ABSTRACT:

The present invention discloses surface modified nanoparticles for targeted delivery of Antimalarial drug specifically to parasitized RBCs with improved therapeutic activity.

We claim,

1. *A pharmaceutical formulation comprises surface modified/functionalized lipid and/ or* protein nanoparticles of Antimalarial drug for targeted delivery, specifically to parasitized RBCs with improved therapeutic activity.

2. The pharmaceutical formulation according to claim 1, wherein the antimalarial drug is selected from Artemether and other artemisinin derivatives such as dihydroartemisinin, arteether and artesunate; lumefantrine, proguanil, atovaquone chloroquine, quinine, mefloquine, amodiaquin, quinine, Sulfedoxine and pyrimethamine.

3. The pharmaceutical pharmaceutical formulation according to claim 1, wherein the formulation comprises antimalarial drug in the range of 0.1 to 100 %w/w; lipid or protein in an amount of 0.1-10% w/w; surfactant/solubilizers in an amount of 0.1 to 10%W/V; surface modification agent and/or functionalizing agent in an amount of 0.001--5% in association with one or more pharmaceutical excipients.

4. The pharmaceutical formulation according to claim 1, wherein the surface modification agent is PEG 200-8000/m-PEG 200-8000 used in an amount of 0.1 to 5% and the functionalizing agent is selected from biotin, pantethenol, dextran or chondriotin sulpahate or any other suitable ligand used in the range of 0.001-1%w/v respectively.

5. The pharmaceutical formulation according to claim 1, wherein the protein is Human Serum albumin used in an amount of 0.1-5%w/w.

6. The pharmaceutical formulation according to claim 1, wherein the formulation is selected from the group consisting of solid, liquid/disperse phase dosage forms.

7. The pharmaceutical formulation according to claim 6, wherein the formulation is selected from the group consisting of tablet, capsule, powder for reconstitution, liquid, disperse phase system, emulsion, Lipid nanocarriers, nanoemulsion, nanocapsules, self-nanoemulsifying drug delivery systems (SNEDDS), polymeric nanocarriers, protein nanoparticles.

8. The pharmaceutical formulation according to claim 3, wherein the lipid is selected from the group consisting of GRAS Lipids including Triglycerides like Trimyristin, Tristearin, Tripalmitin, Tribehanin, trilaurin; Long chain fatty acids like: Stearic acid, Lauric acid, myristic acid; palmitic acid, behanic acid, Capric acid, Caprylic acid, Cerotic acid, archidic acid, lignoceric acid,Glyceryl mono and di-esters like Glyceryl palmitostearate, Glyceryl monostearate, Glyceryl behenate; Glyceryl laurate, Fatty alcohols like Capryl alcohol, Capric alcohol, Cerotyl, archidyl alcohol Cetyl alcohol, Stearyl alcohol, Myristyl alcohol, palmityl alcohol, Benhyl alcohol, lauryl alcohol, lignoceryl alcohol, behnayl alcohol, and Waxes like Ceresine, Hard fat, Microcrystalline waxes The formulation according to claim 3, wherein the surfactants are combination of lipophilic and hydrophilic selected from the group consisting of egg lecithin, phosphatidyl choline, soyabean lecithin, mixed soyabean phosphatides, glycerol Phosphatides, and polaxamers including polaxamer 124, polaxamer 188, polaxamer 237, polaxamer 407,Polysorbates, sorbitan esters,Polyoxytl Stearates, Polyoxyethylene Castor oil derivatives, polyoxyethylene alkyl ethers and the like.

9. The pharmaceutical formulation according to claim 3, wherein the solubilizers are selected from the group consisting of Macrogol-15-Hydroxy stearate, Polyoxyethylene sorbitan fatty acid esters including polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, Caproyl Propylene glycol mono-caprylate, Propylene glycol mono laurate, Polyglyceryl Oleate, Polyoxyl glycerides including Caprylocaproyl macroglycerides, lauryl macrogolglycerides, linoleoyl macrogolglycerides, oleoyl macrogolglycerides, stearoyl macrogol glycerides,Tricaprylin, Caprylic/capric Triglyceride, Trioelin, sorbitan esters, polyoxyethylene stearates, polyoxyethylene castor oil derivatives and polyoxyethylene alkyl ethers and the like.

10. The pharmaceutical formulation according to claim 1, wherein the particle size of the nanocarrier is in the range of 10-200nm.

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FIELD OF INVENTION:

This invention relates to surface modified nanoparticles for targeted delivery of Antimalarial drug specifically to parasitized RBCs with improved therapeutic activity.

BACKGROUND AND PRIOR ART:

Amongst antiparasitic agents, antimalarials are of great interest to researchers as malaria has always put a great burden on economy of tropical countries including India. Malaria is one of the most serious challenges to modern healthcare. Artemisinin and its derivatives are at present, the only effective drugs against drug resistant malaria. Artemether, arteether, artesunate are active against all plasmodium including those which may be resistant to other antimalarials. Artemether and other artimisin derivatives have very rapid blood schizonticidal activity. Schizonticidal activity is mainly due to destruction of the asexual erythrocytic forms of *P.falciparum* and *P. vivax* and active against the erythrocytic stage of multidrug-resistant strains of *Plasmodium falciparum.* There is inhibition of protein synthesis during growth of trophozoites. These drugs reduces gametocyte carriage (the sexual form of the parasite capable of infecting any blood sucking mosquito), but has no sporontocidal actinity.

Solid lipid nanoparticles (SLN) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes, and polymeric nanoparticles. SLN can provide advantages including stabilization of incorporated compounds and controlled release. SLN are conventionally prepared by a melting/solidification process, wherein the lipid is first melted, dispersed in water and then cooled to solidify the lipid particles. Alternatively, SLN are conventionally produced using an emulsion process akin to the formation of polymeric microparticles, wherein the lipids are dissolved in a solvent, emulsified, and then dispersed in an aqueous solution containing an emulsifying agent to harden the solid lipid nanoparticles. The role of the emulsifying agent is to stabilize the SLN; however, it also precludes further functionalization of the SLN. SLN are generally known to those of skill in the art and may be obtained by conventional methods as described in, for example, M. R. Gasco, Nanoparticelle Lipidiche Solide Quali Sistemi Terapeutici Colloidali, NCF nr. 7, 1996, pg 71-73; Kozariara et al., In-situ Blood-Brain Barrier Transport of Nanoparticles, Pharmaceutical Research, vol. 20, no. 11, p.1772

(2003); and Lockman, et al., Brain Uptake of Thiamine-Coated Nanoparticles, Journal of Controlled Release, 93 (2003) 271- 282.

However, SLN prepared by conventional means exhibits some limitations such as limited drug incorporation, crystal growth expulsion to overcome generally surfactants or emulsifiers are used, however this conventional approach typically fails to achieve stable aqueous suspensions, and/or fails to provide satisfactory surface modification. Therefore, there remains a need for methods and compositions that overcome these deficiencies of SLN and that effectively provide higher drug incorporation by addition of suitable solubilizers to modify the lipid matrix and enable surface modification of lipid nanoparticles. The present invention reports surface modified nanoparticles of Artemether, artemisinin derivatives and/or other antimalarial drugs loaded in lipid and/or protein base matrix with specific targeting to the parasitized RBCs.

Literature search on antimalarial drugs and their formulations revealed the following applications.

WO2005030197 discloses novel combination comprising artemisinin, piperaquine and primaquine.

NLC (Joshi *et al.* International Journal of Pharmaceutics, 2008; 364:119–126; Nayak et al. (Colloids and Surfaces B: Biointerfaces 2010;81:263–273.), SMEDDS (Mandawgade et al.,International Journal of Pharmaceutics 2008; 362:179–183) liposomes, (Chimanuka *et al.* J. Pharm. Biomed. Anal. 2002;28:13–22) and pegylated lysine based copolymeric dendritic micelles by (Jain *ét al)* have been studied. However none of the above formulations are target specific. Particulate carriers would be easily taken up by reticuloendothelial system rich kupffer cells (phagocytosis) and thus could be cleared from circulation and its site of action. For therapeutic purpose, it would be advantageous to make drug delivery system long circulating which would result in increased contact time between drug and erythrocytes.

Lipid based carrier systems for drug delivery has been found to be useful for targeted delivery of certain drugs. The features such as reduced dose, subsequent reduction in

toxicity and enhanced therapeutic index enable lipoidal systems to be explored further for development of antiviral, anticancer and antiparasitic drugs.

In recent years biodegradable lipid nanoparticles have been proposed as new drug administration systems. One of the most important features that they offer is the controlled release of the incorporated drug. This leads to greater therapeutic efficacy, provides a more comfortable administration for the patient and allows preventing overdose. Furthermore, drugs with different physicochemical features can be included, enabling improving their stability in biological fluids. This fact is very important in the case of antigens, proteins and macromolecules in general. Furthermore due to their small size, nanoparticles are suitable for the administration of drugs through various routes, such as orally, parenterally and ocularly (Kreuter, Adv. Drug Del. Rev., 7 (1991) 71-86; Gref et al., Science, 263 (1994) 1600-1603; Zimmer and Kreuter, Adv. Drug Del. Rev., 16 (1995) 61-73).

Parenteral administration of nanoparticles provides controlled systemic release that is suitable for drugs with (i) low oral bioavailability, (ii) short biological plasma half-life and (iii) limited stability. Another significant advantage of parenteral nanoparticles is the possibility of concentrating the drug in a certain organ. However, nanoparticles are quickly recognized, uptaken and eliminated from the blood circulation by macrophages of the mononuclear phagocyte system (MPS) after their intravenous administration. This phenomenon limits their function in controlled release as well as the possibility of concentrating the drug in tissues other than MPS.

Therefore, even though nanoparticles are potentially useful by the various administration routes, there are still problems which make their use difficult. Modification of the characteristics of the lipid matrix as well as of their surface may provide the solution to some of the limitations associated with nanoparticles administration. From this point of view, the association or coating of nanoparticles with suitable polymers may modify their physicochemical characteristics, and it may indirectly modify their distribution and interaction with the biological medium. A possible strategy is polyethylene glycol (PEG) binding to the nanoparticles, known as pegylation or obtaining stealthy nanoparticles.

Various nanoparticles coated with polyethylene glycol administered intravenously have demonstrated prolonged circulation (Gref et al., Science, 263 (1994) 1600-1603; Stolnik et al., Pharm. Res., 11 (1994) 1800-1808; Bazile et al., J. Pharm. Sci., 84 (1995) 493-498). Poly(lactic) (PLA) nanoparticles coated with polyethylene glycol have a much longer plasma half-life (t1/2=6 h) than when they are coated with albumin or poloxamer (t1/2=2-3 minutes) (Verrecchia et al., J. Controlled Rel., 36 (1995) 49-61). The presence of hydrophilic polyethylene glycol chains on the surface of the nanoparticles significantly reduces their interaction with blood proteins (known as opsonins). These proteins promote phagocytosis forming a "bridge" between the particles and phagocytes (Frank & Fries, Immunol. Today, 12 (1991) 322-326). However, the hydrophilic properties of polyethylene glycols are not the only important factor providing efficient resistance to opsonization. Other hydrophilic polymers such as polyvinyl alcohol have demonstrated a low protecting ability against opsonization of the nanoparticles (Leroux et al., Life Sci., 57 (1995) 695-703). Therefore, the steric stabilization provided by pegylation would also be due to other physicochemical properties, such as the high flexibility of the PEG chains and a specific structural formation (Mosquiera et al., Biomaterials, 22 (2001) 2967-2979). Oral dosage forms of drug delivery system are very much patient friendly and compliant. At the same time Literature reports enhanced therapeutic activity of drug molecule after oral administration of nanoparticles.

With respect to nanoparticles used by oral administration, the association of polyethylene glycols to conventional nanoparticles allows protection against enzymatic attack in digestive fluids. (Yaméogo et al., European Journal of Pharmaceutics and Biopharmaceutics 80 (2012) 508–517). This is due to the stealthing property of PEG and their ability to turn down proteins (Gref et al., Science, 263 (1994) 1600-1603). This also allows minimizing their interaction with mucin and other proteins present in the lumen of GIT. Similarly Fresta et al. have observed a significant increase of the ocular absorption of acyclovir after its administration in poly(alkylcyanoacrylate) nanospheres coated with polyethylene glycol (Fresta et al., J. Pharm. Sci., 90 (2001) 288-297). The enhanced ocular activity is probably due to greater interaction of the coated nanoparticles with the corneal epithelium. The main drawback with this new strategy is the stability of the association of polyethylene glycols to the surface of the nanoparticles (Peracchia et al., Life Sci., 61 (1997) 749-761). It is known that the ability of

polyethylene glycol to reject proteins depends on the configuration, the charge, the length and the flexibility of the chains (Torchillin, J. Microencaps., 15 (1998) 1-19). The process for modifying the surface of the nanoparticles is mainly carried out by physical adsorption (Stolnik et al., Adv. Drug Del. Rev., 16 (1995) 195-214) or by covalent bonding (De Jaeghere et al., J. Drug Target., 8 (2000) 143-153). However, the drawback of simple adsorption is the quick loss of the coating due to the instability of the interaction. Given that covalent binding is preferable, most pegylated nanoparticles have been prepared using polyethylene glycol copolymers with lactic or glycolic acid. However, the copolymerization process requires the use of several catalysts and specific chemical conditions (Beletsi et al., Int. J. Pharm., 182 (1999) 187-197). Furthermore, the toxic organic solvent residues used in the organic synthesis (methylene chloride, toluene etc.), may be problematic.

Busquets *et al.*(2011) have shown 200-nm liposomes of chloroquin loaded with quantum dots, covalently functionalized with oriented, specific half-antibodies against *P. falciparum* late form-infected pRBCs. The liposomes dock to pRBC plasma membranes and release their cargo to the cell in less than 90 min.

Ghosh *et al* (2011) have used Stearylamine liposomes for preferential targeting to RBCs significantly inhibiting the growth of the parasites depending on the phospholipids composition, maximum inhibition was observed when SA was delivered through Soya phosphatidylcholine (SPC) liposomes. Incorporation of cholesterol or Distearylphosphatidylethanolamine–Methoxy-Polyethylene glycol-2000 (DSPE-mPEG-2000) in Soya phosphatidylcholinestearylamine (SPC-SA) liposomes improved the efficacy.

The main goal of malaria therapy is to obtain maximal drug loading at the parasitophous vacuoles where the plasmodia are hosted in the RBCs. Plasmodium induces new permeation pathways (NPP) in infected erythrocytes (IRBC) that are involved in the transport of small molecules across the erythrocyte membrane. NPP are most prevalent in the trophozoite and schizont stages of infection. An increase in permeability of the membranes of the infected RBC to a wide range of low-molecular-weight solute is found following host infection by Plasmodium with membrane channels appearing 12-16 hours

after Plasmodium invasion Thus antimalarial Nanocarriers (<80nm) could access intracellular compartments of the parasite through NPPs in infected RBCs

Therefore, there is need in the art to provide nanoparticles which can target the antimalarial drugs specifically to pRBCs. Also, there is need that they must be stable, safe, non-toxic, biodegradable and easy to produce in order to be effective.

A cursory review of prior art shows that there are no reports demonstrating surface modified nanoparticles for targeted delivery of Antimalarial drug specifically to parasitized RBCs with improved therapeutic activity.

Therefore, it is an objective of the present invention to provide surface modified Artemether, artemisinin derivatives and other antimalarial drug loaded lipid or protein Nanocarriers with PEG or pantethenol or biotin or suitable ligand, that enhance the interaction between the pRBCs thereby leading to preferential uptake by the parasitized RBCs.

SUMMARY OF THE INVENTION:

In accordance with the above objective, the invention provides a pharmaceutical formulation which comprises surface modified/functionalized lipid and/ or protein nanoparticles of Antimalarial drug for targeted delivery, specifically to parasitized RBCs with improved therapeutic activity. The antimalarial drug is selected from Artemether and or artemisinin derivatives and other antimalarial drugs such as dihydroartemisinin, arteether and artesunate, chloroquine, amodiaquin, quinine, mefloquine atovaquone, proguanil lumefantrine, Sulfedoxine and pyrimethamine.

In a preferred aspect, the invention provides pharmaceutical formulation which comprises antimalarial drug in the range of 0.1 to 100 %w/w; lipid or protein in an amount of 1-10% w/w; surfactant/solubilizers in an amount of 0.25 to 10%W/V; surface modification agent and/or functionalizing agent in an amount of 0.2-5% in association with one or more pharmaceutical excipients.

According to the invention, the surface modification agent is PEG 200-8000 and the functionalizing agent is selected from biotin or pantethenol, Chondriotin, dextran or any other suitable ligand.

According to the invention the protein is selected as Human Serum albumin as it is the main protein of human plasma. Also, it is easily available, biodegradable and can be used parenterally. The concentration range selected for the preparation of nanoparticles was 0.1-3 10%w/w.

The formulation according to the invention may be provided as solid, tablet, capsule, powder for reconstitution, nanopowder,, liquid, disperse phase, emulsion, Lipid nanocarriers, nanoemulsion, nanoparticles, nanocapsules, self-nanoemulsifyng system.

The lipid according to the invention is selected from the group consisting of GRAS Lipids including Triglycerides like Trimyristin, Tristearin, Tripalmitin, Tribehanin, trilaurin; Long chain fatty acids like: Stearic acid, Lauric acid, myristic acid; palmitic acid, behanic acid, Capric acid, Caprylic acid, Cerotic acid, archidic acid, lignoceric acid,Glyceryl mono and di-esters like Glyceryl palmitostearate, Glyceryl monostearate, Glyceryl behenate; Glyceryl laurate, Fatty alcohols like Capryl alcohol, Capric alcohol, Cerotyl, archidyl alcohol Cetyl alcohol, Stearyl alcohol, Myristyl alcohol, palmityl alcohol, Benhyl alcohol, lauryl alcohol, lignoceryl alcohol, behnayl alcohol, and Waxes like Ceresine, Hard fat, Microcrystalline waxes.

The surfactants according to the invention are combination of lipophilic and hydrophilic selected from the group consisting of egg lecithin, phosphatidyl choline, soyabean lecithin, mixed soyabean phosphatides, glycerol Phosphatides, and polaxamers including polaxamer 124, polaxamer 188, polaxamer 237, polaxamer 407,Polysorbates, sorbitan esters,Polyoxytl Stearates, Polyoxyethylene Castor oil derivatives, polyoxyethylene alkyl ethers and the like.

The solubilizers according to the invention are selected from the group consisting of Macrogol-15-Hydroxy stearate, Polyoxyethylene sorbitan fatty acid esters including polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, Caproyl Propylene glycol

mono-caprylate, Propylene glycol mono laurate, Polyglyceryl Oleate, Polyoxyl glycerides including Caprylocaproyl macroglycerides, lauryl macrogolglycerides, linoleoyl macrogolglycerides, oleoyl macrogolglycerides, stearoyl macrogol glycerides,Tricaprylin, Caprylic/capric Triglyceride, Trioelin, sorbitan esters, polyoxyethylene stearates, polyoxyethylene castor oil derivatives and polyoxyethylene alkyl ethers.

The particle size of the surface modified nanoparticles is in the range of 10-200nm.

Description of drawings

Fig 1. Particle size distribution of bare artemether lipid nanoparticles (ALN)

Fig 2. Particle size distribution of surface modified artemether lipid nanoparticles(PALN)

Fig 3. Particle size distribution of bare artemether albumin nanoparticles (AAN)

Fig 4. Particle size distribution of Surface modified artemether albumin nanoparticles (PAAN)

Fig 5. TEM of Artemether Lipid Nanoparticles (ALN)

Fig 6. PEGylated Artemether Lipid Nanoparticles (PALN) respectively

Fig 7. SEM of Artemether Albumin Nanoparticles (AAN)

Fig 8. PEGylated Artemether Albumin Nanoparticles (PAAN) respectively

Fig9: Time dependent Comparitive Antimalarial activity of developed nanoparticles and Std ARM v/s Time of incubation .Data expressed as mean ± S.D (n=3), PALN statistically significant w.r.t ALN and ARM ($P<0.05$).

Fig 10: Confocal Microscopy Images showing Rhodamine images, DIC pictures of RBC and merged images. Fig (a) Uninfected RBCs incubated with ALN at the end of 24h: The nanoparticles are seen at the periphery of the RBCs however they are not uptaken by the RBCs probably due to the lack of NPP in the normal RBCs. (b) Uninfected RBCs incubated with PALN at the end of 48h: The nanoparticles are seen at the periphery of the RBCs however they are not uptaken by the RBCs. (c) Infected RBCs incubated with ALN at the 2h (d) ALN accumulation specifically into the infected RBCs at the end of 24h (e) ALN accumulation into the infected RBCs at the end of 48h (f) Infected RBCs incubated with PALN at the 2h (g) PALN accumulation specifically into the infected RBCs at the end of 24h (h) PALN accumulation specifically into the infected RBCs at the end of 48h

Fig 11(a): shows Z- Stacking images of infected RBCs incubated with ALN at 2h. The nanoparticles are beginning to penetrate into the RBCs, thereby reinstating the

development of NPPs in the parasitized RBCs. Fig 9(b): Z- Stacking images of infected RBCs incubated with ALN at 24h. Fig 9 (c): Z- Stacking images of infected RBCs incubated with ALN at 48h. Fig 9(d): shows Z- Stacking images of infected RBCs incubated with PALN at 2h. The nanoparticles are beginning to penetrate into the RBCs, thereby reinstating the development of NPPs in the parasitized RBCs. Fig 9(e) : Z-Stacking images of infected RBCs incubated with PALN at 24h Fig 9(f): Z- Stacking images of infected RBCs incubated with PALN at 48h. The number of PALN particles seen inside the pRBCs are more as compared to the ALN that shows increased and faster uptake by the ducts in the infected RBCs.

Fig 12 : Z-stack image explaining entry of bare nanoparticles (AAN) in the infected RBCs after (a) 15 min, (b) 1 hour, (c) 2 hours, (d) 4 hours and (e) 24 hours

Fig 13: Z-stack image explaining entry of surface modified nanoparticles (PAAN) in the infected RBCs after (a) 15 min, (b) 1 hour, (c) 2 hours, (d) 4 hours and (e) 24 hours

DETAILED DESSCRIPTION:

In line with the above objective, the present invention discloses surface modified nanoparticles for targeted delivery of Antimalarial drug specifically to parasitized RBCs with improved therapeutic activity

The present invention particularly discloses lipid and/ or protein nanoparticles surface modified with PEG and/or functionalized with biotin / pantethenol/dextran/chondriotin sulphate which acts as a carrier for active principles used for the treatment of malaria. The main goal of malaria therapy is to promote a high drug concentration in the intracellular parasitophorous vacuole where the Plasmodium is hosted inside RBCs and there are multiple membranes that must be traversed by antimalarial drugs. Nanocarriers remain in the blood stream for a long period of time in order to improve the interaction with infected red blood cells and parasite membranes. The present invention provides lipid and/or protein based nanoparticulate system of antimalarial drugs with higher drug payloads incorporated into it, more specifically the invention discloses Artemether lipid nanoparticle (ALN) using solubilizers to aid in incorporation of higher drug payloads. Lipid nanoparticles have been PEGylated (PALN) to provide stealth properties and resistance to opsonization with prolonged circulation time. The present invention also extends to Artemether-Albumin nanoparticle (AAN). These nanoparticles are further

PEGylated (PAAN) and/or functionalized with pantethenol/biotin/ dextran/chondriotin or suitable ligand. The bare and PEGylated lipid nanoparticles specifically target to parasitized RBC (pRBC). They are not uptaken by the normal RBCs.

This has been demonstrated with nanoparticles labeled with fluorescent probe which showed preferential uptake by the infected RBCs in *P.falciparum* culture within 15 minutes to 2h of incubation and accumulation continued upto 24 to 48h as demonstrated by Confocal microscopy. The erythrocyte membrane is extensively modified during the intracellular development of *P. falciparum*. *P. falciparum* induces changes in shape, adhesiveness, permeability, deformability as well as changes in their osmotic fragility. Reports suggest that channels called "New Permeability Pathways" or NPPs appear after 12–16 h of Plasmodium invasion. New permeability pathways in infected RBCs could be responsible for transport of nanoparticles within the RBC and enhanced uptake.

The PEGylated and surface functionalized nanoparticles of the instant invention resulted in improved efficacy in *P.falciparum in vitro* and *P. berghei in vivo*, leading to reduction in drug dose.

In a preferred embodiment, the invention provides surface modified lipid and/ or protein nanoparticles for targeted delivery of Antimalarial drug specifically to parasitized RBCs with improved therapeutic activity.

In another embodiment, the invention provides Formulation of drug loaded lipid nanoparticles (ALN):
According to this embodiment, GRAS lipids and surfactants have been used to formulate lipid nanoparticles. The primary emulsions stirred at high speed using Ultra-Turrex and were homogenized further using high pressure homogenizer at homogenization pressure in the range 200-1200bars and 2-8 homogenization cycles. The nanoparticles were characterized for pH, particle size, creaming, cracking, phase separation, centrifugation and drug content. Factorial designs and response surface methodology have been utilized to optimize the naparticles. Drug is loaded in dose range from 0.1to 100 % of drug dose.

Selection of lipids

GRAS Lipids were used in preparation of Lipid Nanoparticles. GRAS lipids are usually physiologically inert, biocompatible and widely accepted. Lipids including Triglycerides like:

Trimyristin, Tristearin, Tripalmitin, Tribehanin; trilaurin; Long chain fatty acids like: Stearic acid, Lauric acid, myristic acid; palmitic acid, behanic acid, Capric acid, Caprylic acid, Cerotic acid, archidic acid, lignoceric acid,Glyceryl mono and di-esters like Glyceryl palmitostearate, Glyceryl monostearate, Glyceryl behenate; Glyceryl laurate, Fatty alcohols like: Cetyl alcohol, Stearyl alcohol, Myristyl alcohol, palmityl alcohol, Benhyl alcohol, lauryl alcohol, lignoceryl alcohol, behnayl alcohol

Waxes like: Ceresine, Hard fat, Microcrystalline waxes were used for preparation of Nanoparticles. The lipids were used in concentration range of 0.1-10% w/w.

Selection of surfactants

A combination of lipophilic and hydrophilic surfactant was used to stabilize the lipid nanoparticles.

Surfactants included but not restricted to egg lecithin, phosphatidyl choline, soyabean lecithin, mixed soyabean phosphatides, glycerol Phosphatides, and polaxamers including polaxamer 124, polaxamer 188, polaxamer 237, polaxamer 407,Polysorbates, sorbitan esters,Polyoxyl Stearates, Polyoxyethylene Castor oil derivatives, polyoxyethylene alkyl ethers and the like .

Selection of solubilizers/stabilizers

Solubilizers used in formulation of Lipid Nanoparticles included but not restricted to Macrogol-15-Hydroxy stearate, Polyoxyethylene sorbitan fatty acid esters including polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, Caproyl Propylene glycol mono-caprylate, Propylene glycol mono laurate, Polyglyceryl Oleate, Polyoxyl glycerides including Caprylocaproyl macroglycerides, lauryl macrogolglycerides, linoleoyl macrogolglycerides, oleoyl macrogolglycerides, stearoyl macrogol glycerides,Tricaprylin, Caprylic/capric Triglyceride, Trioelin, sorbitan esters, polyoxyethylene stearates, polyoxyethylene castor oil derivatives, polyoxyethylene alkyl ethers. These solubilisers modified the lipid matrix and resulted in lipid nanoparticles with higher payload of drug.

Solubilisers were used alone or in combination in the concentration range of 0.25 to 5%W/V.

Formulation of Artemether loaded albumin nanoparticles (AAN):

Method 1: Phase Separation-Coacervation using either a non-solvent or salt solution:

Human Serum Albumin (HSA) was dissolved in sterile water for injection. Artemether was separately dissolved in sufficient amount of a suitable solvent and mixed with the protein solution under continuous stirring. Coacervating agent, either a non-solvent for protein or 20% Sodium Sulfate (Na_2SO_4), i. e. salt solution, was added to the above mixture till protein is precipitated from the aqueous phase due to its reduced solubility. Thus formed nanoparticles were cross-linked with the aid of heat.

Method 2: Phase separation-Coacervation using a deaggregating agent:

The method was similar to that described in method 1. Before crosslinking the nanoparticles, Mannitol, in the form of a dry powder or solution is added to the dispersion of the nanoparticles. The nanoparticles were then thermally crosslinked by heating at 55-60°C.

Method 3: Solvent Displacement and Interfacial deposition:

Drug was dissolved in the minimum amount of organic solvent. This drug solution was then added to the HSA Solution till it precipitated to form a colloidal dispersion. This dispersion was then slowly injected in the aqueous phase, i.e. sterile water for injection, from which the nanoparticles were precipitated. The formed nanoparticles were then cross-linked with the aid of heat.

Method 4: Solvent evaporation followed by high pressure homogenization (HPH):

HSA was dissolved in sterile water for injection. Artemether was separately dissolved in organic solvent containing solubilizer. The drug solution was then added slowly under high speed stirring to the aqueous solution of Human Serum Albumin. The dispersion formed was further passed through high pressure homogenization (Niro Saovi Panda Plus) for a time sufficient to obtain desired particle size of the nanoparticles. Organic Solvent was removed from the nanoparticle under reduced pressure into Rotavap. The

dispersion was then lyophilized. Finally, the solid Artemether loaded Albumin nanoparticles (AAN) were obtained.

Selection of HSA Concentration: Human Serum albumin (HSA) was used in the preparation of the nanoparticles. HSA constitutes 60% of the total proteins in the body. It is easily available, biodegradable and can be used parenterally. The concentration range selected for the preparation of nanoparticles was 0.1-10%w/w.

Selection of Organic Solvent concentration: The organic solvent was used to fabricate the nanoparticles by precipitation of HSA. The organic solvent was used in the concentration range of 5-50%v/v.

Selection of Surfactant/solubiliser and its concentration: Artemether is practically insoluble in water. Hence to facilitate wetting and solubilization of the drug in the aqueous phase surfactant was incorporated in the formula. Surfactant was selected from Polysorbate 20, Polysorbate 80, Span, 80 Polaxamer and Sorbitan esters, Solutol, Polyoxyethylene Castor oil derivatives, polyoxyethylene alkyl ethers, Caproyl Propylene glycol mono-caprylate, Propylene glycol mono laurate, Polyglyceryl Oleate, Polyoxyl glycerides including Caprylocaproyl macroglycerides, lauryl macrogolglycerides, linoleoyl macrogolglycerides, oleoyl macrogolglycerides, stearoyl macrogol glycerides, sorbitan esters, polyoxyethylene stearates. The surfactant/solubiliser was selected based upon its ability to dissolve the drug in the aqueous phase. Concentration of the surfactant/solubiliser was selected from the range 0.1-10% w/w.

In another preferred embodiment, the invention provides Surface modification of nanoparticles. According to this embodiment, the Surface modification of Bare Lipid nanoparticles may be carried with surface modification agent PEG and/or functionalizing agent such as biotin /pantethenol/ dextran/chondriotin or any other suitable ligand.

In one preferred embodiment, Bare Lipid nanoparticles were suitably surface modified by incubating with polyethylene glycol (PEGs). PEG of molecular weight in the range of 200-8000 was used in concentrations of 0.2-5%w/v. The Nanoparticles were incubated

with PEGs at incubation temperature of 20°C-50°C and incubation period in range of 1hr to 3days.

In another embodiment Bare Artemether-albumin nanoparticles (AAN) were surface modified using Methoxy PEG 200/350/400/1000/1500/2000/4000/6000/8000 or suitable molecular weight and/or pantothenic acid / Biotin dextran/chondriotin or suitable ligand in the concentration range of 0.1-1%w/v and 0.001-0.1%w/v respectively. The nanoparticles were incubated with mPEG at incubation temperature of 20 to 50 $^{0}C^{C}$ and incubation period of 1-5 hours.

In another embodiment, the invention provides particle size determination of the nanoparticles. Accordingly, the Mean particle size and particle size distribution of developed nanoparticles were determined using N5 Beckman Particle Size Analyzer at fixed angle of 90^{0}C at 20^{0}C temperature using double distilled water as dispersant.

In yet another embodiment, the invention provides antimalarial activity of the nanoparticles and its efficacy when compared with conventional Artemether. Accordingly, antimalarial activity was carried out using Peter's four-day suppressive test using healthy male Swiss albino mice weighing between 18-20g as a suitable animal model. Parasitic culture of *Plasmodium bergheii bergheii* maintained in citrated saline was injected intraperitoneally (1x106 parasites per animal) to the test animals.

Bare (ALN) and surface modified (PALN) lipid nanoparticles were selected for evaluation of antimalarial efficacy study. Appropriate dilutions of the formulations were made prior to administration in order to deliver the selected doses.

For comparison, conventional Artemether (ARM) dispersion was prepared by dispersing plain drug in water for injection and appropriately diluted to administer desired dose by i.v. and oral route. The test as well as the conventional formulations was administered at two dose levels100 % and 50 % therapeutic dose. Reduction in parasitemia and improved activity of all the test formulations as compared to marketed Standard at 100% dose as well as at reduced dose (50%) level indicates improved delivery of Artemether in the

form of biodegradable polymeric nano-particulate drug delivery system with reduction in the total dose administered.

This experiment concludes that at the 100% therapeutic dose level PALN showed early onset of action and higher antimalarial activity compared to std ARM and bare lipid nanoparticles and sustained effect till day 30 showing superior antimalarial activity against conventional and bare lipid nanoparticles.

The results of pharmacodyanamic activity suggest that with the help of PEGylation the therapeutic dose of ARM can be significantly reduced to 50%. This study further suggest that significantly higher and prolonged drug concentrations could be obtained in RBC with PALN as compared to bare nanopaticles ALN and reference standard. Also drug was observed to get concentrated more in RBC as compared to plasma.

The following examples, which include preferred embodiments, will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purpose of illustrative discussion of preferred embodiments of the invention.

Examples

Example 1

Examples of Artemether lipid nanoparticles:

Table-1

Example No.	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**
Artemether (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Glyceryl Behenate (%w/v)	2	2	2	2	4	4	4	4	4
Soya Lecithin (%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1	2

Table-2

Example No.	10.	11.	12.	13.	14.	15.	16.	17.
Artemether (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Cetyl alcohol (%w/v)	1	1	1	1	1	1	1	1
Soya Lecithin (%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1
Polysorbate 80	-	-	-	-	1	1	1	1

Table-3

Example No.	18.	19.	20.	21.	22.	23.	24.	25.
Artemether (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Cetyl alcohol (%w/v)	2	2	2	2	2	2	2	2
Soya Lecithin (%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1
Polysorbate 80	-	-	-	-	1	1	1	1

Table-4

Example No.	26.	27.	28.	29.	30.	31.	32.	33.
Artemether (%)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Cetyl alcohol (%w/v)	2	2	2	2	2	2	2	2
Soya Lecithin (%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v))	1	1.5	1.5	1	1	1.5	1.5	1
Macrogol – 15 – Hydroxystearate (%w/v)	1	1	1	1	0.5	0.5	0.5	0.5

Table-5

Example No.	34.	35.	36.	37.	38.	39.	40.	41.
Artemether (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Stearyl alcohol (%w/v)	2	2	2	2	2	2	2	2
Soya Lecithin (%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1
Macrogol – 15 – Hydroxystearate (%w/v)	1	1	1	1	0.5	0.5	0.5	0.5

Table-6

Example No.	42.	43.	44.	45.	46.	47.	48.	49.	50.	51.	52.	53.
Artemether (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Stearic acid (%w/v)	1	1	1	1	2	2	2	2	4	4	4	4
Soya lecithin(%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1	1	1.5	1.5	1

Table-7

Example No.	54.	55.	56.	57.	58.	59.	60.	61.	62.	63.	64.	65.
Artemether (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Stearic acid (%w/v)	2	2	2	2	2	2	2	2	2	2	2	2

Soya lecithin(%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1	1	1.5	1.5	1
Macrogol – 15 – Hydroxystearate (%w/v)	-	-	-	-	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Polysorbate 80 (%w/v)	0.5	0.5	0.5	0.5	-	-	-	-	0.5	0.5	0.5	0.5

Table-8

Example No.	**66.**	**67.**	**68.**	**69.**	**70.**	**71.**	**72.**	**73.**	**74.**	**75.**	**76.**	**77.**
Artemether (%)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Stearic acid (%)	2	2	2	2	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Soya lecithin(%)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%)	1	1.5	1.5	1	1	1.5	1.5	1	1	1.5	1.5	1
Polysorbate 80 (%w/v)	0.5	0.5	0.5	0.5	-	-	-	-	-	-	-	-
Propylene glycol lmonolaurate(%w/v)	0.5	0.5	0.5	0.5	-	-	-	-	-	-	-	-
Caprylocaproyl macrogolglycerides (%w/v)	0.5	0.5	0.5	0.5	-	-	-	-	-	-	-	-
Tricaprylin(%w/v)	-	-	-	-	0.25	0.25	0.25	0.25	-	-	-	-
Caprylic/Capric Triglyceride (%w/v)	-	-	-	-	-	-	-	-	0.25	0.25	0.25	0.25

Table-9

Example No.	**78.**	**79.**	**80.**	**81.**	**82.**	**83.**	**84.**	**85.**	**86.**	**87.**	**88.**	**89.**
Artemether (%)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Stearic acid (%)	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	2	2	2	2

Soya lecithin(%)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%)	1	1.5	1.5	1	1	1.5	1.5	1	1	1.5	1.5	1
Macrogol – 15 – Hydroxystearate (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Polysorbate 80 (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CaproylPropylene glycol mono-caprylate (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol mono laurate. (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Tricaprylin(%w/v)	0.25	0.25	0.25	0.25	-	-	-	-	-	-	-	-
Caprylic/Capric Triglyceride(%w/v)	-	-	-	-	0.25	0.25	0.25	0.25	-	-	-	-

Table-10

Example No.	**90.**	**91.**	**92.**	**93.**	**94.**	**95.**	**96.**	**97.**
Artemether. (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
***Trimyristin* (%w/v)**	2	2	2	2	2	2	2	2
Soya lecithin(%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1
Polysorbate 80 (%w/v)	-	-	-	-	0.5	0.5	0.5	0.5
Caproyl Propylene glycol mono-caprylate (%w/v)	-	-	-	-	0.5	0.5	0.5	0.5

Table-11

Example No.	98.	99.	100.	101.	102.	103.	104.	105.	106	107	108.	109.
Artemether (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Trimyristin (%w/v)	2	2	2	2	2	2	2	2	2	2	2	2
Soya lecithin(%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1	1	1.5	1.5	1
Macrogol – 15 – Hydroxystearate (%w/v)	0.25	0.25	0.25	0.25	0.375	0.375	0.375	0.375	0.5	0.5	0.5	0.5
Polysorbate 80 (%w/v)	0.25	0.25	0.25	0.25	0.375	0.375	0.375	0.375	0.5	0.5	0.5	0.5
CaproylPropylene glycol mono-caprylate (%w/v)	0.25	0.25	0.25	0.25	0.375	0.375	0.375	0.375	0.5	0.5	0.5	0.5
Propylene glycol mono laurate (%w/v)	0.25	0.25	0.25	0.25	0.375	0.375	0.375	0.375	0.5	0.5	0.5	0.5

Table-12

Example No.	110.	111.	112.	113.	114.	115.	116.	117.
Artemether (%w/v)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Tristearin (%w/v)	2	2	2	2	2	2	2	2
Soya lecithin(%w/v)	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
Polaxamer (%w/v)	1	1.5	1.5	1	1	1.5	1.5	1
Macrogol – 15 – Hydroxy stearate (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Polysorbate 80 (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Caproyl Propylene glycol mono-caprylate (%w/v)	-	-	-	-	0.5	0.5	0.5	0.5
Propylene glycol mono laurate (%w/v)	-	-	-	-	0.5	0.5	0.5	0.5

Table 13

Example No.	118.	119.	120.	121.	122.	123.	124.
Artemether (%w/v)	0.02	0.04	0.053	0.08	0.16	0.2	0.32
Tristearin (%w/v)	2	2	2	2	2	2	2
Soya lecithin(%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Polaxamer (%w/v)	1	1	1	1	1	1	1
Macrogol – 15 – Hydroxy stearate (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Polysorbate 80 (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Caproyl Propylene glycol mono-caprylate (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol mono laurate (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 14

Example No.	125	126	127	128	129	130	131
Artemether (%w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Human Serum Albumin (20% w/w solution) (%w/w)	4	2	1	2	2	2	2
Ethanol (%v/v)	40	40	40	15	20	30	40
Polysorbate 80 (%w/w)	0	0	0	0	0	0	0

Table 15

Example No.	132	133	134	135	136	137	138	139	140
Artemether (%w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Human Serum Albumin (20% w/w solution) (%w/w)	2	2	2	2	2	2	3	3	3

Ethanol (%v/v)	40	40	40	40	40	40	40	40	40
Polysorbate 80 (%w/w)	0.15	0.2	0.3	0.5	1	2	3	5	10

Table 16

Example No.	**141**	**142**	**143**	**144**	**145**	**146**	**147**	**148**	**149**
Artemether (%w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Human Serum Albumin (20% w/w solution) (%w/w)	1	1	1	2	2	2	3	3	3
Ethanol (%v/v)	20	30	40	20	30	40	20	30	40
Polysorbate 80 (%w/w)	3	3	3	3	3	3	3	3	3

Table 17

Example No.	**150**	**151**	**152**	**153**	**154**	**155**	**156**	**157**	**158**
Artemether (%w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Human Serum Albumin (20% w/w solution) (%w/w)	1	1	1	3	2	2	3	3	2
Ethanol (%v/v)	40	40	40	40	40	40	40	40	40
Polysorbate 80 (%w/w)	3	5	10	10	5	10	3	5	3

Homogenisation parameters; homogenization pressure and number of homogenization cycles were optimized to obtain mean particle size in the range of 70 to 90 nm.

Table 18: Optimization of homogenization parameters of Artemether lipid nanoparticles

Example No.	**Homogenization Pressure (in bars)**	**Homogenization cycles**	**Mean particle size (nm)**
159	INITIAL	-	134.5

160	200	4	84.7
161	400	4	81.9
162	600	4	68.2
163	800	4	79

Table 19: Optimization of homogenization parameters of Artemether lipid nanoparticles

Example	**Pressure (Bar)** →									**Particle Size (nm)**
	No. of cycles ↓									
	200	**300**	**400**	**600**	**800**	**900**	**1000**	**1200**	**1400**	
164	10	10	5	-	-	-	-	-	-	275.6
165	8	-	8	8	12	-	-	-	-	259.1
166	4	-	4	-	4	-	4	-	-	683.0
167	-	-	-	-	-	-	20	20	20	798.4
168	-	5	-	5	-	5	-	-	-	864.6
169	12	-	12	8	-	-	-	-	-	731.4
170	-	-	-		10	-	20	20	-	707.8
171	-	-	-	-	-	-	20	30	-	851.4
172	-	-	-	-	-	-	10	30	5	715.6
173	-	-	-	-	-	-	-	50	40	316.6
174	-	-	20	-	30	-	-	30	-	752.2
175	8	-	8	4	4	-	4	-	-	383.3
176	20	-	10	5	-	-	-	-	-	238.9
177	15	-	10	-	-	-	-	-	-	265.3
178	30	-	10	-	-	-	-	-	-	246.9
179	15	-	15	-	-	-	-	-	-	161.2
180	5	-	5	5	5	-	5	5	5	136.5
181	5	-	5	-	-	-	-	-	-	75.7
182	10	-	10	-	-	-	-	-	-	247.7
183	10	-	5	-	-	-	-	-	-	66.1

Example 2

Surface modification of nanoparticles: Bare Lipid nanoparticles were suitably surface modified by incubating with polyethylene glycol (PEGs). PEG of molecular weight in the range of 200-8000 was used in concentrations of 0.1-5%. The Nanoparticles were incubated with PEGs at incubation temperature of 20^0C-50^0C and incubation period in range of 1hr to 3days. The nanoparticles were functionalized with biotin/pantethenoic acid/dextran/chondriotin or suitable ligand.

Bare Artemether-albumin nanoparticles (AAN) were surface modified using Methoxy PEG 350 /400/1000/1500/2000 and/or pantothenic acid/Biotin /dextran/chondriotin or suitable ligand in the concentration range of 0.001-5% w/v respectively. The nanoparticles were incubated with mPEG at incubation temperature of 30°C and incubation period of 3-4 hours.

Table: 20

Example No.	Stealthing agent Concentration(%w/v)	Particle size & Size Distribution	
		Particle Size (nm)	Polydispersity Index
PEG300			
184	0.25	98.9	0.256
185	0.5	406.3	0.502
186	1	92.2	0.238
PEG400			
187	0.25	100.2	0.196
188	0.5	106.8	0.291
189	1	93.3	0.354
PEG600			
190	0.25	328.2	0.520
191	0.5	226.5	0.342
192	1	337.5	0.437
PEG1000			
193	0.25	82.6	0.413

194	0.5	86.6	0.272
195	1	120.5	0.513
PEG1500			
196	0.25	128.9	0.646
197	0.5	211.5	0.819
198	1	155.1	0.98
PEG 4000			
199	0.25	171	0.216
200	0.5	406.3	0.502
201	1	262.7	0.525
PEG 6000			
202	0.25	214.7	0.365
203	0.5	66.3	0.263
204	1	229.6	0.370
mPEG-350			
205	0.25	148.0	0.675
206	0.5	81.1	0.808
207	1	205.6	0.754
Pantothenic acid			
208	0.001	121	-
209	0.002	163	-
210	0.005	330	-
211	0.01	379	-
212	0.02	722	-
213	0.08	552	-
214	0.1	576	-
Biotin			
215	0.001	95	-
216	0.002	117	-
217	0.003	136	-
218	0.005	217	-
219	0.008	300	-

220	0.01	456	-

Example 3

Physicochemical characterization

Particle size determination:

Mean particle size and particle size distribution of developed nanoparticles were determined using N5 Beckman Particle Size Analyzer at fixed angle of 90^0C at 20^0C temperature using double distilled water as dispersant.

Table 21: Characterization of selected bare and surface modified nanoparticles using lipid or protein

S. No	PARAMETERS	ALN(Artemether lipid nanoparticle)	PALN(Pegylated Artemether lipid nanoparticles)	AAN(Artemether loaded albumin nanoparticles)	PAAN(pegylated Artemether loaded albumin nanoparticles)
1	Organoleptic properties				
	a)Appearance	Homogenous	Homogenous	Clear, Transluscent	Clear, Transluscent
	b)Odour	Odourless	Odourless	Odourless	Odourless
	c)Colour	Bluish-transparent	Bluish-transparent	Pale yellow-bluish Transparent	Pale yellow-bluish Transparent
2.	Sedimentation or Creaming	Not found	Not found	Not found	Not found
3.	Effect of Centrifugation	No separation	No separation	No separation	No separation
4.	pH	6.55	6.8	7.03	7.15
5.	Syringability	Good	Good	Good	Good
6.	Injectability	Good	Good	Good	Good
8.	Zeta Potential	- 20.8	- 19.8	-14.9	-11.8
9.	Particle Size(nm)	68.2	120.5	66.1	85.9

10.	Polydispersity Index	0.413	0.513	0.090	0.808
11.	Particle size Range	10-200	10-400	10-100	10-150
12.	Assay (%)	99.5%	97.5%	99.5%	98.5%
13.	Drug release at the end of 8h (%)	54%	45%	67%	69%
14.	Drug release at the end of 24h (%)	84%	79%	87%	84%

Example 4

Antimalarial activity of drug loadedLipid Nanaoparticles

Antimalarial activity was carried out using Peter's four-day suppressive test using healthy male Swiss albino mice weighing between 18-20g as a suitable animal model. Parasitic culture of *Plasmodium bergheii bergheii* maintained in citrated saline was injected intraperitoneally ($1x10^6$ parasites per animal) to the test animals.

Treatment

Bare (ALN) and surface modified (PALN) lipid nanoparticles were selected for evaluation of antimalarial efficacy study. Appropriate dilutions of the formulations were made prior to administration in order to deliver the selected doses.

For comparison, conventional Artemether (ARM) dispersion was prepared by dispersing plain drug in water for injection and appropriately diluted to administer desired dose by i.v. and oral route. The test as well as the conventional formulations was administered at two dose levels100 % and 50 % therapeutic dose.

Experimental groups and administration of doses

The animals were divided into 14 groups depending upon the formulation and the dose level to be administered. The experimental groups are summarized in following table:

Table 22: Experimental groups of animals and the doses administered (Lipid Nanoparticles)

Formulations	Route of Administration	Experimental groups based on dose (mg/kg)	
		100%	50%
ARM std	Per oral	A1	A2
ALN		B1	B2
PALN		C1	C2
Arm Std	i.v.	D1	D2
ALN		E1	E2
PALN		F1	F2
Untreated control	-	H	
Healthy control	-	I	

Four hours after the infection, the animals were given different treatments. Groups A1 was administered ARM Std orally at the dose levels of 100 % for four consecutive days respectively. Groups B1 were treated orally ALN at the same dose levels. Groups C1 was given oral PALN at the same dose levels. Group D1, D2, E1, E2, F1 and F2 were treated with ARM Std, ALN and PALN respectively by i.v. route at the same two dose levels. While group H was maintained as an untreated control. Group I was maintained as the healthy control and was neither infected nor given any treatment but was exposed to the same environmental conditions as the rest of the groups.

Table 23: Experimental groups of animals and the doses administered (Human Albumin Nanoparticles):

Formulations	Experimental groups based on the dose	
	100%	50%
Larinate	A1	A2
AAN	B1	B2
PAAN	C1	C2
Blank	D	

Untreated control	E
Healthy control	F

To Groups A1 and A2, Larinate (Artesunate) was administered intravenously at the dose levels of 100 and 50% of therapeutic dose for four consecutive days. Groups B1 & B2 and Groups C1 & C2 were treated by IV route with AAN and PAAN respectively at the same two dose levels for four consecutive days. Group D received Blank Nanoparticles where Group E and F served as untreated and healthy control groups respectively.

In vivo anti-malarial efficacy was evaluated on the following parameters-

- Mean percent parasitemia against time (in days) for different groups
- Percent antimalarial activity against time (in days)

The parasite count were made on day 5 from thin blood smears of tail blood, fixed with methanol and stained with field's stain. Parasitemia was reported as percentage parasitemia after counting 1000 RBCs from each slide. A graph of average percentage parasitemia against time (in days) was plotted for different groups. Percent anti-malarial activity (in days) was calculated by the following formula suggested in the standard protocol by Fidock et. al.and The animals were also observed for their survival till 30 days.

$$\text{Activity} = 100 - \left\{ \frac{\text{Mean Parasitemia of treated group}}{\text{Mean parasitemia of control group}} \times 100 \right\}$$

Statistical analysis: Data was expressed as Mean ± S.D. and assessed by one way ANOVA followed by Tukey's multiple comparison tests using Graphpad instat Demo version. $P<0.05$ was considered as significant.

Peter's antimalarial activity estimated the suppression of parasitemia in the animals infected with *P. berghei* by bare and surface modified lipid nanoparticles of Artemether(ALN and PALN) as compared with conventional formulation standard drug dispersion (Arm std). Percent parasitemia and percent antimalarial activity of control animal group and groups receiving per oral (p.o.) and intravenous (i.v.) were determined.

Table 24: Mean percent parasitemia, % antimalarial activity of control animal groups (oral-Lipid Nanoparticles)

Group*	Mean percent parasitemia(±SD)	
	Day 5	Day 30
H	15.30 (0.988)	19.79 (1.237)

*Group H represent untreated control group

Table 25: Mean percent parasitemia, % antimalarial activity of Treated animals after peroral administration of formulations Artemether standard, ALN, PALN

Group*	Mean percent parasitemia(±SD)		% Antimalarial activity	
	Day 5	Day 30	Day 5	Day 30
A1	12.59 (1.569)	15.22 (1.45)	17.71	23.09
B1	11.41 (0.897)	5.93 (1.89)	25.40	70.04
C1	6.17 (8.97)	2.48 (1.987)	59.65	87.47

*Groups A1, B1, C1 represent ARM Std, ALN, PALN respectively at the 100% dose levels by oral route

Untreated control group (H) showed gradual increase in parasitemia which reached upto 19.79 % by day 30. The animals appeared sick and as the infection progressed, more and more red blood cells (RBCs) were invaded by the parasites which completely ruptured the RBC membrane, thus destroying the RBCs. No survival of the control group animal was observed beyond day 30.

From table 25 it is evident that at 100% therapeutic dose level of the bare and surface modified lipid nanoparticles, ALN, PALN as well as the conventional formulation ARM std administered orally showed at 30th day 70.04%, 87.47% and 23.09% antimalarial activity respectively and complete cure of all the test animals was observed till day 30. Also by the end of 5th day conventional formulation, ALN and PALN showed 17.71, 25.40 and 59.65% antimalarial activity respectively. Thus at the 100% therapeutic dose level PALN showed early onset of action and higher antimalarial activity compared to std ARM and bare lipid nanoparticles and sustained effect till day 30 showing superior

antimalarial activity against conventional and bare lipi d nanoparticles.

Table 26: Mean percent parasitemia, % antimalarial activity of control animal groups (i.v.-Lipid Nanoparticles)

Group*	Mean percent parasitemia(±SD)	
	Day 5	Day 30
H	16.35 (0.762)	20.44 (2.71)

Table 27: Mean percent parasitemia, % antimalarial activity of Treated animal after i.v. Administration of formulations Artemether standard, ALN, PALN

Group	Mean percent parasitemia(±SD)		% Antimalarial activity	
	Day 5	Day 30	Day 5	Day 30
D1	7.87 (0.566)	15.25 (3.55)	51.9	25.4
D2	9.20 (3.69)	16.78 (0.12)	43.7	17.9
E1	6.16 (0.639)	6.54 (0.852)	62.3	68.0
E2	6.51 (6.9)	11.76 (1.023)	60.2	42.4
F1	3.97 (0.132)	2.90 (0.882)	75.7	85.8
F2	4.24 (0.751)	4.70 (2.05)	74.1	77.0

*Groups D1-D2,E1-E2,F1-F2 represent ARM Std, ALN, PALN respectively at the two dose levels (100%, 50%) by i.v. route

In case of I.V administration, at 100% of the therapeutic dose, the PALN showed higher % antimalarial activity (75.7%) on the 5th day as compared to the conventional formulation which showed only 51.9% and bare LN showed value of (62.3%). Whereas on 30th day PALN has showed highest activity 85.8% compared to Std ARM (25.4%) and ALN (68.0%).

Similar results were obtained at 50% dose levels indicating much higher antimalarial activity by PALN 74.1% on day 5 where ALN (60.2%) and ARM Std showed 43.7% antimalarial activity only. Further on day 30 PALN showed 77% of activity which is higher as compared to conventional (17.9%) and ALN (68%)

One way ANOVA followed by Tukey's multiple comparison test applied to mean percent parasitemia of the treatment group showed P<0.001 extremely significant for surface modified lipid nanoparticles of artemether.

In vivo antimalarial efficacy on day 5 depicted that the animals treated with standard ARM solution and ALN showed percent parasitemia of 7.87 & 6.16% respectively as compared to that of control group which showed highest parasitemia (16.35%). However PALN treatment showed lowest percent parasitemia (3.97%) as compared to all other groups (p<0.001) showing the superiority of surface modification approach in improving the delivery of Artemether.

The most striking observation of the investigation is that the highest and comparable Antimalarial activity of PALN (74.1 & 75.7%) at 50 and 100% of therapeutic dose of ARM respectively after i.v. route of administration, as compared to standard ARM solution, which showed only 43.7 and 51.9% activity at 50 and 100% dose levels respectively. Bare ALN showed much lower activity (60.2 & 62.3% at 50 &100 % dose of ARM) as compared to PALN.

Results of pharmacodyanamic activity suggest that with the help of PEGylation the therapeutic dose of ARM can be significantly reduced to 50%.

Table 28: Mean percent parasitemia, % antimalarial activity of control animal groups (oral)

Group*	**Mean percent parasitemia(±SD)**		
	Day 5	Day 10	Day 30
E	64.79	72.13	-

*Group E represent untreated control group

Antimalarial Activity of drug loaded HSA Nanaoparticles

Table 29: Mean percent parasitemia, % antimalarial activity of Treated animal after i.v. Administration of formulations Artemether standard, AAN, PAAN at 100% dose (HSA Nanaoparticles)

Group*	Mean percent parasitemia(±SD)		% Antimalarial activity	
	Day 5	Day 21	Day 5	Day 21
A1	13.72	23.46	86.28	76.54
B1	10.94	13.21	89.06	86.79
C1	20.14	8.29	79.86	91.71
D	68.88	30.48	31.12	69.52

*Groups A1, B1, C1 and D represent Larinate, AAN, PAAN and Blank respectively at the 100% dose levels by i.v. route

Table 30: Mean percent parasitemia, % antimalarial activity of Treated animal after i.v. Administration of formulations Artemether standard, AAN, PAAN at 50% dose(HSA Nanaoparticles)

Group*	Mean percent parasitemia(±SD)		% Antimalarial activity	
	Day 5	Day 21	Day 5	Day 21
A2	37.37	46.34	63.63	53.66
B2	28.51	13.71	71.49	86.29
C2	20.43	11.45	79.57	88.55

*Groups A2, B2 and C2 represent Larinate, AAN, PAAN and Blank respectively at the 50% dose levels by i.v. route

The control group showed highest parasitemia at all-time interval with maximum of 72.13 on day 10. All mice were found to be dead after 10^{th} day.

Marketed formulation at 100% dose showed a parasitemia of 23.46 % on 21^{st} day. The parasitemia was found to higher i.e. 46.34 % at 50% dose level. Hence, the standard

showed reduction in the activity (53.66 %) at 50% dose as compared to 100% dose level (76.5%) when the dose was reduced to half.

Bare Artemether nanoparticles were also tested at two dose levels viz. 100 % and 50%. At the end of 21st Day, mean percent parasitemia of the test group was found to be 13.21%. Interestingly, the test formulation was found to be effective even at 50% dose level. The mean percent parasitemia at 50% dose was 13.71%. Hence, on 21st day, activity at both the dose levels of 100% and 50% were 86.79 and 86.29% respectively.

Surface modified formulations at 100% dose have an antimalarial activity of 91.71% and 88.55 % activity at 50% dose level at the end of 21st day.

Surprisingly, blank nanoparticles (Placebo) also showed considerable reduction in the parasitemia as compared to the control and marketed formulation. The parasitemia was 30.48% on 21st day. The blank formulation therefore showed an antimalarial activity of 69.52%.

Reduction in parasitemia and improved activity of all the test formulations as compared to marketed Standard at 100% dose as well as at reduced dose (50%) level indicates improved delivery of Artemether in the form of biodegradable protein nano-particulate drug delivery system with reduction in the total dose administered.

Example 5

In vitro* efficacy studies of the drug loaded nanoparticles against *P.falciparum

Time dependent in vitro antimalarial efficacy of ALN and PALN was studied on P.falciparum (3D7 strain) and compared with ARM Std. In brief, the parasite culture was grown using Trager Jensen Candle Jar Method for continuous culture. Synchronized cultures were obtained by 5% sorbitol lysis. For growth inhibition assays, parasitemia was adjusted to 1.5% with more than 90% of parasites at ring stage after sorbitol synchronization. 200 µl of this Plasmodium culture was plated in 24-well plates and incubated in the presence of ALN ,and PALN in the conditions described above. The parasite infected RBCs were incubated with the developed ALN and PALN formulations at two dose levels (6.97nM/well equivalent to therapeutic dose, 4mg/kg, 100% of therapeutic dose and 3.48nM/well equivalent to 2mg/kg, 50% of therapeutic dose) at

37°C. Parasitemia was determined at the end of 2,4,6,10,24 and 48h of incubation. The dilutions were carried out using the RPMI media. Blood smears were prepared and fixed with methanol and stained with Giemsa stain diluted 1:10 in Sorenson's Buffer, pH 7.2 and the parasites were counted. Parasitemia was reported as percentage parasitemia after counting 1000 RBCs from each slide. Antimalarial activity was calculated by the following formula suggested in the standard protocol by Fidock et al. (18)

In addition, antimalarial activity was also determined on late stage of parasite whereby the study was conducted as the procedure outlined above except that the Synchronized cultures were incubated for 24 h before addition of ALN/PALN drug to allow for the appearance of late forms and parasitaemia was determined at the end of 2h of incubation.

Fig 11. shows the time dependent antimalarial activity of the developed lipid nanoparticles and standard drug at two drug concentration levels. Std drug ARM showed only 0.39% antimalarial activity against *P. falciparum* at two hours post incubation which further diminished to 0.05% at the end of 6h. ALN showed 0.69 % activity in the ring stage at the end of two hours which increased to 49.69% at the end of 24 hours and then decreased to 12.86% at the end of 48 hours. With 24h synchronous trophozoite late stage culture, ALN showed antimalarial activity of 62.69% at the end of 2h indicating that ALN was highly effective at the late trophozoitic stage of the *P.falciparum* parasite. At lower dose levels, 50% of the therapeutic dose ALN showed negligible activity, maximum being 3.42% at the end of 6h.

In case of PALN, after 2h of incubation, 5.61% antimalarial activity was found which was approximately 5 times higher than the activity shown by ALN at this time point, indicating higher and faster uptake of PALN by the infected RBCs. PALN showed time dependent increase in antimalarial activity, 63.82% at 10h, increasing to 82.88% at the end of 24 h with maximum of 92.69% at the end of 48h. With 24h synchronous late stage culture, PALN showed much higher activity (86.69%) than ALN at the end of 2h. Surprisingly PALN showed 89.48 and 90.23% activity at the end of 24h and 48h at 50% of the therapeutic dose. With PALN showing comparable antimalarial activity of at 100 and 50% therapeutic dose, PEGylation of nanoparticles would lead to reducing Artemether dose by 50% .

At all stages of infection, PALN demonstrated better activity than the bare ALN nanoparticles, clearly demonstrating PEGylation was responsible for enhanced efficacy.

Example 6

Confocal Laser Scanning microscopy to identify uptake of drug loaded nanoparticles specifically by the parasitized RBCs

Fluorescent labeled nanoparticles were prepared by incubating ALN, PALN, AAN and PAAN with Rhodamine B at 25°C for 3h to allow binding of the dye to the nanoparticles. *In vitro* pRBC uptake studies of ALN, PALN, AAN and PAAN was studied on P.falciparum (3d7 strain) using fluorescence microscopy. DAPI was used as the parasite nucleic acid stain. In brief, the parasite culture was grown using Trager Jensen Candle Jar Method for continuous culture. The non- infected and parasite infected RBCs were incubated with Fluorescent dye labeled ALN , PALN, AAN and PAAN formulations in a 24-well cell culture plate, at 37°C. At the end of 0, 2, 24 and 48h the nanoparticle uptake by the infected and non- infected RBCs was studied using CLSM technique. After incubation cells were spun down 2× at 2 min each at 1000 g and washed with RPMI medium before processing for fluorescence microscopy. The cells were wet mounted on a clean glass slide by placing a drop of culture and a glass cover slip was placed on it. The coverslip was sealed on the slide using acetone to avoid any air entrapment. Images were taken using Zeiss Leica 510 microscope at an excitation wavelength of 561nm and emission at 571–625 nm under 100X Oil immersion lens. Images were acquired at 400 Hz in a 512×512 pixels format,8× zoom, and pixel size of 60×60 nm.

Fig 10(a) and (b) shows uninfected RBCs incubated with ALN and PALN at the end of 24h and 48h respectively. Both bare and PEGylated nanoparticles were seen to accumulate at the periphery of the RBCs but did not penetrate the cells. ALN and PALN showed time dependent specific uptake by pRBCs with maximum uptake at 24h. Fig 10(c) shows infected pRBCs incubated with ALN, at end of 2h, nanoparticles are seen clinging to the RBCs but no uptake was seen. This is further confirmed with Z-stacking images (fig. 11a) of infected RBCs incubated with ALN at 2h. The z–stacking images show nanoparticles being adhered onto the surface of the infected RBCs, however are not seen accumulated inside RBCs as seen in middle row of the panel. Again at the lower

surface nanoparticles are found to have adhered to the RBCs. However at the end of 24h (fig 10d) ALN were found to accumulate into the infected RBCs (fig 10e) which is confirmed with Z-stacking images (Fig.11b), where nanoparticles are not seen on the periphery but are seen inside the infected pRBCs incubated with ALN at 24h (Fig.13b middle panel). ALN accumulation in pRBCs decreased with time with fewer nanoparticles seen within pRBC at 48h (fig 10f) and is confirmed with Z- Stacking images (Fig 11c) of pRBCs incubated with ALN at 48h.

PALN showed early uptake by pRBC with few nanoparticles seen accumulating within parasitized RBCs even at 2h (Fig 10f), Z-stacking images (fig. 11d) of infected RBCs incubated with PALN at 2h confirmed this phenomenon. At 24h PALN showed distinct and much higher accumulation into pRBCs (Fig 11h) as compared to ALN which persisted till 48h (Fig 11g). Z-stacking images (Fig. 11e and 11f) of infected RBCs incubated with PALN at 24 and 48h, confirm the higher in-vitro uptake of PEGylated nanoparticles by pRBCs.. This higher accumulation of PALN in pRBC could be responsible for better in *vitro* antimalarial efficacy obtained with PALN at 24 and 48h even at half the dose in *P. falciparum* infected RBCs.

The uptake of the drug loaded HSA nanoparticles was also confirmed by Z-stacking of the confocal images which showed presence of the nanoparticles in various sections of the RBC with respect to time.

15 minutes post incubation, both AAN and PAAN nanoparticles were found to enter inside the pRBC {fig. 12(a) and 13(a)}. Bare nanoparticles, AAN, when incubated with the infected erythrocytes, showed time dependent uptake and hence the nanoparticles were observed inside the pRBCs after 1 hour of incubation were more as compared to 15 min post incubation (Fig. 12(b)). Time dependent uptake was again confirmed at 4 hours as shown with a single cell was focused to observe entry of the nanoparticles in the parasitophorous membrane in Fig. 12(c). Nanoparticles resided in the pRBCs even at 24 hours as observed in Fig. 12(d).

Like AAN, more amount PEGylated nanoparticles, PAAN, were also found to enter pRBCs in 1 hour (Fig. 13(b)) distinctly observed in the parasitophorous vacuole after 4 hours (fig 153c)) and remained in the cells even at 24 hours (Fig. 13(d)).

Z-stacking confirmed maximum amount of nanoparticles enter into the pRBC and into the parasitophorous vacuole in the pRBC. Minimum amount of nanoparticles were observed on the surface of the pRBC. In case of normal RBC, all the nanoparticles were present only on the surface of the nanoparticles and none entered inside the cell.

In vitro pRBCs uptake studies using Confocal Microscopy demonstrated preferential accumulation of nanoparticles in Plasmodium infected RBCs. This could be due to the fact that pRBCs differ widely from the normal RBCs. It is well known that P. falciparum invades and remodels the human erythrocyte; it feeds on haemoglobin, grows and divides, and subverts the physiology of its hapless host. Development of the parasite within the RBC results in remarkable modifications to the RBC that support the growth and multiplication of the parasite.

Our results have proved that the nanoparticles surface modified using PEG have shown increased uptake by pRBCs initially within 15 minutes to 2h of incubation and the accumulation continued for a prolonged period of time as compared to the bare nanoparticles. This could possibly be due to the increased adhesiveness of the PEG cloud around the nanoparticle with the altered pRBC membrane. Infected RBCs incubated with PEGylated nanoparticles at 24 and 48h, confirm the higher in-vitro uptake of PEGylated nanoparticles by pRBCs as compared to bare nanoparticles. While the bare nanoparticles showed maximum accumulation at 24h and declined thereafter, Pegylated nanoparticles showed prolonged accumulation even till 48h. The role of New Permeability Pathway (NPP) could play a significant role in the transport of the nanoparticles to the infected RBCs that are induced in the parasitized cell between 10 and 20 h post invasion. It has been reported that parasite has direct access to extracellular nanosized (80 nm) latex bead particles. The small particle size of developed nanoparticles (80-120nm) contributed to the specific uptake of these particles by the NPPs as shown by Confocal microscopy studies.

We claim,

1. *A pharmaceutical formulation comprises surface modified/functionalized lipid and/ or* protein nanoparticles of Antimalarial drug for targeted delivery, specifically to parasitized RBCs with improved therapeutic activity.

2. The pharmaceutical formulation according to claim 1, wherein the antimalarial drug is selected from Artemether and other artemisinin derivatives such as dihydroartemisinin, arteether and artesunate; lumefantrine, proguanil, atovaquone chloroquine, quinine, mefloquine, amodiaquin, quinine, Sulfedoxine and pyrimethamine.

3. The pharmaceutical pharmaceutical formulation according to claim 1, wherein the formulation comprises antimalarial drug in the range of 0.1 to 100 %w/w; lipid or protein in an amount of 0.1-10% w/w; surfactant/solubilizers in an amount of 0.1 to 10%W/V; surface modification agent and/or functionalizing agent in an amount of 0.001--5% in association with one or more pharmaceutical excipients.

4. The pharmaceutical formulation according to claim 1, wherein the surface modification agent is PEG 200-8000/m-PEG 200-8000 used in an amount of 0.1 to 5% and the functionalizing agent is selected from biotin, pantethenol, dextran or chondriotin sulpahate or any other suitable ligand used in the range of 0.001-1%w/v respectively.

5. The pharmaceutical formulation according to claim 1, wherein the protein is Human Serum albumin used in an amount of 0.1-5%w/w.

6. The pharmaceutical formulation according to claim 1, wherein the formulation is selected from the group consisting of solid, liquid/disperse phase dosage forms.

7. The pharmaceutical formulation according to claim 6, wherein the formulation is selected from the group consisting of tablet, capsule, powder for reconstitution, liquid, disperse phase system, emulsion, Lipid nanocarriers, nanoemulsion, nanocapsules, self-nanoemulsifying drug delivery systems (SNEDDS), polymeric nanocarriers, protein nanoparticles.

8. The pharmaceutical formulation according to claim 3, wherein the lipid is selected from the group consisting of GRAS Lipids including Triglycerides like Trimyristin, Tristearin, Tripalmitin, Tribehanin, trilaurin; Long chain fatty acids like: Stearic acid, Lauric acid, myristic acid; palmitic acid, behanic acid, Capric acid, Caprylic acid, Cerotic acid, archidic acid, lignoceric acid,Glyceryl mono and di-esters like Glyceryl palmitostearate, Glyceryl monostearate, Glyceryl behenate; Glyceryl laurate, Fatty alcohols like Capryl alcohol, Capric alcohol, Cerotyl, archidyl alcohol Cetyl alcohol, Stearyl alcohol, Myristyl alcohol, palmityl alcohol, Benhyl alcohol, lauryl alcohol, lignoceryl alcohol, behnayl alcohol, and Waxes like Ceresine, Hard fat, Microcrystalline waxes The formulation according to claim 3, wherein the surfactants are combination of lipophilic and hydrophilic selected from the group consisting of egg lecithin, phosphatidyl choline, soyabean lecithin, mixed soyabean phosphatides, glycerol Phosphatides, and polaxamers including polaxamer 124, polaxamer 188, polaxamer 237, polaxamer 407,Polysorbates, sorbitan esters,Polyoxytl Stearates, Polyoxyethylene Castor oil derivatives, polyoxyethylene alkyl ethers and the like.

9. The pharmaceutical formulation according to claim 3, wherein the solubilizers are selected from the group consisting of Macrogol-15-Hydroxy stearate, Polyoxyethylene sorbitan fatty acid esters including polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, Caproyl Propylene glycol mono-caprylate, Propylene glycol mono laurate, Polyglyceryl Oleate, Polyoxyl glycerides including Caprylocaproyl macroglycerides, lauryl macrogolglycerides, linoleoyl macrogolglycerides, oleoyl macrogolglycerides, stearoyl macrogol glycerides,Tricaprylin, Caprylic/capric Triglyceride, Trioelin, sorbitan esters, polyoxyethylene stearates, polyoxyethylene castor oil derivatives and polyoxyethylene alkyl ethers and the like.

10. The pharmaceutical formulation according to claim 1, wherein the particle size of the nanocarrier is in the range of 10-200nm.

Dated this the 12th day of September, 2013

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