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# Recent advances in particulate anti-malarial drug delivery systems: A review

Chukwuebuka Umeyor [1]*, Franklin Kenechukwu [2], Emmanuel Uronnachi [1], Salome Chime [3], Joy Reginald-Opara [2], Anthony Attama [2]

***Corresponding author:**

Chukwuebuka Umeyor

[1]Drug Delivery and Nanotechnology Research Unit, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[2] Drug Delivery Research Unit, Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]Drug Delivery Research Unit, Department of Pharmaceutical Technology and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

## Abstract

Malaria remains a tremendous health burden in tropical areas, causing a life-threatening disease and accounts for 1 to 2 million deaths round the globe yearly. Researchers have explored different novel approaches to deliver and improve the biopharmaceutical performance of drugs used in malaria chemotherapy. These novel drug delivery systems (NDDS) enhanced bioavailability of these drugs and also may offer controlled release of these drugs. The major aim of the NDDS is to improve the efficacy of these drugs, and at the same time to eliminate their toxicity. These NDDS include: micro/nanoparticulate DDS, emulsion based DDS, dendrimers and liposomes among others. The development of these particulate carriers as vehicles for the delivery of active compounds is a novel area of research that provides a new hope in malarial chemotherapy. The work presents various trends in malarial chemotherapy, as well as an exhaustive screening of different particulate drug delivery systems (PDDS) and the recent advances in the delivery of anti-malarial drugs using the novel particulate drug delivery systems (NPDDS).

**Keywords:** Malaria, dendrimers, SEDDS, NLCs, SLNs, artesunate, ACTs

## Introduction

In recent years, there has been an upsurge in interest in the development of particulate drug delivery systems (PDDS) among researchers who are actively involved in drug delivery. This high interest is largely due to the potential of these systems to improve the biopharmaceutical performance of drug entities employed in disease therapy compared to conventional systems such as tablets, capsules and others. Designing and formulating these PDDS such as niosomes, liposomes, dendrimers, microparticles, nanoparticles, for the delivery of actives have proven to be challenging especially considering the vast formulation excipients needed and their cost implications. These challenges notwithstanding, formulation scientists have successfully designed, formulated and patented many particulate drug products for therapeutics and diagnostics (theranostics) purposes. Some of these products already in the market for clinical use are listed in table 1

The development of these particulate carriers as vehicles for the delivery of active compounds is a novel area of research that provides with a new hope, the technology leading to the creation of devices and delivery systems with fundamentally new properties and functions. PDDS are designed and formulated by the incorporation of the drug compound into inert lipid vehicles containing surfactants and co-surfactants [1]. These particulate carriers offer a number of advantages making them ideal drug delivery systems. The advantages include:

Better drug delivery profile across physiological barriers and membranes of the bodyDue to their small size, chemistry and distribution, they have bridged the gaps between structure and function of biomoleculesTheir micron and sub-micron size ranges enable them to be good potential carriers of biological molecules such as proteins, vaccines and other peptides

They are used to target drug compounds to body tissues and sites with reduced or no untoward effectsThey increase drug solubilization especially practically insoluble drugs, and hence solve their bioavailability problemsTargeted delivery of drugs using these carriers provides more efficient drug distribution [2]

Table 1.Some particulate drug formulations available in the market

| Brand Name | Drug | Formulation Type | Route of Administration | Application | Company |
|---|---|---|---|---|---|
| Rapamune® | Rapamycin | Nanoparticles | Oral | Immunosuppressant | Wyeth Pharma, USA |
| Intelectol® | Vinpocetine | Liposomes | Oral | Cerebrovascular disorders | Menory Secret Inc., USA |
| Nurofen® | Ibuprofen | Nanocapsules | Oral | NSAIDs | Abbott AG, USA |
| Lipofen® | Fenofibrate | Liposomes | Oral | Hypercholesterolemia | Kowa Pharma Inc., USA |
| Ambisome® | Amphotericin B | Liposome | Intravenous Infusion | Fungal Infections | Astellas Pharma Inc., USA |
| Mevacor® | Lovastatin | SLNs | Oral | Hyperlipidaemia | Merck and Co. Inc., USA |
| Procardia® | Nifedipine | Nanosuspension | Oral | Hypertension | Pfizer Labs Inc., USA |
| Cesol® | Praziquantel | SLNs | Oral | Antihelmintic | Merck KGaA, Germany |
| Abraxane® | Paclitaxel | Nanoparticles | Intravenous Injection | Metastatic breast cancer | American Biosciences, USA |
| Efudex® | N3-o-toluyl-Fluorouracil | Liposomes | Oral | Tumour Inhibition | Valeant Pharma. Intl, USA |

Note: SLNs - Solid Lipid Nanoparticles

It is very interesting to note that the majority of the PDDS formulated are lipid- and/or polymer-based systems. Lipid-based formulations have been shown to enhance the bioavailability of drugs, especially drugs administered orally [3-6]. 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 [7]. The proven safety (biocompatibility) of lipid based carriers makes them attractive candidates for the formulation of pharmaceuticals. For poorly water soluble drug molecules, whose dissolution in water is likely the limiting step of overall oral absorption, the primary role of ingested lipids and their lipolytic products is to impact the drug dissolution step by forming – with bile components – different colloidal particles, which are able to maintain a larger quantity of hydrophobic drugs in solution via micellar solubilization [8]. 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 [9].

Drug development and formulation experience have very low success rates with regards to drugs that enter the market. These shortfalls are due to factors such as toxicity of the therapeutic compounds, poor solubility leading to lowered bioavailability and reduced efficacy. These challenges are even more important in poverty related diseases (PRDs) especially malaria, due to the high prevalence of resistance and patient non-compliance to available drugs used in malaria chemotherapy.

# Malaria

## A parasitic killer disease

Malaria is a very prominent parasitic disease because of its immense global significance. Malaria is a life-threatening disease and accounts for 1 million to 2 million deaths round the globe every year [10]. Malaria is a major public health problem in several tropical countries causing about 500 million clinical cases especially in children and pregnant women. The severe and complicated stages have a mortality rate between 20 and 50 % [11]. The geographical extent and clinical severity of malaria is increasing across much of sub-saharan Africa [12]. Approximately 80 % of malaria cases in Africa were in 13 countries and over half of them occurred in Nigeria, The Democratic Republic of Congo, Tanzania, Ethiopia and Kenya. Among the cases that occurred outside Africa, 80 % occurred in India, Myanmar, Bangladesh, Indonesia, Papua New Guinea and Pakistan [13]. In humans, malaria is caused by four distinct species of parasites viz: *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium falciparum* and *Plasmodium ovale*. Among these, the most severe malaria is caused by blood-borne apicomplexan parasite *P. falciparum* which is responsible for almost all the malaria-related deaths [14].

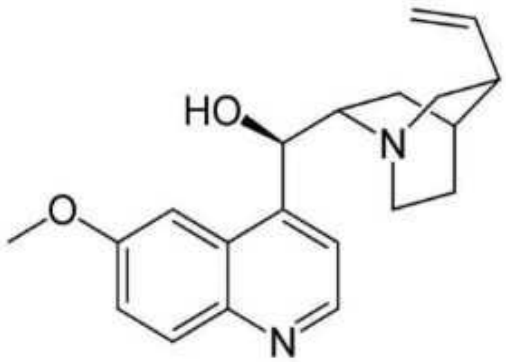
The elimination of malaria is now considered a realistic goal because of good surveillance and high intervention coverage between 2000 and 2007 which have resulted in the reduction of malaria cases and deaths by 50 % or more in some countries and regions of African countries [15]. Existing treatments for malaria include a limited number of clinically effective anti-malarial agents such as chloroquine, amodiaquine, sulphadoxine and pyrimethamine, etc. However, the clinical utility of most of the anti-malarial agents is hampered due to problems such as poor oral bioavailability and the emergence of drug-resistant parasite strains. The coincidental proliferation of resistance to the commonly deployed first-line therapeutics poses a major threat to national and international targets to reduce child mortality due to malaria by one-third by the year 2015 [16]. In view of this, the entire world is looking for global expertise to improve the delivery and biopharmaceutical profiles of the current chemical entities deployed in malaria therapy worldwide, and especially in the endemic countries and regions. This would go a long way to cut down the high mortality rate associated with malaria.

## Chemotherapy of malaria

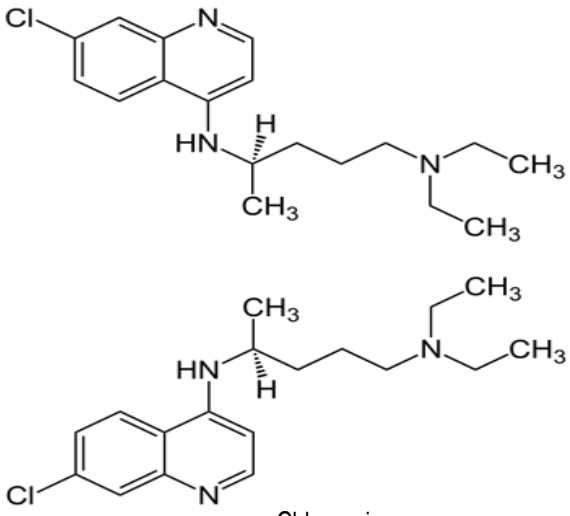


Currently, there is no patented, approved and clinically effective vaccine for use in the fight against malaria although committed efforts are being put in place to find one. Against this backdrop, chemotherapy is the mainstay of malaria treatment. Effective utilization of anti-malarial drugs have produced good results in the past since the clinical use of the majority of them has been reduced due to problems such as poor oral bioavailability and the emergence of drug-resistant parasite strains.

Chloroquine (CQ) was one of the most useful drugs ever discovered [17]. CQ has several pharmacokinetic and pharmacological advantages over all the other anti-malarial drugs, which accounts for its excellent performance over eight decades of malaria therapy [18-20]. The main advantages of CQ therapy are the fast action in blood parasite stages, low toxicity, and good bioavailability from oral dosage form, water solubility, and high volume of distribution in the body [18-21]. Above all, CQ is cheap, relatively safe, easy to administer and was extremely effective. Wide use of CQ after the 1960s resulted in a wide decline of death among infants by 18 % compared to the pre-1960s [18, 21, 22]. This continued until the emergence of CQ resistance in the late 1980s, which cause is still being studied, but non-compliance to drug regimen being the major pointer. The decline in CQ efficacy led to the introduction of Sulphadoxine-Pyrimethamine (SP) as the recommended first-line drug for uncomplicated malaria in several countries [23]. SP is a convenient single dose drug and within the same price range as CQ. However, the useful therapeutic life of SP has been shown to be short with reported treatment failure rates as high as 40 % after only a few years of widespread use and frequent adverse effects to the drug [18, 24]. Second and third line treatment are Amodiaquine (AQ) and Quinine (QN) [25]. QN is the drug of choice for complicated malaria such as cerebral malaria but resistance to the drug has been widely reported and also the drug produces high toxicity when administered intravenously. Another drug of choice is primaquine (PQ) which is characterized by poor oral bioavailability and drug related side effects (SEs) that can lead to haemolytic anaemia, gastrointestinal disturbances, heart failure and abdominal cramps [18, 26].




Quinine




Chloroquine

**Figure 1: Some of the Aminoquinoline compounds used in malaria treatment [27, 28]**

Resistance to these drugs by the Plasmodium strains, and the high toxicity effects associated with them has reduced the popularity of these drugs in malaria treatment. This has led to the need to device new methods of malaria therapy and the search for the most effective drugs, and/or drug combinations that would reduce morbidity and mortality of the disease.

## Current trend in the treatment of malaria

In 1967, the government of the People's Republic of China embarked on a systematic examination of indigenous plants used in traditional remedies as sources of drugs. One such plant, a pervasive weed with a long history of use is known as *qing hao* (*Artemisia annua* L., sweet wormwood, annual wormwood) [29, 30]. Artemisinin (ART) is proven to be a sesquiterpene, a natural product composed of fifteen carbon atoms based on three isoprene molecules usually joined head-to-tail, with additional oxygen atom functionality. The really striking feature of ART was the peroxide bridge spanning one of the molecule's rings, which was shown to be its active ingredient, responsible for its activity against plasmodium [31].

The artemisinins including artesunate (AS), artemether (ARM), arteether (AE) and dihydroartemisinin (DHA) are the most effective anti-malarial drugs known today. They possess a remarkably wide therapeutic index. They have the ability to rapidly kill a broad range of asexual parasite stages at safe concentrations that are consistently achievable through standard dosing regimens [13]. ART and its derivatives are considered the keystones of the treatment of *P. falciparum* malaria due to their high potency and

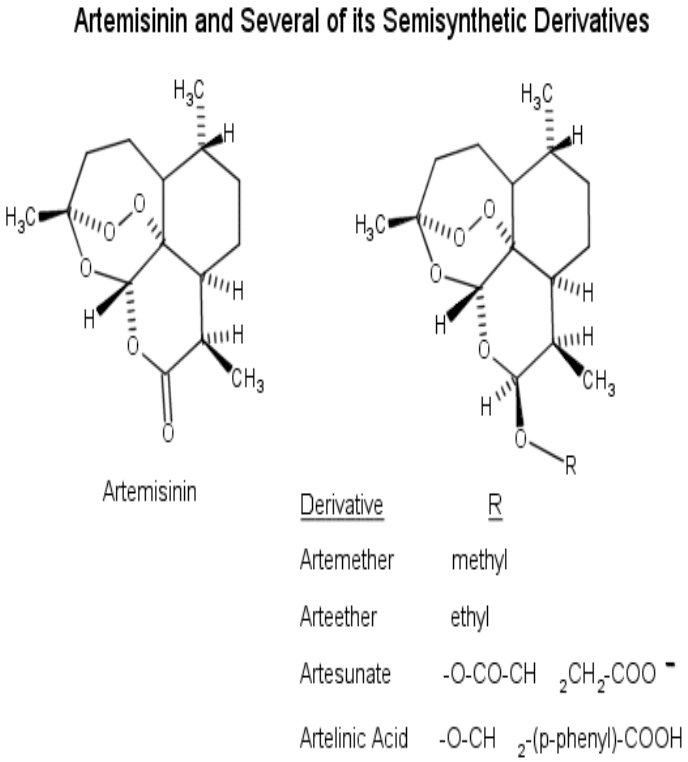



**Figure 2: Artemisinin and its derivatives [29 and 31].**

rapid action [32]. They have gametocytocidal properties by inhibiting parasite transmission which probably reduces the development of resistance [33]. Despite these achievements, there are reported cases of *in vitro* tolerance in South America [34], and in South-East Asia [18, 35]. Furthermore, stable resistance to ART drugs has been achieved independently in two different rodent malaria models [36, 37]. To minimize resistance, the World Health Organization, WHO has recommended ART to be used to treat uncomplicated malaria in combination with other anti-malarial drugs in the so-called artemisinin-based combination therapy (ACT). 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 [38, 18]. 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. There are also physicochemical and biopharmaceutical problems of the artemisinins (ARTs) such as short half-life, poor oral bioavailability and low solubility [39]. Furthermore, the reported advances recorded by the ACTs are now being threatened by low sensitivity of the parasites to ACTs in South-East Asia [40, 17, 18].

Against these backdrops, there is an urgent need to develop highly efficacious formulations of the ARTs, and especially the ACTs, to enhance their oral solubility and pharmacokinetic profiles. This would reduce the high trend of malaria morbidity and mortality, and save the world from the catastrophe of non-availability of highly efficacious ACT formulations to combat this deadly parasitic disease.

## Particulate drug carriers in malaria chemotherapy

Due to lack of economic incentives, there are not many initiatives for the development of new anti-malarial agents. This situation led to the smart and effective utilization of the current anti-malarial agents with the help of novel drug delivery systems (NDDS) [14]. The main goal of malaria therapy is to promote a high drug concentration in the intracellular parasitophorous vacuoles where the plasmodium is hosted [13]. Thus, the major setbacks of conventional malaria chemotherapy is the development of multiple drug resistance and the non-specific localization to intracellular parasites, resulting in high dose requirements and subsequent intolerable side effects which eventually lead to patient non-compliance [26, 13]. Hence, to improve the delivery of anti-malarials drugs researchers have developed and evaluated many particulate drug carriers which are mainly lipid-based (e.g. liposomes, nanoparticles, microparticles), and polymer-based (e.g. dendrimers and nanocapsules) [14]. These drug carriers are known to improve the efficacy of currently available anti-malarial drugs and also contribute to the formulation and delivery of new chemical entities. Drug targeting might have great advantage in malaria since malaria parasites frequently develop drug resistance due to the administration of drugs in concentrations which might be low in the presence of a high parasite load. Furthermore, particulate drug carriers have the potential to restore the use of old and toxic drugs by modifying their biodistribution, improve their bioavailability and reduce their toxicity [13, 18]. These advantages are of immense importance to malaria chemotherapy, since the development of new dosage forms for delivering drugs to parasite infected cells is urgently needed, especially for the anti-malarials in clinical use [41, 18].

### Particulate Drug Delivery Systems (PDDS) used in malaria

Early treatment with effective anti-malarial drugs is the main life-saving intervention in malaria therapy. PDDS such as liposomes and nanoparticles have been studied for intracellular infections because they are able to deliver the drug to the specific target in the human body, where the parasite is located, such as tissues (spleen and liver) and cells (macrophages and kupffer cells)[42-44]. Some of these particulate systems applied in the delivery of anti-malarial drugs will be discussed below.

### Liposome systems as anti-malarial carriers

Liposomes, first described in 1976, were the first type of particulate drug delivery system applied in disease therapy [45, 18]. These are self-assembling spherical, closed colloidal structures composed of phospholipid bilayers that surround a central aqueous space. These amphiphilic phospholipid molecules form a closed bilayer sphere, shielding the hydrophobic groups from the aqueous

environment, while maintaining contact with the aqueous phase through the hydrophilic head groups [46, 18]. Liposomes are classified into three basic types based on their size and number of bilayers. Multilamellar vesicles (MLVs) consist of several lipid bilayers separated from one another by aqueous spaces. These entities are heterogeneous in size, often ranging from a few hundred to thousands of nanometers in diameter. On the other hand, both small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) consist of a single bilayer surrounding the entrapped aqueous space. SUVs are less than 100 nm in size whereas LUVs have diameters larger than 100 nm [47, 48]. The predominant physical and chemical properties of a liposome are based on the net properties of the constituent phospholipids, including permeability, charge density and stearic hindrance. Drug loading into liposomes can be achieved through:

Liposome formation in an aqueous solution saturated with soluble drug
The use of organic solvents and solvent exchange mechanisms
The use of lipophilic drugs
pH gradient methods [49]

Liposomes have been widely reported to be used for drug delivery and drug targeting [51-53]. Some biologically active compounds have been encapsulated using liposomes [54, 55]. It has also been reported by Allison *et al* [56] and Alving *et al* [57] to be used as immunological adjuvants in vaccination.

Anti-malarial drugs such as Chloroquine (CQ), Quinine (QN), Primaquine (PQ), Artesunate (AS), Artemether (ARM), Arteether (AE) and very recently, a combination of Artemisinin (ART) and curcumin have been encapsulated in neutral conventional or long-circulating liposomes using different preparation techniques [58-65]. From the findings of these studies, the pH gradient technique seems to be the best for enhancing the encapsulation efficiency of anti-malarials. Investigations into the possibility of encapsulating drugs like QN and CQ in neutral large unilamellar vesicles (LUVs) applying the pH gradient method have been concluded and reported [58, 59, 13]. In the study, uptake of 148 and 104 nmol/μmol were reported after 15 min, and 81 and 88 nmol/μmol were also reported after 2 h for QN and CQ respectively. This finding showed that drugs such as QN and CQ were able to accumulate within these LUVs which exhibit a proton gradient. Bayomi *et al*, encapsulated AE for oral administration in neutral multilamellar liposomes [64]. In their study, multilamellar liposomes prepared with dibehenoylphosphatidylcholine (DBPC), cholesterol (CHOL) and AE at a ratio of 1:1:2 presented a mean size of 3.20 ± 1.03 μm and entrapment efficiency (EE) of 82.3 %. The daily release rate of AE from liposomes prepared with mixtures of DBPC and dipalmitoylphosphatidylcholine (DPPC) at a ratio of 1:1 was 0.818 %/day while it was 0.783 %/day when CHOL was added to DBPC at a ratio of 1:1, and 0.616 % when CHOL was used at a ratio of 1:2. These findings showed that the increase in the length of acyl chain of phospholipids as well as the addition of CHOL led to a decrease in the release rate of AE. This might be due to the ability of CHOL to induce drug/phospholipid interactions in the bilayer, leading to a decrease in drug release. Its *in vivo* evaluation compared with that of an oral aqueous suspension showed that orally administered liposomes of AE gave a relative bioavailability of 97.91 % while its oral aqueous suspension gave 31.83 %. This is shown in fig. 5. Gabriels and Plaizier-Vercammen have reported the encapsulation of AS in neutral liposomes using a pH 5 buffer solution as aqueous phase to prevent the aqueous instability of AS [62]. The EE of the AS-loaded liposomes was approximately 100 % and the liposomes remained stable for 10 days at 25 ºC. They reported that the release of the drug from the liposomes was influenced by the lipid content as the release rate decreased with increase in lipid concentration.

Furthermore, Chimanuka *et al*. reported the encapsulation of β-artemether (βAM) in neutral liposomes, and also evaluated its therapeutic efficacy in mice infected with *Plasmodium chabaudi* [63]. They reported an EE of about 100 % for their formulations, which also retained their stability for 3 months at 4 ºC. When administered to the infected *mice*, a 100 % cure was observed after 22 days of infection. Benedetta *et al*. reported the encapsulation of ART and a combination of ART and curcumin in conventional and PEGylated liposomes using the film hydration method [65]. In the study, ART conventional liposomes (A-CL) were formulated using Phospholipon® 90G (P90G), CHOL and ART. ART-loaded PEGylated liposomes (A-PL) were formulated using polyethyleneglycol-2000-distearoylphosphatidylethanolamine (PEG2000-DSPE), P90G, CHOL and ART. ART-Curcumin-loaded conventional liposomes (AC-CL) were formulated using P90G, CHOL, ART and curcumin while ART-Curcumin-loaded PEGylated liposomes (AC-PL) were formulated using PEG2000-DSPE, P90G, CHOL, ART and Curcumin. They reported that the mean diameter of all the ART-based vesicles was 200 nm and suitable for intraperitoneal administration. HPLC analysis of the vesicles gave EE of 78 % and 68 % for conventional liposomes and PEGylated liposomes respectively. The EE of curcumin showed that the EE for AC-PL was smaller than that of AC-CL. This could be due to the

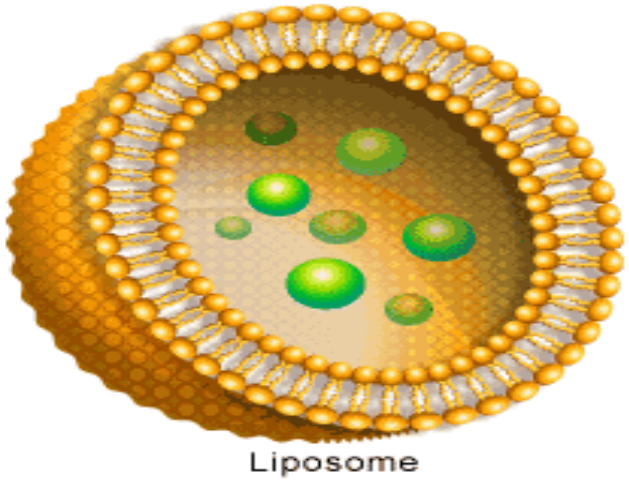



**Figure 3: Schematic representation of a liposome [50]**

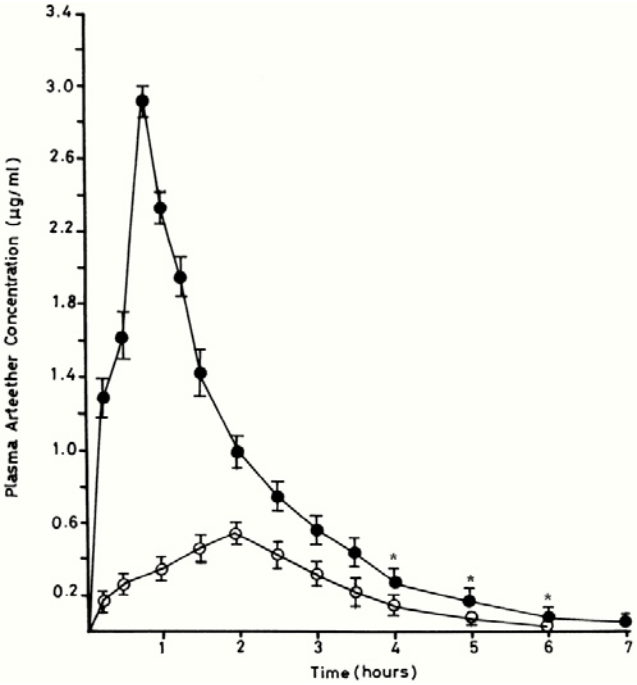



**Figure 4: Mean plasma concentration versus time profile of oral arteether liposomes and its aqueous suspension [64]**

smaller size of PEGylated liposomes which decreases the bilayer capacity of solubilizing lipophilic drugs. All the ART-loaded vesicles remained stable for a period of 1 month. When the ART-loaded vesicles were administered in mice infected with *Plasmodium berghei,* parasitaemia was reduced faster with AC-PL to about 60 % after only 3 days. In all liposomal treatments, parasitaemia was reduced more than 95 % from day 5. Interestingly, the infection was almost totally reverted in mice treated with A-CL after 7 days and with AC-CL, A-PL and AC-PL after 5 days. This is as shown in table 2.

On the basis of the above studies, liposomes have been extensively used as an effective carrier of anti-malarial drugs in the treatment of experimental malaria. This could be explained from their ability to reduce the toxicity profile of these chemical entities, show improved experimental therapeutic efficacy against the *Plasmodium* strains, modify the bioavailability of these drugs as well as ensure prolonged *in vivo* release.

**Table 2. Percentage reduction of parasitaemia in *P. berghei* infected mice treated with various ART-loaded liposomes [65].**

| Day | Positive Control (%) | A (%) | A-CL (%) | AC-CL (%) | A-PL (%) | AC-PL (%) |
|---|---|---|---|---|---|---|
| 3 | 67.39 | 60.86 | 35.86 | 11.95 | 22.82 | 60.86 |
| 5 | 100.00 | 37.68 | 96.01 | 98.84 | 97.39 | 97.24 |
| 7 | 94.79 | 80.59 | 99.49 | 98.79 | 97.04 | 98.05 |
| 9 | 96.47 | 51.94 | 100.00 | 98.68 | 98.26 | 95.61 |
| 12 | 99.02 | 90.92 | 98.36 | 98.95 | 93.73 | 99.00 |

Notes: A: ART, A-CL: ART conventional liposomes, A-PL: ART-loaded PEGylated liposomes, AC-CL: ART-Curcumin-loaded conventional liposomes, AC-PL: ART-Curcumin-loaded PEGylated liposomes

## Nanocarriers for anti-malarial drug delivery

Nanotechnology-based drug delivery systems for malaria have been evaluated since they are able to deliver the drug to the specific target in the human body where the malaria parasite is located. Some of these novel drug delivery systems (NDDS) could be used for both the active and passive targeting of the anti-malarials to the site where the parasite is located. Conventional nanocarriers such as liposomes, polymeric nanoparticles, and surface-modified long-circulating nanocarriers like polyethyleneglycol (PEG)-coated particles, could be employed in passive targeting [66, 13]. Nanocarriers are useful tools to improve the pharmacokinetic profile of effective drugs that have limited pharmacotherapeutic application due to high toxicity, low bioavailability and poor water solubility [67-69]. The major objective in using nanocarriers as drug delivery systems (DDS) is to promote drug or vaccine protection against extracellular degradation, to improve selectivity in relation to the target, to reduce the frequency of administration and the duration of the treatment and to improve the pharmacokinetic profile of the drug [68, 70, 71, 13]. The most important property of a nanocarrier in malaria therapy is the ability to remain in the blood stream for a long period of time in order to improve the interaction with infected red blood cells (RBCs) and parasite membranes, protection of unstable drugs, cell-adhesion properties, and the ability to be surface-modified by conjugation of specific ligands [72, 73, 13].

Lipid nanocarriers such as nanostructured lipid carriers (NLC) have been reported to be very effective and efficient for the parenteral delivery of artemether (ARM) [74]. NLC are lipid nanocarriers based on the mixture of biocompatible solid lipid and liquid lipid (oil). They are very good alternatives to liposomes and nanoemulsions due to their ease of manufacture, particulate nature, high drug loading and ability to sustain the release of the drug [75]. Also, the aqueous nature of NLC, their nanostructure and the biocompatibility of the excipients would enable intravenous delivery of active drugs with concomitant reduction or abolishment of pain on injection. Furthermore, their ability to sustain the delivery of therapeutic agents could be useful in combating the recrudescence which is commonly observed with ARM therapy [76]. Joshi *et al.* formulated NLC of ARM and tested it *in vivo* using *P. berghei* infected-mice [74]. The NLC prepared by the microemulsion template technique [77] using mixtures of the lipids and surfactants had an average particle size of 63 ± 28 nm, while the EE was 30 ± 2 %. This result indicated that the encapsulated drug would be released in a sustained manner which may help to prevent recrudescence. The *in vivo* studies clearly demonstrated that both administered doses of the nanoject, ND I and ND II (Nanoject Dose I and II), were significantly more effective compared to the marketed formulation (Larither®). ND I showed very quick onset of action (~95 % anti-malarial activity) compared

to the marketed formulation (~45 % anti-malarial activity) on the 8th day.

Very recently, Yameogo *et al*. reported the intravenous delivery of artemisinin (ART) using self-assembled bio-transesterified cyclodextrin (CD)-based nanocarriers [78]. The carriers were used to deliver ART due to their size and ability to deliver lipophilic drugs appropriately. Their study was guided by the need to improve ART dosage by encapsulating it in CD esters-based nanocarriers, and to ensure a sufficient blood circulation time of the nanocarriers through their surface modification for targeted delivery to infected erythrocytes after systemic administration. However, *in vitro* study of ART-loaded nanocarriers against 3D7 (CQ-sensitive) and K1 (CQ-resistant) strains of *P. falciparum* indicated that the ART-loaded vesicles exhibited satisfactory *in vitro* activity against the different strains of *P. falciparum*. The findings suggested that neither additive nor antagonistic effect was observed when ART was nanoencapsulated in CD-based nanocarriers.

Lipophilic anti-malarial drugs have also been delivered using nanocarriers in form of nanoemulsions. Singh and Vingkar reported that Primaquine (PQ) nanoemulsions have been delivered orally against *P. berghei* infected-wistar rats [79]. Nanoemulsions are heterogenous systems comprised of two immiscible liquids in which one liquid is dispersed as droplets in another liquid [80]. The ingredients of nanoemulsions are well tolerated by the body owing to their structural and functional similarity with physiological lipids [81, 82]. The idea behind their study was that since nanoemulsions are easily taken up by lipoprotein receptors in the liver, they can be exploited for the encapsulation of PQ for targeted delivery to the liver. Lipid nanoemulsion was prepared using Ovathin 160 (egg lecithin), Topcithin 300 (soyabean lecithin liquid) and PQ. The formulation gave an encapsulation efficiency, EE of 95 %, and an *in vitro* release > 90 % in phosphate buffer pH 7.4. On oral administration to rats at a dose of 1.5 mg/kg/d, the parasitaemia was completely cleared in the animals leading to increased survival time of the test animals than those receiving the plain drug solution. Thus, lipid nanoemulsion of PQ could achieve significant higher drug levels in the liver after oral administration. While in circulation, the lipid nanoemulsion associates with Apo E due to its narrow size (20 – 200 nm), which would enhance the drug uptake by liver parenchymal cells, thereby leading to improved anti-malarial activity.

The above studies showed clearly that the fabrication of nanocarriers for the delivery of available anti-malarial drugs for the treatment of malaria (both CQ-sensitive and CQ-resistant) could be promising, and could serve as veritable alternative to enhance the pharmacokinetic and biodistribution performance of these drugs. These have rekindled the hope that malaria could be properly treated when antimalarial drugs are formulated and delivered in particulate nanocarriers, pending the discovery and availability of effective malaria vaccine for clinical use.

## Microparticulate carrier systems for anti-malarial drug delivery

One of the novel systems for the enhanced delivery of anti-malarial drugs is the microparticulate systems, which might be in the form of microemulsion or microspheres. For some years now, lipid microemulsions have been designed and formulated as a commercially feasible NDDS with the capability to improve oral bioavailability and therapeutic efficacy of several drugs. These microcarriers are thermodynamically stable, transparent, isotropic, low viscosity colloidal dispersions consisting of microdomains of oil and/or water stabilized by an interfacial film of alternating surfactant and co-surfactant molecules [83]. Microspheres on the other hand, are characteristically free-flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm [84]. Several anti-malarial drugs have been encapsulated into microcarrier systems. They include Halofantrine (Hf) [85] and Primaquine (PQ) [86, 14], however, they were not tested *in vivo*. Nishi and Jayakrishnan formulated PQ-conjugated gum Arabic microspheres using thermal denaturation process and dehydration which yielded as much as 75 % of PQ-loaded microspheres [86]. Thus, this formulation approach could be said to be reproducible. The microspheres were spherical, free-flowing with an average size below 2 µm. *In vitro* release of PQ from the microspheres in phosphate buffer system (PBS) at 37 ºC showed that matrices having a higher degree of oxidized gum Arabic experienced slow release due to the better drug conjugation onto the polymer because of the large number of aldehyde groups present e.g. only 100 % of PQ was released from 50 % oxidized matrix having a drug payload of 4.7 % while 30 % of drug was released from 20 % oxidized matrix with similar payload. Attama and Igbonekwu reported enhanced *in vitro* release of highly lipophilic drug Hf in three biorelevant media: simulated gastric fluid (SGF pH 1.2), simulated intestinal fluid (SIF pH 7.2) and phosphate buffer (pH 6.8) [85]. The model drug, Hf at different concentrations was loaded into surface-modified solid lipid microspheres using lipid matrix formed from goat fat and the phospholipid, phospholipon® 90H. The formulation gave discrete and spherical microspheres with particle size range of 33-34 µm and EE between 87 and 91 % after 3 months. The *in vitro* release showed sustained drug release from the lipid microspheres compared to the commercial drug, Halfan®.

The importance of NDDS is to deliver drug entities in such a manner that their pharmacokinetic profile such as solubility and bioavailability would be enhanced. From the above reported experimental studies on the delivery of anti-malarial drugs using NDDS, it could be rightly stated that microparticulate systems formulated using polymeric and lipidic materials could be good alternative for the improved delivery of these drugs. However, these studies should be stretched further to elicit the possible mechanism of anti-malarial activity. Studies should also be done to encapsulate other available anti-malarial drugs used in the clinic for malaria chemotherapy. This would make it easier to determine formulations that could be taken up for possible clinical trials.



## Dendrimers as nanoscopic carriers of anti-malarial drugs

Dendrimers are globular repeatedly branched macromolecules that exhibit controlled patterns of branching with multiple arms extending from a central core. They are used in drug delivery and imaging at a size range of 10 to 100 nm in diameter, and have improved the pharmacokinetic and pharmacodynamic properties of encapsulated drug, making them less susceptible to uptake by the reticuloendothelial system (RES) [87, 88, 18]. Unique structures of dendrimers include highly branched and well-defined globular structures with controlled surface functionality, adding to their potential as new scaffolds for drug delivery [89, 90]. Some dendrimers are formulated by incorporating carbohydrates in their structures. These glycodendrimers are of immense benefit for drug encapsulation and delivery. They can be classified basically as carbohydrate-coated, carbohydrate-centered and carbohydrate-based [91].

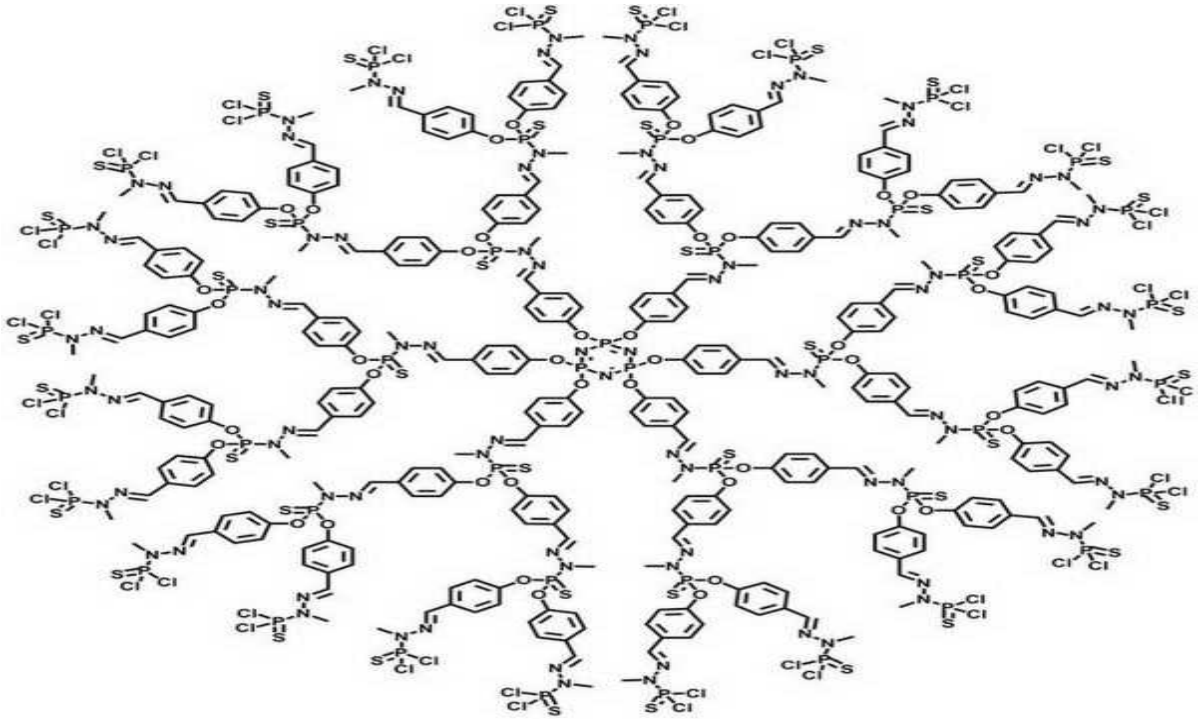


**Figure 5 : Schematic representation of a Dendrimer [92]**

Bhadra *et al.* have reported the formulation of glycodendrimers for hepatic targeting of PQ phosphate [91]. The glycodendrimers were obtained by the formulation of polypropyleneimine (PPI) dendrimers with ethylenediamine (ED) as the core coating it with galactose, and finally loading the drug by the equilibrium dialysis method. The haemolytic toxicity study of the dendrimers showed that carbohydrate coating drastically reduced the haemolysis of RBCs, which is a major limitation in the use of polycationic dendrimers in drug delivery. The reduction was due to covering of the cationic amine terminations responsible for haemolysis with carbohydrates. The biodistribution study (BDS) showed compared to 2.3 %, 1.5 % and 18.5 % of the free PQ found in the liver, spleen and blood after 2 h, carbohydrate coated PPI PQ dendrimers gave 50.7 %, 5.5 % and 7.8 % of the drug in the liver, spleen and blood after 2 h of administration. This significant delivery of PQ in the liver might be due to galactose coating because galactose receptors are found in the liver, which localized the coated systems and ensured sustained release of the drug from the dendrimers up to 2 h. In another study, CQ was loaded in PEGylated-poly-lysine type of dendrimers for sustained and controlled delivery of the drug through intravenous route of administration [93]. In that study, there was also a significant reduction in the *in vitro* levels of the trophozoite stages of *P. falciparum* when treated with the PEGylated-poly-lysine based dendrimers. When evaluated *in vivo* using albino rats, the system controlled and prolonged the blood levels of CQ in the rats after intravenous administration. From their study, the PEGylated dendrimers are also suitable for use as a safe and effective carrier for intravenous administration of CQ. Furthermore, ARM, an ART derivative was encapsulated in a PEGylated-lysine based copolymeric dendritic micelles for solubilization and delivery of ARM [94]. Transmission Electron Microscopy (TEM) of the dendrimers showed formulations in nanometric size range (5-25 nm), spherical and uniform shaped. Experimental *in vitro* release studies gave significant ($p<0.05$) release rates for the 5000D and 2000 D series respectively, but with the 5000 D series been slightly higher. This higher drug release might be due to the lower weight fraction of hydrophobic core in these polymers. This study showed that PEGylated-lysine

dendrimer systems could act as effective nanocarriers of anti-malarials.

These studies are very promising and have the potential of creating new dimensions in the use of anti-malarials chemotherapy, however more anti-malarial drugs should be encapsulated using dendrimers, especially the ACTs.

## Self-emulsifying carrier systems for anti-malarials

Self-emulsifying drug delivery systems (SEDDS) have gained significant interest due to their ability to increase solubility and bioavailability of poorly soluble drugs. SEDDS have been described as homogenous mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents [95-98]. The principal characteristic of these systems is their ability to form fine oil-in-water (o/w) emulsions or microemulsions upon mild agitation following dilution by aqueous phases. This property renders SEDDS as good candidates for the oral delivery of lipophilic drugs with adequate solubility in oil or oil/surfactants blends. SEDDS can be administered in soft or hard gelatin capsules, and will produce fine oil droplets/micelle dispersion upon capsule disintegration and aqueous dilution. Self emulsifying/dispersing formulations spread readily in the GIT, while the digested motility of the stomach and intestine provide the agitation necessary for self-emulsification/dispersion [99]. SEDDS formulations are characterized by *in vitro* lipid droplet sizes of 100 nm and above, and the dispersion has a turbid appearance [100]. The potential benefits of the use of SEDDS and self-microemulsifying drug delivery systems (SMEDDS) for improving the extent and reproducibility of the oral absorption of anti-malarial drugs have been reported.

Furthermore, a solid microemulsion pre-concentrate formulation loaded with artemether (ARM) known as NanOsorb, was recently formulated by Joshi *et al.* and its anti-malarial activity was evaluated in *P. berghei* (ANKA strain) infected-mice, and also compared with the activity of the marketed formulation of ARM (Larither®) and ARM solution [14]. ARM-NanOsorb system were formulated using the required amounts of surfactants, Gelucire 44/14® (Lauroyl macrogol glycerides) + Labrasol® (Caprylocaproyl macrogol-8-glycerides), and oil, capmul® MCM (Glyceryl mono-, di-caprylate) in the ratio of 3:2 after a formulation development study using the pseudo-ternary phase diagram. The *in vivo* anti-malarial study showed that ARM-NanOsorb system has an activity 2.6-fold and 2.3-fold higher than that of ARM solution and Larither® respectively. This clearly demonstrated the effectiveness of the microemulsion system in improving the therapeutic efficacy of ARM, and the animals treated with NanOsorb-ARM showed higher survival rate than that of ARM solution and Larither®.

This higher anti-malarial activity of the NanOsorb-ARM is a combined result of the nanosize (183 nm) of the microemulsion instantaneous dissolution of ARM which would facilitate quick

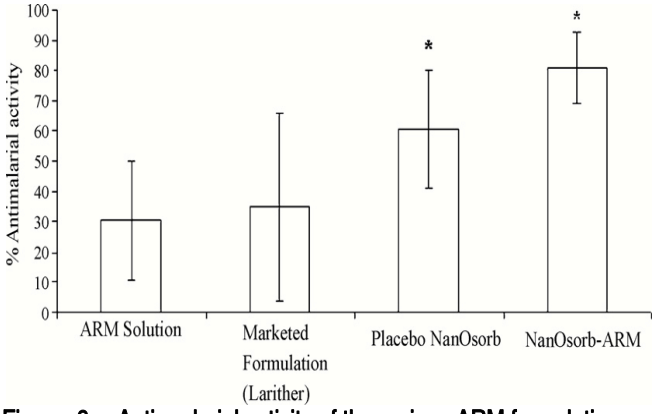



**Figure. 6: Anti-malarial activity of the various ARM formulations. (*) indicates significantly ($p<0.05$) higher than ARM solution and Larither® [14]. Note: ARM-Artemether.**

absorption, probable enhancement in bioavailability due to lipidic nature of the drug and protection of ARM from the acidic environment of the stomach. The bioavailability of Hf has been evaluated in beagle dogs using SEDDS and SMEDDS [101]. The particle sizes of the SMEDDS and SEDDS were 50 nm and 100 – 200 nm respectively. After administration to the dogs, the mean absolute bioavailability of Hf for all formulations administered ranged between 52 and 67 % representing approximately 6-to 8-fold improvement compared to the 8.6 ± 3 % bioavailability obtained from the commercial tablet formulation. The ability of the lipid-based formulations to deliver Hf in a solubilised and dispersed manner resulted in significant improvements in the bioavailability of Hf. Mandawgade *et al.* formulated SMEDDS-loaded with β-Artemether (βAM) using a natural lipophile or natural long chain triglyceride (N-LCT) [102]. N-LCT is a refined vegetable oil obtained from pressed fruit seed kernel; an edible oil with 1:2.37:1.36 ratio of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and triglycerides of $C_{16}$-$C_{18}$ fatty acids. The concept of this formulation and trial was that upon oral administration of the SMEDDS, they rapidly transform into microemulsions (MEs). The formulation was tested in *P. berghei* infected-mice and the results compared with that of the commercial brand, Larither®. *In vivo* results showed that there was very significant ($p < 0.05$) improvement in the anti-malarial activity of SMEDDS-loaded βAM against the lethal ANKA strain of *P. berghei* with an average parasitaemia of 35.11 ± 4.16 on day 20 of infection compared to Larither® with an average parasitaemia of 42.35 ± 4.18. To the best of our knowledge, the only particulate carrier system reported for the encapsulation of an ACT for experimental treatment of malaria was the one designed and developed by Kuentz *et al.* in collaboration with GlaxoSmithKline (GSK), UNICEF, the World Bank, Medicines for Malaria Venture and the WHO [103]. They formulated SEDDS-loaded with Chlorproguanil + Dapsone + Artesunate (CDA). After oral administration in rats, the self-emulsifying systems enhanced the bioavailability of the chemical entities, and proved promising in experimental treatment of malaria.

These studies have shown, interestingly, the potential of self-emulsifying systems to improve the oral solubility and bioavailability of lipophilic drugs. More anti-malarial drugs should be encapsulated and evaluated using this drug delivery system (DDS). However, to the best of our knowledge, no study have reported the encapsulation and delivery of anti-malarial drugs using self-nanoemulsifying drug delivery systems (SNEDDS) or solid-SNEDDS (s-SNEDDS) for the improvement of the bioavailability of these drugs, especially the combined therapy as in ACTs against experimental malaria. The results from the reported PDDS are very promising especially in the continued effort to halt resistance of *plasmodium* strains to available anti-malarial drugs until an effective malarial vaccine is discovered. However, further studies should be extended into determining the possible or exact mechanisms of action of these PDDS when used to encapsulate anti-malarial drugs so that it would be easier to solve any untoward effect(s) that might occur especially when possible clinical trials of the most promising of these formulations would be commenced.

## Conclusion

PDDS could be the future of malarial chemotherapy. This is because of the numerous opportunities it could provide for improving the efficacy of the current anti-malarial drugs used in malaria therapy, as well as possible new drugs characterized by poor solubility, bioavailability and high toxicity profile. There is urgent need to try possible modifications of the properties of already reported carrier systems so as to enhance their encapsulation and delivery properties. ACTs also have not been widely featured in the various studies reported. Since the WHO standard for malaria chemotherapy is the use of the ACTs, they should be extensively studied using any of the PDDS. Furthermore, the use of formulation excipients, especially natural materials that could be easily sourced from a particular locality should be encouraged. This would help to reduce the cost of formulation design and development, reduce or eliminate any possible untoward effect(s) that might result from the use of highly synthetic excipients, thereby leading to the development of smart, well-tolerated, cost effective and efficacious therapeutic particulate carrier systems for targeted delivery to treat malaria in the clinics.

## Conflict of interest

The authors wish to state that there is no conflict of interest and have not received any funding for this work.

## Authors' contributions

CU participated in conception of work, design of work, collection of data, analysis and interpretation of data, and drafting of manuscript. FK contributed in conception of work, data collection and analysis. EU assisted in data collection and analysis. SC helped in design of work and data collection. JA contributed in data collection and analysis. AA contributed in conception of work, analysis and interpretation of data, critical revision of manuscript, and approving the final version for publication.

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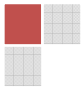

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