ABSTRACT

The present invention relates to novel nanodrug delivery system, with the targeted approaches for treating infectious diseases using combination of more than one pharmaceutical active ingredients formulated as single unit dosage form. The invention further relates to combining actives with potent activity, completely vivid host target sites and distinct kinetic profiles with the aim of improving delivery of these pharmaceutical active ingredients. More particularly the developed novel nanodrug delivery system involves engineered nanostructured carriers of lipophilic active drugs like antimalarial agentalong with a hydrophilic class of antibiotics. The invention thus relates to development of nano coformulation preconcentrate based on combination therapy for treating infections ranging from uncomplicated as well as severe malaria to cerebral malaria.

Dated this 13th day of November 2013

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CLAIMS

I Claim

1) A low dose pharmaceutical composition for treatment of malarial infection comprising a combination of ;
    d) lipophilic pharmaceutically active antimalarial agent ranging from 1% to 10 % w/w.
    e) One or more hydrophilic and/or lipophilic pharmaceutically active antimalarial agent ranging from 1% to 60 % w/w.
    f) lipidic nanocarriers system ranging from 1 % to 20 % w/w.

    Wherein the active drugs are entrapped in lipidic nanocarriers system to form aqueous based formulation when reconstitute in suitable solvent for targeted delivery of the drugs.

2) A low dose pharmaceutical composition as claimed in claim 1, wherein lipophilic antimalarial agent is preferably selected from the class of artemisinin.
3) A low dose pharmaceutical composition as claimed in claim 1, wherein at least one other antimalarial agent is selected from the group consisting of either lincomycin class of antibiotics and or with 4 –aminoquinolone, 8 –aminoquinolone, arylaminoalcohol, antifolates, respiratory chain inhibitors, aza acridine derivatives, diamidines, arylaminoalcohol class of antimalarials or any other class of drug showing potent in vitro antimalarial activity thereof class of antimalarials and or with antibiotics active against malaria parasites.
4) A low dose pharmaceutical composition as claimed in claim 1, wherein lipidic nanocarriers system comprising mixture of mono-,di- and triglycerides and of C8 - C18 fatty acids and of polyethylene glycol monoesters and di-esters with a hydrophilic/lipophilic balance (HLB) of less than 16; the preferred oils are medium chain mono/di/ triglyceride lipids Caproyl 90 (Propylene glycol monocaprylate), Capmul MCM (mono and diglycerides of medium chain fatty acids; caprylic and capric acids), Lauroglycol 90 (Propylene glycol monolaurate), Miglyol 812 (Triglyceride of fractionated coconut oil; C8-C10 fatty acids), Migloyl 840 (Propylene Glycol Di-

Caprylate Caprate), Captex 200 P(Propylene Glycol Dicaprylate/Dicaprate) , Captex 355(Triglyceride of Caprylic and Capric acid).

5) A low dose pharmaceutical composition as claimed in claim 4, wherein proportion of mixture of lipid is preferably in the ratio of 1:1.
6) A low dose pharmaceutical composition as claimed in claim 4, wherein Lipids are stabilized by mixture of Polysorbate esters and stearate based surfactants and co-surfactants, preferably in ratio of 1:1.
7) A low dose pharmaceutical composition as claimed in claim 6, wherein concentration of stabilizer is ranging from 1% to about 50 % of the lipid system.
8) A low dose pharmaceutical composition as claimed in claim 1, wherein the formulation is in the form of single unit preconcentrate for reconstitution with suitable solvent and administered by various routes like intravenous, intramuscular, oral or rectal.
9) A low dose pharmaceutical composition as claimed in claim 8, wherein the suitable solvent for reconstitution is selected from sterile water for injection, 0.9% sodium chloride and 5 % Dextrose injection.
10) A low dose pharmaceutical composition as claimed in claim 1, wherein Artemether when loaded in the preconcentrate exhibited double entrapment efficiency without second antimalarial agent load.
11) A low dose pharmaceutical composition as claimed in claim 1, wherein the second antimalarial agent is selected from a hydrophilic agent, it exhibiting sustained drug release and slower clearance.
12) A low dose pharmaceutical composition as claimed in claim 1, wherein the second hydrophilic antimalarial agent is selected from Antibiotic agent such as Clindamycin phosphate.
13) A low dose pharmaceutical composition as claimed in claim 1, wherein the ratio of first lipophilic antimalarial to second antimalarial agent is ranging between 1:1 to 1:10, preferably 1: 6 and the ratio of first to second to third antimalarial ranging from 1:1:1 to 1: 10 :1 preferably 1:6:1.

14) A low dose pharmaceutical composition as claimed in claim 1 to 13, wherein the dose of artemether is range from 1 mg to 50 mg and dose of Clindamycin range from 10 mg to 250mg.

15) A low dose pharmaceutical composition as claimed in claim 1 to 13, wherein the dose of artemether is range from 1 mg to 50 mg, dose of Clindamycin range from 10 mg to 250 mg and dose of Lumefantrine range from 6 mg to 120 mg.

16) A low dose pharmaceutical composition as claimed in claim 1 to 13, wherein the dose of artemether is range from 1 mg to 50 mg and dose of Lumefantrine range from 6mg to 120 mg.

17) A low dose pharmaceutical composition as claimed in claim 1 to 16, wherein the formulation is preferably administered once a day or as divided doses, once for four successive days whichever is applicable

18) A process for preparation of a low dose pharmaceutical composition as claimed in claim 1, comprising the steps of ;

e) Addition of lipophilic antimalarial drug in to the mixture of lipids and stabilizers
f) Melting the mixture with constant stirring,
g) Second antimalarial drug is added and vortexes to reconstitute the preconcentrate dispersion loaded with both drugs in suitable solvent.
h) Optionally freeze dried to form dry solid power.

Dated this 12th day of November 2014

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Applicant's Agent

FIELD OF INVENTION:

The present invention relates to novel nanodrug delivery system targeted for treating infectious diseases using combination of more than one pharmaceutical active ingredient formulated as single unit dosage form. The invention further relates to combining actives with potent activity, completely vivid host target sites and distinct kinetic profiles with the aim of improving delivery of these pharmaceutical active ingredients. More particularly the developed biocompatible novel nanodrug delivery system involves engineered nanostructured carriers of lipophilic active antimalarial along with a hydrophilic class of antibiotics formulated together for the treatment of malaria in patients with uncomplicated to severe malaria as well as in malaria infected pregnant women.

BACKGROUND OF INVENTION:

Although the existing treatments for malaria includes limited number of effective antimalarial drugs, most of which are water insoluble, with faster elimination half-life and resistant to most of the *Plasmodium* species affecting human population like *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae.* Out of these, *Plasmodium falciparum* and *Plasmodium vivax* are wide spread and cause significant mortality and morbidity associated with these types of infections. The implementation of judicious combination of such drugs is yet to fall in place, WHO malaria guidelines 2011 has adopted the use of chemotherapeutic agents in combinations wherein simultaneous administration of two or more antimalarial drugs with independent modes of action and different biochemical targets in the parasite along with different pharmacokinetic profiles - is thought to improve treatment efficacy and to delay the emergence of drug resistance to the individual components of the combination. In order to combat drug resistance issues, WHO has proposed a few commonly used antimalarial drug combinations like Artesunate plus Mefloquine, Artesunate plus Amodiaquine, Artesunate plus Sulfadoxine-pyrimethamine, Artemether plus Lumefantrine, Dihydroartemisinin plus Piperaquine and Artesunate plus Tetracycline or Doxycycline or Clindamycin (Guidelines for the treatment of malaria, second edition). The clinical trial data has shown that a total of 47 trials met the inclusion criteria and all five ACT combinations were shown to have failure rates of < 10% in line with WHO recommendations in uncomplicated *P. falicparum* malaria. In addition, most of these combinations have their own drawbacks. Clinical trials of these have also shown

that the use of Artesunate–Mefloquine in African children has caused excessive vomiting (associated with Mefloquine at the recommended dose of 25 mg/Kg) restricting its use. Moreover, reuse of Mefloquine within 60 days of first treatment is associated with an increased risk of neuropsychiatric reactions resulting in its exclusion from the second-line treatment regimen. Another clinical trial study indicates slowing of parasitological response to Artesunate and the associated increase in gametocyte carriage is of particular concern for combining Artesunate with Mefloquine (Carrara et al, 2009). Amodiaquine and Sulfadoxine-Pyrimethamine have low levels of resistance in some parts of Africa proving to be an effective option. However, Amodiaquine and Sulfadoxine-Pyrimethamine remain widely available as monotherapies providing continued selection pressure, and it is likely that resistance will continue to worsen despite the deployment of corresponding antimalarial combinations. Artesunate plus Tetracycline or Doxycycline or Clindamycin has a dosing regimen for seven days which is not well tolerated and adherence is likely to be poor. Doxycycline is preferred to other Tetracyclines because it can be given once daily, and does not accumulate in renal failure. But considering the side effects and high doses, treatment with Doxycycline only starts when the patient has recovered sufficiently, thus not making it an eligible candidate in cases of severe malaria. Clindamycin in that case proves to be safe and preferred drug for combination therapy.

Indian patent no 248977discloses a novel process of preparation of complex mixture of lipid nanoparticles to deliver actives from the class of antimalarial and anti-infective. It reports of single drug in nano formulation.

Another important drawback of the available antimalarial combinations is that none of them employ prolonged drug release technologies. Such prolonged release could help in reducing patient non-compliance responsible for disease recrudescence. Thus there is a need to formulate a composition that with an aim of developing newer drug combinations will reduce the drug dosage as well as enhance drug delivery.

Currently, since no product is available that enables intravenous delivery of ACTs, there is a need to have an aqua based intravenous formulation of ACTs that enables its quick availability to the body that can lead to quick eradication of the malarial infection with concomitant reduction in the pain on injection. Combination of atovaquone and proguanil (U.S 5,998,449) describes a

method for the treatment of malaria. Combination of fenozan with another anti-malarial agent selected from artemisinin, sodium artesunate, chloroquine, mefloquine (U.S. Pat. No. 5,834,505) is described for the prophylactic and curative treatment of malaria.With the emergence of *P. falciparum*strainsresistant to chloroquine and quinine, alternative antimalarial is required. The artemisinin, obtained from the plant *Artemisia anua,* and its derivatives are rapidly effective in severe malaria. Artemisinin drugs are very effective in causing rapid parasite clearance and fever resolution in malaria patient.

US20060141024 discloses synergistic combination kit of alpha,beta-arteether, sulfadoxin and pyrimethamine for treatment of severe/multi-drug resistant cerebral malaria wherein individual dose has to be administered.

WO2013018069 relates to combination of antimalarial agents from the group consisting of Artemisinine and derivatives thereof, such as Artemisinine, Dihydroartemisinine, Artemether, Arteether, Artesunate, Artelinate, Artemisone, Artelinic acid and other antimalarial agent is selected from the group consisting of antibiotics active against malaria parasites such as Rifampicin, Doxycycline, Clindamycin, and Azithromycin.

1353/MUM/2006 discloses the development of lipid nanoparticulate based dosage forms of antiparasitics and anti-infective, which are suitable by oral, transmucosal, rectal and parenteral routes of administration to patients.

Another drawback with antimalarial dug therapy is that malaria treatment has been neglected for pregnant women being particularly vulnerable to malaria as pregnancy reduces a woman's immunity to malaria, making her more susceptible to malaria infection and increasing the risk of illness, severe anemia and death. However till date there is no safe combination for treating severe malaria in pregnant women. Preclinical studies have consistently shown that artemisinin and its derivatives do not exhibit mutagenic or teratogenicity activity, (Assessment of the safety of artemisinin compounds in pregnancy).In light of these reports, co-formulating Artemether (a potent and rapidly acting antimalarial agent which is enlisted in WHO List of Essential medicines for the treatment of severe multi resistantmalaria) along with Clindamycin phosphate (a lincosamide antibiotic with anti-malarial activity against *P.falciparum)*or even as monotherapy as a target specific lipidic pharmaceutical composition with increased drug bio

disposition, no recrudescence ,increased safety and painless intravenous nano co-formulation was the need of the hour.

As evident from the prior art, it becomes challenging to formulate a composition with nano formulation containing combination of effective anti-malarial agents. Thus, the present invention holds an advantage of combining actives with potent activity, completely vivid host target sites and distinct kinetic profiles with the aim of improving delivery of these pharmaceutical active ingredients. Hence the present inventor has developed a biocompatible novel nanodrug delivery system which involves engineered nanostructured carriers of lipophilic active antimalarial along with a hydrophilic class of antibiotics formulated together for the treatment of malaria in patients with uncomplicated to severe malaria as well as in malaria infected pregnant women.

The said pharmaceutical composition is formulated in single unit dry sterilized powder to be reconstituted with suitable solvent/excipients to administer it by various routes like intravenous, intramuscular, oral or rectal. The preferred solvents for reconstitution include sterile water for injection, 0.9% sodium chloride and 5% Dextrose injection.

The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

OBJECTIVES OF THE INVENTION

- The main objective of the present invention is to develop Nano formulation with combination of drugs for treatment of parasite infection.
- Another objective of the present invention is to develop a nano formulation that can be administered by various routes like intravenous or intramuscular or oral or rectal to achieve better patient compliance.
- Another objective of the present invention is to develop a formulation with increased drugs bioavailability.

- Yet another objective of the present invention is to develop a nano-coformulation of Artemether with increase in stability of drug thereby increasing the shelf life.
- Yet another objective of the present invention is to develop a nano-coformulation of Artemether with reduced dosing frequency and improving patient compatibility over the present conventional dosage forms
- Further objective of the present invention is to develop a nano-coformulation of Artemether with a novel system wherein two drugs are formulated in an aqueous based nanostructured lipid carrier.
- Further objective of the present invention is to develop a nano-coformulation of Artemether with sustained release of drugs from the nanocarriers matrix thus maintaining plasma drug concentrations for longer periods.
- Further objective of the present invention is to develop a nano-coformulation of Artemether thereby reducing dose related side-effects and achieves complete parasite clearance.
- Further objective of the present invention is to develop a nano-coformulation of Artemether by dose reduction of both the drugs, thus being effective in all types of malaria.

SUMMARY OF INVENTION

The present invention relates to novel nanodrug delivery system, targeted for treating infectious diseases using combination of more than one pharmaceutical active ingredient formulated as single unit dosage form. More particularly the developed biocompatible novel nanodrug delivery system involves engineered nanostructured carriers containing combination of lipophilic active antimalarial along with another hydrophilic and/or lipophilic class of antimalarial formulated together for malaria treatment.

According to the present invention, there is provided an anti-malarial pharmaceutical composition comprising combination of;

a) lipophilic pharmaceutically active antimalarial agent, preferably from class of artemisinin
b) One or more hydrophilic and/or lipophilic pharmaceutically active antimalarial agent

c) Mixture of mono-,di- and triglycerides and of C8 - C18 fatty acids and of polyethylene glycol monoesters and diesters with a hydrophilic/lipophilic balance (HLB) of less than 16.

The invention also describes the process of manufacturing these compositions. As per the present invention, Artemether (ARM) is dissolved in molten lipid mix to yield desired concentration in lipid load along with at least one more anti-malarial agent not limiting to but are selected from the group consisting of either lincomycin class of antibiotics like clindamycin and or with 4 – amino quinolone, 8 –aminoquinolone, arylaminoalcohol, antifolates, respiratory chain inhibitors, azaacridine derivatives, diamidines, arylaminoalcohol class of antimalarials, at a required concentration, further added and vortexed to reconstitute the preconcentrate dispersion loaded with both drugs.

Artemether when loaded in the preconcentrate exhibited enhanced entrapment efficiency, which is nearly double as compared to the nano redispersion without second agent load. The hydrophilic antibiotic agent such as Clindamycin phosphate was also entrapped in the lipid matrix thus exhibiting sustained drug release and slower clearance.

The fabrication technique is amenable to scale up for commercialization and possessed size <100 nm with poly dispersity index below 0.5 and neutral surface charge. Artemether loaded in the preconcentrate exhibited entrapment efficiency nearly double (range being 20-70 %) as compared to the nanoredispersion without Clindamycin load. Clindamycin phosphate was also entrapped in the range of 10-50 % in the lipid matrix thus exhibiting sustained drug release and slower clearance.XRD studies showed amorphization of both the actives loaded in the Nanocomposition.

STATEMENT OF INVENTION

The present invention relates to a low dose pharmaceutical composition for treatment of malarial infection comprising a combination of;

a) lipophilic pharmaceutically active antimalarial agent ranging from 1% to 10 % w/w, preferably selected from the class of artemisinin.

b) One or more hydrophilic and/or lipophilic pharmaceutically active antimalarial agent ranging from 1% to 60 % w/w.
c) lipidic nanocarriers system ranging from 1 % to 20 % w/w.

Wherein the active drugs are entrapped in lipidic nanocarriers system to form aqueous based formulation when reconstitute in suitable solvent for targeted delivery of the drugs.

At least one other antimalarial agent is selected from the group consisting of either lincomycin class of antibiotics and or with 4 –aminoquinolone, 8 –aminoquinolone, arylaminoalcohol, antifolates, respiratory chain inhibitors, aza acridine derivatives, diamidines, arylaminoalcohol class of antimalarials or any other class of drug showing potent in vitro antimalarial activity thereof class of antimalarials and or with antibiotics active against malaria parasites. The lipidic nanocarriers system comprising mixture of mono-,di- and triglycerides and of C8 - C18 fatty acids and of polyethylene glycol monoesters and di-esters with a hydrophilic/lipophilic balance (HLB) of less than 16; the preferred oils are medium chain mono/di/ triglyceride lipids Caproyl 90 (Propylene glycol monocaprylate), Capmul MCM (mono and diglycerides of medium chain fatty acids; caprylic and capric acids), Lauroglycol 90 (Propylene glycol monolaurate), Miglyol 812 (Triglyceride of fractionated coconut oil; C8-C10 fatty acids), Migloyl 840 (Propylene Glycol Di- Caprylate Caprate), Captex 200 P(Propylene Glycol Dicaprylate/Dicaprate) , Captex 355(Triglyceride of Caprylic and Capric acid). The proportion of mixture of lipid is preferably in the ratio of 1:1. Lipids are stabilized by mixture of Polysorbate esters and stearate based surfactants and co-surfactants, preferably in ratio of 1:1. The concentration of stabilizer is ranging from 1% to about 50 % of the lipid system. The formulation is in the form of single unit preconcentrate for reconstitution with suitable solvent and administered by various routes like intravenous, intramuscular, oral or rectal. The suitable solvent for reconstitution is selected from sterile water for injection, 0.9% sodium chloride and 5 % Dextrose injection. Artemether when loaded in the preconcentrate exhibited double entrapment efficiency without second antimalarial agent load. The second antimalarial agent is selected from a hydrophilic agent, it exhibiting sustained drug release and slower clearance. The second hydrophilic antimalarial agent is selected from Antibiotic agent such as Clindamycin phosphate. The ratio of first lipophilic antimalarial to second antimalarial agent is ranging between 1:1 to 1:10, preferably 1: 6. and the ratio of first to second to third antimalarial ranging from 1:1:1, to 1:10:1 preferably 1:6:1. The

dose of artemether is range from 1 mg to 50 mg and dose of Clindamycin range from 10 mg to 250 mg.The dose of artemether is range from 1 mg to 50 mg, dose of Clindamycin range from 10 mg to 250 mg and dose of Lumefantrine range from 6 mg to 120 mg. The dose of artemether is range from 1 mg to 50 mg and dose of Lumefantrine range from 6 mg to 120 mg. The formulation is preferably administered once a day or as divided doses, once for four successive days whichever is applicable.

A process for preparation of a low dose pharmaceutical composition comprising the steps of ;

a) Addition of lipophilic antimalarial drug in to the mixture of lipids and stabilizers
b) Melting the mixture with constant stirring,
c) Second antimalarial drug is added and vortexes to reconstitute the preconcentrate dispersion loaded with both drugs in suitable solvent.
d) Optionally freeze dried to form dry solid power.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures and in which:

Figure No 1: Drug-free developed nanocarriers are selectively taken up by iRBCs. Colocalization of the nuclear stain (DAPI) and tagged nanocarriers shows the selective uptake by infected RBCs unlike uninfected RBCs.

Figure No 2: Graph of Intravenous efficacy for ARM-CP Co-formulation of Example 1. The% Parasitemia observed in *P.berghei* infected mice. All values are expressed as Mean ± SD. n=8. One-way ANOVA followed by Bonferroni's test is applied for statistical analysis (*$p<0.05$).

Figure No 3: Graph of Oral efficacy for ARM-LFN Co-formulation of Example 2. The% Parasitemia observed in P. berghei italics infected mice. All values are expressed as Mean ± SD. n=8. One-way ANOVA followed by Bonferroni's test is applied for statistical analysis (***$p<0.001$ for ARM-LFN NLC 1/20, ARM-LFN NLC 1/10, ARM-LFN NLC 1/5, compared to control).

Figure No 4: Drug Release profile of Artemether from ARM-CP Co-formulation of example 1. All values are expressed as Mean ± SD. n=6. One-way ANOVA followed by Bonferroni's test is applied for statistical analysis.*$p<0.05$

Figure No5: Drug Release profile of Clindamycin from ARM-CP Co-formulation of example 1. All values are expressed as Mean ± SD. n=6. One-way ANOVA followed by Bonferroni's test is applied for statistical analysis.*$p<0.05$.

DETAILED DISCRIPTION OF INVENTION

While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as "a", "an" and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

Many antimalarial drugs have poor aqueous solubility but are soluble in lipids. Hence they have poor and erratic bioavailability when given orally. If administered as oily injection they cause pain at the site of injection. In the present invention the preconcentrate is modified to deliver both lipophilic as well as hydrophilic pharmaceutical actives to patients with uncomplicated to severe malaria as well as in malaria infected pregnant women. The present invention provides an aqua based intravenous formulation of ACTs that enables its quick availability to the body that can lead to quick eradication of the malarial infection with concomitant reduction in the pain on injection.

Accordingly in one preferred embodiment, the present invention of nano-coformulation increases antimalarial efficacy of individual drug, it also effects to dose reduction and is well-tolerated in murine studies. The present invention also focuses on the selectively and target specificity of pharmaceutical composition and is therefore particularly suited for an effective combination based antimalarial therapy.

In one of the embodiment artemisinin includes class of antimalarial along with other antimalarial agent not limited to but are selected from the group consisting of either lincomycin class of antibiotics and or with 4 –aminoquinolone, 8 –aminoquinolone, arylaminoalcohol, antifolates,respiratory chain inhibitors, azaacridine derivatives, diamidines, arylamino alcohol class of antimalarials or any other class of drug showing potent *in vitro* antimalarial activity thereof, class of antimalarials and or with antibiotics active against malaria parasites.

In one of the embodiment Artemisinin is combined with different class of drug not limiting to but are selected from the group consisting class of antibiotics preferably clindamycin, Also combined with other antimalarial preferably chloroquine, Amodiaquine, like aminoquinolone preferably Primaquine, Tafenoquine, like arylaminoalcohol preferably quinine, mefloquine, halofantrine ,Lumefantrine, like antifolates preferably Sulfadoxine-pyremethamine, like respiratory chain inhibitors preferably Atovaquone /proguanil , like antibiotics preferably Clindamycin, Doxycycline ,tertracyclins, azithromycin, like azaacridine derivatives ,diamidines or any other class of drug showing potent *in vitro* antimalarial activity thereof.

In another embodiment, the lipophilic phase of preconcentrate comprises of a mixture of mono-,di- and triglycerides and of C8 - C18 fatty acids and of polyethylene glycol monoesters and di-esters with a hydrophilic/lipophilic balance (HLB) of less than 16; the preferred oils are medium chain mono/di/ triglyceride lipids Caproyl 90 (Propylene glycol monocaprylate), Capmul MCM (monoand diglycerides of medium chain fatty acids; caprylic and capric acids), Lauroglycol 90 (Propylene glycol monolaurate), Miglyol 812 (Triglyceride of fractionated coconut oil; C8-C10 fatty acids), Migloyl 840 (Propylene Glycol Di- Caprylate Caprate), Captex 200 P(Propylene Glycol Dicaprylate/Dicaprate) , Captex 355(Triglyceride of Caprylic and Capric acid), or it can

be a mixture of any two or more , preferably in the ratio of 1:1. The amount of lipophilic phase used in accordance with the present invention from about 1% to about 20 %.

Lipids are stabilized by mixture of Polysorbate esters and stearate based surfactants and co-surfactants, preferably in the ratio of 1:1. The amount of stabilizer used in accordance with the present invention from about 1% to about 50 %

According to the present invention, there is provided an anti-malarial pharmaceutical composition comprising combination of ;

a) lipophilic pharmaceutically active antimalarial agent, preferably from class of artemisinin
b) One or more hydrophilic and/or lipophilic pharmaceutically active antimalarial agent
c) Mixture of mono-,di- and triglycerides and of C8 - C18 fatty acids and of polyethylene glycol monoesters and diesters with a hydrophilic/lipophilic balance (HLB) of less than 16.

The fabrication technique is amenable to scale up for commercialization and possessed size <100 nm with polydispersity index below 0.5 and neutral surface charge.

The invention also describes the process of manufacturing these compositions. As per the present invention, Artemether (ARM) is dissolved in molten lipid mix to yield desired concentration in lipid load along with at least one more anti-malarial agentnot limiting to but are selected from the group consisting of either lincomycin class of antibiotics like clindamycin and or with 4 – aminoquinolone, 8 –aminoquinolone, arylaminoalcohol, antifolates, respiratory chain inhibitors, azaacridine derivatives, diamidines, arylaminoalcohol class of antimalarials, at a required concentration, further added and vortexed to reconstitute the preconcentrate dispersion loaded with both drugs.

Artemether when loaded in the preconcentrate exhibited enhanced entrapment efficiency, which is nearly double as compared to the nanoredispersion without second agent load.The hydrophilic Antibiotic agent such as Clindamycin phosphate was also entrapped in the lipid matrix thus exhibiting sustained drug release and slower clearance.

In an embodiment of the present invention wherein the ratio of Artemether and other antimalarial agent is ranging between 1:1 to 1:10, preferably 1: 6.and the ratio of first to second to third antimalarial ranging from 1:1:1, to 1: 10 :1 preferably 1:6:1

In another embodiment of the present invention the composition comprises a therapeutically effective amount of first lipophilic pharmaceutically active antimalarial agent or a pharmaceutically acceptable salt thereof at a concentration in the range from 1% to about 10 %, Whereas second hydrophilic and/or lipophilic pharmaceutically active antimalarial agent is present in said composition at a concentration in the range from about 1 % to 60 %.

The anti malarial product disclosed in the preferred embodiment of this invention is once a day product.

In accordance with one preferred embodiment of the invention, the average period of treatment with Artemether and Clindamycin combination equaled to 4 days (minimum 2 days to maximum 8 days).

Any reference herein before or hereinafter to pharmaceutical active compound Artemether, Clindamycin, Lumefantrine is to be understood as referring also to salts, especially pharmaceutically acceptable salts, of a compound as appropriate and expedient.

In particular the term "combination" according to the present invention means a fixed combination with defined amounts of the combinations partners. The combination of the present invention can be used as medicaments , e.g in the form of pharmaceutical composition for enteral (such as oral, rectal) or parenteral and including topical application or inhalation administration, and are suitable for the treatment and/or prevention of the diseases mentioned herein, such as especially malaria.

A novel feature of the present invention is the combination of Artemether and at least one antibiotics agent such as Clindamycin phosphate, which is being co-formulated in nanodrug delivery system with targeted delivery of drugs for treating infectious diseases.

Another novel feature of the present invention is that by using the combination of Artemether and at least one antibiotics agent such as Clindamycin phosphate and / or another anti malarial

agent such as Lumefantrine, the duration of treatment of patient is reduced thereby reducing hospitalization time of patients.

It has also been observed by the inventor that the low dose nano-coformulationof Artemether of the present invention has higher rate of success than the administration of component individually or in mere admixing co-formulations.

A low dose nano-coformulation of Artemether of the present invention comprises of dose of artemether is range from 1 mg to 50 mg and dose of Clindamycin range from 10 mg to 250 mg.

A low dose nano-coformulation of Artemether of the present invention comprises of dose of artemether is range from 1 mg to 50 mg, dose of Clindamycin range from 10 mg to 250 mg and dose of Lumefantrine range from 6mg to 120 mg.

A low dose nano-coformulation of Artemether of the present invention comprises of dose of artemether is range from 1 mg to 50 mg and dose of Lumefantrine range from 6 mg to 120 mg.

It has further been studied by the inventor that the formulation of present invention has better stability. Experimental study details also provided.

The present invention is further described with the help of the following examples, which are given by way of illustration all the parts, percent's and ratios are by weight unless otherwise indicated and therefore should not be construed to limit the scope of the invention in any manner.

EXAMPLES:

Ingredients of composition in weight percentage range or in other unit in following conditions:

Example 1:Preparation of Artemether- Clindamycin co-formulation

Formula (actives/excipients)	**% w/w**
Capmul MCM	12.43 %
Glyceryldilaurate	12.43 %
Tween 80	31.06 %
Solutol HS15	31.06 %
Artemether	1.87 %
Clindamycin	11.19 %

Artemether (ARM)is dissolved in molten lipid mix to yield desired concentration in lipid load along with Clindamycin Phosphate (CP), this is further added and vortexed to reconstitute the preconcentrate dispersion loaded with both drugs in suitable vehicle.

Example 2:Preparation of Artemether- Lumefantrine co-formulation

Formula (actives/excipients)	**% w/w**
Capmul MCM	10.31
Glyceryldilaurate	10.31
Oleic acid	20.62
Tween 80	25.77
Solutol HS15	25.77
Artemether	1.03
Lumefantrine	6.18

Artemether (ARM)is dissolved in molten lipid mix to yield desired concentration in lipid load along with Lumefantrine this is further added and vortexed to reconstitute the preconcentrate dispersion loaded with both drugs in suitable vehicle.

Example 3:Preparation of Artemether- Lumefantrine-Clindamycin co-formulation

Formula (actives/excipients)	**Range in % w/w**
Capmul MCM	1-20%
Glyceryldilaurate	1-20%
Oleic acid	2-40 %
Tween 80	2-50%
Solutol HS15	2-50%
Artemether	1-5%
Lumefantrine	1-20%
Clindamycin	1-20 %

Artemether (ARM) is dissolved in molten lipid mix to yield desired concentration in lipid load along with Lumefantrine and clindamycin this is further added and vortexed to reconstitute the preconcentrate dispersion loaded with drugs in suitable vehicle.

Example 4: Comparativedata for increased drugs bioavailability.

In vivo pharmacokinetics experiments were carried out in Wistar rats (n=6) of either sex were taken in each group. Animals were injected (**single dose**) i.v. via tail vein and blood was withdrawn at definite time points i.e. 0.08h, 0.17h, 0.67h, 1h, 2h, 6h and 12h post i.v. administration

a. *In vitro* **pharmacokinetics parameters of ARM from ARM-CP Co-formulation and ARM solution**

		pharmacokinetics parameters of Artemether (ARM)	
Parameter	**Units**	**ARM-CP Co-formulation of example 1**	**ARM Solution**
Cmax	ng/ml	402	275
Ke	h^{-1}	0.2	0.33
$t_{1/2}$	H	3.3	2.0
AUC	µg-L h^{-1}	1952	815
Vd	L	6.7	9.8
CL	L h^{-1}	1.3	3.3

Table No. 1

b. *In vitro* **pharmacokinetics parameters of CP from ARM-CP co-formulation and CP solution**

		pharmacokinetics parameters of Clindamycin (CP)	
Parameter	**Units**	**ARM-CP Co-formulation of example 1**	**CP Solution**
Cmax	ng/ml	238464	443316
Ke	h^{-1}	0.2418	0.47
$t_{½}$	H	2.86	1.47
AUC	µg-L h^{-1}	987.8	947.36
Vd	L	0.0679	0.0365
CL	L h^{-1}	0.0164	0.0171

Table No. 2

Estimates of pharmacokinetic parameters after intravenous dosing of ARM-CP NLCs are depicted in **tables** at doses of ARM and CP in example 1. The plasma concentration of ARM from NLCs (C_{max} = 402 ng/ml) was significantly higher as compared to that from solution (C_{max}= 275 ng/ml) as was observed at (t_{max}=0.08 h) first sampling point. The area under the curve (AUC) of ARM was also significantly higher in case of NLCs (1952 μg-L h^{-1}) as compared to solution. This indicated that ARM concentration in plasma in case of NLCs was significantly higher throughout as compared to ARM solution. Elimination half-life of ARM circulating in plasma was prolonged almost 1.68 folds in case of NLCs (3.4 h) as compared to its solution form (2h). Since the plasma concentration was higher for ARM in case of NLCs, the volume of distribution of ARM-CP NLCs was found to be lowered. Lower V_d with comparatively more plasma circulation of ARM in case of NLCs indicated more partitioning of drug in whole blood components especially RBCs. The total plasma clearance (K_e x V_d) of ARM was faster in case of solution (3.311 h^{-1}) as compared to NLCs (1.38 h^{-1}) indicating that the plasma residence time for ARM loaded in NLCs has increased significantly. Cmax of CP was found to be two folds higher in its solution form as compared to its NLC formulation because of its good water solubility which could easily attain high plasma concentration as against the delay developed by the entrapped CP for its release from lipidic matrix of NLCs. Half-life of CP was also significantly prolonged in NLCs (2.86 h) as compared to its solution form (1.47 h).

Therefore our fabricated nanocarriers of example 1 increased bioavailability of drugs (artemether /clindamycin) loaded in nanosystem because of their reduced particle size and improved absorption pattern as compared to individual drug solutions.

Example 5: <u>Stability Studies of formulation (Accelerated conditions).</u>

ARM-CP NLCs (example 1) were subjected to various storage conditions of temperature and humidity as per ICH guidelines as depicted in table. There were no observable physical or chemical changes as they appeared as transparent, homogenous liquids without any signs of phase separation on dilution with no signs of drug precipitation. Particle size evaluation and poly dispersity index was same and statistically insignificant as compared to samples evaluated on day 0 indicating shelf life of 2 years.

Data of Stability studies of ARM-CP Co-formulation (example 1) with respect to drug content (assay) and particle size

	Drug Content (%)					Globule Size (nm) (PDI)				
Storage Condition	**0 Days**	**30 Days**	**60 Days**	**90 Days**	**180 Days**	**0 Days**	**30 Days**	**60 Days**	**90 Days**	**180 Days**
25^0C/60 % RH	99. 01 ± 2.15	99.51± 1.98	99.0 ± 3.35	99.12 ± 2.95	98.01 ± 1.90	45 (0.358)	45 (0.317)	49 (0.351)	50 (0.375)	51 (0.372)
5^0 C	99 ± 2.15	99.83 ± 1.94	98.97 ± 2.24	98.11 ± 1.56	97.25 ± 1.22	43 (0.358)	50 (0.322)	50 (0.387)	46 (0.398)	54 (0.355)

Table No. 3

Example 6: <u>Data for reduced dosing frequency</u>

***A.* Intravenous efficacy data of formulation in example 1**

As shown in Figure 2; *In vivo* efficacy studies following clinical simulation of malaria in mice showed complete parasite clearance at 1/5th (T.D) ARM -1/5th (T.D) CP NLCs with 100 % antimalarial activity. This treatment group also showed significant antimalarial efficacy and potent activity as compared to cosolution of Artesunate and Clindamycin phosphate (therapeutic dose and $1/5^{th}$of therapeutic dose) and also against$1/5^{th}$ (T.D) ARM– NLCs and plain CP drug solution.

$1/5^{th}$ (T.D) ARM– 1/5th (T.D) CP NLCs exhibited significantly prolonged survival as compared to $1/5^{th}$(T.D) CP solution, Artesunate(T.D)–CP(T.D) solution and 1/5th (T.D) Artesunate–1/5th (T.D) CP solution (log rank, p=0.001). Thus $1/5^{th}$ (T.D) ARM– 1/5th (T.D) CP NLCs could

effectively clear parasitemia and prolong survival proving to be an effective nanocoformulation therapy for malaria treatment. Thus 20 % dose of ARM and CP both loaded in NLCs (1/5th (T.D) ARM -1/5th (T.D) CP NLCs group) could also be of potential benefit in treatment of malaria and complete parasite clearance thus claiming to be effective in early parasite clearance, improved drug bioavailability and reduced dosing frequency.

***B.* Oral efficacy data of formulation in example 2**

As shown in figure 3; The anti-malarial efficacy of formulations was tested in *Plasmodium berghei* infected murine model after oral administration. The animals were dosed once daily with the formulation as opposed to the currently recommended oral dosage regimen which includes twice a day dosing. Control mice received no treatment, whereas the marketed group received treatment with commercially available Artemether-Lumefantrine tablets. Blank NLC group received the NLC vehicle without any drug. The developed nanolipid formulation (ARM LFN NLC) containing Artemether and Lumefantrine showed complete parasite clearance at 1/20 of the oral dose of the drug combination thus resulting in 95 % dose reduction of both the drugs. Thus the disclosed nanolipid formulation reduced both dose of drugs and dosing frequency which would result in better patient compliance.

Example 7: <u>Dissolution Testing</u>

Release study of Artemether and Clindamycin from ARM-CP Co-formulation (example 1): As shown in figure 4 and 5: *In vitro* release test was performed using the dialysis bag technique for ARM-CP NLCs. *In vitro* dissolution profile of the ARM-CP NLCs exhibited a release profile with 49% ARM and 88.4% CP release in dissolution medium at the end of 24 h, indicating sustained release of drugs as compared to only ARM-CP solution wherein the release of both the drugs was much faster (ARM~ 62% and CP ~ 100%) in the same medium at the end of 24 h. Thus the fabricated nano-coformulation yielded a sustained drug release pattern for both the potent anti-malarial drugs having extreme physicochemical properties viz. ARM is a BCS class IV drug and CP being BCS class I drug with log P values of 3.46 and 1.04 respectively.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Dated this 12th November 2014.

phoke

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CLAIMS

I Claim

1) A low dose pharmaceutical composition for treatment of malarial infection comprising a combination of ;
    d) lipophilic pharmaceutically active antimalarial agent ranging from 1% to 10 % w/w.
    e) One or more hydrophilic and/or lipophilic pharmaceutically active antimalarial agent ranging from 1% to 60 % w/w.
    f) lipidic nanocarriers system ranging from 1 % to 20 % w/w.

    Wherein the active drugs are entrapped in lipidic nanocarriers system to form aqueous based formulation when reconstitute in suitable solvent for targeted delivery of the drugs.

2) A low dose pharmaceutical composition as claimed in claim 1, wherein lipophilic antimalarial agent is preferably selected from the class of artemisinin.
3) A low dose pharmaceutical composition as claimed in claim 1, wherein at least one other antimalarial agent is selected from the group consisting of either lincomycin class of antibiotics and or with 4 –aminoquinolone, 8 –aminoquinolone, arylaminoalcohol, antifolates, respiratory chain inhibitors, aza acridine derivatives, diamidines, arylaminoalcohol class of antimalarials or any other class of drug showing potent in vitro antimalarial activity thereof class of antimalarials and or with antibiotics active against malaria parasites.
4) A low dose pharmaceutical composition as claimed in claim 1, wherein lipidic nanocarriers system comprising mixture of mono-,di- and triglycerides and of C8 - C18 fatty acids and of polyethylene glycol monoesters and di-esters with a hydrophilic/lipophilic balance (HLB) of less than 16; the preferred oils are medium chain mono/di/ triglyceride lipids Caproyl 90 (Propylene glycol monocaprylate), Capmul MCM (mono and diglycerides of medium chain fatty acids; caprylic and capric acids), Lauroglycol 90 (Propylene glycol monolaurate), Miglyol 812 (Triglyceride of fractionated coconut oil; C8-C10 fatty acids), Migloyl 840 (Propylene Glycol Di-

Caprylate Caprate), Captex 200 P(Propylene Glycol Dicaprylate/Dicaprate) , Captex 355(Triglyceride of Caprylic and Capric acid).

5) A low dose pharmaceutical composition as claimed in claim 4, wherein proportion of mixture of lipid is preferably in the ratio of 1:1.
6) A low dose pharmaceutical composition as claimed in claim 4, wherein Lipids are stabilized by mixture of Polysorbate esters and stearate based surfactants and co-surfactants, preferably in ratio of 1:1.
7) A low dose pharmaceutical composition as claimed in claim 6, wherein concentration of stabilizer is ranging from 1% to about 50 % of the lipid system.
8) A low dose pharmaceutical composition as claimed in claim 1, wherein the formulation is in the form of single unit preconcentrate for reconstitution with suitable solvent and administered by various routes like intravenous, intramuscular, oral or rectal.
9) A low dose pharmaceutical composition as claimed in claim 8, wherein the suitable solvent for reconstitution is selected from sterile water for injection, 0.9% sodium chloride and 5 % Dextrose injection.
10) A low dose pharmaceutical composition as claimed in claim 1, wherein Artemether when loaded in the preconcentrate exhibited double entrapment efficiency without second antimalarial agent load.
11) A low dose pharmaceutical composition as claimed in claim 1, wherein the second antimalarial agent is selected from a hydrophilic agent, it exhibiting sustained drug release and slower clearance.
12) A low dose pharmaceutical composition as claimed in claim 1, wherein the second hydrophilic antimalarial agent is selected from Antibiotic agent such as Clindamycin phosphate.
13) A low dose pharmaceutical composition as claimed in claim 1, wherein the ratio of first lipophilic antimalarial to second antimalarial agent is ranging between 1:1 to 1:10, preferably 1: 6 and the ratio of first to second to third antimalarial ranging from 1:1:1 to 1: 10 :1 preferably 1:6:1.

14) A low dose pharmaceutical composition as claimed in claim 1 to 13, wherein the dose of artemether is range from 1 mg to 50 mg and dose of Clindamycin range from 10 mg to 250mg.

15) A low dose pharmaceutical composition as claimed in claim 1 to 13, wherein the dose of artemether is range from 1 mg to 50 mg, dose of Clindamycin range from 10 mg to 250 mg and dose of Lumefantrine range from 6 mg to 120 mg.

16) A low dose pharmaceutical composition as claimed in claim 1 to 13, wherein the dose of artemether is range from 1 mg to 50 mg and dose of Lumefantrine range from 6mg to 120 mg.

17) A low dose pharmaceutical composition as claimed in claim 1 to 16, wherein the formulation is preferably administered once a day or as divided doses, once for four successive days whichever is applicable

18) A process for preparation of a low dose pharmaceutical composition as claimed in claim 1, comprising the steps of ;

e) Addition of lipophilic antimalarial drug in to the mixture of lipids and stabilizers
f) Melting the mixture with constant stirring,
g) Second antimalarial drug is added and vortexes to reconstitute the preconcentrate dispersion loaded with both drugs in suitable solvent.
h) Optionally freeze dried to form dry solid power.

Dated this 12th day of November 2014

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