based on ethylcellulose and polyvinyl chloride, compressed into tablets with LUM has been demonstrated [19]. To the limit of our knowledge, co-formulation of ATM-LUM microparticles by emulsion-solvent evaporation technique using Phospholipon® 90 H (P90H) has not been reported. The aim of this study was to explore the use of P90H in the formulation of ATM-LUM microparticles by emulsion-solvent evaporation method for improved oral co-delivery.

MATERIALS AND METHODS

Materials

Pure samples of ATM and LUM were received as gift samples from May and Baker, Nigeria. P90H was received as a kind gift from Lipoid GmbH, Ludwigshafen, Germany. Polyethylene glycol 4000 (PEG) and commercial ATM-LUM tablets were purchased from AC drugs Ltd, Enugu, Nigeria. All

other reagents and solvents used were of analytical grade.

Preparation of microparticles

Microparticles containing ATM-LUM were formulated by emulsion-solvent evaporation method [18, 20] with modifications. Initially, preformulation studies were carried out to obtain the optimal formular for the formulation. Nine batches (1-9) were prepared (Table 1), varying the drug:P90H ratio and stirring rate. The emulsion consistency and observance of microparticles under optical microscope as well as pH stability were considered factors for choosing the optimized batch.

The aqueous phase of the emulsion was prepared by dispersing 0.3 mg of PEG in 200 ml of the solvent mixture (10% propylene glycol) and allowing it to dissolve. The organic phase of the emulsion was prepared by dispersing a mixture (ratio 1:6) of the drugs (300.0 mg of ATM and 1800.0 mg of LUM) and the P9OH (2100.0 mg) giving a drug-P9OH mixture ratio of 1:1 in 10 ml of chloroform. The aqueous phase was gradually incorporated (in fine sprays, using a syringe and needle) into the organic phase in a beaker, and homogenized (Silverson mixer) at low stirring rate (600 rpm) to form an emulsion. After addition of the last portion of the aqueous phase, the dispersion was further stirred (600 rpm) for 1 h and allowed to stand for 12 h for complete evaporation of the chloroform. The microparticles formed were filtered out from the dispersion, washed with distilled water three consecutive times then air-dried at room temperature (29 ^{o}C). The procedure was repeated twice but at varying stirring rates, medium (800 rpm) and high (1200 rpm), each for batches 2 and 3, respectively. The drugs, (ATM (300.0mg) and LUM (1, 800.0 mg)) and P90H (4, 200.0 mg) were weighed out thrice and mixed separately, giving drugs–P90H mixtures ratio of 1:2, each for batches 4, 5 and 6. Also, 300.0 mg ATM and 1800.0 mg LUM, and 8,400.0 mg P90H were weighed out thrice and mixed separately, giving drugs–P90H mixtures ratios of 1:4, each for batches 7, 8 and 9. Then the procedure for formulating the other batches was repeated.

Evaluation of the formulated microparticles

After initial assessments of the formulations, batch 5, (M^m) was selected as the optimal formular based on consistency of the emulsions, observance of clear spherical microparticles and pH stability. The microparticle formulation was evaluated for particle morphology, pH, percentage yield, drug content, EE and *in vitro* drug release.

Morphology

The morphology of the microparticles were analyzed using an optical microscope (Hund® Wettzlar, Germany) attached with motic camera (Moticam 2.0 MP CMOS, China) after drying of the formulation at room temperature. Approximately 5.0 mg of the microparticles from each batch was dispersed in distilled water and a drop of the dispersion smeared on a microscope slide using a glass rod. The smear was viewed on the microscope at x 40 magnification and the micrographs captured using themotic camera.

pH stability studies of the formulation

The formulation was subjected to pH analysis for one month to check the effect of storage on the parameters. The pH of dispersions of the microparticles from each batch was determined using a pH meter (6305, Jenway, USA) after one week and a month of storage, respectively.

Yield determination

The dried microparticles from each batch were weighed to get the yield of the formulation. The percentage yield was calculated using equation 1:

Percentage (%) recovery = $\frac{W1}{W2+W3.}$ x 100 ….. 1

where: W_1 is the weight of the microparticles formulated (g), W_2 is the weight of the drugs added in the formulation (g), and W_3 is the weight of the P90H and PEG 4000 (g).

Determination of drug content of the microparticles

Ultraviolet-visual (UV-Vis) spectrophotometric simultaneous multicomponent analysis technique was adopted [21]. A 100.0 mg quantity of each formulation was weighed out, dissolved in 10 ml of the solvent (ethanol – SGF mixture ratio of 1:3, respectively) and diluted to 100 ml. The solutions were filtered (Whatmann No. 1) and their absorbances taken spectrophotometrically (1800, Shimadzu, Germany) at earlier established wavelengths of maximum absorption (λ_{max}), 236.5 and 240.0 nm for LUM and ATM, respectively. The drug contents were determined with references to the standard Beer's plot using the simultaneous equation method for multicomponent analysis [21].

At a particular λ_{max} (λ_1), absorbance of the mixture (the formulation solution) is the sum of the individual absorbances of the 2 drugs, *x* and *y* (being ATM and LUM), respectively. Two equations are simultaneously constructed thus [21]:

$A_1 = a_{x1}bC_x + a_{y1}bC_y$…….. 2

$A_2 = a_{x2}bC_x + a_{y2}bC_y$ 3

where b = part length of the curvet used in the measurement = 1 cm.

Rearranging equation (3) gives,

$$C_y = \frac{(A_2 - a_{x2}\,Cx)}{a_{y2}} \quad \text{..... 4}$$

Substituting for C_y in equation (2) and rearranging it gives,

$$C_x = \frac{(A_2 a_{y2} - A_1 a_{y1})}{a_{x2}a_{y1} - a_{x1}a_{y2}} \quad \text{..... 5}$$

Also

$$C_y = \frac{(A_1 a_{x2} - A_1 a_{x1})}{a_{x2}a_{y1} - a_{x1}a_{y2}} \quad \text{..... 6}$$

where a_{x1} and a_{x2} = absorptivities (i.e., *K*) of *x* (ATM) at λ_1 (= 240 nm) and λ_2 (= 236.5 nm)

a_{y1} and a_{y2} = absorptivities (i.e., *K*) of *y* (LUM) at λ_1 and λ_2, respectively,

A_1 and A_2 are the absorbances of the diluted samples (of the formulation solution) at λ_1 and λ_2, respectively.

C_x = concentration of *x* (ATM) in the diluted samples,

C_y = concentration of y (LUM) in the diluted samples.

Encapsulation efficiency

The encapsulation efficiency (EE) of the formulation was determined after preparation. The amount of drug encapsulated or entrapped in the microparticles was calculated from results obtained from the drug content with reference to the standard Beer's plot for ATM and LUM to obtain the percentage EE. The EE was calculated using the equation:

$$\text{EE (\%)} = \frac{\text{actual drug content}}{\text{theoretical drug content}} \text{ X 100 ..... 7.}$$

In vitro drug release

The drug release study was done by the dialysis method and the UV-Vis spectrophotometric simultaneous multicomponent analysis technique used to determine the amounts [21]. The *in vitro* drug release of ATM-LUM was studied for time period of 8 h. This study was performed on the optimized batch of microparticles (5, M^m) and the commercial sample (AC Drugs Ltd., Enugu, Nigeria), using two dissolution media, namely simulated gastric fluid (SGF), and simulated intestinal fluid (SIF). The dissolution times for the samples in both media were 3 h and then 5 h, respectively. A 100.0 mg sample of the formulation was weighed out, placed in a pocket (basket) made of muslin cloth (which served as dialysis membrane).Using thread and needle, the basket with sample was placed in 200 ml of the medium (SGF), which was maintained at 37 ± 0.5 °C on a magnetic stirrer rotating at speed of 75 rpm [18]. Samples (5 ml) were withdrawn at different time intervals of 0.17, 0.33, 0.5, 1.0, 1.5, 2.0 and 3 h. Equal volume (5 ml) of fresh dissolution medium (SGF) was introduced into the beaker to replace withdrawn sample each time. The microparticles-containing basket was removed from the SGF and transferred into 200 ml of another medium, SIF (PBS, pH 6.8) which was maintained under the same condition with the SGF. Samples (5 ml) were withdrawn at different time intervals of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 h, which when added cumulatively with the time intervals of drug release in SGF became 3.5, 4.0, 5.0, 6.0, 7.0 and 8 h, respectively. Equal volume (5 ml) of fresh dissolution medium (PBS) was introduced into the beaker to replace withdrawn sample each time. The above procedure was repeated for the commercial sample (AC Drug Ltd. Enugu Nigeria). Each sample was filtered using filter paper (Whatmann No. 1). Then 1.0 ml of the filtrate was diluted suitably with fresh dissolution medium and analyzed spectrophotometrically (1800, Shimadzu, Germany) at the respective λ_{max} of the drugs (240 and 236.5 nm, for ATM and LUM, respectively).

Data analysis

The measurements were done in replicates and data analyzed by descriptive statistics and Student *t*-test using Excel Microsoft Office, version 2007 and Graph Pad Prism, version 6.

RESULTS

ATM-LUM microparticles were produced using P90H by the emulsion solvent evaporation method.

Figure. 2 shows the photomicrographs of the formulations as observed under the microscope. The results showed that the microparticles produced at low stirring speed (low rotation rate) were irregular in shape. However, as the rotation speed increased, more regular, spherical and smooth particles were produced.

The results of the preliminary pH stability assessments of the different batches of the microparticle formulations at room temperature and at different time intervals are shown in Table 2. The results showed that there was a little change in the pH over the one-month period. Batch 5, $(M)^m$ was considered the optimized formulation batch because it had the most pH stability.

The results obtained for the yield, drug content and EE of the optimized microparticle formulation are shown in Table 3. The yield was 85.71 ± 1.2 %.

The results showed that the drug content of the microparticles formulated were generally low for both ATM and LUM. The microparticles had higher EEs for ATM than for LUM. However, the values were generally low for both drugs.

Fig. 3 shows the release profiles of the drugs from the test formulation (ATMt and LUMt), and the reference commercial tablet (ATMr and LUMr). The microparticle formulation gave maximum release of 1.097 and 1.116 % in SGF at 3 h for ATM and LUM, respectively, and 1.155 and 1.102 % in SIF at 5 h for ATM and LUM, respectively. The commercial tablet sample gave maximum release of 0.230 and 0.310 % for ATM and LUM in SGF at 3 h, respectively, and 0.240 and 0.290 % in SIF at 5 h for ATM and LUM, respectively. Thus, the ATM-LUM-loaded P90H-based microparticles released the drugs slowly over several hours but comparatively at higher rates than the reference commercial sample. However, nonparametric two-tailed *t*-test and Mann-Whitney post-test statistics showed that the test formulation had significantly ($p< 0.05$) ($P = 0.0002$ and 0.0009 for ATM and LUM, respectively) higher amounts of the drugs released compared to the reference, despite the generally low levels.

DISCUSSION

The observation of the microparticles shapes being stirring speed-dependent was in agreement with earlier reports from work done by some researchers [19, 20], where the solid lipid microparticles were found to be spherical in shape. The P90H-based ATM-LUM microspheres had spherical and smooth surfaces. Spherical shape encourages easier permeation of the particles through biological membranes in the course of delivering the encapsulated drug particles. The pH stability of the optimized microparticles implied that there was probably little or no degradation of the drugs and excipients in the formulation within the period of test. According to a previous report, when the ATM was loaded into the formulations, the pH increased to neutrality but there were swings within the neutral range at day 7 to 30 days which indicated that the SLMs would require a buffer to keep it pH-stable [18]. The low drug content and EE obtained could be due to the inability of the drugs to dissolve completely in the dissolution media used. Baring human experimental errors, the findings were, however, contrary to high EE earlier reported for ATM-containing solid-lipid microparticles (SLM) in similar media [18]. The drug-lipid ratio used in the formulation of the microparticles could also have influenced results of the EE and drug content.

As observed under the microscope, the P90H formed spherical microparticles encapsulating the drug particles. The drug particles were then released gradually from the lipid (P90H) microcapsule carriers. Good sustained release properties were reported from an *in vitro* evaluation of ATM-containing solid-lipid microparticles (SLMs) [18]. The results obtained in this study also showed that the formulated microparticles had pH-dependent release due probably to possible variations in the profiles in the acidic (SGF) and alkaline (SIF) media.

As earlier postulated under the EE subsection, the very low EE, drug content and amounts of drug released obtained in this study might have been influenced by the type and pH of the media used. This calls for some detailed investigation on the most suitable solvent(s) and other conditions to be employed in the analysis of ATM and LUM formulations.

Having established the feasibility of producing P90H-based microparticles co-formulation for oral delivery of ATM-LUM using the emulsion-solvent evaporation technique, the plan for the next stage of this research is to carry out preclinical kinetics, pharmacokinetics and pharmacodynamics studies on the product.

CONCLUSION

This study has demonstrated the feasibility of formulating P90H-based microparticles by the emulsification-solvent evaporation technique for oral co-delivery of ATM-LUM. The microparticles exhibited relatively better *in vitro* release profiles than a reference conventional tablet formulation with significant differences ($p< 0.05$). The plan is to deepen the research on the product, including carrying out kinetics, pharmacokinetics and pharmacodynamics studies.

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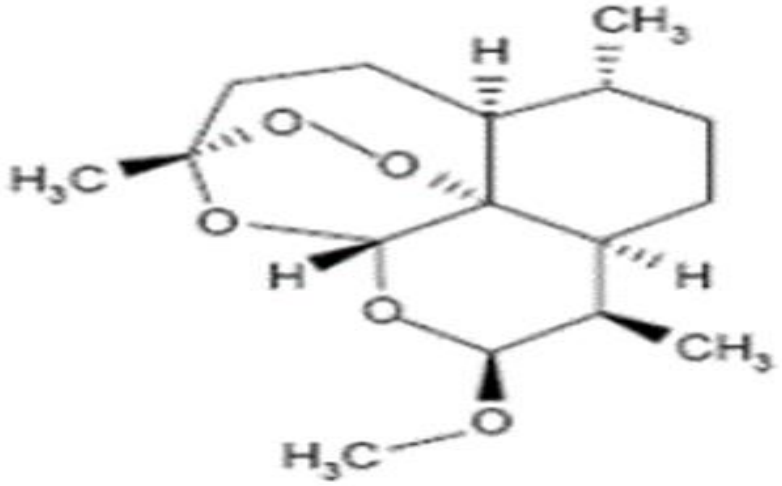
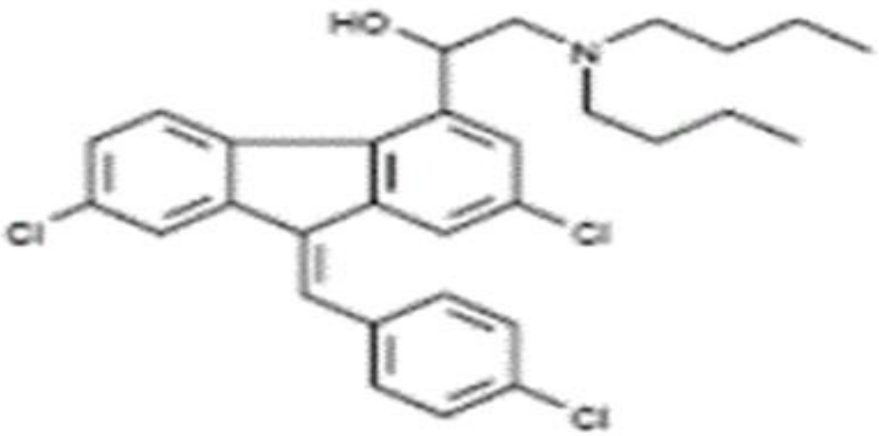


Artemether (ATM) ($C_{16}H_{26}O_5$; molecular weight: 298.4).



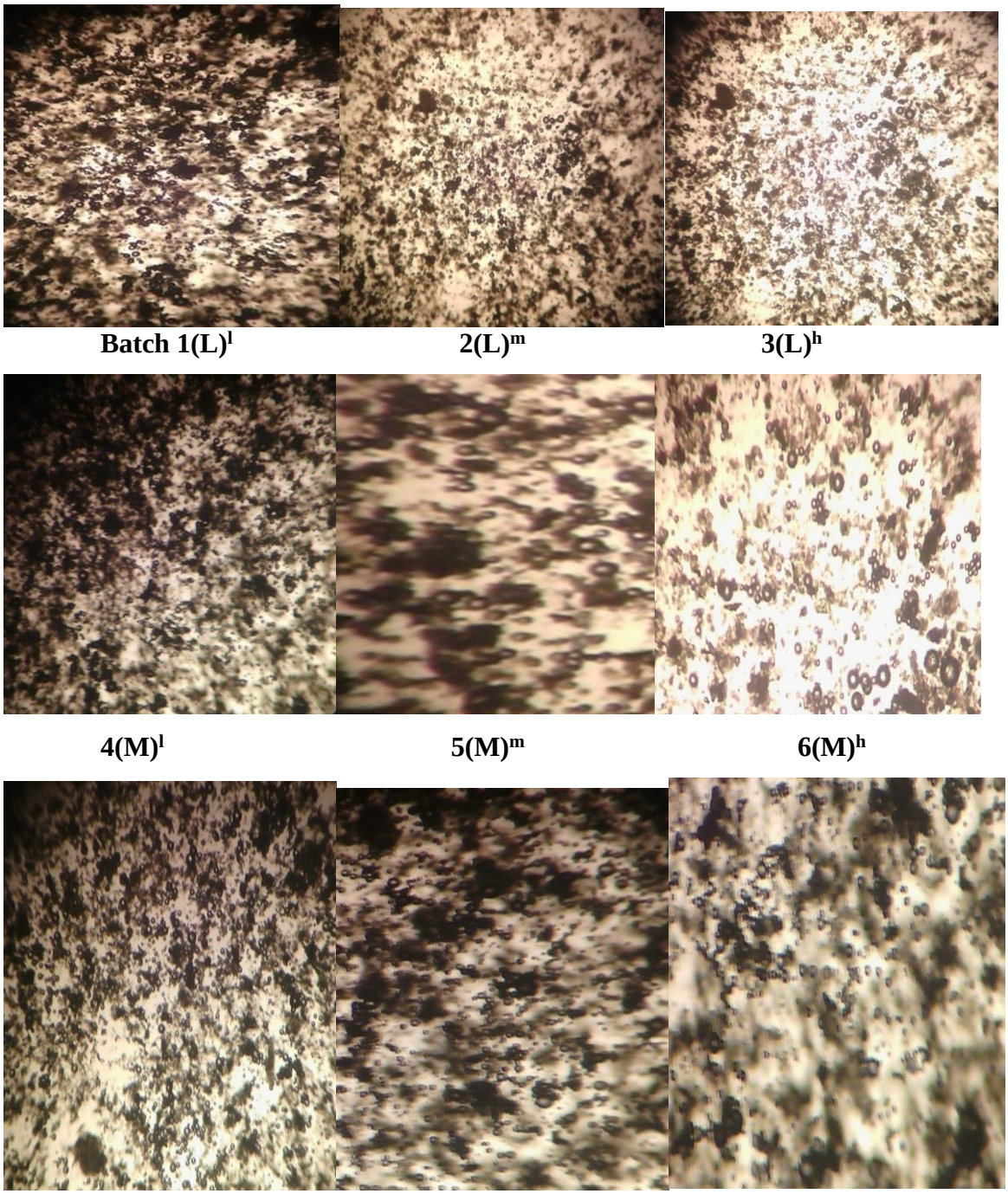
Lumefantrine (LUM) ($C_{30}H_{32}C_{l3}NO$; molecular weight: 528.9).

Figure 1: Chemical structures of artemether and lumefantrine [15,16].

Table 1: Formulae for preparation of the microparticles.

Ingredient	Quantities per unit dose for the batches								
	1(L)^{l}	2(L)^{m}	3(L)^{h}	4(M)^{l}	5(M)^{m}	6(M)^{h}	7(H)^{l}	8(H)^{m}	9(H)^{h}
ATM (mg)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
LUM (mg)	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
P90H (mg)	70.0	70.0	70.0	140.0	140.0	140.0	280.0	280.0	280.0
Chloroform (ml)	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
PEG 4000 (mg)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Propylene glycol (ml)	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33
Distilled water (ml)	*q.s* 6.67	*q.s* 6.67	*q.s* 6.67	*q.s* 6.67	*q.s* 6.67	*q.s* 6.67	*q.s* 6.67	*q.s* 6.67	*q.s* 6.67

Key: *L = low (1:1), M = medium, (1:2), and H = high drug-P90H ratio; l = low stirring rate (600 rpm), m = medium stirring rate (800 rpm), and h = high stirring rate (1, 200 rpm).*



Batch 1(L)^{l} **2(L)^{m}** **3(L)^{h}**

4(M)^{l} **5(M)^{m}** **6(M)^{h}**

7(H)^{l} **8(H)^{m}** **9(H)^{h}**

Figure 2: Photomicrographs of the microparticle formulations x 40 magnification.

Table 2: pH of the formulations

S/N	Formulation	pH	
		After 1 week	After 1 month
1	L^1	5.36 ± 0.00	3.37 ± 0.01
2	L^m	5.12 ± 0.01	5.61 ± 0.12
3	L^h	5.06 ± 0.02	4.98 ± 0.10
4	M^l	5.42± 0.13	5.60 ± 0.00
5	M^m	5.34 ±0.01	5.34 ± 0.11
6	M^h	5.40 ± 0.15	4.70 ± 0.15
7	H^I	4.96 ± 0.01	5.01 ± 0.01
8	H^m	5.10 ± 0.12	5.83 ± 0.10
9	H^h	5.21 ± 0.00	5.15 ± 0.07

Table 3: Results of the Yield, drug content and EE of the optimized formulation

Formulation	Yield (%)	Drug content (mg/unit dose)		EE (%)	
		ATM	LUM	ATM	LUM
$5M^m$	85.71 ± 1.2	0.342 ± 0.05	0.576 ± 2.0	3.99 ± 0.5	1.12 ± 0.8

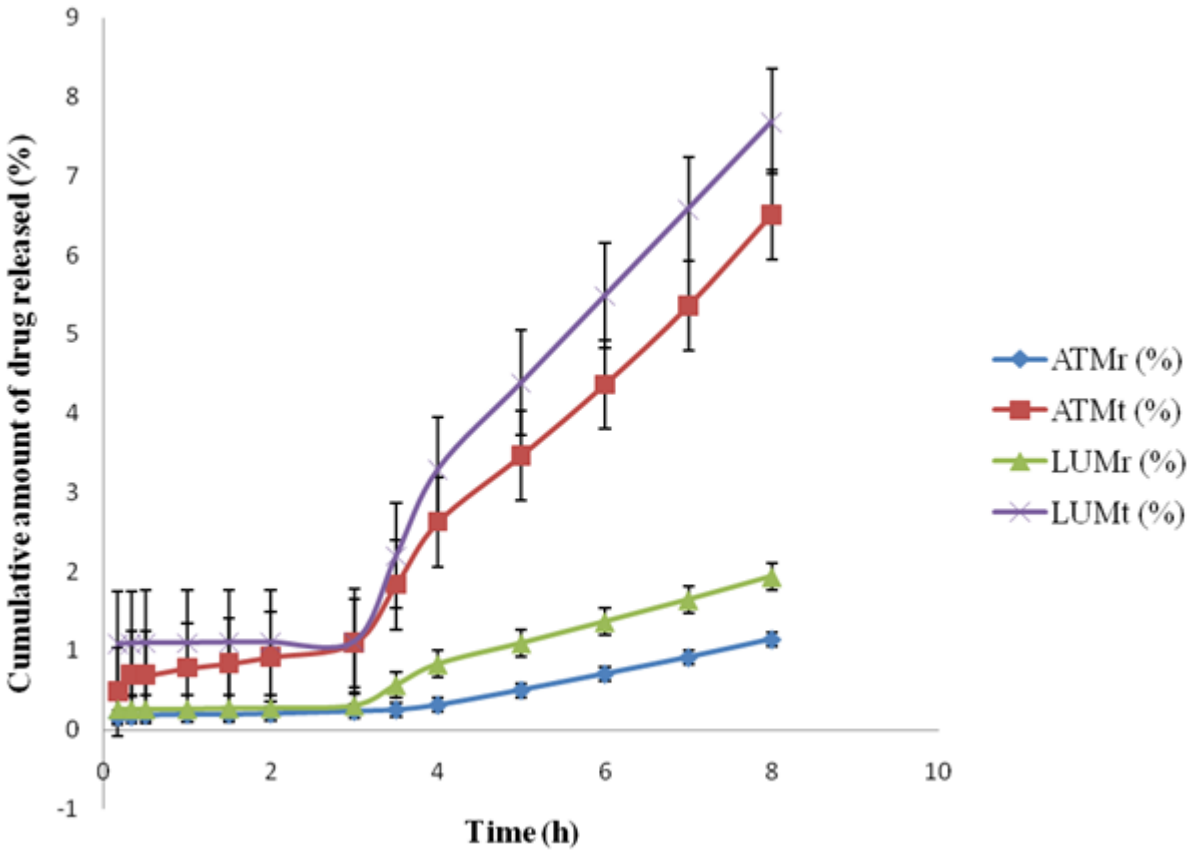


Figure 3: Cumulative drug released (%) in both SGF and SIF for the optimized batch (5, $(M)^m$) and the commercial sample. There were significant differences ($p < 0.05$) between the release profiles from the P90H-based microparticles and the reference commercial tablet formulation for both drugs.

REFERENCES

1. WHO, 2006. World Health Organization Guidelines for the treatment of malaria. Geneva: ISBN 92-4-154694-8, (2006). Accessed on May 19, 2018.
2. White NJ. *Plasmodium knowlesi*: the fifth human malaria parasite. *Clinical Infectious Diseases*.46, 2008: 172–3.
3. Perkins DJ, Were T, Davenport GC, Kempaiah P, Hittner JB, Ongecha JM. Severe malarial anaemia: innate immunity and pathogenesis. *International Journal of Biological Sciences*. 7(9), 2011: 1427-1442.
4. Perlmann P, Troye-Blomberg M. Malaria blood-stage infection and its control by the immune system. *Folia Biologica*. 46(6), 2000: 210-8.
5. Karbwang J, Na-Bangchang K, Congpuong K, Molunto P, Thanavibul A. Pharmacokinetics and bioavailability of intramuscular artemether. *European Journal of Clinical Pharmacology*., 52, 1997:307-10.
6. Voileta M-A, Rosa A, Michel C. Pregnancy-associated malaria and malaria in infants: an old problem with present consequences. *Malaria Journal*, 13(271), 2014:1-10.
7. Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. *New England Journal ofMedicine.* 370(23), 2014: 2211-2218.
8. Sunil P, Maru O, Chan M. Novel lipid-based formulations enhancing the in vitro dissolution and permeability characteristics of a poorly water-soluble model drug, piroxicam. *International Journal of Pharmaceutics*. 301, 2005: 209–16.
9. Nnamani PO, Ugwu AA, Ibezim EC, Kenechukwu FC, Akpa PA, Ogbonna JDN, Obitte NC, Odo AN, Windbergs M, Lehr C-M, Attama AA, Sustained-release liquisolid compact tablets containing artemether–lumefantrine as alternate-day regimen for malaria treatment to improve patient compliance. *International Journal of Nanomedicine*.11, 2016: 6365–6378.
10. Cannon JB, Long MA. "Emulsions, microemulsions, and lipid-based drug delivery systems for drug solubilization and delivery, part II, Oral Applications, In: Water-insoluble drug formulation, 2nd ed., vol. 16, Taylor & Francis LLC, 2008, pp. 227–254. DOI: 10.1201/9781420009552.ch11.
11. Humberstone AJ, Charman WN. Lipd-based vehicles for the oral delivery of poorly water-soluble drugs. *Advanced Drug Delivery Reviews*.25, 1997:103-128.
12. Schneider M. *Investigation of the transport of lipophilic drugs in structurally diverse lipid formulations through caco-2 cell monolayer using mathematical modeling.* Doctoral Thesis, No. 8561, University of Basel, Faculty of Science, 2008, pp172. DOI: 10.5451/unibas-004761454. Available at https://edoc.unibas.ch/903/. Accessed on May 20, 2018.
13. Phosphorex. Microsphere nanoparticles and drug delivery. Available at https://phosphorex.com/nanoparticles-vs-microsphere-forr-drug-delivery. Accessed on November 30, 2018.
14. Taneja A. Lipids Types: Simple, compound and derived lipids. Available at www.biologydiscussion.com/lipids-types-simple-compound-and-derived lipids. Accessed on November 19, 2018.
15. Amin CN, Fabre H, Blanchin M. Determination of artemether and lumefantrine in anti-malarial fixed–dose combination tablets by microemulsion electrokinetic chromatography with short-end injection procedure. *Malaria Journal*.12(1), 202, 2013.
16. Novatis Pharma Ag. Coartem (artemether/lumefantrine) tablet description. Available at https://www.rxlist.com/caortem-drug.htm. Accessed on September 29, 2018.
17. Abhay ML, Verma SS, Rekha F. Formulation and characterization of microspheres of artemether. *Literati Journal of Pharmaceutical Drug Delivery Technologies*.01(02),2015: 65-69.
18. Salome A, Onyishi I, Obitte N, Onunkwo G, Odo G. Sustained release artemether-loaded solid lipdmicroparticles based on solidified reverse micellar solution (SRMS). *Innovare Journal of Sciences*.1(2), 2011: 1-7.
19. Vinchurkar K, Mane S, Balekar N, Jain DK, Formulation and evaluation of tablets containing artemether microspheres and lumefantrine, *Journal of Drug Delivery and Therapeutics*. 7(7), 2017:4-7.
20. Attama AA, Okafor CE, Builders PF, Okorie O. Formulation and *in vitro* evaluation of a PEGylated microscopic lipospheres delivery system for ceftriaxone sodium. *Drug Delivery*.16, 2009: 448–616.
21. Kamal AH, EL-Malla SF, Hammad SF. A review on UV spectrophotometric methods for simultaneous multicomponent analysis.

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