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Original Research Article

Development and Evaluation of Artemether-loaded Microspheres Delivery System for Oral Application in Malaria Treatment

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ABSTRACT

The application of artemether for the treatment of malaria is limited by its poor solubility leading to low bioavailability. The current study formulated sustained-release artemether mucoadhesive microspheres for oral delivery to prolong oral artemether delivery and improve the low bioavailability. Microspheres were formulated with mixtures of Eudragit®RS100 and Eudragit®RL100 in ratios of; 1:1 (A1), 1:3 (A2), and 3:1 (A3) by solvent evaporation techniques. The percentage yield, particle sizes, encapsulation efficiency (EE%), flow property, differential scanning calorimetry (DSC), Scanning electron microscopy (SEM), bioadhesion study, *in vitro* and *in vivo* studies of the microspheres were evaluated and characterized. Results show that microspheres exhibited an overall high percentage yield of up to 98%. Particle sizes were between 29.40 ± 0.18 - 41.42 ± 0.12 µm. EE (%) obtained were 93.0, 94.5, and 95.0% for A1, A2, and A3, respectively. Flow characteristics indicated that the microspheres had good flowability. Thermal analysis of the drug and the microspheres showed sharp melting peaks which indicated that the drug was pure and crystalline. Morphological characteristics exhibited fairly spherical in shape. Bioadhesion properties depicted that microspheres exhibited good mucoadhesion properties on the bovine ileum. Drug release in simulated gastric fluid (SGF) ranged from 2.24 to 19.3% as compared to 60.43-83.31% in simulated intestinal fluid (SIF). The decreased in parasitemia levels are 91.78 ± 0.53%, 87.35 ± 0.23%, and 81.82 ± 0.31% for A3, A2 and A1, respectively. This method shows a promising result for possible delivery of artemether with improved sustained release activity.

***Keywords*:** Antimalaria, Artemether, Mucoadhesion, Methacrylic acid, Copolymer.

Introduction

Malaria is considered a threat to human existence as it affects people in many Africa countries especially children and mothers with pregnancy.[1] Four distinguished kinds of plasmodium include: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*. However, *P. falciparum* is mainly responsible for nearly all malaria-related deaths globally. Medically, malaria is a preventable and treatable disease, if all the preventive measures are observed and properly implemented.[1] Among the measures is chemoprevention and treatment.[2] However, these means are hampered as a result of poor drug oral bioavailability and resistance to the parasite strains.

Artemisinin and its analogues (artesunate, artemether, dihydroartemisinin) are the most potent and clinically approved antimalarial drugs. They lower the infecting plasmodium biomass as compared to what is obtained in other antimalarials.[3]

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Recently, the artemisinins are notably well tolerated and are considered to be the most potent antimalarial in clinical practice. Structurally, artemisinin is a sesquiterpene 1, 2, 4- trioxane extracted from the Chinese medicinal herb qinghao (*Artemisia annua L.*). Chloroquine-resistant strains of *P. falciparum* have been treated using this molecule and are vastly used in clinical settings. This activity has been attributed to the functional group on the structure of the compound. However, it is a comparatively lipophilic and unstable drug. They are effective against both chloroquine-resistant/-sensitive strains of *P. falciparum*, and can be used to treat cerebral malaria. Aside from creating reactive free radicals, it disrupts the membrane transport system of the plasmodium organism.[4]

Artemether, a potent rapidly-acting schizonticide is commonly a hydrophobic drug. In the biopharmaceutical classification system (BCS), it is a class II agent with oral bioavailability of about less than 40% due to its poor water solubility. Currently, there are reported cases of resistance to this drug.[5-6] Additionally, there are also physicochemical and biopharmaceutical problems associated with formulations of artemisinin derivatives currently used in clinical practice; these problems include short half-life, poor oral bioavailability, poor stability, and low solubility profiles.[6] Hence, to improve the delivery of antimalarial drugs with minimal side effects, some researchers have developed novel particulate drug carriers.[7] The potential use of old and toxic drugs has been reestablished by modifying biodistribution and improve bioavailability with low toxicity using particulate drug delivery systems.[8] These benefits are of huge importance to malaria treatment since the development of novel

drug delivery systems for managing parasite-infected cells is paramount.[9]

Physiologically, the pH of the gastrointestinal tract (GIT) increases bit by bit as the GI tract declines from the stomach to the ileocecal region with a pH range of 1.5 - 3.0 and 5.0 - 8.0, respectively.[10-11] Hence, this higher pH improves site-specific delivery of drug formulation with high pH levels to disintegrate and release at this site. Methacrylic acid copolymers are one of the pH-dependent polymers with the pKa of approximately 4. This pka suggests that at neutral pH the acid groups in the chemical structure are nearly deprotonated producing a negative surface which enhances certain properties of hydrophobic drugs.

This research aimed at preparing mucoadhesive artemether-loaded microspheres based on methacrylic acid copolymer for improved oral bioavailability of artemether and to investigate the *in vivo* antimalarial attributes of the formulations in mice.

Materials and methods

Materials

Methacrylic acid copolymer (Eudragit RL100 and RS100) (BASF Chemical Industry Germany), sorbitan monostearate (Span® 60), acetone (analytical grade), concentrated hydrochloric acid (Sigma Aldrich, USA), artemether (Visa Pharm. Limited, India), magnesium stearate, n-hexane, monobasic potassium phosphate, sodium hydroxide, liquid paraffin (BDH, Poole, England) and distilled water (Biochemistry Lab., UNN, Nigeria).

Determination of melting point of the drug sample

A 5 mg quantity of artemether samples were packed in the sealed end of a thin-walled capillary tube and introduced into a melting point apparatus (Gallenkamp, England), and heated. The temperature at which the drug completely melts was read and recorded. The experiment was repeated thrice and the average melting point was calculated and compared with the manufacturer's stipulated melting point. Differential scanning calorimetry (DSC) was also carried out to further confirm the result of melting point and purity of the drug.

Preparation of microspheres

Artemether-loaded microspheres were prepared by the oil-in-oil emulsion solvent evaporation method. Eudragit RS100 and RL100 (RS100:RL100) in the ratios of 1:1, 1:3 and 3:1 (designated A1, A2, and A3, respectively) were accurately weighed using an analytical balance (Ohaus Adventurer, China) and dissolved in a beaker containing acetone (12.5 mL); 50 and 100 mg of artemether and magnesium stearate (100 mg), respectively were added and stirred for 3 min. The dispersion was homogenized using a magnetic stirrer (Remi Equipment Pvt. Ltd.) for 1 min at 700 rpm. Span® 60 (1% v/v) was added to liquid paraffin (125 mL) in a beaker and was also homogenized as above. Artemether dispersion containing the polymers was then drop wisely added into a beaker containing the liquid paraffin mixture and stirred. The mixture was homogenized using a Gallenport mechanical stirrer with a double blade (4 cm in diameter) at 700 rpm for 2 min. The resulting emulsion was further stirred at room temperature for 1.5 h at 700 rpm until the acetone evaporated completely. The microspheres were harvested by filtration using filter paper (Whatman no.1) and washed several times with n-hexane until no traces of liquid paraffin were observed. Microspheres were air-dried at room temperature for 48 h, packed in a tight cover container, and stored at 4^{o}C in a refrigerator until used. The unloaded microsphere (A4, RS/RL without drug) was similarly prepared.

Differential scanning calorimetry (DSC)

DSC plot of the microspheres and physical mixtures was done a DSC 204 F1 equipment (Netzsch, Germany). The equipment was graduated with indium and sapphire before samples analysis at 20 mL/min of a nitrogen atmosphere. In brief, approximately 5 mg of the materials were packed in an aluminum pan and sealed hermetically. Then, the heating was done at ten degrees Celsius per min in the range of 30 – 250^{o}C, for 3 min at the same temperature to permit complete melting and the thermograph recorded. All the experiment was conducted in triplicates.

Percentage practical yield of microparticles

The total amount of microparticles obtained was weighed and the percentage yield was calculated for each batch using the formula in equation 1:

$$Yield\ (\%) = \frac{Actual\ weight\ of\ product}{Total\ weight\ of\ excipient\ and\ drug} \times 100 \qquad (1)$$

Microspheres morphology using scanning electron microscopy and particle size analysis

Morphological study of the microspheres (A1 and A2) was evaluated using a scanning electron microscope (SEM 1000, Miniscope, Japan) using a method by Momoh *et al*.[12] Microspheres particles sizes were determined using a Hund® binocular microscope (Wetzlar, Germany) connected to a Motic image analyzer (*Moticam, China)*. Microspheres were assembled on a glass slide and placed on the microscope for observation at a magnification of x 40.

Determination of encapsulation efficiency

Approximately 20 mg of microsphere was dissolved in methanolic HCl, properly diluted, and filtered using a non-adsorbent filter paper (Whatman No 1). Then, about 5 mL filtrate was analyzed with a spectrophotometer (Jenway 6305 spectrophotometer, UK) at 254 nm. The artemether content and its encapsulation efficiency (EE%) were determined with reference to Beer's plot using equation 2:

$$Encapsulation\ Efficiency\ (\%) = \frac{Actual\ drug\ loading}{Theoretical\ drug\ loading}\ x\ 100 \qquad (2)$$

Micromeritics properties of the microspheres

Bulk and Tapped Densities

The bulk volume was determined using a 25 g quantity of the microspheres laid inside a 10 ml measuring cylinder. Bulk volume was obtained as the volume occupied by the formulation was noted as the bulk volume and the bulk density (ℓ_B) was calculated using the formula in equation 3.[13,14]

$$Bulk\ density\ (\ell_B)\ \frac{Mass\ of\ powder\ (M)}{Bulk\ volume\ of\ powder} \qquad (3)$$

The tapped volume was evaluated by tapping the cylinder on a wooden flat surface from a height of one inch at 2 seconds intervals until constant volume. The tapped density (ℓ_T) was calculated using the formula in equation 4:

$$Tapped\ density\ (\ell_T)\ \frac{Mass\ of\ powder\ (M)}{Tapped\ volume\ of\ powder} \qquad (4)$$

Hausner's ratio and compressibility index

Hausner's ratio of the formulations was calculated using equation 5:

$$Hausner's\ ratio\ (\ell_T)\ \frac{\ell_T}{\ell_B} \qquad (5)$$

Note: ℓ_T (tapped density); ℓ_B (bulk density)

In vitro drug release analysis

In vitro release was studied using the USP apparatus type II (Veego, India). The dissolution medium consisted of 250 mL of freshly prepared simulated physiological fluids; SGF (pH, 1.2) without pepsin for 2 h and SIF (pH, 7.2) without pancreatin for 12 h were kept at 37 ± 1^{o}C. The polycarbonate dialysis membrane (MWCO 6000 - 8000, Spectrum Labs, Breda, The Netherlands) was previously soaked in the physiological fluid for 24 h before use. A 50 mg quantity of the artemether-loaded microspheres was enclosed in the membrane, centralized in the medium and the paddle rotated at 100 rpm. At a time interval, a 5 mL portion of the fluid was collected and filtered (filter paper, Whatman no. 1). Then, analyzed with a UV spectrophotometer (Jenway 6305 spectrophotometer, UK) at a previously determined wavelength of 273 nm. The experiment was repeated thrice for all the microparticles.

Kinetic analysis of in vitro release profiles

The *in vitro* release data were analyzed for kinetic and mechanism of release using three different models. The first order model (Eq. 6), Higuchi (Eq. 7) and Korsmeyer (Eq. 8).[15,16] To carry out this study, the 60% cumulative artemether release was fitted in these equations.

$$Log\ Q_0 - \log Q_t = \frac{K_{1t}}{2.303} \quad (6)$$

$$Q = K_2 t^{1/2} \quad (7)$$

$$\frac{M_t}{M_\infty} = K3t^n \quad (8)$$

Note: Q is the quantity of artemether released at time t, Q0 is the initial concentration of artemether, k1, k2 and k3 are first-order, Higuchi and Korsmeyer-Peppas kinetic constants, respectively. Mt/M∝ is a fraction of an artemether released at time t, n is diffusion exponent and is an indicator of the mechanism of transport of artemether through the carrier.14 Log cumulative of per cent artemether remaining vs. time (first order kinetic model), cumulative per cent artemether release vs. square root of time (Higuchi model) and the integral form of Higuchi, log cumulative percent artemether release vs. log time and log fraction of artemether release versus log time (Korsmeyer-Peppas model) were plotted.

Bioadhesion study

A mucoadhesion study was performed using bovine ileum obtained from an abattoir in Nsukka, Nigeria. Approximately 200 mg of artemether-loaded microspheres were correctly weighed accurately and laid on a 12 cm bovine ileum and microspheres were permitted to cling to the surface of the ileum for 10 min. A funnel was fixed to a retort stand and 100 ml of SIF (pH 7.2) was permitted to overrun over the bovine ileum treated with the formulations. The drug formulation that separated from the ileum tissue were gathered, dried, and weighed. This procedure was repeated with other batches and % bio-adhesion was calculated using the formula:

$$\text{Bio-adhesion (\%)} = \frac{Wo - WI}{Wo} \times 100 \quad (3)$$

Key: Wo and Wi are mass of microspheres applied and mass of microspheres detached, respectively.

Anti-malarial evaluation

A modified Peter's 4-day suppressive investigation using *Plasmodium berghei* infection in mice was adopted with little modification.[17] In this study, thirty-six healthy mice with an average weight of 30.5 g of either sex were carefully and randomly shared into six groups of six (n = 6). This study was accomplished in conformity to guidelines of the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, the University of Nigeria, Nsukka in line with the National Code of Conduct for Animal Research Ethics (NCARE), with reference DOR/UNN/17/00012, and EU Directive 2010/63/EU for mice investigation.[18] *Ab initio*, the mice were separately caged and allowed to acclimatize for 14 days. They had allowed free access to food and water during the experiment. A blood sample equivalent to 10^8 *Plasmodium berghei* cells/mL was collected from plasmodium donor mice and diluted with normal saline.

The six groups of mice were carefully inoculated with 0.2 ml of V_2 (equivalent to 10^8 cells/mL) intraperitoneal administration and supervised for three days before the administration of the drug product. After the three days period, approximately 4 mg/kg microspheres were dispersed in an aqueous medium (0.5 mL) and given orally to the mice. A placebo group received normal saline 5 mL/kg while, the standard (reference) group was given artemether market brand (4 mg/kg) and chloroquine phosphate tablets (10 mg/kg), dispersions in 0.5 mL aqueous medium. At the end of 4 days, a blood sample (1 mL) was collected at the retro-orbital venous plexus of the experimental animals. Microscopy of the malaria parasite in the animal blood was done using Giemsa stained thin-film quadruplet field view. Hence, the % parasitemia in the blood was determined in accordance with the earlier researcher as in equation 10.[19,20]

$$\text{Parasitemia (\%)} = \frac{\text{Parasitemia in negative control} - \text{Parasitemia in study group}}{\text{Parasitemia in the negative control}} \times 100 \quad (10)$$

Statistical analysis

All experimental study was replicated (n = 3) for statistical analysis. Data were conveyed as mean ± SD using ANOVA and Student *t*-tests and taken as significant for p values < 0.05.

Results and Discussion

Melting point determination

The result of melting determination is in agreement with the requirement of the manufacturer and indicates that the drug is authentic. The melting point of the artemether sample gave 89.0°C and was within the manufacturer's specification, 86.0 – 92.0°C. The result of DSC showed a sharp peak at 89.5°C (Fig. 1a), which further confirmed the purity of the drug and suitability of the drug for the study. The DSC of the microspheres is shown in Figure 1 (a-e) and showed that the thermograms of the artemether pure sample exhibited a sharp endothermic peak at 89.5°C (Figure 1a). The thermograms of the artemether-loaded microspheres showed melting endotherms of the methacrylic acid copolymers at 63°C (Fig. 1b) for batch A1 formulated with polymer ratios 1:1. However, batches A2, A3 (c, d) showed endotherms at 63.5°C. The endothermic peak of the artemether was seen clearly at 89°C in batches A1 and A2 (Figure 1 b, c, and e), showing that the drug was not denatured by any form of treatment. The presence of small peaks of artemether in the thermogram of some batches may indicate that a portion of the drug was present in its crystalline form. The results of the thermal analysis of the drug and the microspheres show that artemether exhibited sharp endotherm at 89.5°C, this sharp melting peak indicated purity and crystallinity of the drug. The DSC thermograms of the microspheres showed an endothermic peak of the methacrylic acid copolymers (Eudragit RS100: RL100) at 63, 63, and 63.5°C (A1, A2, A3 and A4). More so, an endothermic peak of artemether was observed at 89°C, and the peak depicted the presence of artemether with decreased crystallinity due to a reduction in the sharpness of this melting peak. However, the existence of a low measure of active pharmaceutical ingredient (API) in the polymer matrix when the microparticles are melting decrease the accuracy in detecting the melting peak of an encapsulated drug.

Percentage practical yield and encapsulation efficiency (EE%)

Practical yields of microspheres are presented in Table 1 and showed the overall high% yield of up to 98%. This high percentage recovery certifies the reproducibility of the formulation, cost-effectiveness, and reliability of the procedure. The EE% as presented in Table 1 indicated that the microsphere had good encapsulation of 93.0, 94.5, and 95.0% for A1, A2, and A3, respectively. Encapsulation efficiency is the quotient of the quantity of encapsulated API and the entire quantity of the preparation components. Microsphere had EE% which may be due to the high lipophilic nature of the drug[21].

Particle size, SEM and morphology

The Particle size of microspheres is presented in Table 1. The result ranged between 29.40 ± 0.18 to 41.42 ± 0.12 µm for the artemether-loaded microspheres formulated with 1:1 and 3:1 (A1 and A3 Eudragit® RS100:RL100) respectively, while the unloaded or drug-free microspheres (Batch A4) had a particle size of 23.68 ± 0.09 µm. The photomicrographs and SEM of representative artemether-loaded microspheres are shown in Figsure 2 and 3, respectively. The morphological characteristics indicated fairly spherical microspheres whereas the particle size was within the micrometre limits for microspheres. Particle size was influenced by the combination ratio of polymer employed, batch A3 formulated with Eudragit RS100:RL100 (3:1) had a higher particle size significantly different from microspheres formulated with polymer ratio 1:1 (p < 0.05). Particle size could be a function of formulation excipient, method of formulation amongst other factors. Particle size increased with the incorporation of drugs.

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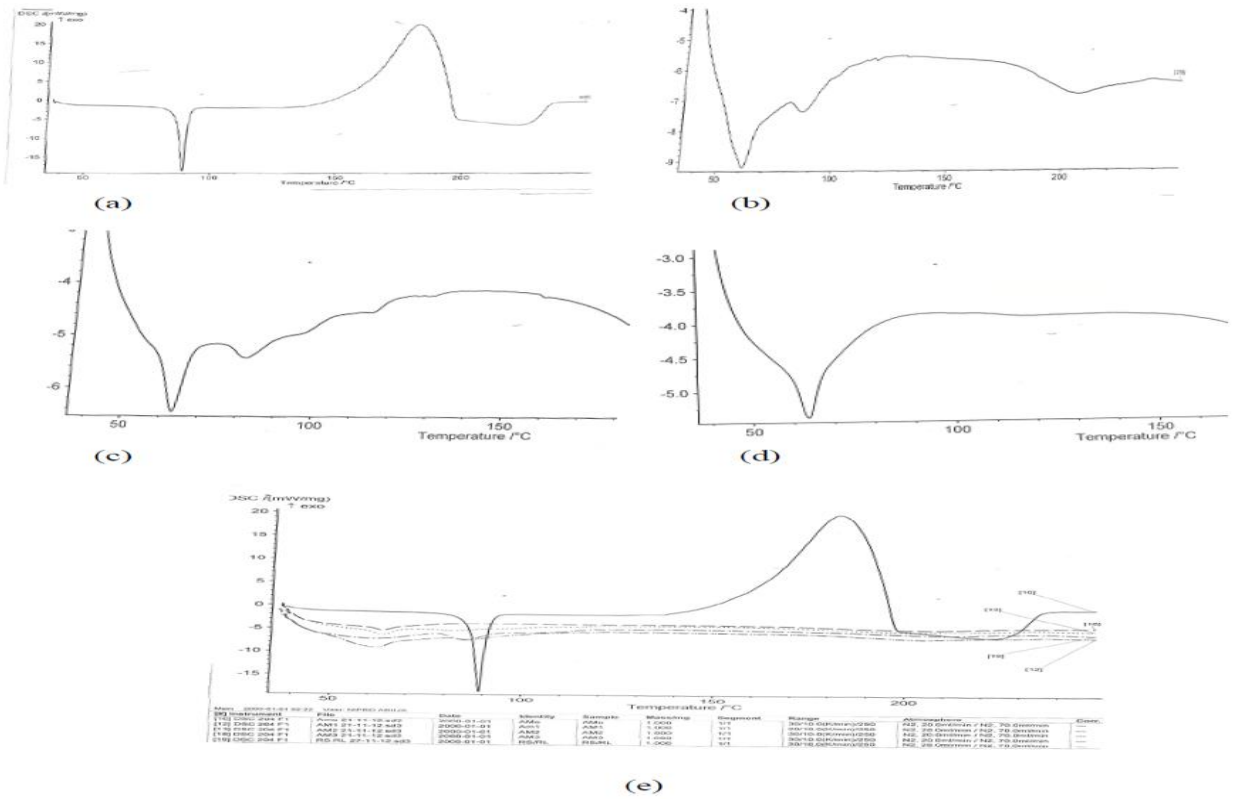

Figure 1: DSC thermograms of (a) artemether, (b) A1, (c) A2 (d) A3, (e) super imposed thermograph of all composition.

Table 1: Physicochemical properties of artemether-loaded microspheres

Formulation code	Particle size (µm)* †	Yield (%)*	Encapsulation efficiency (%)*
A1	29.40 ± 0.18	97.12 ± 0.11	93.0
A2	37.15 ± 0.16	98.15 ± 0.21	94.5
A3	41.42 ± 0.12	98.02 ± 0.02	95.0
A4	23.68 ± 0.09	97.03 ± 0.12	---

Note; *Mean ± standard deviation, †n = 3.

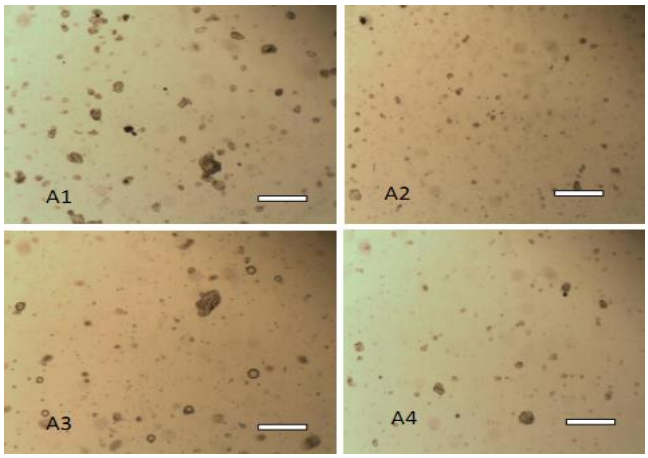

Figure 2: Photomicrographs of representative batches of microspheres containing (A1) 1:1, (A2) 1:3, and (A3) 3:1 of Eudragit RS 100: RL 100 loaded with 50 mg of arthemter, and A4 (blank).

Key: - (white bar) represents 50 µm.
Microspheres (A1, A2 and A3) loaded with artemether were significantly higher ($p < 0.05$) in size than the unloaded microspheres (A4).

Micromeritic properties of microspheres
The flow characteristics of formulations are shown in Table 2 and show that they had low bulk and tapped density. Hausner's ratios were within the limits for good powder flow. However, batch A4 (1:1 without artemether) showed poor flow with Hausner's ratio of 1.38 and compressibility index of 26.91%. The results of the flow characteristics of the microspheres show that the microspheres had good flowability.

In vitro release of artemether from microspheres
Artemether release profiles from microspheres are represented in Figure 4 (a-b). The amount of drug release in SGF pH 1.2 (Figure a) was significantly lower ($p < 0.05$) than that in SIF (pH 6.8). Drug release in SGF between 0.5-3 h ranged from 2.24 to 19.3% in all the batches, however when the formulations were transferred into SIF pH 6.8 (4-12 h), a significant increase ($p < 0.05$) in drug release was seen with about 60.43, 72.41, and 83.31% drug release at 4, 7, and 12 h, respectively, from microspheres, formulated with polymer combination ratio 1:1 (batch A1). The release of drug in SIF (Figure b) shows that about 25.33, 42.13, 58.02, and 79.32 at 1, 5, 8, and 12 h, respectively, from batch A1, formulated with polymer ratio 1:1 (RS100:RL100), while 43.5, 81.45, 84.35, and 99.21% at 1, 5, 8 and 12 h, respectively, of artemether, were released from batch A3 formulated with polymer ratio 3:1 (RS100:RL100). Therefore, batches A1 showed more sustained drug release, significantly higher than those of batches A2 and A3 ($p < 0.05$). This study was done in three bio-relevant media. This is because the drug was targeted to adhere in the mucosal layer of the intestine and formulated in such a way that they release in the intestinal pH. The results showed that artemether was released at a very low concentration in the SGF (0.5 – 3 h) and when the drug was placed in the intestinal region, a high release of artemether was exhibited by the formulation and the release was maintained over time between 4 -12 h study. This attested that the microspheres achieved a sustained release effect which is advantageous with improved oral bioavailability of artemether thus maintaining an effective concentration in the blood over time.

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Eudragit RS100 and Eudragit RL100 are ammonium methacrylate copolymers and their insolubility in water may have caused the microspheres to show sustained and prolonged release characteristics since the polymers form the matrices of these formulations. Drug release was, however, affected by the polymer concentration with batch A3 formulated with 3:1 (ERS100:ERL100) exhibiting the highest drug release in all the media over time. This was proceeded by batch A2 formulated with 3:1 (ERS100:ERL100), while batch A1 exhibited more sustained release of drug over time. The differences in drug release observed across the batches could be related to the nature of the matrices. Eudragit RS100 has 5% of functional quaternary ammonium groups while Eudragit RL100 has 10% of the ammonium groups.[22] The hydrophilic nature of these quaternary ammonium groups may imply that the lipophilic Span® 60 interacted more with the less hydrophilic Eudragit RS100 during the formulation process, creating pores in the microspheres that facilitated drug release.

Release kinetics of the microspheres

The release kinetics studied using three kinetic models are represented in Table 3. First order plots show a high range of linearity in their plots (0.939 - 0.969) indicating that artemether release observed this order. Release rate (k) constants were significantly higher ($p < 0.05$) than all other models studied (53.95 -140.60). The Higuchi plots were also linear ($r^2 = 0.9$), showing that drug release was also diffusion-controlled as shown in Table 3. The Korsmeyer-Peppas plots were linear ($r^2 \approx 0.9$) as shown in Table 3. However, Higuchi plots gave n values of 0.43 for batch A3 indicating that drug release in this batch followed Fickian diffusion-controlled process, unlike batches A1 and A2 that followed non-Fickian diffusion ($n > 0.5$).[14-16] Korsmeyer-Peppas also seconds the integral form of Higuchi in the analysis.

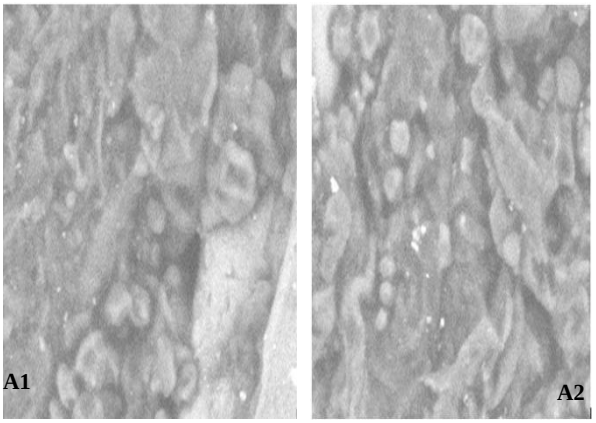
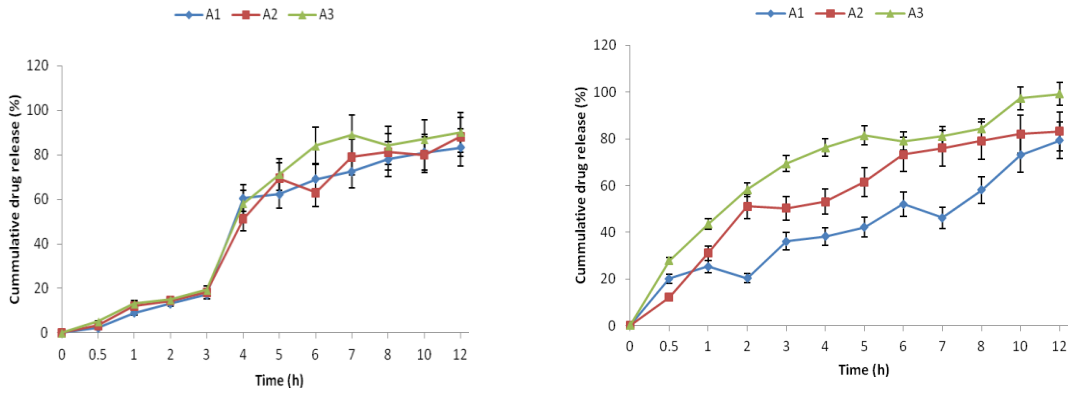


Figure 3: Photomicrographs of representative batches of microspheres containing (A1) 1:1, and (A2) 1:3.

Table 2: Micromeritic properties of the microspheres

Formulation code	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's ratio
A1	0.3128 ± 0.0124	0.3230 ± 0.0900	1.03
A2	0.5800 ± 0.0130	0.5810 ± 0.0120	1.00
A3	0.4230 ± 0.0100	0.5101 ± 0.0520	1.21
A4	0.3548 ± 0.0110	0.4905 ± 0.0120	1.38

Note: A1, A2 and A3 are artemether-loaded microspheres formulated with polymer ratio of 1:1, 1:3 and 3:1 (RS100:RL100); A4: Bland microspheres with polymer ratio 1:1.



a. **b.**

Figure 4 (a, b): *In vitro* release of artemether in (a) SGF pH 1.2 (0-3 h) and (b) SIF pH 7.2 (3-12 h); A1, A2 and A3 are artemether-loaded microspheres formulated with polymer ratio of 1:1, 1:3 and 3:1 (RS100:RL100**.**

Table 3: *In vitro* release kinetics of artemether-loaded microspheres

Formulation code	First order		Higuchi			Korsmeyer-Peppas		
	r^2	K (h^{-1})	r^2	N	K (h^{-1})	r^2	N	K (h^{-1})
A1	0.968	53.95	0.886	0.976	7.79	0.894	0.987	0.10
A2	0.939	140.60	0.901	0.609	24.49	0.898	0.814	0.30
A3	0.969	53.95	0.964	0.430	40.83	0.985	0.463	0.49

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Results showed that batches A1 and A2 followed non-Fickian or anomalous release (diffusion and erosion process) ($0.5 < n < 1$). Therefore, drug release from microspheres followed mechanisms of dissolution, erosion, and diffusion-controlled processes.

Bio-adhesion properties of the preparations

Bio-adhesion attributes of the artemether-loaded formulations are shown in Figure 5 and showed that bio-adhesion was not significantly ($p > 0.05$) affected by polymer combination ratio and ranged from 95 to 98%. *In vitro* bioadhesion properties of artemether-loaded microspheres show that they exhibited good mucoadhesion properties on the bovine ileum. Mucoadhesion was not dependent on the polymer combination ratio (Figure 5). The high bio-adhesion observed imply that the microspheres have a high affinity for mucosal surface components. Furthermore, Eudragit RS100 and Eudragit RL100 are water-insoluble, and this prevented the formulations from dissolving or losing their affinity with the biomaterial upon contact with the aqueous medium (SIF). Mucoadhesive drug delivery systems have various benefits that develop from localization at a specific site, prolonged residence time at the site of drug absorption and an intensified contact with the mucosa increasing the drug concentration gradient.[23] Thus, absorption and bioavailability of the drug is high and frequency of dosing decreased, resulting in an increase in patient compliance.[23]

Antimalarial properties

Antimalarial properties of artemether-loaded microspheres formulated with different ratios of Eudragit RS100:RL100 are shown in Table 4, and batch A3 (3:1 ERS100:ERL100) showed the highest parasitemia reduction of 91.78 ± 0.53%, followed by A2 (87.35 ± 0.23) and then A1 (81.82 ± 0.31) formulated with 1:3 and 1:1 ERS100:ERL100, respectively. The animal groups that received reference sample as control (market brand of artemether and CQ) also showed high parasitemia reduction compared with the test samples as shown in Table 4. The results of *in vivo* antimalarial properties of artemether-loaded microspheres depicted that the microspheres had anti-malaria activities comparable to that of the reference drugs used and varied significantly from control ($p < 0.05$). This formulation being a sustained-release preparation would circumvent or prevent the problem of variability in the blood level of drugs thereby, maintaining the effective dose for a prolonged period of time. Additionally, relapse of malaria or resurface of the symptom after treatment could be averted as has to be the case when malarial are poorly treated in children and adults with a view of eradicating deaths due to malaria. Consequently, therapeutic failure will be averted and early drug-resistance that has been a trend in the malaria case as per the development of resistance to newer molecules will be avoided.

Table 4: Anti-malarial properties of artemether-loaded microspheres

Formulation code	Dose administered	Reduction in parasitaemia (%)
A1	4 mg/kg	81.80 ± 0.34
A2	4 mg/kg	87.35 ± 0.27
A3	4 mg/kg	91.78 ± 0.53
CQ	10 mg/kg	90.86 ± 0.57
MKT	4 mg/kg	87.39 ± 0.31
NS	5 ml/kg	0.00 ± 0.00

Note: A1, A2 and A3 are artemether-loaded microspheres formulated with polymer ratio of 1:1, 1:3 and 3:1 (RS100:RL100); chloroquine phosphate (CQ), MKT: artemether market brand, NS: normal saline.

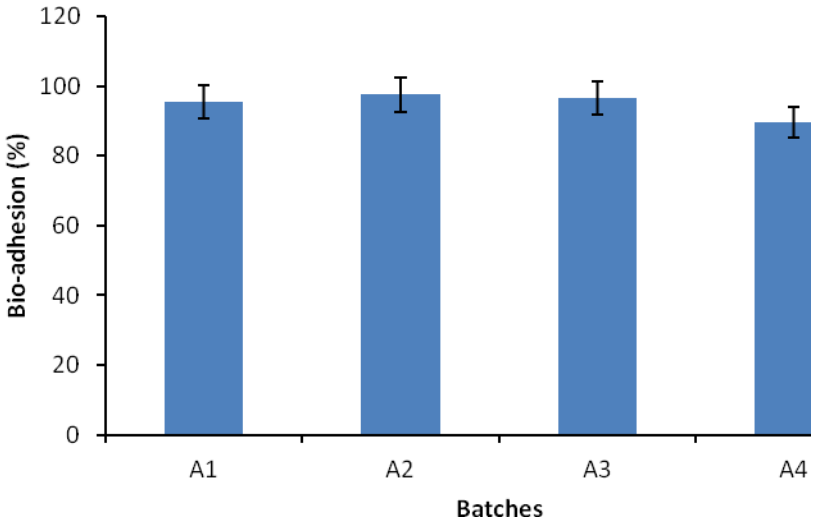

Figure 5: Bioadhesive properties of artemether-loaded microspheres formulated with Eudragit RS100:RL100 polymer ratio of 1:1 (A1), 1:3 (A2) and 3:1 (A3), and unloaded microspheres with polymer ratio 1:1 (A4).

Conclusion

Artemether-loaded microspheres based on methacrylic acid copolymers have demonstrated that conventional low oral bioavailability of artemether as a result of poor aqueous nature could be enhanced when delivered as sustained drug delivery. The formulation exhibited a significant ($p < 0.05$) prolonged drug release over a period of time (12 h). The *in vitro* bioadhesion and *in vivo* antimalarial properties of artemether-loaded microspheres showed that they exhibited good mucoadhesion properties on the bovine ileum and a good antimalaria effect when compared to the reference ($p < 0.05$). Thus, artemether-loaded microspheres formulated with methacrylic acid copolymers have the potential to deliver an effective quantity of artemether in a sustained form by achieving the required antimalarial activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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