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Research Article

ARTEMETHER-LUMEFANTRINE-LOADED LIPOSPHERES: EVALUATION OF PROPERTIES OF SOLUTOL® HS 15 AND SOLUPLUS® ON THE *IN VITRO* PROPERTIES

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ABSTRACT

Objectives: To formulate artemether-lumefantrine-loaded lipospheres and to evaluate the effect of excipients on the in vitro properties.

Materials and methods: Lipospheres were formulated using goat fat (70 %) and Phospholipon® 90H (70 %) as the lipid matrix, Solutol® HS 15 and Soluplus® were used respectively as surfactants. The lipospheres were formulated by melt homogenization and analysed for drug content, encapsulation efficiency (EE%), particle size and pH stability. In vitro release was studied in simulated gastric fluid (SGF, 1.2) and simulated intestinal fluid (SIF, 7.2).

Results: Lipospheres formulated with Solutol® had particle size range of 24.16 to 30.89 µm, while those formulated with Soluplus® had particle size range of 24.72 to 74.16 µm. The formulations showed a decline in pH at 30 days. The EE of artemether range from 71.80 to 75.30 % for lipospheres formulated with Soluplus®, while those formulated with Solutol® had EE% of 65.30 to 75.02 %. Also, the EE% of lumefantrine ranged from 76.36 to 88.99 % for lipospheres formulated with Soluplus®, while those containing Solutol® had EE range of 73.22 to 85.06 %. Formulations exhibited sustained release properties with maximum release of at 6 h, however, lumefantrine exhibited higher release than artemether in SIF ($p < 0.05$) and significantly lower release in SGF ($p < 0.05$).

Conclusion: Lipospheres exhibited good properties as a delivery system for artemether-lumefantrine.

Keywords: Goat fat, phospholipid, antimalaria, melt homogenization, loading capacity

INTRODUCTION

Artemether is an effective anti-malarial drug that possesses a remarkably wide therapeutic index and has the ability to rapidly kill a broad range of asexual parasite stages at safe concentrations that are consistently achievable through standard dosing regimens [1]. However, there have been reported cases of resistance to artemisinins[2-3]. Also, some physicochemical and biopharmaceutical problems such as short half-life, poor oral bioavailability and low solubility have been reported [4]. To minimize resistance, the World Health Organization (WHO) has recommended that artemisinins should be used as combination therapy with other anti-malarial drugs in the so-called artemisinin-based combination therapy (ACT), especially in treatment of uncomplicated malaria[5-7]. With their deployment in 2005 and 2006 as first-line treatments in several endemic countries of the world, morbidity and mortality associated with malaria decreased [7-9]. However, major limitations of ACTs have been ascribed to the imbalance between demand and supply, comparatively high cost, dosing complexity and the lack of clinical experience. Also, advances recorded by the ACTs are now being threatened by low sensitivity of the parasites in South-East Asia [8,10-11]. Therefore, there is an urgent need to develop lipid based formulations of ACTs in order to enhance their oral solubility and pharmacokinetic profiles [7].

Lipid-based drug delivery is an accepted, proven, commercially viable strategy to formulate pharmaceuticals. Lipid formulations can be tailored to meet a wide range of product requirements[12]. Lipid-based formulations can be used to influence the absorption of active ingredients through different mechanisms to modify the release of active ingredients thus, improving bioavailability. They can affect the intestinal environment, stimulate the lymphatic transport of active ingredients, and interact with enterocyte based transport [13]. Lipid formulations in general, provide increased drug solubilization for water – insoluble drugs[14-16]. This could be due to the ease of wetting of the hydrophobic drug particles in the presence of lipid matrix. The presence of surfactant in the formulation may ease the wetting further. Also entrapment of drug in the micelles may be enhanced due to the presence of lipidic matrix[17]. The primary mechanism of action which leads to improved bioavailability is usually avoidance or partial avoidance of slow dissolution process which limits the bioavailability of hydrophobic drugs from conventional solid dosage form [18].

Lipospheres are restricted to the stabilizing material of a phospholipid layer[19]. These have been utilized in the delivery of anti-inflammatory compounds, local anesthetics, antibiotics, anticancer agents, insect repellents, vaccines, proteins and peptides [20-24]. Agents for agricultural application such as herbicides, fungicides and fertilizers can also be incorporated into lipospheres [24-25]. The lipospheres are distinct from micro droplets, vesicles or liposomes since the lipospheres have solid inner core at room temperature. The lipospheres are distinct from microspheres of uniformly dispersed material in homogenous polymer since they consist of at least two layers of phospholipid [25].

In view of the problems recently encountered with most anti-malarial drugs such as poor oral bioavailability and the emergence of drug resistant parasite strains, there is need to improve the delivery and biopharmaceutical profile of these drugs in order to reduce the high mortality rate associated with malaria [7]. Hence, the aim of this work is to formulate artemether-lumefantrine loaded lipospheres and to evaluate the properties of the formulations.

MATERIALS AND METHODS

Materials

Artemether, lumefantrine (Hangzhou Dayang Chem., China), artemether-lumefantrine tablets (Coartem, Novartis), Phospholipon ® 90H (Phospholipid Köln, Germany), Soluplus ®, Solutol ® (BASF, Germany), sorbitol, sorbic acid (Fischer scientific company, New Jersey), sodium chloride, hydrochloric acid, sodium hydroxide pellets (BDH Chemicals Ltd, Pooles, England) and distilled water (STC, UNN).

Extraction and purification of goat fat

The adipose tissues of Capra hircus was obtained from an abattoir in Nsukka market, Enugu, Nigeria. It was grated and subjected to moist heat by boiling with about half its weight of water in a water bath for 45 min. Water was separated from the molten fat by means of a muslin cloth. Further purification was carried out by heating 2 % suspension of activated charcoal and bentonite (1:19) in the lipid at 80 °C for 1 h. The suspension was finally vacuum filtered using a Buchner funnel[26].

Preparation of lipid matrix carrier

The lipid matrix carrier was prepared by fusion method using goat fat (70 %) and Phospholipon 90H, a purified and completely hydrogenated soy phosphatidylcholine (30 %). The lipids were melted together in a beaker using a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India) and stirred until they melted completely. They were finally allowed to cool and solidified.

Preparation of lipospheres

The lipospheres were prepared by melt homogenization using an Ultra-Turrax homogenizer (T25 Basic, Digital, Ika Staufen, Germany). Soluplus® and Solutol® HS 15, were used respectively as the surfactants and the effect of the individual surfactants was studied. Sorbitol was used as a lyoprotectant, while sorbic acid was used as a preservative. The lipid matrix was melted using the magnetic stirrer hot plate and artemether-lumefantrine was dispersed in the molten lipid (1, 3 and 5 %. A solution of sorbitol, sorbic acid and the surfactant at the same temperature with the lipid was transferred into the lipid dispersion of the drug and homogenized using an Ultra-Turrax homogenizer (T25 Basic, Digital, Ika Staufen, Germany) at 5000 rpm for 5 min. The lipospheres (o/w) was formed by phase inversion and was stored in an air tight bottle for further studies [27].

Table 1: Composition of artemether-lumefantrine-loaded lipospheres

Batch	LM (%)	Solutol® *(%)*	Soluplus® (%)	Sorbic acid (%)	Sorbitol (%)	Artemether (%)	Lumefantrine (%)	Distilled water qs (%)
A	7.0	-	0.75	0.05	4	1	1	100
B	7.0	-	0.75	0.05	4	3	3	100
C	7.0	-	0.75	0.05	4	5	5	100
D	7.0	0.75	-	0.05	4	1	1	100
E	7.0	0.75	-	0.05	4	3	3	100
F	7.0	0.75	-	0.05	4	5	5	100
G	7.0	-	0.75	0.05	4	-	-	100
H	7.0	0.75	-	0.05	4	-	-	100

Batches A, B, C, and G contains Soluplus® and 1, 3, 5 and 0 % API, while batches D, E, F and H contains Solutol® HS 15

CHARACTERISATION OF LIPOSPHERES

Analysis of particle size and morphology

The particle size of the lipospheres was determined by computerized image analysis using a microscope (Weltzlar, Germany) attached with a digital image analyzer (Moticam, China). The projected particle diameters as well as the morphology were determined [28]. The mean particle size for each batch was calculated (n = 100).

Analysis of encapsulation efficiency and loading capacity

Quantitative determination of the amount of artemether and lumefantrine respectively that were encapsulated in each formulation was determined using UV-spectrophotometer (Jenway 6305 Spectrophotometer, UK) at a predetermined wavelength of 296 nm for lumefantrine and 293 nm for artemether in simulated gastric fluid (SGF, pH 1.2) containing Tween 80 (98:2). A 10 ml quantity of the lipospheres was centrifuged at 1,252 × g for 30 min (Chem. Lab. Instrument, UK). The supernatant was diluted and the absorbance readings were determined. The actual amount of drug encapsulated was determined by subtracting the actual mass of drug in the supernatant (Wf) from the total amount incorporated into the formulation (Wi).

Encapsulation efficiency (EE %) was calculated from the equation below:

$$EE\ (\%) = \frac{Wi - Wf}{Wi} x\ 100 \qquad (1)$$

LC was determined using the formula:

$$LC = \frac{Wa - Ws}{Wa - Ws + Wl} \times 100 \qquad (2)$$

Where Wl is the weight of lipid in the formulation, Wa is the weight of active pharmaceutical ingredient (API) added to the formulation and Ws is the actual amount of API encapsulated (Ws = Wi - Wf) in the lipospheres.

Analysis of pH of lipospheres over time

The pH was determined using a pH meter (HANNA, Padova, Italy) at 1, 7 and 30 days.

In vitro release studies

In vitro release was studied in 900 ml of freshly prepared simulated gastric fluid (SGF, pH 1.2) without pepsin and simulated intestinal fluid without pancreatin (SIF, pH 7.2) containing Tween 80 (98:2) and maintained at 37 ± 1 °C using the USP apparatus type II (Veego, India). The polycarbonate dialysis membrane (MWCO 6000 - 8000, Spectrum Labs, Breda, The Netherlands) used was soaked in the dissolution medium for 24 h before use. A 10 ml quantity of the artemether-lumefantrine-loaded lipospheres was used and the paddle was rotated at 100 rpm. About 5 ml portion of dissolution medium was withdrawn at 0.3, 0.45, 1, 2, 3, 4, 5, 6, 7, 8 h and filtered using a filter paper (Whatman no 1). The medium withdrawn was replaced with a fresh one. The absorbance readings were obtained using UV spectrophotometer (Jenway 6305 spectrophotometer, UK) at wavelength of 296 nm for lumefantrine and 293 nm for artemether.

In vitro release kinetics

Various kinetic models were used to describe the release kinetics and mechanisms. The first order model (Eq. 3) describes the release from systems where release rate is concentration dependent. Higuchi [29] described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion (Eq. 4). To find out the mechanism of drug release, the first 60 % drug release data were fitted in Ritger–Peppas model [30].

$$\log Q_0 - \log Q_t = k_1 t/2.303 \quad (3)$$

$$Q = K_2 t^{1/2} \quad (4)$$

$$M_t/M_\infty = K_3 t^n \quad (5)$$

where Q is the amount of drug released or dissolved at time t, Q_0 is the initial concentration of drug, k_1, k_2 and k_3 are first order, Higuchi and Ritger-Peppas kinetic constant. M_t/M_∞ is fraction of drug released at time *t*, *n* is diffusion exponent and is indicator of the mechanism of transport of drug through the polymer [23]. The following plots were made: log cumulative of % drug remaining versus time (first order kinetic model), cumulative % drug release vs. square root of time (Higuchi model) and log fraction of drug release versus log time (Ritger-Peppas model) [29-31].

Statistical analysis

Data were analyzed by one-way ANOVA. Differences between means were assessed using student's t-test, $p < 0.05$ was considered significant.

RESULTS

Particle size and morphology

The results of the particle size and morphology of artemether-lumefantrine-loaded lipospheres are shown in Fig. 1 and show that lipospheres formulated with Solutol® HS 15 had particle size range of 24.16 to 30.89 µm, while those formulated with Soluplus® had particle size range of 24.72 to 74.16 µm. The results of particle morphology show that the particles were smooth and spherical.

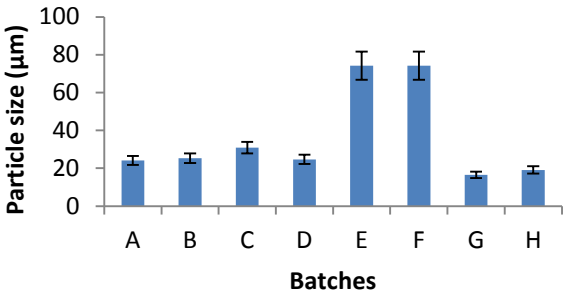

Fig. 1: Particle size of artemether-lumefantrine-loaded and unloaded lipospheres, batches A, B, C, and G contains Soluplus® and 1, 3, 5 and 0 % API, while batches D, E, F and H contains Solutol® HS 15

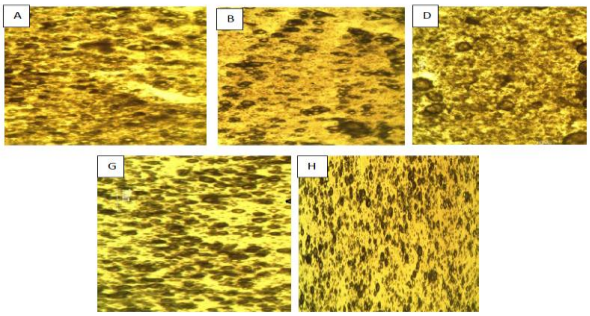

Fig. 2: Particle morphology of artemether-lumefantrine-loaded and unloaded lipospheres; magnification x 100; batches A, B and H contain Soluplus® and 1, 3 and 0 AP1 while, D and G contain Solutol® HS 15 and 1 and 0 % API

The pH of lipospheres

The results of the pH of artemether-lumefantrine-loaded lipospheres are shown in Fig. 3 and show that the pH of the unloaded lipospheres formulated with Soluplus (batch G) decreased from 5.78 at day 1, to 4.81 at 30 days. Also, the pH of the artemether-lumenfantrine-loaded lipospheres formulated with Solutol HS 15 also declined from 5.63 to 4.32 for batch C containing 5 % of API. This pH decline over time was significant in most of the formulations ($p < 0.05$). Formulations containing Soluplus also had pH decrease over time as shown in Fig. 3.

Encapsulation efficiency (EE%) and loading capacity

The results of the EE% of artemether-lumefantrine-loaded lipospheres are shown in Fig. 4 and show that the formulations generally had high encapsulation of both drugs. The EE of artemether ranged from 71.80 to 75.30 % for lipospheres formulated with Soluplus® as surfactant, i.e. batches C and D containing 5 and 3 % API, while batches F and E containing 5 and 3 % of both anti-malarial and Solutol® had EE range of 65.30 to 75.02 %. Also, the EE% of lumefantrine ranged from 76.36 to 88.99 % for lipospheres formulated with Soluplus® as surfactant (batches C and D), while batches F and E containing 5 and 3 % of both anti-malarial and Solutol® had 73.22 to 85.06 %. Lumefantrine showed significantly higher EE% than artemether in batches B and E containing 3 % of both anti-malarials ($p < 0.05$). The results of LC showed that it increased significantly with increased drug loading as shown in Fig. 4, ($p < 0.05$).

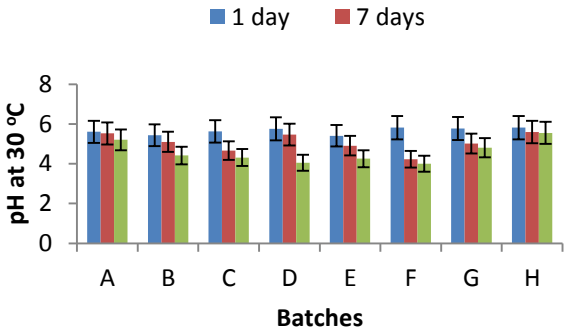

Fig. 3: The pH of artemether-lumefantrine-loaded and unloaded lipospheres; batches A, B, C, and G contains Soluplus® and 1, 3, 5 and 0 % API, while batches D, E, F and H contains Solutol® HS 15

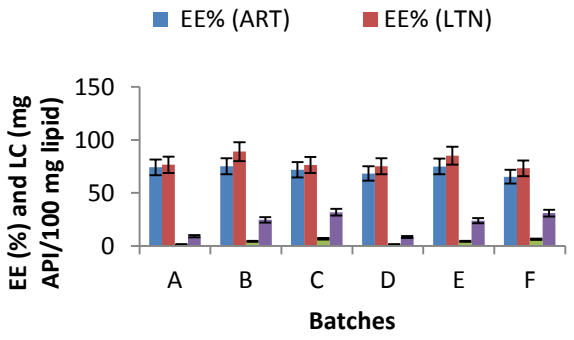

Fig. 4: The encapsulation efficiency and loading capacity of artemether-lumefantrine-loaded lipospheres; batches A, B and C contain Soluplus® and 1, 3 and 5 API, while batches D, E and F contain Solutol® HS 15; ART: Artemether, LTN: Lumefantrine, LC: Loading capacity, EE: encapsulation efficiency

***In vitro* drug release**

The results of the *in vitro* release of artemether in SIF (pH, 7.2) are shown in Fig. 5 and show that the lipospheres exhibited good release of the loaded drugs. About 14.8, 23.2, 11.6, 20.8 and 42.3 % of artemether were released from batches A (1 % artemether and Soluplus), B (3 % artemether and Soluplus), D (1 % artemether and Solutol), E (3 % artemether and Soluplus) and the commercial sample respectively at 0.3 h. However, at 3 h, about 53.2, 59.2, 75.6, 48.8 and 89.4 % of artemether were released from batches A, B, D, E and the commercial sample respectively, while 96.8, 98.8, 97.2 and 79.14 % artemether were released from batches A, B, D, E and the commercial sample respectively at 6 h.

The lipospheres also showed good release of lumefantrine in SIF (pH, 7.2) as shown in Fig. 6. At 0.3 h, about 9.72, 19.9, 3.3, 15.7 and 74.3 % of lumefantrine were released from batches A (1 % artemether and Soluplus), B (3 % artemether and Soluplus), D (1 % artemether and Solutol), E (3 % artemether and Soluplus) and the commercial sample respectively, while at 6 h 83.27, 79.7, 94.7, 97.4 and 34.5 for batches A (1 % artemether and Soluplus), B (3 % artemether and Soluplus), D (1 % artemether and Solutol), E (3 % artemether and Soluplus) and the commercial sample respectively.

Also the results of the *in vitro* release of artemether in SGF (pH, 1.2) are shown in Fig. 7, while the results of the release of lumefantrine in SGF (pH, 1.2) are shown in Fig. 8. Artemether showed significantly higher release in SGF (pH, 1.2) than lumefantrine ($p < 0.05$). However, the commercial sample (CM) exhibited 98.4 and 93.7 % release of artemether and lumefantrine respectively in SGF (pH, 1.2) at 2 h, while the formulations could not reach maximum release at 8 h in SGF (pH, 1.2).

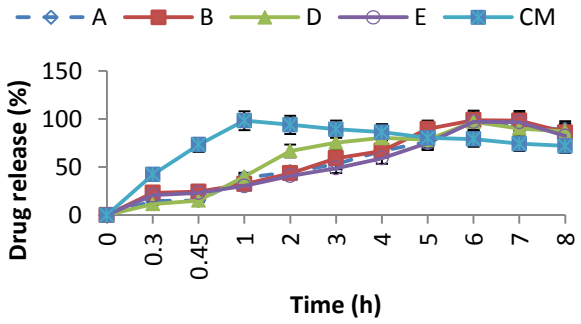

Fig. 5: Drug release profile of artemether in SIF, pH 7.2; batches A, B and C contain Soluplus® and 1, 3 and 5 API, while batches D, E and F contain Solutol® HS 15, CM: Commercial sample

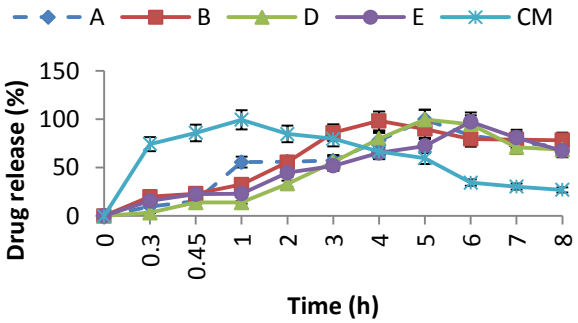

Fig. 6: *In vitro* release of lumefantrine in SIF (7.2); batches A, B and C contain Soluplus® and 1, 3 and 5 API, while batches D, E and F contain Solutol®, CM: Commercial sample

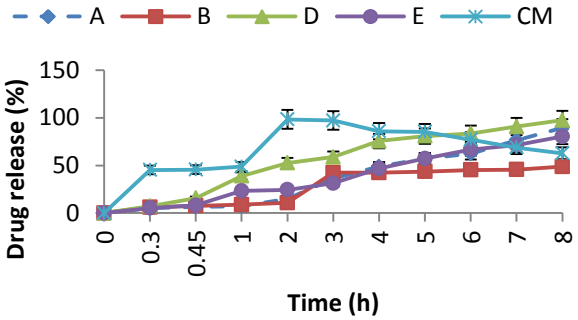

Fig. 7: *In vitro* release of artemether in SGF (pH, 1.2); batches A, B and C contain Soluplus® and 1, 3 and 5 % of artemether-lumefantrine, while batches D, E and F contain Solutol®, CM: Commercial sample

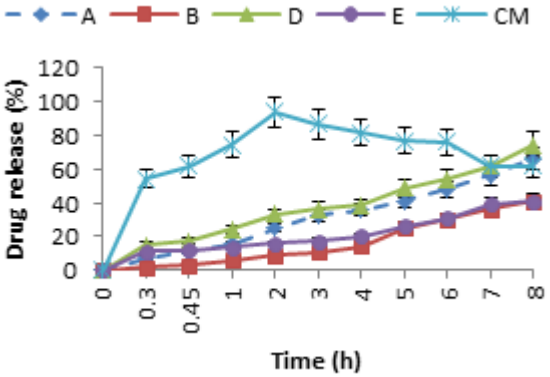

Fig. 8: *In vitro* release of lumefantrine in SGF (pH, 1.2); batches A, B and C contain Soluplus® and 1, 3 and 5 API, while batches D, E and F contain Solutol®, CM: Commercial sample

***In vitro* release kinetics**

The results of the *in vitro* release kinetics of artemether-lumefantrine-loaded lipospheres in SIF (pH, 7.2) are shown in Table 2. The results show that the first order plots were linear showing that drug release kinetics followed first order release (r^2 ranged from 0.864 to 0.973). Higuchi models were also linear confirming that drug release followed diffusion controlled process.

The Ritger-Peppas models were linear with *n* value range of 0.404 to 0.6. The results indicated that drug released followed mixed mechanism of release as shown in Table 2.

Table 2: *In vitro* release kinetics of artemether-lumefantrine-loaded lipospheres

Batch	First order	Higuchi		Ritger-Peppas	
	r^2	r^2	*n*	r^2	*n*
A	0.973	0.961	8.351	0.960	0.600
B	0.864	0.954	4.709	0.976	0.404
D	0.965	0.938	6.920	0.944	0.492
E	0.994	0.917	4.269	0.970	0.430

Batches A, B and C contain Soluplus® and 1, 3 and 5 % of artemether-lumefantrine, while batches D, E and F contain Solutol® HS 15 and 1, 3 and 5 % respectively

DISCUSSION

The photomicrographs of artemether-lumefantrine-loaded lipospheres show that they are crystalline in nature, spherical and uniform with a smooth surface. The particles appeared to be aggregate in nature without evidence of any collapsed particles. It should be noted that the micrograph presents the particles in two dimensions and particles viewed edge on may not appear spherical [28]. The particle size of artemether-lumefantrine-loaded lipospheres showed that they were within the micrometer limits and increased significantly with increased drug loading ($p < 0.05$) in agreement with previous works [14-16, 26-28]. However, particle size may be a function of either one or more of the following: formulation excipients, degree of homogenisation, homogenisation pressure, rate of particle size growth, crystalline habit of the particle etc.[14-16].

The results of the pH of the artemether-lumefantrine loaded and unloaded lipospheres showed that batch A containing 1 % API and Soluplus and batch H (unloaded lipospheres formulated with Soluplus as surfactant) exhibited a fairly stable pH with an insignificant decline over time ($p < 0.05$). However, all the other batches showed significant decline in pH at 30 days compared to day one ($p < 0.05$). This decline in pH over time may be due to release of free fatty acids over time since there was also a corresponding pH decline in the unloaded formulations [15].

The results of encapsulation efficiency showed that lumefantrine exhibited higher EE values than artemether. This may be as a result of the lipophilic nature of this drug. However, the lipospheres exhibited high encapsulation efficiency of both anti-malarials. The results of the loading capacity of the lipid matrix for both drugs revealed that lumefantrine generally exhibited significantly higher LC values than artemether ($p < 0.05$), which may be due to the lipophilic nature of this drug. However, the surfactant used in the formulations affected both the EE and the LC. Lipospheres formulated with Solutol® HS 15 showed higher EE and LC than those formulated with Soluplus®. Both EE% and LC are dependent on several parameters, such as the lipophilic properties of the drug, the screening of the most appropriate lipid composition/ratio and surfactant combination, as well as the production procedures used [15].

The results of the *in vitro* release of artemether and lumefantrine respectively was studied in SIF and SGF for 8 h and results showed that the lipospheres exhibited good sustained release of both drugs with maximum release of artemether at 6 hours in SIF. This formulation is recommended for twice daily administration in order to effectively achieve the goal of maximum parasitaemia eradication. The formulations showed no burst release in both media however, batches B and E containing 3 % of API, Soluplus and Solutol respectively showed up to 20 % release of artemether in SIF at 0.3 h, which may be due to presence of encapsulated drug in the periphery of the lipospheres with increased drug loading. However, batch CM which is the reference commercial sample exhibited burst release of both anti-malarials in the two media used. Drug release in all the formulations was not clearly affected by the surfactant type used. The results also revealed that lumefantrine exhibited higher release from the lipospheres in SIF than artemether ($p < 0.05$) and significantly lower release in SGF than lumefantrine ($p < 0.05$).

The results of the *in vitro* release kinetics showed that drug release kinetics were both diffusion and dissolution controlled with high linearity for both first order and Higuchi models. However, the *n* values of Higuchi models were significantly higher than 0.5, showing that drug release was by non-Fickian diffusion controlled process. Ritger-Peppas models also seconds Higuchi models and showed that batches B and E containing 3 % of API exhibited Fickian diffusion release mechanisms ($n \leq 0.43$). However, batches A and D containing 1 % API followed non-Fickian diffusion release mechanisms, showing that drug release was by diffusion and erosion ($0.43 < n < 1.00$) [29-31].

CONCLUSION

Lipospheres based on lipid matrix consisting of goat fat and phospholipid presented good carrier for the delivery of artemether-lumefantrine. The results revealed that the formulations had sustained release properties in addition to high encapsulation efficiency. The two surfactants Solutol® HS 15 and Soluplus® used in the formulations exhibited good properties for the preparation of artemether-lumefantrine-loaded lipospheres. Lipospheres have advantages of high carrier capacity, high stability, biocompatible, and improve oral bioavailability. Further research into this novel area is highly encouraged in order to effectively study all aspects of this formulation for possible scale up.

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COMPETING INTERESTS

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