Nanocarriers to Enhance Solubility, Bioavailability, and Efficacy of Artemisinins

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Abstract

The therapeutic potential of artemisinin (ART) and its derivatives (ARTs) is not limited to malaria but has been recently expanded to other infections with protozoans, trematodes, or viruses as well as to cancer. Due to their limited poor water and oil solubility, rapid degradation by the liver, and short half-life, they have a low bioavailability after oral administration. Consequently, there is a pressing necessity to formulate new ART preparations to raise its bioavailability and efficacy. Nanosized drug delivery systems represent important tools in modern medicine with wide clinical applications, because of their potential modulation of pharmacokinetic and biodistribution. This review focuses on polymer-based systems, lipid-based systems, and inorganic nanoparticles loaded with ARTs. The overall goal of this field of research is to enhance their solubility and stability to improve bioavailability at much lower doses and to increase long-term safety. In addition, the opportunity to reach highly specific site-targeted delivery by these nanocarriers confers a high medicinal value. Remarkably, most of the reported nanoparticulate drug delivery systems are biologically inactive or marginally immunogenic, generating no antigenic or pyrogenic reactions but only partial intrinsic toxicity. As clinical studies in human patients are available so far, there is a pressing need to translate preclinical results on ART-based nanosystems into clinical settings.

Keywords: Artemisinin, Asteraceae, derivatives, inorganic nanocarriers, lipid nanocarriers, nanotechnology, polymeric nanocarriers, sesquiterpene lactones

INTRODUCTION

Recently, new clinical perspectives were opened for artemisinin (ART) and its derivatives (ARTs), not only limited to malaria,[1] but with potential application toward infections with protozoans,[2] trematodes,[3] or viruses[4] and toward cancer.[5,6] In most cases, however, their therapeutic value is narrow due to low aqueous and oil solubility. This poor bioavailability after oral intake is due to fast metabolization by the liver and a little half-life (about 2.5 h).[7] Consequently, there is a crucial necessity to formulate new ART preparations to enhance its bioavailability, selectivity, and clinical use.

Currently, nanosized drug delivery systems represent important factors in modern medicine with wide clinical applications, because of their potential modulation of pharmacokinetics and biodistribution.[8] Their size is correlated to the cellular uptake ratio (the best values of diameter are between 200 and 300 nm) and to the period they persist in the bloodstream (if less than 10 nm, clearing is by glomerular filtration in the kidneys).[8-10] These nanovehicles can modulate pharmacokinetics and biodistribution of drugs, thus reducing possible side effects by leaving the normal sensitive cells uninjured.[9] They can increase the therapeutic index of drugs by enhancing their localization to specific tissues, organs, or cells because of a possible active targeting.[9] In addition,
they can sustain drug release over an extended period and are usually appropriate for different delivery routes, namely oral, ocular, parental, mucosal, dermal, pulmonary, and transdermal. Finally, nanocarriers, if conveniently designed, are capable to cross biological barriers, namely blood–retinal barrier, blood–brain barrier, blood–cerebrospinal fluid barrier other barriers of the eye (corneal, tear film, and aqueous).[10]

Nanocarriers are formulated using different materials, which can successfully deliver hydrophobic drugs or hydrophilic drugs, as in the case of vesicles. Generally, nanocarriers are classified as polymer-based systems, lipid-based systems, and inorganic nanoparticles [Figure 1].[8,9]

This review focuses on nanocarriers based on ARTs developed to enhance solubility, bioavailability, and efficacy that have already advanced in comparison with traditional formulations, as stated by in vivo or in vitro experimentation. The presentation of the studies is based on nanocarriers’ chemical composition, i.e., lipid nanovectors, polymeric nanovectors, and inorganic nanovectors.

**Lipid NanoveCTors**

Lipid-based nanoveCTors comprise vesicles, micelles, emulsions having nanometric scale (microemulsion and nanoemulsions [NEs]), and nanoparticles, classified as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs).[8,9]

**Vesicles**

Vesicles are aggregates of amphiphilic lipids that spontaneously form by layer structures [Figure 2]. Liposomes, first proposed as drug carriers in 1971, are the most widely used vesicles and consist of synthetic or natural phospholipids and cholesterol. They are flexible vectors suitable for loading both hydrophilic drugs (solvilized in the aqueous compartments) and lipophilic drugs (hosted into the bilayer). According to their size and the number of lipid bilayers, liposomes are classified as large multi-lamellar liposomes (MLV), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). A further group of vesicles is represented by niosomes, constituted of nonionic surfactants, structurally comparable to liposomes. Generally, niosomes are more stable than liposomes and offer some important advantages over liposomes, because they are stable at room temperature without necessity of inert atmosphere for the protection or storage.[8,9]

First liposomal formulations were reported by Al-Angary and colleagues,[12] who developed liposomes loaded with arteether and evaluated their stability and release properties. In particular, the authors demonstrated that the acyl moiety length as well as the addition of cholesterol produced a reduction of the arteether release rate. Two years later, an in vivo study of arteether-loaded liposomes to New Zealand rabbits at a dose of 50 mg/kg (orally given) demonstrated that liposomal formulations had better pharmacokinetic parameters, if compared with an aqueous suspension of micronized arteether. High bioavailability was found with a faster rate and a better absorption of arteether. Higher \( C_{\text{max}} \) and shorter \( T_{\text{max}} \) as well as a higher value of area under the curve (AUC) were obtained with liposomes, and a relative bioavailability of 97.91% compared with 31.83% was obtained with the oral suspension.[13]

Few years later, Chimanuka et al.[14] reported an in vivo study to evaluate the therapeutic efficacy of \( \beta \)-artemether liposomes in mice against *Plasmodium chabaudi* infection. The formulation was characterized by extraordinary encapsulating capacity (ca. 100%) and huge stability even after 3 months of storage at 4°C. Mice infected with the virulent rodent malaria parasite and treated with 1.5 mg liposomal \( \beta \)-artemether cured completely without recrudescent parasitemia.

In the same period of time, Gabriëls and Plaizier-Vercammen[15] developed liposomes loaded with artesunate and evaluated their properties. Liposomes were loaded with 1 mg/ml

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**Figure 1:** Classification of nanocarriers
artesunate and had a great encapsulation efficiency (ca. 100%) and high stability with a maximum value using phosphate buffer of pH 5. The *in vitro* release test proved that artesunate was reversibly encapsulated, and the release was influenced by several factors. For instance, the content of lipids or the amount of artesunate enhanced the release velocity. A method for sterile production of liposomes at laboratory-scale level was also reported.

During the past decade, a huge amount of studies have been published dealing with the possible use of liposomes as suitable carriers for ARTs. Isacchi *et al.*[7] developed conventional and long-circulating, polyethylene glycol (PEG)-decorated ART-loaded liposomes. Both liposomal formulations showed an encapsulation efficacy of >70% and a suitable size for parental administration (130–140 nm) with a suitable homogeneity (polydispersity index of 0.2–0.3). The main pharmacokinetic parameters were estimated in mice after intraperitoneal injection. Unformulated ART was quickly cleared from the blood circulation and almost not detected 1 h after administration, while ART loaded in conventional and long-circulating liposomes was still detectable after 3 and 24 h after the administration, respectively. Furthermore, the AUC (0–24 h) values were improved by roughly six times in both liposomal preparations, if compared with unformulated ART. On average, the half-life of ART was enhanced by more than five-fold by long-circulating liposomes.

The same authors also investigated the efficacy of the developed liposomes in *Plasmodium berghei* NK-65-infected mice. ART at the dosage of 50 mg/kg/day alone or plus curcumin was administered at the dosage of 100 mg/kg/day to the mice. Free ART reduced parasitemia levels only 7 days after the beginning of the treatment, and this treatment had fluctuant blood concentrations of ART. By contrast, all other treatments (conventional liposomes loaded with ART, conventional liposomes loaded with ART plus curcumin, PEGylated liposomes loaded with ART, and PEGylated liposomes loaded with ART plus curcumin) had a rapid antimalarial effect and cured all malaria-infected mice within the identical postinoculation period. In particular, ART-loaded PEGylated liposomes gave the most pronounced and statistically significant therapeutic effects in this murine model of malaria.[16]

Analogous PEGylated liposomes loaded with ART but with a mean diameter of 455 nm were developed by Dadgar *et al.*[17] and evaluated against MCF-7 cells. Encapsulation efficiency and drug release were approximately 92% and 5%, respectively. The cytotoxicity was analyzed by means of the MTT method. PEGylated liposomes had a lower IC$_{50}$, if compared to the standard drug (1.58 µg/ml vs. 2.3 µg/ml).

Jin *et al.*[18] developed vesicles loaded with artesunate, which were estimated for their cytotoxicity against HepG2 cells. The IC$_{50}$ rates of artesunate loaded into vesicles and free drug were 16 and 20 µg/ml, respectively. In addition, the liposomal formulation of artesunate was tested against L-O2 normal human liver cells obtaining IC$_{50}$ values of 100 and 106 µg/ml, respectively. Artesunate-loaded in liposomes had an inhibitory effect on tumor growth of 32.7% while that of the free drug was 20.5%. A dose-dependent apoptosis was found in HepG-2 cells treated with artesunate loaded in liposomes. Artesunate loaded in liposomes was more effective on human hepatoma HepG2 cells than the unformulated artesunate.

Zhang *et al.*[19] formulated liposomes loaded with ART dimers–piperazine conjugates. These liposomes efficiently released the drugs at acidic pH values characteristic of solid tumors and endosomes and lysosomes. The nanosystem downregulated the antiapoptotic protein surviving, cyclin D, the oncogenic protein human epidermal growth factor receptor 2, and its counterpart, human epidermal growth factor receptor 3 after incubation in a HER2$^+$ cell line.

Righeschi *et al.*[20] developed dihydroartemisinin-loaded conventional and long-circulating liposomes, which were tested for the cytotoxic effects against the MCF-7 cell line. Both formulations were suitable for parental administration in terms of size with good values of encapsulation efficiency (ca. 70% for both). Physical and chemical stabilities were high under storage conditions and in the presence of albumin. Cellular uptake studies by flow cytometry showed high internalization, greater in the conventional liposomes, if compared with long-circulating vesicles. The lack of toxicity of blank preparations in MCF-7 cells indicated that liposomes may be suitable vector for delivery of dihydroartemisinin circumventing the usage of organic solvents. The cytotoxicity of dihydroartemisinin and the formulations gave the following IC$_{50}$ values: 12.1, 48.2, and 77.0 µM, respectively, for free drug, conventional, and long-circulating liposomes.

In a further study by Li *et al.,*[^21^] paclitaxel plus arteremether liposomes were developed and decorated with mannose -Vitamin E derivative denominated mannose-Vitamin E derivative conjugate (MAN-TPGS1000) and dequalinium -lipid derivative called dequalinium-lipid derivative conjugate (DQA-PEG2000-DSPED). The crossing blood–brain barrier properties of the carrier were due to receptor-mediated endocytosis by MAN-TPGS1000 and adsorptive-mediated endocytosis by DQA-PEG2000-DSPED. The liposomes were tested on human glioma cells *in vitro* and on brain glioma-bearing rats *in vivo*. Destruction of vasculogenic
mimicry (VM) channels and the induction of apoptosis in brain cancer cells due to the activation of apoptotic enzymes and proapoptotic proteins and inhibition of antiapoptotic proteins were the mechanisms of action of the investigated drugs.

Gharib et al.\[22\] settled ART and transferrin-loaded magnetic liposomes in thermosensitive and nonthermosensitive forms evaluating the antiproliferative activity against MCF-7 and MDA-MB-231 cells. The encapsulation efficiencies of ART, transferrin, and magnetic iron oxide in the nonthermosensitive liposomes were approximately 89%, 85%, and 78%, respectively. The developed liposomes had a suitable polydispersity index, size uniformity, as well as a worthy stability at pH 7.4 (citrate-phosphate buffer) within 12 h at 37°C. Furthermore, the thermosensitive preparation had appropriate characteristics for thermal drug release at 42°C. These liposomes displayed dose (12.5–100 μg/ml ART) and time-dependent (12–48 h) antiproliferative activity against MCF-7 and MDA-MB-231 cells in the presence of a magnetic field.

Zhang et al.\[23\] formulated ART dimers—piperazine conjugates liposomes having high encapsulation efficiency (>90%) and a size of 70–76 nm. More than 50% of the conjugates were released in 48 h at pH 4.0, while in neutral conditions, the released conjugates were 20%. The uptake of liposomes was by endocytosis followed by the release of core-trapped marker through the cytosol at 37°C. The liposomes were well tolerated by nontumorigenic cells. In addition, the developed liposomes were more effective than paclitaxel in controlling cancer cells due to the activation of apoptotic enzymes and the induction of apoptosis in brain cancer cells. Tumor necrosis factor-α immunity toward protective helper T type 1 cells was also evident. Increased AUC values and prolonged residence time of drugs loaded into liposomes were assessed compared with conventional liposomes. Transferrin-conjugated liposomes were also more cytotoxic if compared with the other liposomal formulations. The IC_{50} values of ART-loaded into transferrin-decorated liposomes were 69 μM versus 121 μM of long-circulating liposomes.

Kang et al.\[27\] produced liposomes decorated with mannose for co-delivery of dihydroartemisinin and doxorubicin to overcome multidrug resistance in human colon tumor HCT8/ADR cells, which overexpressed mannose receptors. The liposomes had a size of 158.8 nm and a zeta potential of −15.8 mV. Liposomes decorated with mannose in the surface displayed a great accumulation of doxorubicin in the nuclei and had the maximum cytotoxicity (IC_{50} = 0.073 μg/ml). Liposomes administered to an HCT8/ADR tumor xenograft model, caused a decrease of the tumor of 88.59%, compared to the values of 47.46% or 70.54%, respectively, for the treatment with free doxorubicin or free doxorubicin plus dihydroartemisinin.

Want et al.\[28\] developed ART-loaded liposomes to evaluate their efficacy in vitro and in a murine model of experimental visceral leishmaniasis. The liposomes were characterized by a size of 83 nm, a polydispersity index of 0.2, and a zeta potential of −27.4 mV, while the drug loading was 33.2%. The liposomes significantly decreased the intracellular infection of Leishmania donovani amastigotes, and the number of infected macrophages with an IC_{50} value of 6.0 and 5.1 μg/ml, respectively. In vivo studies demonstrated that liposomes were more effective than free ART having an infection decrease of 82.4% in the liver and 77.6% in the spleen when 20 mg/kg body weight ART was administered. A modulation of cell-mediated immunity toward protective helper T type 1 cells was also found.

In a further study, Shackle et al.\[29\] prepared liposomes co-encapsulated with artemether and lumefantrine. Entrapment efficiency of artemether was 66.18% and that of lumefantrine was 53.46%. The liposomes had a size of 125.3 nm and were stable at 4°C for 60 days. In vitro drug release studies showed a first burst effect followed by a sustained release process over a time period of 30 h. Liver and kidney function tests as well as histopathological examinations assessed in vivo safety properties of liposomes. A low hemolytic potential was found, and no significant modification (P > 0.05) in biochemical parameters between control and treated group of animals was evident. Increased AUC values and prolonged residence time of drugs loaded into liposomes were assessed compared with a solution of the two drugs. In addition, enhanced uptake in organs of the reticuloendothelial system was found for drug loaded in liposomes, in particular, in the liver and the spleen. Tian et al.\[30\] developed liposomes of artemether with a size...
of 187 nm. Encapsulation efficiency and drug loading were 94% and 11%, respectively. An in vitro release study showed a sustained release property of the liposome according to a first-order kinetics equation. After intravenous injection to mice, the t½, the AUCs of arteether loaded into liposomes were 8.38-, 3.38-, and 3.11-fold to those of arteether solution, respectively. In the pharmacodynamic studies, the tumor doubling time, growth inhibition rate, and specific growth rate of tumor of arteether loaded into liposomes were 1.97 times, 1.54 times, and 0.51 times higher than arteether solution.

Ismail et al.[31] developed liposomes loaded with dimeric arteesunate and liposomes containing dimeric artesunate conjugated with glycophosphorylcholine. The size of developed liposomes dimeric artesunate conjugated with glycophosphorylcholine was 190 nm with a negative zeta potential of −20.35 mV. Drug loading was 77.6%. An in vivo pharmacokinetic study revealed that liposomes with artesunate dimers conjugated with glycophosphorylcholine had a longer retention half-life in the bloodstream. The liposomes loaded with dimeric artesunate had an IC₅₀ value 0.39 nM. The liposomes containing the conjugate had an IC₅₀ value of 1.90 nM, and both did not cause hemolysis of erythrocytes. The IC₅₀ value of free artesunate was 5.17 nM while that of conventional liposomes loaded with artesunate was 3.13 nM. Moreover, liposomes containing the conjugate resulted in improved in vivo the parasites killing in P. berghei-infected mice with delayed recrudescence and enhanced survivability compared to free artesunate administration.

A unique noisome formulation was reported in literature. Dwivedi et al.[32] described the development of vesicles prepared with sorbitan monostearate: cholesterol (3:1 ratio) and artemisone. These vesicles were evaluated for their effects in human A-375 melanoma cells and human HaCaT keratinocytes. The encapsulation efficiency was 67%, with a size and zeta potential of 211 nm and −38 mV, respectively. After 7 h of the test, the drug release was 85%. The MTT assay showed that niosomes considerably inhibited melanoma cells (P ≤ 0.05) in a dose-dependent manner. Unloaded artemisone (0.06 mg/ml) inhibited 50% of melanoma cell growth, while artemisine (0.06 mg/ml) loaded in niosome almost completely inhibited melanoma cell growth. Niosomes were very discriminating cytotoxic to the melanoma cells with insignificant toxicity toward the normal skin cells.

**Micelles**

Micelles are aggregates (generally 50–100 monomer units) of amphiphilic molecules containing a polar “head” and a nonpolar hydrocarbon chain “tail” in a liquid medium, generally represented by water. Aggregation occurs, if the critical micellar concentration (CMC) is reached, to minimize the interaction of the hydrophobic tail with water. Generally, micelles have a spherical structure leaving only the water-soluble ionic heads exposed to solution. However, at increasing concentrations, they can form elongated columns that can pack into hexagonal arrays or other structures. Micelles can represent useful nanovectors for the delivery of drugs scarcely soluble in water, because of their loading into the hydrophobic inner core. The small size (10–100 nm) of these vectors render efficient their accumulation in pathological tissues with leaky vasculature, such as tumors and infarcts, by improved permeability and retention effects.[8,12]

Bilia et al.[33] prepared octanoyl-6-O-ascorbic acid (ASC8) micelles to solubilize ART. ASC8 is a surfactant with radical scavenger properties. When ART added to ASC8 micelles, it was efficiently solubilized without significant perturbation of micellization. The CMC of ASC8 (approximately 6 mM) was evaluated using diffusion-ordered NMR spectroscopy experiments. ART water solubility was approximately 0.21 mM and resulted approximately 1 mM in the presence of 60 mM ASC8. ART and curcumin separately and in the mixture were also solubilized in micelles of sodium dodecyl sulfate (SDS). 40 mM SDS increased approximately 25-fold the aqueous solubility of ART, while 81 mM SDS enhanced about 50-fold ART water solubility and 40-fold the water solubility of curcumin (from 2 mM in water to 81 mM in SDS). In the same study, the solubility properties of ASC8 were combined with its antioxidant activity. Micelle prepared with 40 mM SDS and 60 mM ASC8A increased ART water solubility by 16-fold. ASC8 increased the stability of ART in the presence of 60 mM of hydrogen peroxide, suggesting possibly smart nanocarriers based on SDS and ASC8.[34]

Micelles based on tri-block copolymers of poly(e-caprolactone)-poly(ethylene glycol)-poly(e-caprolactone) (PCL-PEG-PCL) were also applied to improve the bioavailability, water solubility, and antiplasmodial property of ART. The zeta potential of the polymeric micelles (PMs) loaded with ART was −8.37 mV and the size was 91.87 nm. The resulting loading capacity was 19.33% ± 0.015% and the encapsulation efficacy was 87.21% ± 3.32%. Finally, the in vivo antiplasmodial activity of ART loaded in the PMs was measured in P. berghei-infected Swiss albino mice. Multiple injections of ART-loaded micelles prolonged the circulation time and increased the therapeutic efficacy of ART.[35]

In another study, PMs of PCL-PEG-PCL tri-block copolymers were synthesized and evaluated for ART delivery in in vitro and in vivo studies. ART was encapsulated into micelles by a nanoprecipitation method (ART/PCL-PEG-PCL). The obtained micelles had an average size of about 83.22 nm. The encapsulation efficacy was 89.23% ± 1.41%. This formulation significantly increased drug accumulation in tumors, as proved by in vivo results. Pharmacokinetic study in rats revealed that in vivo ART-loaded micelles increased and prolonged drug exposure, if related to the same dose of free ART. The MTT assay showed that unloaded PCL-PEG-PCL micelles were nontoxic to MCF7 and 4T1 cancer cells, whereas when loaded with ART, they had a definite toxicity to both cancer cell lines.[36]

To specifically deliver artemisinin to both highly metastatic tumour and its lymphatics, tumour- and tumour lymphatics-
Solid lipid nanoparticles and nanostructures lipid carriers

SLNs are very stable nanovectors with sizes ranging from 50 to 1000 nm. SLNs are easily to produce on a large scale, and they have been widely proposed by numerous researches to carry therapeutic agents and diagnostics.

They are mainly composed of lipids that are in the solid-state at room temperature, stabilized by surfactants. Due to their small size and biocompatibility, these vectors can be used for different routes of administration, mainly oral, parenteral, and percutaneous. If administered orally, they can be absorbed through different mechanisms such as mucoadhesion, nanoparticle internalization, and permeation-enhancing effects. The advantages of SLN include a controlled drug release and drug targeting. In addition, the loaded drugs are protected from chemical degradation.\textsuperscript{[41]}

NLCs are a secondary generation of lipid nanoparticles, overcoming some drawbacks of SLNs. NLC contains a mixture of liquid and solid lipids and an aqueous phase containing a surfactant or a mixture of surfactants. This disorganized matrix ameliorate the loading capacity of the carrier.\textsuperscript{[8,11]}

Other advantages are long-term stability, improved bioavailability of loaded drug, a controlled or targeted release, and possibility to load both lipophilic and hydrophilic drugs. Finally, they are made of biocompatible and biodegradable lipids, decreasing the possibility of acute or chronic toxicity.\textsuperscript{[41]}

Solid lipid nanoparticles

SLNs prepared with tween 80, pluronic F68, and soya lecithin were loaded with arteether gave homogeneous particle sizes of about 100 nm and loading efficiency nearly 69%. The release of arteether from SLNs was gentle but time-dependent. This was required, as it would aid to inhibit the hydrolysis of arteether in the stomach. No cytotoxicity of blank SLN was found. Pharmacokinetics studies showed that the absorption and the relative bioavailability of arteether formulated as SLN has been considerably improved in rats, in comparison to aqueous arteether and arteether in groundnut oil.\textsuperscript{[42]}

The same authors reported the preparation of SLNs using a solid lipid and 2:1 monostearate: lecithin, which were loaded with artemisone (10-amino-artemisinin). The study investigated the effects of SLNs against human melanoma A-375 cells and human HaCaT keratinocytes. The loading capacity was about 80% with an average particle size of approximately 295 nm and a zeta potential of −12 mV. The drug release was 85% after 7 h. The formulation significantly suppressed melanoma cells (\( P \leq 0.05 \)) in a dose-dependent manner, as shown by the MTT assay. Free artemisone (0.06 mg/ml) suppressed almost 50% of the melanoma cell growth, whereas loading into SLNs resulted in a complete suppression of melanoma cell growth.\textsuperscript{[22]}

Artesunate was loaded into a polymer lipid hybrid nanovector and investigated for the anticancer activity. Chitosan was used to coat the lipid nanoparticles which was loaded with artemesate (chitosan-coated lipid nanoparticle [ART-CLN]). ART-CLN had a positive charge (+13.2 ± 0.87 mV), a small particle size (160.9 ± 3.5 nm), and a spherical shape. Extraordinary drug loading (95.49% ± 1.13%) and sustained homing peptide (LyP-1) conjugated PEG-PCL micelles (LyP-1-PM) were developed. Both polymeric micelles and LyP-1-PM had similar physiochemical properties, about 30 nm in size with uniform distribution. Highly metastatic breast cancer MDA-MB-435S cells and lymphatic endothelial cells (LEC) were applied as cell models. LyP-1-PMs loaded with ART had greater antitumor effects than PMs against these two cell lines using cell apoptosis, cell cycle, and cytotoxicity tests. PMs accumulated near blood vessels, while those decorated with LyP-1 was more located to tumor lymphatic vessels. As a consequence, these micelles displayed greater antitumor efficacy in vivo with little toxicity. Both in vitro and in vivo studies demonstrated that LyP-1 decoration improved the specific delivery of ART in both tumor cell lines, evidencing a powerful use of these micelles for therapeutic or imaging nanovectors.\textsuperscript{[37]}

In a further study, dendritic micelles based on methoxypolyethylene glycol (MPEG) 2000 and 5000 linked to di-fluorene methoxycarbonyl-L-lysine and to the two amino groups of L-lysine were developed up to 2.5 generations. The 0.5 G, 1.5 G, and 2.5 G dendritic micelles of MPEG 2000 and 5000 were used to load arteether. An up to 3–15 times increased solubility of arteether was found to depend on concentration, generation, and type of the dendritic micelles used. Moreover, the formulations improved the stability of the drug and also extended the release of arteether up to 1–2 days in vitro.\textsuperscript{[38]}

Arteether was formulated into biodegradable and pH-sensitive polyurethane nanomicelles to increase its quantity in tumor microenvironment. The nanovector had a negative zeta potential of −26.2 mV, a size of about 42.30 nm, and an extraordinary loading capacity (92%). The release profile showed a faster rate of drug liberation at pH 5.4 when compared to that obtained at pH 7.4.

After the administration of the vector, the levels of interferon-gamma (IFN-\( \gamma \)) and IL-4 cytokines of mice splenocytes were assessed by ELISA, and significant inhibitory effects on the growth of the 4T1 cell line with increased IFN-\( \gamma \) levels were observed.\textsuperscript{[39]}

Artesunate was MPEGylated PEG monomethyl ether, and the obtained formulation was a nanocapsule. They had an average particle size of 88.7 nm. The vesical morphology was confirmed by transmission electron microscopy. The zeta potential was around −12.4 mV. The release of artemesate from the nanocapsules was related to the hydrolysis of the ester bond, and more than 80% of the loaded artemesate in the delivery system was released during 10 h. The in vitro cytotoxicity of the MPEG-ART nanocapsules was evaluated in murine L1210 leukemia cells using the MTT assay. The cytotoxicity of the prodrug showed an essential decrease compared with the free artemesate. These results offer a new strategy to design antitumor artemesate nanocapsules for targeting tumor cells.\textsuperscript{[40]}

Nanocarriers for artemisinins

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release properties were observed. The anticancer activities were confirmed toward different cancer cell lines by MTT assay. Finally, ART-CLN displayed higher anticancer activity than free artesunate against breast cancer cells (MCF-7 and MDA-MB-231). The loading of artesunate into lipid core of polymer lipid hybrid carrier increased the activity and physical stability of artesunate and improved its cancer chemotherapy.\[43\]

**Nanostructures lipid carriers**

NLCs were applied to improve the biopharmaceutics characteristics of artemether (Nanoject). The average particle size of Nanoject was about 63 nm, with an encapsulation efficiency of about 30% and a sustained release profile. Nanoject displayed minor hemolytic potential (approximately 13\%) when compared to the single components. Nanoject displayed considerably greater antimalarial activity ($P < 0.005$) when compared to the marketed formulation based on injectable oily intramuscular preparation. The antimalarial activity of Nanoject continued for more than 20 days, indicating that Nanoject may be long-circulating in vivo. Nanoject displayed considerably greater survival rate (60\%) even after 31 days when compared to the oily formulation which showed 0\% survival (100\% mortality).\[44\]

Dihydroartemisinin was also formulated as NLCs. The carrier was made of glycerol monostearate and Miglyol\* 812 as solid–liquid and liquid–lipid material, respectively. Surfactants were Tween 80 (1\%) and Poloxamer 188 (1\%). The pharmacokinetic and tissue distribution were compared after intravenous administration of dihydroartemisinin in solution and loaded in NLCs. Following intravenous administration of a dihydroartemisinin solution, the mean measured peak plasma concentration was 917.51 ng/ml for dihydroartemisinin and 289.28 ng/mL dihydroartemisinin-NLCs. After 2 h, the plasmatic concentration was inferior for the dihydroartemisinin solution than that for dihydroartemisinin, because of its solubility in the plasma resulted in a rapid distribution, elimination, and slower release of dihydroartemisinin from NLCs, leading to poorer clearance. AUC (0\text{--}\infty) increased from 633.97 ng/ml/h for the free drug to 1382.45 ng/ml/h for the dihydroartemisinin-NLCs. The mean residence time value of the dihydroartemisinin-NLCs was greater than that of free drug. The distribution half-life of both formulations was similar, while the volume of distribution of drug loaded in NLC was 14.91 mg/kg (ng/ml), considerably greater than that of the drug solution (5.21 mg/kg [ng/ml]). Furthermore, the clearance (CLs) of the dihydroartemisinin solution were higher than that of dihydroartemisinin-NLCs, which suggested that NLCs caused a sustained release of the drug. By evaluating the amount of dihydroartemisinin in the tested organs, the NLCs increased the amount of drug in liver, spleen, lung, brain, and muscle and decreased the drug in heart and kidney, respect to solution.\[45\]

NLCs based on glyceryl trimitystate and soybean oil and loaded with 10\% artemether were used as a combination of nonionic, cationic, or anionic surfactants to modify the surface. Particle size, zeta potential, and encapsulation efficiency were approximately 120 nm, $-38$ mV, and 97\%, respectively. The hemolytic activity was in the tolerable range revealing a little toxicity risk of NLCs for parenteral artemether administration. Biocompatibility was evaluated by hepato- and nephron-toxicity investigations. \textit{In vivo} antimalarial studies showed an improved activity of NLCs in comparison to the free drug and to a marketed formulation.\[46\]

NLCs were developed to enhance the oral efficacy of artemether-lumefantrine (ARM-LFN), which is a fixed-dose combination approved by the World Health Organization with low solubility and poor oral bioavailability. The therapeutic dose for adults is 80 mg for ARM and 480 mg for LFN twice a day. NLCs were safe in rats and displayed sustained release of the drugs. ARM-LFN NLCs given once a day at 1/5 of therapeutic dose (16 mg ARM and 96 mg LFN) showed complete parasite clearance and 100\% survival in \textit{P. berghei}-infected mice. Only 33\% of the mice treated with marketed tablets twice a day at the therapeutic dose showed late-stage recrudescence. NLC displayed improved efficacy at 1/10 of the daily dose of ARM-LFN. The ARM-LFN NLCs were formulated in soft gelatin capsules, which showed good stability at room temperature for 1 year, being an opportunity for oral malaria therapy.\[47\]

Tran et al.\[48\] prepared artesunate-loaded NLCs (ART-NLCs). The ART-NLCs had a diameter of 117.5 $\pm$ 6.1 nm and a zeta potential of $-19.47 \pm 0.9$ mV. Drug entrapment efficiency was 92.93\% $\pm$ 1.47\%. ART-NLCs showed good cellular uptake in breast cancer cells and a significantly higher \textit{in vitro} cytotoxicity against human MCF-7, MDA-MB-231 breast cancer cells than free ART. Hoechst 33342 staining indicated that ART-NLCs induced higher apoptosis rates in MCF-7 and MDA-MB-231 cells than free ART.

**Nanoeumulsions**

Recently, there has been considerable interest on microemulsion and NE formulations for the delivery of hydrophilic as well as lipophilic drugs and for their enhanced drug solubility, bioavailability, extensive shelf life, and the simple formulation.\[49\]

Microemulsions are homogeneous thermodynamically stable and transparent dispersions of two immiscible liquids stabilized by surfactants. The droplet size is between 10 and 500 nm and needs only little energy to prepare the emulsion, since they form spontaneously when the aqueous, lipid, and surfactant components are put together. Further, their production costs are lower when compared to NE, which are nonequilibrium systems with a natural propensity to separate into the two phases. NEs require inferior surfactant amounts with respect to microemulsions. However, NEs may have a relatively great kinetic stability even for several years due to their very small size droplets, as a consequence of substantial steric stabilization between droplets. The size of droplets is between 20 and 500 nm and is stated as mini-emulsions, ultrafine emulsions, and submicrometric emulsions.
A further formulation is represented by the self-micro-emulsifying drug delivery system or self-nanoemulsifying drug delivery systems (SNEDDS). This is a stable mixture of drug, oil, surfactant, and co-surfactant, which form reasonable oil-in-water droplets with a diameter less than 100 nm, simply under slight agitation of the gastrointestinal tract without the dissolution process. Self-emulsifying drug delivery systems have the potential to deliver poorly water-soluble drugs, because microemulsion and NE droplets provide 100% capability to load a drug and protect it from gastrointestinal degradation. Moreover, the droplets are quickly distributed in the blood as well as lymph, avoiding the first-pass effect. Furthermore, self-emulsifying drug delivery systems are appropriate for filling in gelatin capsules.\[53\]

SNEDDS containing medium-chain triglycerides (MCTs) and long-chain triglycerides (LCTs) were prepared for increasing the biopharmaceutical and antimalarial potential of artemether. CapteX 355 and ethyl oleate were selected as triglycerides, cremophor RH 40 and tween 80 as tensides, and transcutol HP as co-solvent.

MCT-SNEDDS prepared with CapteX 255 (412 mg), cremophór RH40 (688 mg), and transcutol HP (154 mg) had a size of 51.7 nm, whereas LCT-SNEDDS containing ethyl oleate (154 mg), tween 80 (604 mg), and transcutol HP (113 mg) had a size of 91 nm. The negative values of the zeta potential (−28 mV and −36 mV) confirmed high stability of NE system. In vitro drug release profiles showed marked improvement in the drug release rate formulated as SNEDDS. Artemether release was almost 100% within 20 min for the optimized formulations, while free drug showed only 41.73% release over a time period of 3 h.

In situ single-pass intestinal perfusion studies in Wistar rats showed a significant increase in the absorption and permeation parameters of MCT and LCT-SNEDDS compared to the free artemether. In vivo studies in P. berghei-infected mice exhibited higher reduction in parasitemia, SGOT, SGPT, and bilirubin. In addition, a superior survival rate of the animals was found principally with MCT-SNEDDS and at less extended with LCT-SNEDDS. These results were confirmed by histopathological examination of liver tissues.\[50\]

Laxmi et al. developed an NE of artemether with enhanced solubility, stability, and oral bioavailability. The oil phase contained artemether solubilized in coconut oil and span 80, while the external phase was tween 80 and ethanol solubilized in water. As analyzed by transmission electron microscope, the globules of the NE had a size of 79.0 nm and a zeta potential of −15 mV, with an expected good electokinetic stability. It had a clear and transparent appearance with 98.2% transmittance. The release artemether from the NE formulation was reasonably important when compared to the free artemether. Stability studies were carried out for a period of 90 days. In vivo studies of the formulation with Wistar rats showed that the bioavailability was 2.6-fold higher than the plain drug (about 40%).\[51\]

Five NEs loaded with arteether were applied against malaria parasites. The aim of the study was to development of an oral formulation of arteether, which increases the bioavailability and reduces the arteether doses. The maximal achieved artemether loading was 93% ± 7.4%, the globule size was 156 ± 10.2 nm, and the zeta potential was −23.3 ± 3.4 mV. The developed NEs were stable at different pH values. The in vitro release profile of the artemether-loaded NE showed 62% drug release within 12 h. The pharmacokinetic study was conducted using a NE containing 1% w/w arteether, 5 ml of groundnut oil (GNO), 0.5% w/w tween 80, 0.5 w/w span 80, and 45 ml water. This NE showed significantly enhanced bioavailability of the drug. NE had a high antimalarial efficacy and survival cure rate (80% at 12.5 mg/kg for 5 days) in mice, when compared with arteether in GNO at the same daily dose, and it was also comparable to the 100% cure rate at 12.5 mg/kg for 5 days given by arteether administered intramuscularly.\[52\]

### Polymeric Nanoparticles

Polymeric nanoparticles are extensively used for the encapsulation of various drugs, both hydrophilic and hydrophobic ones. According to the structure, nanoparticles are roughly divided in nanospheres (matrix systems) and nanocapsules, where the drug is confined to a cavity as reservoir system.

In polymeric nanoparticles, drugs are bound to surface, linked to the polymer, physically and uniformly dispersed in the matrix or encapsulated inside. Polymeric nanoparticles are made of natural (proteins and polysaccharides), semisynthetic, or synthetic polymers. Biodegradable polymers are highly preferred because of their high biocompatibility with tissues and cells. Among natural polymers, the most used ones are albumin, gelatin, chitosan, hyaluronan, proteins, such as silk fibroin, collagen, and others. Synthetic derivatives include poly(alkylcyanoacrylates), poly(lactic acid), poly(glycolic acid), poly(ε-caprolactone), and their copolymers.\[53\]

Yaméogo et al.\[53\] developed nanosystems based on cyclodextrins linked to decanoic alkyl chains (CD-C10). Nanosphere or nanoreservoir-type systems with a size of 70–220 nm were obtained and loaded with 0.3 and 1.6 mg/ml ART, for the nanospheres and reservoir-type nanovectors, respectively. Nanoparticles decorated with PEG did not influence the ART bioavailability. Both types of ART-loaded nanovectors showed a sustained in vitro release profile over 96 h (nanoreservoirs) and 240 h (nanospheres). Finally, the formulations inhibited the growth of cultured Plasmodium falciparum, both multi-resistant K1 and susceptible 3D7 strains with IC\(_{50}\) values of 2.8 and 7.0 ng/ml, respectively.

The same authors prepared long-circulating micelles, which were tested in vitro toward the immune system (complement activation and macrophage uptake assays) and for their biodistribution in mice. In vitro plasma protein adsorption and phagocytosis by macrophage cells were significantly reduced, if the micelle surface was decorated with PEG1500-stearate, DMPE-mPEG2000, or polysorbate.
80. A prolonged blood circulation time was assessed for both γ-CD-C10-based nanovectors (nanoreservoirs and nanospheres) containing DMPE-PEG2000 and polysorbate 80, respectively. Micelles were nonhemolytic at the concentration range used in vivo.\(^{[54]}\)

Chadha \textit{et al.}\(^{[55]}\) prepared chitosan/lecithin nanoparticles (size less than 300 nm) loaded with artesunate and artesunate complexed with β-cyclodextrin to enhance the antimalarial activity. The drug loading capacity was 90% for nanoparticles containing 100 mg artesunate. Enhanced in vivo antimalarial activity was found in infected \textit{P. berghei}-infected mice after the oral administration of both nanoparticle formulations.

Sun \textit{et al.}\(^{[56]}\) developed gelatin or hyaluronan nanoparticles (30–40 nm) loaded with dihydroartemisinin. The dihydroartemisinin loading was approximately 13% and 35% for the gelatin and hyaluronan nanoparticles, respectively. The proliferation of A549 cells was inhibited by both nanoparticles. Annexin V-FITC and propidium iodide stains dramatically increased, when the cells were incubated with gelatin and hyaluronan nanoparticles with better antiproliferative activity than dihydroartemisinin alone in A549 cells.

Want \textit{et al.}\(^{[57]}\) developed ART-loaded poly lactyl co-glycolic acid nanoparticles. Their size was 220 nm with a polydispersity index, zeta potential, drug loading, and entrapment efficiency of 0.1, −9.07 mV, 28%, and 68%, respectively. Microscopy studies confirmed a spherical shape. Drug release was investigated both at pH 7.4 and 5.5 and exhibited an initial burst release during the first 24 h followed by a sustained release during the following 3 days. Stability was proved for 1 month at 4°C without significant changes (\(P > 0.05\)) of the physical characteristics. Unloaded nanoparticles had no toxicity against murine macrophages, while ART exhibited significant toxicity at 200 µg/ml with a drop of 40% in cell viability. Pentamidine served as positive antileishmanial control. Nanoparticles were not active; however, if loaded with ART, they significantly inhibited the growth of intracellular amastigotes. The IC\(_{50}\) value of nanoparticles for intracellular amastigotes was 2.9-fold lower than free ART (11.9 vs. 3.93 µg/ml). Formulation had a significant reduction in the percentage of infected macrophages resulting in a, IC\(_{50}\) value of 3.6-fold inferior when compared with free ART (14.86 vs. 4.16 µg/ml).

The same authors tested the ART-loaded poly lactic co-glycolic acid nanoparticles in visceral leishmaniasis. The nanoparticles administered in a BALB/c model of visceral leishmaniasis, at doses of 10 and 20 mg/kg body weight had greater antileishmanial efficacy when compared with free ART. A significant reduction in hepatosplenomegaly as well as in parasite presence in the liver (85.0% ± 5.4%) and spleen (82.0% ± 2.4%) was found at a dose of 20 mg/kg body weight while in free ART was 70.3 ± 0.6% in liver and 62.7 ± 3.7% in spleen. Furthermore, nanoparticles repaired the faulty immune response in mice with recognized visceral leishmaniosis infection.\(^{[58]}\)

Ibrahim \textit{et al.}\(^{[59]}\) developed ART-loaded albumin. Their size was 339 nm, while the zeta potential was −43.8 mV. If freeze-dried, the nanoparticles had a size of 612 nm, and the entrapped efficiency of ART was 97.5%. After reconstitution, the nanoparticles showed an acceptable physical stability if stored at 4°C for four days. They slightly increased the size of about 5.8% with a polydispersion index: <0.21. ART showed good chemical stability. After storage of lyophilized nanoparticles for 1 month at 4°C, the percentage of ART after lyophilization and reconstitution was 98.4%. The activity of the formulation was tested in a chloroquine-resistant strain of \textit{P. falciparum} (FcB1). IC\(_{50}\) of the formulation was 3.5 versus 11.4 nM of free ART. A 4-day treatment with 10 mg/kg nanoparticles suddenly reduced parasitemia (96%), as measured 1 day after the end of the treatment. Furthermore, mice survived for more than 18 days with no recrudescence until the end of the experiment.

Ma \textit{et al.}\(^{[60]}\) developed poly(lactic co-glycolic acid) nanoparticles coated with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine. These were loaded together with dihydroartemisinin and doxorubicin as poly-chemotherapy. Doxorubicin was linked to the polymer with an efficiency of 56%. The resulting nanovecrors had a size of 150 nm, a zeta potential of +50 mV, and a polydispersity index of 0.13. Doxorubicin loading was 0.54%. Dihydroartemisinin and phospholipid were used to coat the nanoparticles. The resulting size was 220 nm, the zeta potential was −12 mV, and the polydispersity index 0.15. Final doxorubicin loading was 0.23% while that of dihydroartemisinin was 1.95%. A colorimetric cell viability test revealed a synergistic effect of loaded drugs, while experiments on cellular uptake proved that the nanovector increased doxorubicin accumulation in cell nuclei, thus improving cytotoxicity.

Kumar \textit{et al.}\(^{[61]}\) developed novel biodegradable poly(organophosphazenes) loaded with primaquine and dihydroartemisinin and evaluated their antimalarial properties. Their size was in a range between 137.4 ± 5.7 and 240.16 ± 2.0 nm. Zeta ranged from −24.63 mV to −41.22 mV. Their degradation behavior was investigated in phosphate buffers pH 5.5, 6.8, and 7.4 at 37°C. The polymers degraded fastest at pH 5.5 (acidic media) and at less extent at pH 6.8 and pH 7.4 at different rates according to the different structures. These polymers exhibited hydrolytic degradability, which are applicable to a variety of drug delivery systems. The release of both drugs from the nanoparticle formulations was characterized by two phases: A burst release for the first 12 h, followed by a sustained release. All designed formulations fitted best toward the Korsmeyer–Peppas model in a two-step release process. The combined drug–nanoparticle formulations were evaluated for their antimalarial potential in \textit{P. berghei}-infected mice. The nanoparticles showed 100% antimalarial activity.

Natesan \textit{et al.}\(^{[62]}\) developed magnetic nanoparticles loaded with ART using chitosan as matrix. The nanoparticles were

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spherical with a size in the range between 349 and 445 nm. The polydispersity index and zeta potential were in the range of 0.373–0.908 and −9.34 to −33.3, respectively. The drug encapsulation efficiency ranged from 55% to 62.5% and the drug-loading capacity from 20% to 25%. About 62%–78% ART was released from the nanoparticles over a period of 48 h. ART magnetic nanoparticles conjugated with FITC as a fluorescent probe displayed an improved accumulation of nanoparticles in the 4T1 breast tumor tissues of BALB/c mice model after application of a physiologically acceptable external magnetic field.

Ho et al.[63] developed artemesate/poly(lactic-co-glycolic) acid/chitosan nanoparticles. Their size was 303 nm and the entrapment efficiency was 80.5%. The release study showed an initial rapid release within 2 h followed by very slow extended-release according to the Korsmeyer–Peppas model.

Liu et al.[64] developed artemesate-loaded bovine serum albumin nanoparticles with a spherical shape. The polydispersity index was 0.016 with a size of 99.9 nm and a zeta potential of −25.6 mV. The release profile showed a smooth and sustained release. More than 85% of artemesate were released within the first 6 h, while the total percentage within a 48 h period was 78.9%. The nanoparticles showed high cytotoxicity and apoptotic effects. The artemesate-loaded bovine serum albumin nanoparticles injured the mitochondrial integrity and activated apoptosis by upregulating apoptosis-related proteins and facilitating the rapid release of cytochrome C.

Shi et al.[65] developed fibers composed of ART dispersed in cellulose acetate as core material and poly(vinyl pyrrolidone) as shell material. The size was about 436 nm. The fraction of entrapped ART was 20% w/w, but after 6 months, storage remained only in very low amounts. Ex vivo permeation studies suggested the ART smoothly permeated through the stratum corneum, providing a potential application of the nanosystem for transdermal patches.

**INORGANIC NANO PARTICLES**

Inorganic nanoparticles are made of inorganic materials that explain their fluorescent, magnetic, electronic, and optical properties. Silver and gold particles are more attractive materials both for diagnostic and therapeutic purposes, generally known as plasmonic nanoparticles. Iron oxide nanoparticles are approved for human use in glioblastoma therapy in Europe and as contrast enhancer for magnetic resonance imaging. Mesoporous silica nanoparticles are versatile biodegradable nanoparticle carriers with tunable pore and particle size. They are highly biocompatible because silica is classified as “Generally Recognized as Safe.” In addition, they possess high loading capacity and a surface which can be easily functionalized. Finally, carbon nanotubes belong to the family of fullerenes and are formed of coaxial graphite sheets (<100 nm) rolled up into cylinders. These structures are single-walled (one graphite sheet) or multi-walled nanotubes (several concentric graphite sheets). They exhibit excellent strength and electrical properties and are efficient heat conductors. Owing to their metallic or semiconductor nature, nanotubes are often used as biosensors. Carbon nanotubes can be rendered water soluble by surface functionalization. Therefore, they are also used as drug carriers and tissue-repair scaffolds.[66,67]

Wang et al.[68] prepared Fe₃O₄ nanoparticles loaded with artemesate. The activity of nanoparticles was evaluated using MTT assay, and the apoptosis rate of K562 cells was investigated using flow cytometry. The protein expression levels of bcl-2, bax, bcl-rambo, caspase-3, and survivin in K562 cells were determined by Western blotting. Nanoparticles significantly inhibited K562 cell growth compared with K562 cells treated with artemesate (P < 0.05). Similarly, the apoptosis rate was significantly increased after treatment with nanoparticles with respect to artemesate. Nanoparticles increased the expression of bcl-2, bax, bcl-rambo, and caspase-3 proteins, with respect to cells treated with artemesate.

Chen et al.[69] prepared Fe₃O₄@C/Ag@mSiO₂ loaded with ART (484 mg/g). These nanoparticles demonstrated pH-responsive Fe⁺⁺ release, reaching a value of 2.765 nmol/l in HeLa cells. The antitumor efficacy of the nanoparticles evaluated by MTT assay was significantly enhanced compared with free ART due to increased internalization of nanoparticles by HeLa cells. They were placed in the endosomes and lysosomes, releasing Fe⁺⁺ ions converting ART to toxic products able to kill cancer cells.

Wang et al.[70] developed Fe₃O₄@C@MIL-100(Fe) nanoparticles for the synchronous delivery of both dihydroartemisinin and iron (III) for cancer therapy. Their structure was based on iron (III) carboxylate materials MIL-100 (Fe) and showed a high dihydroartemisinin loading capacity (805 mg/g) and loading efficiency (80.5%). A549 and HeLa cancer cells were incubated with dihydroartemisinin-loaded nanoparticles, free dihydroartemisinin, and a mixture of dihydroartemisinin and FeSO₄. Free dihydroartemisinin had the highest cytotoxicity toward both A549 and HeLa cells. In vivo studies showed an improved the intracellular accumulation of dihydroartemisinin in tumors and an activation mechanism by co-release of both dihydroartemisinin and Fe (III).

Letchmanan et al.[71] encapsulated ART into pore channels of mesoporous silica, SBA-15. A significant improved dissolution rate and supersaturation was found compared to free ART. Good biocompatibility was also found using Caco-2 cells after 24 h. The nanoparticles had an exceptional physical stability, but their chemical stability was affected by humidity during storage.

The same authors investigated nanoparticles loaded with both ART and mefloquine. The formulations showed a high dissolution improvement with a burst release of >95% of drugs within 30 min. In addition, the combination formulation exhibited a stable supersaturation enhancement which
was two-fold higher than that of the untreated crystalline counterparts. The physicochemical stability was 6 months under different storage conditions.[72]

Zhang et al.[73] loaded ART in hyaluronic acid-derivatized multi-walled carbon nanotubes (HA-MWCNTs). The surface was decorated with transferrin as targeting ligand. This delivery system not only retained cytotoxicity of ART but also demonstrated synergistic cytotoxicity due to transferrin targeting. Extraordinarily enhanced antitumor efficacy of this delivery system was found both in MCF-7 cells in vitro when compared with free ART. Similar results were obtained in a tumor-bearing murine model in vivo, due to increased intracellular accumulation of ART. The formulation with laser irradiation demonstrated the highest inhibition effect compared to the other groups.

A multi-functional tumor-targeting drug delivery system was constructed employing HA-MWCNTs as nanovectors. In addition, transferrin was decorated in the surface as targeting ligand, and loaded with ART. This delivery system (HA-MWCNTs/Tf@ART) not only retained the optical property of MWCNTs and the cytotoxic properties of ART but also demonstrated synergistic antitumor effects compared to ART and transferrin.[74]

Zhang et al.[73] generated particles grafted with hyaluronic acid to obtain a water-soluble biomaterial. Artesunate was adsorbed on the nanosystem with a high loading efficacy. After laser irradiation, the tumor volume declined by half due to the functionalized fullerenes, from approximately 1.72 to approximately 0.84.

Liu et al.[75] developed nanoscale graphene oxide dual-dressed with dihydroartemisinin and decorated with transferrin. Comparing with free dihydroartemisinin, the nanosystem revealed improved tumor specificity and cytotoxic properties, achieving complete tumor cure in mice with minimal side effects.

**Concluding Remarks**

Undoubtedly, the discovery of ART and the development of related derivatives represent the most stimulating and fruitful breakthroughs in natural drug development for the control of malaria. In addition, some others conceivable therapeutic approaches are related to their antiprotozoal, antibacterial, antiviral, and antitumor properties.

The challenge of low bioavailability, short half-life, and poor water solubility may be dealt with approaches from pharmaceutical technology to obtain formulations with improved biopharmaceutical characteristics.

The application of nanotechnology to ART will have a significant impact on developing appropriate therapeutic treatments of ART in the foreseeable future. So far, the results obtained from nanencapsulated natural products are very promising with regard to improved solubility and stability, modified release, enhanced bioavailability at considerably inferior doses, and increased long-term safety of these constituents. In addition, the opportunity to impart highly specific site-targeted delivery of these nanocarriers confers a high medicinal value. Remarkably, most of the reported nanoparticulate drug delivery systems are biologically inactive or faintly immunogenic, producing no antigenic or pyrogenic reactions, with a narrow intrinsic toxicity.

Most of the reported studies evaluated the performance of nanocarriers loaded with ARTs in vitro, but a huge number of publications also dealt with in vivo investigations giving promising results in many cases. No studies in humans have been reported so far, but there is a pressing need to reach clinical trials and establish ART-based nanocarriers in therapy.

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**Conflicts of interest**

There are no conflicts of interest.

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