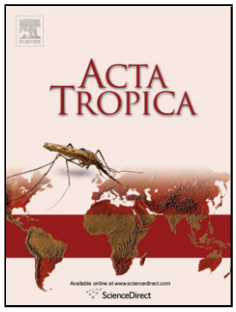

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Nanopharmaceuticals as a solution to neglected diseases: Is it possible?

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Graphical Abstract

Nanopharmaceuticals as solution to neglected diseases: Is it possible?

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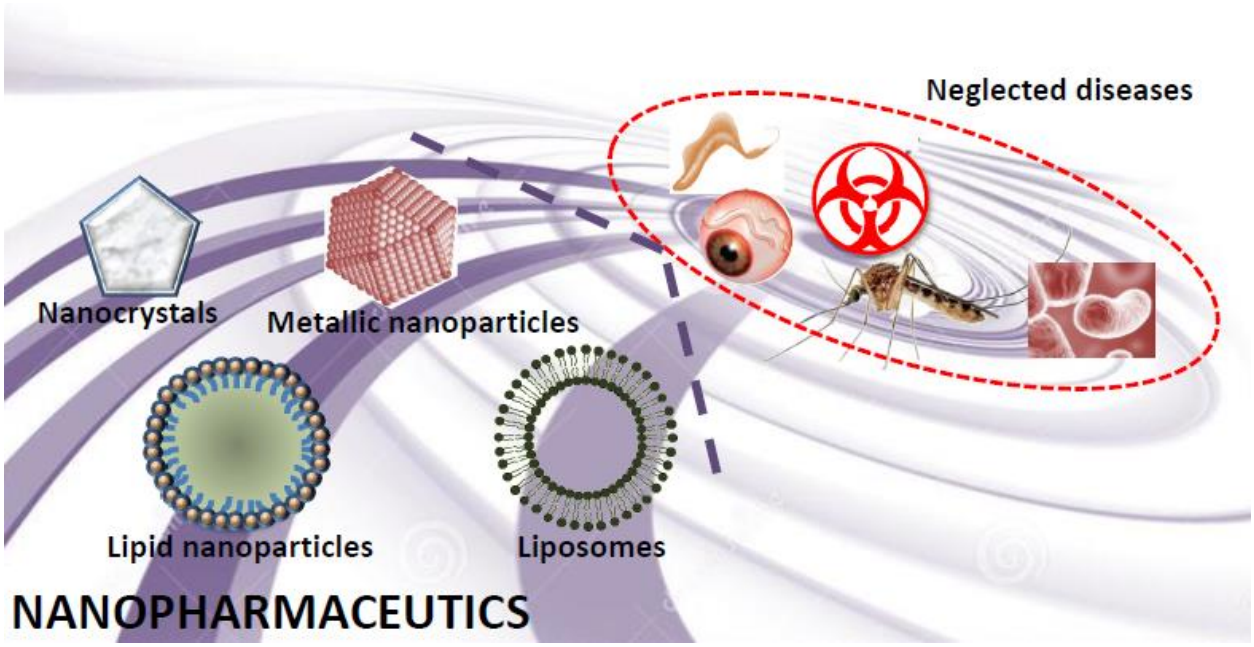
Nanotechnology appears to bring novel therapeutic devices using available drugs in the market to efficiently treat Neglected Diseases with less toxicity, high efficacy and improved bioavailability of drugs with extended release.



Highlights

- Neglected Tropical Diseases affect large populations with little visibility and low political voice.
- Few new drugs are in pipeline to treat 17 reported Neglected Tropical Diseases
- Nanocarriers are promising devices for the treatment of Neglected Tropical Diseases

Abstract

The study of neglected diseases has not received much attention, especially from public and private institutions over the last years, in terms of strong support for developing treatment for these diseases. Support in the form of substantial amounts of private and public investment is greatly needed in this area. Due to the lack of novel drugs for these diseases, nanobiotechnology has appeared as an important new breakthrough for the treatment of neglected diseases. Recently, very few reviews focusing on filiarasis, leishmaniasis, leprosy, malaria, onchocerciasis, schistosomiasis, trypanosomiasis, and tuberculosis, and dengue virus have been published. New developments in nanocarriers have made promising advances in the treatment of several kinds of diseases with less toxicity, high efficacy and improved bioavailability of drugs with extended release and fewer applications. This review deals with the current status of nanobiotechnology in the treatment of neglected diseases and highlights how it provides key tools for exploring new perspectives in the treatment of a wide range of diseases.

1. Introduction

1.1. General aspects of neglected diseases

The WHO Special Programme for Research and Training in Tropical Diseases (WHO-TDR) defines a disease of poverty (DoP) as a disease that affects mainly the poor in developing countries, and it is divided into two classes (WHO, 2010a). The first class includes the "big three" DoPs: malaria, HIV/AIDS, and tuberculosis. These diseases have received considerable attention from the community and investment to eradicate them. Around 70% of drug development focuses on these diseases (Ponder, 2012). The other one comprises neglected tropical diseases (NTD). There are seventeen NTD, and these diseases affect populations with little visibility and low political voice. They cause discrimination and stigma and have a strong impact on morbidity and mortality; these diseases are practically neglected by the research community but can be prevented, controlled, and probably eliminated using adequate solutions (WHO, 2010b).

In addition to the "big three" and NTD, BIO Ventures for Global Health, a nonprofit organization that specializes in accelerating research on medicines for developing countries, classifies diseases such as diarrheal diseases, cholera, and typhoid fever as DoP (Ponder, 2012). Policy Cures, another health nonprofit organization, lists 31 neglected diseases. This nonprofit organization considers an illness a disease of poverty if it meets three conditions: the disease strongly affects people in low-income countries; there is a need for different and new products; and the allocation of goods and services is not efficient (Moran et al., 2010). Table 1 lists the DoP identified by WHO, Policy Cures, and BioVentures for Global Health (Woodson, 2014).

A very recent review gives examples of Training in Tropical Diseases (TDR), approaches and contributions to drug discovery research and development (R&D), and the optimization of known

treatments against the background of immense changes in the R&D landscape for infectious diseases of poor people (Olliaro et al., 2015).

Between 1975 and 1990, only ten drugs were registered for tropical diseases: benznidazole and nifurtimox for Chagas' disease; pentamidine for human African trypanosomiasis (HAT); oxamniquine and praziquantel for schistosomiasis; ivermectin for onchocerciasis; pyrazinamide, halofantrine and mefloquine for malaria; and albendazole for soil-transmitted nematodes (STNs). It is important to note that all these drugs, except halofantrine, are still in use. All of these drugs were developed 25 to 40 years ago; however, they continue to be the cornerstones of strategies in disease control.

Drug development between the public and private sector has been rare in the last years, but there are some cases. Mefloquine was studied in collaboration between the United States Army Medical Research and Development Command, La Roche, and TDR.

During the 2000-2011 period, TDR participated actively in the development of three products, including an injectable artemotil [arteether], rectal artesunate for malaria and oral miltefosine for visceral leishmaniasis [VL]), and played an important role in contributing to the identification and development of a number of other drugs, such as artesunate-amodiaquine and artesunate-mefloquine for malaria and paromomycin for VL.

Trouiller et al. (2002) reported the development of six products for stage 2 of human African trypanosomiasis, eflornithine for visceral leishmaniasis, liposomal amphotericin B for severe malaria, injectable artemether for uncomplicated malaria, and atovaquone and rifapentine for tuberculosis. TDR provided the coordination and funding for the pivotal clinical trials that supported their registration. Currently, TDR continues to fund research on new targets, screening, identification of leads, and development of new candidates.

The current challenges are completely different from those at TDR's start. Now, there are different players and proportionally more funding than before, and the primarily public or nonprofit organization, devoted to research and the search for new drugs and diagnostics, still requires coordination to optimize resources. This active environment requires new and innovative solutions, but there is a need for continuity and also an adaptation to produce adequate results (Olliaro et al., 2015).

The new trends in the last decades suggest that the most innovative strategy at this time should be the use of nanotechnology in the search of new uses for old pharmaceuticals or new development of innovative and intelligent nanomedicines for neglected diseases.

2. Nanostructures most commonly used in neglected diseases

The relevance of nanostructures in biomedical applications is enormeous. Then, the characteristics and aspects of these nanostructures will be briefly described in this section **(Figure 2).**

2.1. Liposomes

Liposomes are sphere-shaped vesicles formed by phospholipid bilayers reported in the mid-60s that have currently made their way to the market. Currently, they have progressed from conventional vesicles to 'second-generation liposomes', in which long-circulating liposomes are obtained by modulating the lipid composition, size, and charge of the vesicle and functionalized with molecules, such as glycolipids or sialic acid. Liposomes are promising intracellular delivery systems for antisense molecules, ribosomes, proteins and/or peptides, and DNA. Probably the most important function of liposomes is to promote targeting of particular diseased cells within the disease site. Liposomal drugs also exhibit reduced toxicities and retain enhanced efficacy compared with free complements (Akbarzadeh et al., 2013). Examples of liposomal formulation in the treatment of negelted diseases are shown in **Table 3**.

2.2. Polymeric and natural macromolecule nanoparticles

Polymeric nanoparticle-based materials have gained an important position due to advancements in polymer science and technology. These nanoparticles are solid colloidal systems in which the therapeutic agent is dissolved, entrapped, encapsulated, or adsorbed onto the constituent polymer matrix (*e.g.*, nanosphere-matrix systems in which the drug is dispersed throughout the particles); they are nanocapsules (vesicular reservoir systems in which the drug is confined to an aqueous or oily cavity surrounded by a single polymeric membrane). Several polymers such as poly(lactide-co-glycolide) (PLGA), polylactide (PLA), polyglycolide, polycaprolactone (PCL), poly(d,l-lactide), chitosan, and PLGA–polyethylene glycol (PEG) have been developed for passive and ligand-targeted delivery of therapeutic moieties (Prabhu et al., 2015) (**Table 4**).

2.3. Solid lipid nanoparticles and nanostructured lipid carriers

Solid lipid nanoparticles (SLN) are nanoparticles prepared from a lipid matrix that is solid at body and room temperature, stabilized by suitable surfactants. Nanostructured lipid carriers (NLC), *i.e.*, nanoparticles composed of a mixture of a solid and a liquid lipid whose lipid matrix is solid at room and body temperature, are a second generation of solid lipid-based colloidal carriers, with improved stability, drug encapsulation ability and controlled drug release (**Figure 3**). Furthermore, *in vitro* tolerability of SLN and NLC appears to be much higher than that of polymeric nanoparticles (Hirlekar et al., 2011; Doktorovova et al., 2014; Dolatabadi et al., 2015; Islan et al. 2016) (**Table 3**).

2.4. Nanoemulsions

Nanoemulsions are nano-sized emulsions that are manufactured for improving the delivery of active pharmaceutical ingredients for nelgelted disease therapies (**Table 5**). The ability of nanoemulsions to dissolve large quantities of hydrophobics, along with their mutual compatibility and ability to protect the drugs from hydrolysis and enzymatic degradation, makes them ideal vehicles for

the purpose of parenteral transport (Lovelyn and Attama, 2011; Kela and Kaur, 2013; Jaiswal et al., 2015).

2.5. Nanocrystals

Nanocrystals from bulk pharmaceuticals or chemicals are carrier-free colloidal delivery systems in the nano-sized range that are important for poorly soluble drugs. Nanocrystals exhibit several features including enhancement of saturation solubility, dissolution velocity and adhesiveness to surface/cell membranes. Many different methologies are applied for nanocrystal production, including precipitation, milling, high pressure homogenization and combination methods such as Nano-Edge™, SmartCrystal and precipitation-lyophilization-homogenization (PLH) technology. Nanocrystals for oral administration were reported to be useful for improving *in vivo* performances, *i.e*., pharmacokinetics, pharmacodynamics, safety, and targeted delivery (**Table 5**). An interesting benefit of drug nanocrystals is that they can provide smaller dose administration to achieve moderate blood level and thus reduce the side effect from giving a larger dose. Furthermore, nanocrystal drugs can be delivered through various routes of administration such as oral, parenteral, ocular, pulmonary and dermal delivery (Durán et al., 2010a; Salazar et al., 2012; Bhuyan et al., 2014; Junyaprasert and Morakul, 2015).

2.6. Metal nanoparticles

Metal nanoparticles, which are a cluster of metal atoms, play an importat role due to their unique optoelectronic and physicochemical properties, which depend strongly on their size, shape, crystallinity and structure. These properties have led to a wide range of potential applications in diverse areas such as molecular diagnostics, electronics, catalysis, drug delivery or sensing. Different physical and chemical methods have been employed for the synthesis of metal nanoparticles. The use of biological organisms in the synthesis and assembly of nanoparticles has received increasing attention, since they are a clean, cost-effective and efficient synthesis technique (Cauerhff and Castro, 2013). In the biomedical sector especially, the metal nanoparticles will play a crucial role in diagnostics, drug delivery, cosmetics, agriculture, band aids, etc. Although metallic/metal nanoparticles are important in remediation through pollution absorption, water filtering, disinfection, etc., the best example is the use of inorganic nanoparticles as antimicrobial agents. Silver nanoparticles are being exploited as new generation antimicrobials because of their significant activity against many types of pathogens including multidrug-resistant organisms (Islan et al., 2015). Although a concern due to large applications, their potential toxicities and the properties forcing such toxic responses must also be studied and understood (El-Nour et al., 2010; Durán et al., 2010b, 2011; Rai and Durán, 2011; Rai et al., 2014ab; Castro et al., 2014). In this area, there have been many misunderstandings about silver nanoparticle characterization, which sometimes has made it hard to

correlate the biological activities with the structure (Durán et al., 2016c). Some examples of the use of metallic nanoparticles for the therapy of neglected diseases are listed in **Table 6**.

2.7. General comments about previous knowledge related to neglected diseases treated through nanotechnology

Since 2007, there has been concern about the slow development of new antiparasitic drugs, and suggestions have been made for an effective management of the current drugs using new strategies, including the modulation of their delivery. Accordingly, Date et al., (2007) published a review focusing on biological and biopharmaceutical topics considering the design of a delivery strategy to treat parasitic infections such as leishmaniasis, malaria, and trypanosomiasis. They pointed out the importance, at that time, of polymeric nanoparticles, liposomes, lipid nanoparticles including lipid drug conjugates (LDC) and solid lipid nanoparticles. Pimentel et al. (2007) emphasized the potential of liposomes and polymeric nanocapsules acting on malaria parasites and as a promising alternative for malaria control and treatment.

However, after 30 years of research in the field of antileishmaniasis, currently AmBisome (liposomes) is the only drug delivery system used against the visceral form, and most of the experimental development only involves parenteral administration (Meyerhoff, 1999).

Durán et al. (2009) commented that for a real development in this area, there is a need for significant funding from private and public partners to apply nanobiotechnology to the treatment of neglected diseases. Their review focused on neglected diseases (*e.g.*, malaria, schistosomiasis, leishmaniasis, trypanosomiasis, tuberculosis, leprosy, filiarasis, and onchocerciasis), where many different nanomaterials, such as polymeric nanoparticles, liposomes and nanostructured lipid carriers, have been applied. But again, the negative aspects were that up to 2009, very few nanomaterials had been used in clinical tests.

Different aspects of nanotechnology applied to neglected diseases were discussed by El-Tonsy (2010). He pointed out that nanomedicine is a wide area with applications in many aspects, such as diagnosis, monitoring, treatment and control of biological systems in leishmaniasis, toxoplasmosis and malaria. Similar comments pointed out that drug delivery formulations involved low-cost research compared to that for the development of new molecules (Qureshi et al., 2011). Khalil et al. (2013) described the roles of colloidal drug carriers, such as polymeric nanoparticles, liposomes, and solid lipid nanoparticles, in optimizing the delivery of drugs against neglected diseases. Malaria was one of the privileged diseases using these particulate carriers as vehicles for the delivery of active compounds.

Since several nanoscale materials in delivery systems have already proved to be efficicient in animal models for the treatment of malaria, strategies to release antimalarials using different nanocarriers (e.g., solid lipid nanoparticles, liposomes, and nano- and microemulsions and polymer-based nanocarriers) and mechanistic aspects that direct their targeting to *Plasmodium* spp.-infected

cells were discussed by Santos-Magalhães and Mosqueira (2010). Singh (2012) also published an interesting chapter that highlights the available antimalarial therapies, as well as the potential benefits of nanotechnology platforms in malaria treatment. A review by Umeyor et al. (2013) presented various possibilities in malarial chemotherapy, as well as an important and detailed screening of different nanoparticle drug release systems and the new aspects in the delivery of antimalarial drugs in that period. In a similar way, Aditya et al. (2013), Ranjita et al. (2011) and Banyal et al. (2013) discussed the importance of nanomedicine in the treatment for drug-resistant parasites in malaria as well as in tuberculosis.

A review by Kaur et al. (2014) discussed the entire global scenario of tuberculosis by introducing promising nanocarrier delivery systems or by modifying existing systems such as chemotherapy protocols for tubercuosis. This review pointed out that nanotechnology provided an immense possibility for the design of a new, minimum-dose, and effective treatment system to control tuberculosis. In 2014, it was suggested that inhaled formulations could be promising for pulmonary and systemic non-pulmonary diseases. These nozzles can produce nanocomposite particles in one step, and their spray-drying system is suitable for scaling up. Nanocomposite particles are useful for improving drug absorption and delivery efficacy against alveolar macrophages. In addition, recent studies on several pulmonary diseases (tuberculosis, lung cancer, cystic fibrosis, pneumonia, and others) and related inhaled formulations have also been reviewed (Ozeki and Tagami, 2014).

Ali et al. (2014) reported the connotations of drug delivery systems in targeting lymphatic filaroids or wolbachia and systemic microfilaria (e.g., liposomes and solid lipid nanoparticles for the treatment of lymphatic filariasis).

One review discussed several nanocarriers (*e.g.*, solid lipid nanoparticles, liposomes, polymeric nanoparticles, inorganic nanoparticles, etc.) on the basis of approaches that have been applied in the literature and in the clinic to combat the different challenges faced by antiviral therapy. Important developments in this area with solid lipid nanoparticles, liposomes, polymeric nanoparticles and inorganic nanoparticles have been reported for lectin receptors, and cell-penetrating peptides for site-specific delivery of antiviral agents (Mehendale et al., 2013).

The advances in chemoinformatics, genomics, bioinformatics and proteomics have brought great opportunities for the fast and cost-effective discovery of new bioactive compounds against these tropical diseases. Neves et al. (2015) reviewed the most important contributions of nanomaterials in drug discovery and in the treatment of schistosomiasis, and pointed out how integration promotes specific strategies (*e.g.*, virtual screening) and may contribute to the development of new schistosomidal leads, mainy through the identification of new biologically active chemical scaffolds and optimization of nanoparticle structures with previously known activity.

3. Considerations and analysis of nanotechnology applications in neglected diseases

3.1. Malaria

Malaria in humans is caused by five Plasmodium species: P. malariae, P. knowlesi, P. ovale, P. falciparum, and *P. vivax*. In general, the current research is focused on the latter two strains, because they are the deadliest and most widespread. Many efforts to control and eradicate malaria through insecticides and antimalaria treatments (*e.g*., artemisine-combined therapies) contributed to a 42% decrease in malaria death. Benelli and Mehlhorn (2016) stated that the emerging scenario highlights that a more effective and green control of mosquito vectors, especially highly invasive species, such as *Aedes aegypti* and *Aedes albopictus*, is crucial. However, one of the challenges is still drug resistance, and currently no effective malaria vaccine exists (Table 2). The only malaria vaccine in phase III testing is the Glaxo Smith KlineRTS, S/AS01 vaccine, but its vaccine efficacy is only ~30% (Siu and Ploss, 2015).

An important challenge to malaria prevention and control is the mosquito strains resistant to synthetic pesticides. Based on the malaria disease complexity, innovative methods will be welcome to combat growing drug resistance among parasites. Effective malaria treatment is critical for controlling the disease. Some of the currently used antimalarials are chloroquine, artemether, arteether, artesunate, artemether/lumefantrine, sulphadoxine/pyrimethamine, chlorproguanil/dapsone, mefloquine, atovaquone/proguanil and primaquine (Date et al., 2007; WHO, 2014). But the current therapy has drawbacks such as *Plasmodium* resistance to drugs, poor bioavailability and extremely low solubility in both aqueous media and oils. The main drawback is related to arteminsins and poor patient compliance at high doses, and also to the need for long-term treatment and high toxicity (Date et al., 2007; Isacchi et al., 2012; Tripathy and Roy, 2014).

To overcome these undesirable and adverse effects, it is possible to encapsulate antimalarial drugs in liposomes. This colloidal carrier protects the drug from degradation with controlled release and fewer adverse effects (Date et al., 2007).

Since the late 1970s, antimalarials encapsulated in liposomes have been studied to decrease the toxicity of the drug. In experimental murine malaria Pieson et al. (1979) observed that liposome encapsulation reduced primaquine toxicity 3.5 times compared with the free drug. In another study in mice, using radiolabeled free and liposome-entrapped primaquine, these researchers demonstrated that the incorporation of primaquine in liposomes reduced the accumulation of the drug in nontarget tissues (*e.g*., lungs, heart, kidneys, and brain), thereby reducing primaquine toxicity. Moreover, primaquine encapsulated in liposomes was detected mainly in spleen and liver, giving better hypnozoitocidal efficiency (Pieson et al., 1979, 1982).

Primaquine in liposomes depend on lipid composition, drug-to-lipid incubation ratio, internal buffer capacity, and the presence of cholesterol and liposome charges. Lyophilized and sterilized (gamma irradiation) liposomes exhibit long-term stability and sterility. Low chemical degradation is obtained when the pH gradient is maintained (Stensrud et al., 2000).

Other nanoformulations of primaquine with an appropriate drug carrier system have been studied to enhance bioavailability, increasing its activity with fewer doses and lower toxicity. Owmoyo et al. (2014) designed, synthesized and characterized primaquine-loaded solid lipid nanoparticles (SLNs) (PQ-SLNs). The average particle size, charge (zeta potential), drug loading, and encapsulation yield of the PQ-SLNs were 236 nm, +23 mV, 14% and 75%, respectively. The particle size was small enough to provide absorption in the gastrointestinal tract. This small size and the moderately positive surface charge could trigger an elevated concentration of primaquine in the liver. The nanoformulated PQ was 20% more effective compared with the conventional oral dose in *Plasmodium berghei*-infected Swiss albino mice. Since the drug was highly effective against hypnozoites concentrated in the liver, one of the stages of malaria, this formulation was found to be effective for antimalarial drugs. Chloroquine is a common drug for malaria treatment, but at present showing resistance in most parts of the malaria endemic regions. To overcome this problem, Owais et al. (1995) studied the liposome/chloroquine carriers in drug-resistant malaria treatment. They tagged antibody on the liposome/chloroquine against infected erythrocytes and assayed their efficacy in chloroquine-resistant *P. berghei* infection in mice. These liposomes recognized only the infected cells and not normal erythrocytes. This nanostructure was active when applied intravenously at 5 mg/kg body weight per day.

The animals with chloroquine-resistant *P. berghei* infections were completely cured (75% to 90%). The reasons for parasite resistance to chloroquine were attributed to the enhanced efflux of chloroquine from the resistant parasites, hindering intracellular concentrations at toxic levels. These results indicated the effectiveness of liposomes with chloroquine against malaria-infected erythrocytes, possibly curing chloroquine-resistant malarial infections with low doses.

There is clinical evidence of the effectiveness of chloroquine-chlorpheniramine coadministration in overcoming drug resistance. Chloroquine resistance is possibly due to effects on P-glycoprotein (Pgp)-mediated drug efflux, which makes attainment of adequate drug levels impossible. Nzekwe et al. (2015) included a P-gp inhibitor, chlorpheniramine, and chloroquine in a lipid-based nanoparticle carrier with the aim of ensuring that adequate drug levels were attained, so as to overcome drug resistance. They concluded that coformulation of chloroquine and chlorpheniramine in lipid-based nanoparticles was feasible using a simple hot emulsion-dilution method. Further studies will be devoted to toxicological tests and *in vivo* testing in both resistant and sensitive strains of *Plasmodium falciparum*.

Other strategies to deliver chloroquine using nanocarriers have been developed. Tripathy et al. (2012, 2013) developed chitosan-tripolyphospate (CS-TPP) nanoparticles (NPs) combined with chloroquine. Chitosan is a natural polysaccharide that currently is extremely useful for the synthesis of nanoparticles and is widely studied. Chitosan nanoparticles are prepared by ionotropic gelation between chitosan and sodium tripolyphosphate. The synthesis of chitosan-tripolyphosphate nanoparticles conjugated to chloroquine was effective against *P. berghei* infection in Swiss mice and more potent than only chloroquine to attenuate the parasitemia and host pathology also. Lymphocytes

are also susceptible to lethal *P. berghei* infection due to a significant oxidative stress that leads to decreased antioxidant status of the mammalian cells. This nanochloroquine formulation was able to increase protection of lymphocytes. These researchers suggested that this combination is more prospective than free chloroquine for protecting the host from parasitic infection by diminishing free radical generation, lipid oxidation, and DNA damage probably by increasing antioxidant levels. It is possible to conclude from this study that this nanopharmaceutical may be administered as a potent therapeutic agent compared to free chloroquine (Tripathy and Roy, 2014).

Artemisinin, isolated from *Artemisia annua L*., a plant derived from traditional Chinese medicine (qinghaosu), has been used for 2,000 years in the treatment of malaria (Isacchi et al., 2011; Tripathy and Roy, 2014). It is an extremely active antimalarial drug, active even against chloroquine- and quinine-resistant *Plasmodium* sp. However, its therapeutic efficiency is limited due to its low bioavailability. To overcome this drawback, Isacchi et al. (2011) prepared conventional artemisinin-loaded and PEGylated liposomes. In both cases, the encapsulation efficacy was >70%; diameters were also similar (130–140 nm). Both liposomal formulations exhibited longer blood-circulation time than free artemisinin. There was a 5-fold increase in the half-life of artemisinin in the case of PEGylated liposomes.

Other formulations were designed with artemisinin and derivatives. Free artemisinin, or combined with curcumin as before, was encapsulated through a conventional way or in PEGylated liposomes. Artemisinin alone had an effect on parasitaemia levels only 7 days after the initiation of treatment. On the contrary, treatments with conventional artemisinin-loaded liposomes or artemisinin-loaded PEGylated liposomes with or without the presence of curcumim appeared to have a short-term antimalarial effect. Besides, all formulations showed low variability in artemisinin plasma concentrations, compared to the free one, showing a regular antimalarial effect over time (Isacchi et al., 2012).

Artemether (a derivative of artemisinin) and lumefantrine are well-accepted in combination therapy for uncomplicated malaria treatment. Artemether first acts on the malaria parasites and reduces the parasite burden instantly, and later lumefantrine acts on the remaining parasites or the parasites that come out of hibernation, which are mostly merozoites. However, the current formulation is available as tablets for oral administration and has several pharmacokinetic problems such as drug degradation in the gastrointestinal tract and low absorption (Parashar et al., 2014). Therefore, Parashar et al. (2014) designed and prepared artemether and lumefantrine co-loaded injectable nanostructured lipid carriers. Artemether and lumefantrine co-loaded NLCs had a hydrodynamic diameter of ~145 nm with a surface charge of -66 mV. This formulation showed effective antimalarial activity with respect to progression of parasitemia and survival time in the treatment of *P. berghei*-infected mice.

Semisynthetic derivatives of artemisinin, artemether and arteether (ART) have also been studied as treatments for uncomplicated *P. falciparum* malaria in most malaria-endemic countries. The problem associated with these derivatives includes its poor aqueous solubility and low stability in the gastric

medium (they decompose at gastric pH 1.2 at high temperature or with long incubation), which results in poor bioavailability. Nanoemulsions (NEs) loaded with arteether have been prepared using a high pressure homogenization (HPH) technique with the aim of improving arteether solubility and thus, its bioavailability. Maximum drug loading was achieved up to 93.0 ± 7.4% with a globule size of 156.0 ± 10.2 nm and zeta potential of -23.3 ± 3.4 mV. The ART-NEs were found to be stable in terms of globule size and size distribution at different pH values, and the *in vitro* release profile showed 62% drug release within 12 h. Therefore, ART-NE can be a promising oral delivery system for ART (Dwivedi et al., 2015).

Innovative methods are a welcome resource to combat growing drug resistance among malaria parasites. Hemozoin nanocrystals can be used to detect and mechanically kill the malaria parasite in theranostic applications. The blood-stage malaria parasite uses hemoglobin as a nutrient source, releasing large amounts of potentially toxic heme, which generates reactive oxygen species, causing death of the parasite. To circumvent this internal toxicity, the parasite packages the heme groups into biochemically inert nanocrystals called hemozoin particles that are located in food vacuoles inside the parasite. The high optical absorbance of the hemozoin nanocrystal can be used to generate transient localized vapor nanobubbles in response to a short laser pulse, and this vapor nanobubble generation around hemozoin supports the efficient detection and destruction of malaria parasites in a theranostic procedure at the nanosecond scale. Laser-induced nanobubbles acting on human blood *in vitro* demonstrated a destruction of up to 95% of parasites after a single procedure, with 8-fold better parasiticidal efficacy compared to standard chloroquine treatment, being highly selective against malaria-infected red cells and not against uninfected erythrocytes (Hleb and Lapotko, 2014).

The use of nanocarrier systems, liposomes, nanostructured lipid carriers, polymeric nanoparticles, and nanocrystals shows promising results in the treatment of malaria, with low toxicity and high efficacy, along with prolonged release associated with a reduced number of doses (Durán et al., 2009; Hleb and Lapotko, 2014; Najer et al., 2014) (**Figure 4**).

Murugan et al. (2015a, 2016ab) reported that silver nanoparticles were effective against *Plasmodium falciparum* and its vector *Anopheles stenensi*. However, in these cases, the main nanostructure is not silver nanoparticles but silver chloride nanoparticles, as in many biogenic syntheses with plants (Durán et al., 2016c). Using biogenic gold nanoparticles, Subramaiam et al. (2016) reported interesting results against malaria.

3.2. Leishmaniasis

Leishmaniases are vector-borne zoonotic diseases caused by various species of protozoa of the genus *Leishmania*. These pathogens are transmitted by sandflies (e.g., phlebotomine) and infect humans that are exposed to ecosystems where the vectors and reservoirs coexist. Anthroponotic cycles have been documented for some species of *Leishmania* and for defined geographical areas such as that of *Leishmania donovani* in the Indian subcontinent and *Leishmania major* in Afghanistan.

Leishmaniases are prevalent in tropical and subtropical areas, with two million new cases occurring yearly. Visceral leishmaniasis (VL) is caused by *L. donovani* in the Indian subcontinent and East Africa and by *L. infantum* in other parts of Asia, Europe, Africa and the New World (formerly referred to as *L. chagasi*).

Tegumentary leishmaniasis (TL) is caused by many species of parasites: *L. major, L. tropica, L. aethiopica* and sometimes *L. infantum* in the Old World and *L. (Viannia) braziliensis*, *L. amazonensis, L. (V.) guyanensis, L. (V.) panamensis, L. mexicana, L. pifanoi, L. venezuelensis, L. (V.) peruviana, L. (V.) shawi, and L. (V.) lainsoni* in the New World. Among species causing TL, different species of subgenus *Viannia and L. amazonensis* are found from Mexico to Argentina, and the largest variety is present in Brazil, mainly in the Amazon region. *L. mexicana* is present in Mexico and some Central American countries (Lindoso et al., 2012).

Accordingly, the control of these parasites is of important public health concern. The development of new technologies is imperative to deal with VL to control epidemics and obviously to reduce its impact on society (Marinho et al., 2015). The difficulty of assessing these strategies has been due to the gap between intervention and the control of transmission, which has only been evaluated by three studies, namely, indication and epidemiologic impact of linking intervention and epidimiologic impact. Most recent treatments for VL using miltefosine and paramomycin have not been evaluated enough and should be evaluated in different scenarios (e.g., epidemiological ones). In the future, investigations should consider a longer time horizon, in such a way that the infectious disease properties and peculiarities of VL could be efficiently expressed and accounted for (Marinho et al., 2015).

Leishmaniasis accounts for the second highest burden of parasitic disease after malaria. Depending on the causative species of the parasite, different types of the disease can occur. The two most common are visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). VL is the most severe one, being fatal in some cases, most of the time enhanced by parasite migration to vital organs resulting in several strong and harmful symptoms such as hypergammaglobulinemia and pancytopenia. On the other hand, the cutaneous infection is the most common form of the disease and can exhibit a high range of manifestations such as cutaneous nodules and gross mucosal tissue destruction. Moreover, it had been reported that CL causes high morbidity levels because of the continued presence of skin ulcers and the terrible psychological effects, especially in children patients (Bern et al., 2008).

Parenteral administration of pentavalent antimony compounds still represents the most common therapeutical option for all leishmaniasis forms (Desjeux, 2004). However, resistance and high frequency of side effects are major problems with these drugs. Amphotericin B (AmB), a highly hydrophobic drug, is the most used second-line therapy. The drug is highly active, but its clinical use is limited due to its toxicity and the high treatment cost (Rodrigues Caldeira et al., 2015). Later, its liposomal formulation (LAMB) displayed a significant reduction in treatment toxicity. This was followed by the introduction of miltefosine (MF) and paromomicyn (PM) in the clinical market.

LAMB continued being the first choice because of its effectiveness and low side effects. However, the excessive high cost of the treatment (AmBisome®, Astellas Pharma US, Inc.) represents a big limitation for its widespread use, especially for the majority of the population in poor countries. Some different formulations of these drugs have been prepared, but VL treatment still relies on the traditional AmB administration worldwide. Despite its severe side effects, AmBisome® has been recommended as first-line treatment of leishmaniasis by the World Health Organization Expert Committee on the Control of Leishmaniasis (Balasegaram et al., 2012). Moreover, there has been a similar tendency for CL treatment. Currently, meglumine antimoniate (Glucantime) is the first drug of choice for local or systemic injection and it has been used for more than four decades despite not being approved by the FDA and presenting high toxic effects on human organs (Momeni et al., 2013).

In this scenario, there is an urgent need for new effective drugs or formulations that allow (in a first step) a wide reduction of toxicity. Patients with an advanced disease present physiologic problems in several organs as usual consequence. If the established cure for the disease is as aggressive as the parasite infection, the whole treatment has no sense. Another point to take into consideration is the high cost of the treatments. As mentioned before, leishmaniasis is endemic especially in those regions with development problems and with a big population of poor families. It is clear that an excessively expensive treatment like AmBisome® is not a satisfactory alternative despite its effectiveness and well tolerance by VL patients. In this part of the review, we will show why nanotechnology is a tool with the potentiality to solve these problems in leishmaniasis treatment.

Many articles of clinical relevance envisioning promising outcomes for leishmaniasis patients have been published in recent years. Nanomedicines generally consist of nanoparticulate drug delivery systems based on liposomes, solid lipid nanoparticles, polymeric nanoparticles and others. These nanocarriers enhance drug efficacy, while reducing drug toxicity by optimizing pharmacokinetics and pharmacodynamics (Henriques et al., 2014; Moreno et al., 2015) (**Figure 5**).

Antimonial liposomes against visceral and cutaneous forms of leishmaniasis have been studied since 1984. As mentioned before, AmBisome and Amphotec are the most used commercial formulations for VL. As a consequence of liposomal encapsulation, toxicity of amphotericin B was reduced by a factor of 50- to 70-fold. The problem with AmBisome is still its cost (estimated at 800 euros per 1 injection per day) (Durán et al., 2009). Many efforts have been made by modulating liposome composition and structure in order to achieve better biocompatibility and encapsulation rates. Besides, different active principles were tested in liposomal systems either as encapsulated drugs or lipids with some therapeutic influence. Hexadecylphosphocholine (HePC) is a perfect example of a phospholipid that, being an important structural ingredient in liposome formulation, has an effective antiparasitic activity against leishmaniasis. The liposomal form of HePC showed to be less toxic than plain phospholipid (Papagiannaros et al., 2005). In contrast, there are not many reports about the uses of liposomes for CL. Glucantime® was one of the effective drugs tested for encapsulation in liposomes, but its hydrophilic nature allows only low encapsulation rates (Ribeiro et al., 2008). Later,

a freeze-drying double emulsion (FDE) method was utilized to prepare liposomes with Glucantime®, miltefosine and paromomycin payloads. Formulations were prepared with 90% encapsulation efficiency (EE). However, a common characteristic of the FDE method is the bimodal size distribution of the liposomes, which poses a problem at the moment of sterilizing the formulation. After the sterilization by filtration, the EE was reduced to 50%, which is still acceptable for hydrophilic drugs. Moreover, *in vivo* tests showed a potential therapeutic effect against *Leishmania major* infection for CL treatment (Momeni et al., 2013). More recently, another liposomal formulation was prepared by the fusion method plus homogenization for the encapsulation of Glucantime®. The advantage of this method is based on its simplicity and no use of organic solvents, exhibiting an EE of around 50%. The final formulation was tested for topical application by cell diffusion studies using mouse skin and vertical Franz cell diffusion. The results indicated that only 1% of the drug penetrated through the skin. This is a key point since *Leishmania* amastigotes live in the macrophages deep the in dermal layer (Moosavian et al., 2014).

Accordingly, the anticancer drug doxorubicin encapsulated in immunoliposome (immunodoxosome) produced total parasite elimination after 45 days compared with a similar dose of the free drug (doxosome). Both immunodoxosome and doxosome exhibited less toxicity than the free doxorubicin, showing the potential of encapsulated doxorubicin as an effective therapy against leishmaniasis (Mukherjee et al., 2004).

Another nanocarrier that has gained interest in the last decade consists of solid lipid nanoparticles. Although the application of SLNs in leishmaniasis is still not as important as liposomal formulation, the number of articles on this subject has been growing in the last years. For example, it has been demonstrated that this system allows the high encapsulation efficiency of hydrophobic molecules like AmB (Jung et al., 2009). SLNs were prepared with AmB for oral administration, since the avoidance of intravenous route could prevent the toxic effects of this drug. However, AmB is not absorbed orally, and that is why this nanoformulation was taken into consideration. In *in vivo* tests, these authors demonstrated a 40-time increment in AmB bioavailability in comparison with free AmB. However, they could not elucidate the SLN absorption mechanism (Amarji et al., 2007). Recently, Lopez et al. (2012) reported a comparative study between liposomes and SLN formulation with the incorporation of a dinitroaniline, oryzalin (ORZ). This compound is a microtubule inhibitor with selectivity to bind only to parasite tubulin. *In vivo* studies in a murine model of visceral leishmaniasis have shown no significant differences between both formulations. Nevertheless, liposomal and SLN formulations led to a significant parasitic burden reduction in liver and spleen as compared to the control group (84% to 91%) and similar to that of Glucantime® (Lopes et al., 2014). Like many active principles, ORZ causes high toxicity in several organs. It was also demonstrated that the encapsulation of this drug in SLNs decreased its cytotoxicity (Lopes et al., 2012). In another study, paromomycin was incorporated into SLNs in order to enhance skin penetration of the drug for CL treatment. Paromomycin clinical use is restricted because of its poor capability to penetrate skin layers. Despite

the drug hydrophilic nature, stearic acid nanoparticles were formulated with an EE of 39%, which is a reasonable value for this kind of molecule. Then, these drug delivery systems showed a sustained drug release profile for more than 24 h. It is interesting to notice that molecules of hydrophilic nature can be incorporated into SLNs (Ghadiri et al., 2011).

In addition to these SLN systems designed for the delivery of pharmaceutical molecules against leishmaniasis, it is worth mentioning that in other scientific studies SLN formulations were tested for the design of vaccines. Among the several antigens potentially capable of inducing protective immunity, cathepsin L-like cysteine proteinases (CPs) have received considerable attention for experimental visceral leishmaniasis. CPs are commonly found from prokaryotes to mammal's organisms. In *Leishmania*, CPs are involved in parasite survival, replication and the onset of disease, which makes them attractive vaccines and/or drug targets. However, the major obstacle for this kind of vaccine is its quick degradation. In this sense, Doroud et al. designed a SLN formulation for the delivery of P-DNA for CPs type I and II. P-DNAs must reach the nucleus of the target cells in order to express the foreign gen. In this case, SLNs worked as a protector yield for DNA preventing its degradation and also modulating the release profile. The cytotoxicity and gene expression effect of SLN-pDNAs was evaluated and compared to linear PEI (25-kDa)-pDNA polyplexes as a standard control (Doroud et al., 2010). Furthermore, in a consecutive research article, the incorporation of recombinant CPs (type 1) into SLNs to be utilized as a vaccine against *Leishmania major* infection in C57BL ⁄ 6 mice was reported. After intraperitoneal vaccination, an antigen-specific T-helper type 1 (Th1) immune response was induced compared to control groups. Lymph node cells from immunized mice displayed lower parasite burden, higher IFN-c, IgG2a, and lower IL-4 production, indicating that robust Th1 immune response was induced (Doroud et al., 2011).

Colloidal nanocarrier polymeric nanoparticles also exhibit relevant properties such as stability, biodegradability and high EE for many active molecules. In this sense, anti-leishmania drugs are not excluded from the list. PLGA NPs were prepared for β-aescin encapsulation. *In vitro* results demonstrated that the drug incorporation in the polymeric nanocarrier allowed a reduction in the cytotoxicity and maintenance of the antileishmanial efficacy (Van de Ven 2011). In addition, a recent study evaluated the use of AmB loaded PLGA NPs as a drug delivery system. *In vivo* tests showed high uptake of nanoparticles in the lungs, liver and spleen, demonstrating the potentiality of the formulation for anti-leishmania treatments (Souza et al., 2015).

Studies have probed the effectiveness of silver nanoparticles as an alternative therapy for leishmaniasis, specifically by subcutaneous intralesional administration for CL. Silver NPs can be synthesized by chemical, physical or biological methods (Durán et al., 2011). Biological synthesis is an eco-friendly alternative and is carried out by using microorganisms, enzymes, and plants. Besides, these synthesis methods generate more effective NPs in medicinal applications due to their surface biocoatings (Prasad et al., 2011). In addition, both chemically and biologically synthesized NPs were compared first by *in vitro* experiments against *Leishmania amazonensis* promastigotes. Biologically

generated silver NPs (Bio-AgNPs) showed to be four times more effective than chemically generated ones (Chem-AgNPs). Then *in vivo* studies in infected mice demonstrated that a Bio-AgNP dose was equally effective as 300-fold higher doses of amphotericin B, and more effective than 3-fold higher doses of Chem-AgNP. Furthermore, no hepato- or nephrotoxicity was detected in comparison with amphotericin B and Chem-AgNPs (Rossi-Bergmann et al., 2012).

3.3. Schistosomiasis

Schistosomiasis is a disease caused by parasitic flatworms of the genus *Schistosoma*, with three species (*S. mansoni, S. haematobium, and S. japonicum*) accounting for the majority of human infections. These parasites cause a chronic and often debilitating infection that impairs development and productivity, and exposure to these parasites is unfortunately strongly linked to extreme poverty. Recent estimates indicate that in around 80 endemic countries (e.g., sub-Saharan Africa, the Middle East, the Caribbean, and South America) more than 250 million people are infected annualy, resulting in 200,000 deaths (Neves et al. 2015). Unfortunately, lack of knowledge related to the disease, deficient sanitation, and the absence of effective health policies promote the spread of schistosomiasis in endemic countries. Praziquantel (PZQ) is the only drug that is being used, since an effective vaccine is lacking. PZQ has high efficacy, tolerability, very few side effects, and a competitive cost. However, the occurence of PZQ resistance in patients and in the laboratory is increasing, so new schistosomicidal drugs are required (Neves et al. 2015).

Praziquantel is a broad-spectrum anthelmintic developed in the 1970s and recommended by WHO (2006) for the treatment of schistosomiasis, that has been used for decades worldwide, mainly in endemic areas. Praziquantel has poor water solubility and resistance for the treatment of *Schistosoma mansoni* and *S. japonicum* (Wang et al., 2012). In addition, the treatment of infections caused by *S. haematobium* with praziquantel failed. Thus, different strategies against worms and eggs of *Schistosoma* spp. using nanotechnology have been examined to improve drug effectiveness.

Liposomes containing anthelmintic have been used against *S. mansoni*, and their antiparasitic activities have been demonstrated in some studies. Some time ago, Frezard and Melo (1997) encapsulated oxamniquine, which is an anthelmintic, and these liposomes were effective against *S. mansoni* in a murine model when applied 3 days after infection. Liposome-entrapped oxamniquine reduced the number of parasites by 97% when injected subcutaneously one day before the infection, displaying excellent therapeutic efficacy.

Liposomes with tartar emetic showed reduction of antimony toxicity and effective delivery in a murine model. Tartar emetic was entrapped in pegylated liposomes (11 mg Sb/kg intraperitoneally) and resulted in a 55% reduction in worm burden compared to the control group. However, the same liposomes with tartar emetic at a higher dose (27 mg Sb/kg) given subcutaneously and intraperitoneally reduced worms by 67% to 82% (Melo et al., 2003).

Liposomal praziquantel decreased the amount of schistosome eggs and worms in preclinical assays (Mourao et al., 2005; Freeza et al., 2013). Freeza et al. (2013) treated mice orally with praziquantel-containing liposomes and only praziquantel 30 and 45 days following infection with *S. mansoni* BH strain. Liposomal praziquantel at 300 mg/kg resulted in a 68.8% decrease in the number of worms, 79% in the number of eggs in intestine, and 98.4% in the number of hepatic granulomas compared to the controls. Yang et al. (2009) incorporated praziquantel into solid lipid nanoparticles and showed that noralpraziquantel bioavailability in rats increased significantly compared to free prazinquantel, suggesting the use of these nanoparticles as drug carriers. Similar results on oral bioavailability were observed in a study targeting the intestinal lymphatic system (Mishra et al. 2014). Souza et al. (2014) showed that solid lipid nanoparticles containing prazinquantel decreased toxicity in HepG2 cells when compared to free prazinquantel.

Cationic nanoemulsions containing the schistosomicidal agent 2-(butylamino)-1-phenyl-1-ethanethiosulfuric acid (BphEA) showed *in vitro* activity, indicating that cationic nanoemulsions can be used as delivery drugs against *S. mansoni*. BphEA has low water solubitity, hampering the application of this compound. These formulations have anionic and cationic interfacial charges with a droplet size ranging between 200 nm and 252 nm (zeta potential of 25.7 ± 3.9 mV). In this study, the schistosomicidal activity of cationic nanoemulsions was higher compared to that of free BphEA, indicating that a delivery system with this nanoemulsion could be interesting in the control of *S. mansoni* (Araujo et al., 2007).

Vaccine candidates in nanoparticles have been studied in some neglected diseases. Fuaad et al. (2013, 2015) developed a vaccine candidate containing cathepsin D-derived epitope and a lipid core peptide system against schistosomes, which induced high levels of IgG without adjuvant, suggesting the use of nanoparticles as an immunogenic vaccine. This study also suggested the combined use of drug and vaccination with synergistic application as an interesting alternative to the conventional treatment of schistosomiasis.

Polymeric nanoparticles such as poly(*d,l*-lactide-co-glycolide) acid (PLGA) and poly (methylmethacrylate) (PMMA) containing prazinquantel have been prepared and described as promising drug carriers. The drug encapsulation in polymeric nanoparticles increases water solubility and chemical stability (Mainardes and Evangelista, 2005; Fonseca et al., 2013ab). Mainardes et al. (2006) showed that PLGA nanoparticles with praziquantel had a more localized effect on intestinal membranes for a prolonged period of time. These physicochemical characteristics of polymeric nanoparticles lead to the control and gradual delivery of drugs.

Another strategy for controlling schistosomiasis is by combating the mollusk vector *Biomphalaria glabrata* through the use of metal nanoparticles. Silver nanoparticles have been studied as a molluscicidal with low toxicity to other aquatic organisms (Yang et al., 2011; Guang et al., 2013). Thus, in controlling schistosomiasis, nanotechnology has been often reported as a great potential alternative for the treatment of the disease by improving the efficacy of drugs.

3.4. Trypasomiasis

Human African trypanosomiasis (HAT) is caused by infection by *Trypanosoma brucei gambiense* or *T. b. rhodesiense* and transmitted to humans via the tsetse fly. In 1995, around 70 million people worldwide were under the possibilty of infection and currently around 20,000 people in Africa are infected with HAT (Sutherland et al., 2015; Nagle et al., 2014).

Chagas' disease, or American trypanosomiasis, is caused by *Trypanosoma cruzi* and occurs in 21 Latin American countries. Chagas' disease is endemic and primarily a vector disease affecting working people, on whom it has a strong economic impact. Approximately 10 million people are infected, and in endemic countries more than 25 million people are probably at risk. The heart is the most commonly compromised organ; symptoms include arrhythmias, cardiomyopathy, and thromboembolism. Death usually occurs from heart failure (Rodrigues-Morales et al., 2015)

The drug pipelines for both diseases are minute: very few compounds are being developed, and drug discovery efforts are extremely limited. In clinical trials for HAT there are only two compounds (nifurtimox, SCYX-7158) indicating the need for enriching the pipeline with novel chemical entities of critical importance (Nagle et al., 2014).

Benznidazole and nifurtimox are the only drugs with efficacy against Chagas' disease in human trials, and benznidazole is better tolerated and favored as the first-line treatment for Chagas' disease. Benznidazole is still used in Brazil (Pereira and Navarro, 2013). However, some patients tolerate nifurtimox better than benznidazole. Nevertheless, limited human data, supported by animal model data, suggest that *T. cruzi* strains may vary with respect to drug susceptibility (Bern, 2015).

Nifurtimox and benznidazole (BNZ) are currently available drugs for the treatment of trypanosomiasis. Nevertheless, classical liposome encapsulation failed to improve the *in vivo* efficacy of nifurtimox and BNZ (Lamas et al., 2006). However, endovenous administration of liposomal etanidazole (ETZ) caused a substantial decrease in parasitemia levels of *T. cruzi*-infected mice. In fact, there is urgent ongoing research on new nanomedicines and nanostructured materials against trypanosomiasis (Salomon, 2011).

It is known that amphotericin B binds to sterols of eukaryotic cell membranes, producing alterations in their permeability and also cell death. Its encapsulation in a liposomal formulation minimized the toxic side effects of this active compound. Several studies have demonstrated its *in vitro* trypanocidal activity (Haido and Barreto-Bergter, 1989; Castro et al., 1993), and a few reports reported the *in vivo* effect of amphotericin B formulations in mice infected with *T. cruzi.* Cencig et al. (2011) investigated the action of AmBisome (amphotericin B liposome) treatment, injected in six intraperitoneal applications at different times during acute and/or chronic phases of *T. cruzi* infection in mice, studying survival rates and parasitic loads in many tissues. These authors concluded that AmBisome therapy failed to completely cure mice infected with *T. cruzi*. However, it prevented mortality and effectively decreased parasitic loads in most tissues (*i.e.*, liver, heart, spleen, adipose

tissues, muscle, and blood). Earlier administration of AmBisome (one day after parasite inoculation) produced a better response in diminishing parasite loads in the liver and spleen, where repeated treatment in the chronic phase reduced parasite load in the liver and heart. Therefore, this beneficial effect of AmBisome, observed with administration over a short time, should promote strategies for using nanoencapsulated amphotericin B in combination with other drugs (e.g., BZN) for a shorter recovery from *T. cruzi* infection (Cencig et al., 2011).

SLNs are candidates in the treatment of neglected diseases, including trypanosomiasis (Olbrich et al., 2002). SLNs offer many advantages: they are biocompatible, solid at 25^{o}C and body temperature (~37^{o}C), protect the active compound against degradation, provide prolonged drug release and can be manufactured at large scale. Accordingly, Carneiro et al. (2014) reported *in vitro* and *in vivo* trypanocidal activity of 5-hydroxy-3-methyl-5-phenyl-pyrazoline-1-(S-benzyl dithiocarbazate) (H2bdtc) encapsulated in SLNs. H2bdtc is a potential drug lead to develop new agents against the trypomastigote form of Tulahuen strains of *T. cruzi* (Maia et al., 2010). Encapsulation did not change the *in vitro* activity of H2bdtc as a trypanosidal, which was more effective than BNZ. Indeed, treatment of mice with H2bdtc-SLN exhibited very promising results: (a) effective parasitemia reduction in mice at a concentration 100 times lower than that currently employed for BNZ clinical application; (b) decreased inflammation and lesions of heart and liver; and (c) overall survival of mice infected with *T. cruzi* (Carneiro et al., 2014).

Allopurinol, violacein, grandisin, bis-triazole DO870, and megazol encapsulated in various biodegradable polymeric nanoparticles have shown interesting *in vitro* trypanocidal activity (Gonzalez-Martin et al., 2000; Durán et al., 2001; Molina et al., 2001; Stecanella et al., 2013; Lima and Albuquerque, 2012). Nanoemulsions of essential oils of andiroba (*Carapa guaianensis*) and aroeira (*Schinus molle*) were highly effective against *T. evansi in vitro* (Baldissera et al., 2013). Adeyemi and Whiteley (2014) performed a thermodynamic and spectrofluorimetric study on the interaction of metal nanoparticles (*i.e.*, gold and silver) with arginine kinase (AK). Silver and gold nanoparticles bind tightly to the arginine substrate through a sulfur atom of a cysteine residue (Cys271). This interaction controls the electrophilic and nucleophilic profile of the substrate arginine guanidinium group, absolutely important for enzyme phosphoryl group transfer from ATP. This phosphotransferase is absent in humans, and the substrates that selectively inhibit AK are needed and should become candidates for trypanocidal drug studies (Miranda et al., 2006). However, all these reported studies need support from *in vivo* studies to validate the success of the proposed nanosystems. Besides, the scene could be different *in vivo*, since the nanomaterials used did not show any surface specific modification (*e.g.*, biofunctionalization), which leads to their rapid clearance by macrophages, resulting in impaired trypanosomiasis treatment (Date et al., 2007; Kroubi et al., 2015). Moreover, in an *in vivo* study with mice infected with *T. cruzi*, it was demonstrated that bis-triazole DO870 encapsulated in polyethyleneglycol-polylactide nanospheres affected a significant cure in these animals (Urbina, 2001; Molina et al., 2001).

Biofunctionalization consists of the surface chemical modification of nanocarriers with strategic biomolecules (*e.g*., folic acid, RGD peptide, sugars, proteins, antibodies, etc.) to achieve specific cell surface molecular interaction. This approach has been effective for targeted *in vivo* drug delivery with antitumor properties (Shao et al., 2015). Exploring the biofunctionalization approach, Arias et al. (2015) developed a new drug delivery system (polyvalent) for the treatment of African trypanosomiasis based on polymeric nanoparticles (PLGA) combined with nanobodies (single-domain antibody fragment obtained from functional heavy-chain antibodies of camelids) that on the parasite surface recognizes conserved cryptic epitopes. Pentamidine nanoparticles (first-line drug) were loaded for *T. b. gambiense* acute infection treatment. The IC50 value was 7-fold lower for the nanoparticles than for the free drug. Moreover, *in vivo* therapy in mice with African trypanosomiasis demonstrated that nanoparticles cured all infected mice at a 10-fold lower dose than the minimal fully curative dose of free pentamidine. In summary, this study demonstrated that the trypanosome surface chemistry is an important therapeutic target. Considering that the polyvalent drug delivery system was prepared using only components approved for their use in humans, including nanobodies, it is a promising nanosystem for practical uses in the treatment of human African trypanosomiasis (Arias et al., 2015). In another investigation, the trypanocidal drug pentamidine was also encapsulated in chitosan nanoparticles coated with nanobodies that specially reach the surface antigen of African trypanosomes. This chitosan-based nanosystem was effective in diminishing the minimal effective therapeutic dose of pentamidine *in vivo*, increasing its efficacy, significantly diminishing toxicity and overcoming drug resistance mechanisms (Unciti-Broceta et al., 2015).

3.5. Leprosy

Leprosy is still an important global health problem, since around 220,000 new cases are diagnosed annually. In Australia, the disease continues to be reported with an average of 10 new diagnoses annually, predominately in migrants from endemic areas, or from indigenous communities. *Mycobacterium leprae* infects the skin and peripheral nerves causing a chronic inflammation and neuropathy in a very slow-growing infection with an average of 12 years (Turner et al., 2015).

Many different agents such as fluroquinolones, macrolides and minocycline have been tried in various formulations and different periods. Selective cytokine inhibitors, thalidomine analogues, and pentoxufylline have proved effective in controlling type-2 reaction in leprosy patients. However, new drugs are desirable for leprosy medical treatment (Prasad and Kaviarasan, 2010).

In the case of paucibacillary (PB or tuberculoid) cases, treatment with dapsone (daily) and rifampicin (monthly) is recommended for six months. However, for multibacillary (MB or lepromatous) cases, treatment with daily dapsone and clofazimine along with monthly rifampicin for twelve months is suggested (Susuki et al, 2012).

Multidrug therapy (MDT) is still highly effective, because of patients are not infectious after doses applied monthly. This procedure is safe and easy to use under any conditions due to the ease of

calendar blister packs, regression rates are low, and no resistance to the combined drugs has been observed (WHO, 2015a).

Leprosy is a curable disease caused by the *Mycobacterium leprae* with well-defined etiology, but lacks better diagnostic tools and therapeutic strategies. There is no specific vaccine against *M. leprae,* and it is still a great challenge for research development. In this sense, nanobiotechnology is a promising strategy during the fabrication of selective biosensors and better drug delivery systems against this neglected disease (Goulart and Goulart, 2008). However, there are currently scarce reports in the literature involving the use of nanosystems for leprosy treatment. Liposomes containing clofazimine, an antibacterial agent active against *M. leprae*, was effective in reducing the time needed for leprosy treatment. In this case, liposomal formulation gel was tested on human cadaver skin demonstrated that liposomes not only prolong the drug release but also promote drug retention on the skin and reduce the healing time of external lesions (Patel et al., 1999ab). Liposomes were also effective in delivering antigens (*i.e.,* murabutide and Trat peptide) against peripheral blood mononuclear cells derived from leprosy patients (Chattree et al., 2007). Besides, it was demonstrated that the expression of apoptotic markers (*i.e.,* CD95 and CD95L) in T cells of leprosy patients increased after 5 days of *M. leprae* antigen stimulation. However, the percentage of expression of these apoptotic markers decreased significantly when the liposomal formulation was used. These results open the possibility of liposome application for the development of vaccines against *M. leprae* by activating the Th1 mediated immune response (Bisht et al., 2005).

The therapeutic potential of leprosy drugs is greatly limited due to their insolubility in water. Schwinté et al. (2003) demonstrated that the solubility of clofazimine can be improved by a factor of 30- to 50-fold after inclusion in cyclodextrin. Since 1943, dapsone (4,4'-diaminodiphenylsulfone) has been the drug of choice in the treatment of leprosy because it has bacteriostatic action against *M. leprae.* Despite its obvious therapeutic efficacy, the clinical use of dapsone is limited due to its low solubility in water (Santos et al., 2012). Moreover, it was recently demonstrated that it is possible to incorporate dapsone into nanoemulsion systems (from 6.0 to 12.0 nm) containing than can be used for topical and oral administration in humans. These nanoemulsions containing dapsone were prepared using isopropyl myristate, N-methyl-pyrolidone, propyleneglycol, Tween® 80 or Span®, and *in vitro* kinetics studies indicated the effective permeation of dapsone in porcine epidermis and Caco-2 human intestinal cell models (Borges et al., 2013, Monteiro et al., 2012).

Recently, a rapid and low-cost assay used for screening the toxicity of nanoparticles (*i.e*., Ag, Cu(II), and ZnO) against pathogenic mycobacteria was reported (Donnellan et al., 2016). Fluorescence was used to monitor mycobacterial growth using nanoparticle concentrations in the range of 6.25 µg/mL to 100 µg/mL for 7 days. The toxicity of NPs was ranked according to their specific composition in the following order: Ag> Cu(II)> ZnO. This new proposed bioassay is an interesting methodology to screen nanopharmaceuticals for leprosy treatment.

3.6. Tuberculosis

In 2014, it was estimated that there were 9 million new tuberculosis (TB) cases in the world and around half a million of them were cuased by multidrug-resistant (MDR) mycobacterium tuberculosis strains (WHO, 2014). MDR-TB has *in vitro* resistance to isoniazid and rifampicin, while extensively drug-resistant (XDR)-TB is resistant to fluoroquinolone and one injectable second-line anti-TB drug, and to isoniazid and rifampicin (D´Ambrosio et al. 2015; Yuen et al., 2015). The evolution of anti-TB drugs is depicted in Figure 1.

TB is still considered a severe disease, with 1.3 million TB deaths (including TB deaths in HIV-positive individuals) being reported in 2012. TB is the major killer among adults in the economically productive age groups and people living with HIV. However, those cured from TB can exhibit sequelae that can substantially modify the government's agenda implemented since the early 1990´s, (Glaziou et al. 2015). Data showed that chemotherapy is one of the most cost-effective health-care interventions. The enormeous impact of the HIV epidemic on TB in Africa and the concern about the growth of MDR-TB has stressed the need to enhance TB prevention and control (Glaziou et al., 2015).

Tuberculosis is an old complex contagious and chronic microbial infection widespread in human civilization since ancient times and first identified by Robert Koch on March 24, 1882. The etiological agents are the slow-growing actinomycetes *Mycobacterium* spp., particularly *M. tuberculosis* and *M. bovis*. In 1993, WHO declared global public health emergency for TB; later in 2006, it launched the Stop TB strategy linked to the Millennium Development Goal with the aim of reversing the trend by 2015 (Zumla et al., 2013). TB is generally caused by the invasion of the alveolar macrophages of the lungs but also other organs in humans by the Gram-(+) bacteria *Mycobacterium tuberculosis* (Mtb). In 2014, the global count for TB was estimated at 9 million and 1.5 million human new infections and deaths, respectively, and it is considered by the WHO as one of the most lethal diseases. Particularly, MDR-TB was detected in about a half million people (WHO, 2014; Uplekar et al., 2015). In addition, TB re-emergency is complex because it is sometimes associated with other pathologies such as HIV in asymptomatic patients (Gandhi et al., 2010; Lalloo and Ambaram, 2010). TB is considered a top killer among adult people during the most economically productive ages, also in people having HIV, and at present the quality of life of TB-cured patients is substantially reduced. This evidence has made TB a priority in the world health agenda since the 90`s (Glaziou et al. 2015).

Anti-TB drugs were developed in many laboratories, distributed and widely administered after the Second World War. However, no novel anti-TB drugs have appeared in the market since 1952, (Lalloo and Ambaram, 2010; Sosnik et al., 2010) (**Figure 2**). The most effective anti-TB drugs available in the market are rifampicin, pyrazinamide, isoniazid, and ethionamide (Mehanna et al., 2014; Kaur and Singh, 2014). However, two major problems associated with the drug still prevail, namely, reaching high drug concentration inside the lung macrophages and the MDR and extensively drug resistant (XDR) *M. tuberculosis*, which are making the therapy ineffective (Pandey and Khuller, 2005). In TB, *in vitro* microbial resistance to at least isoniazid and rifampicin is considered MDR, while extensively

drug-resistant TB occurs when the microorganism is resistant to at least one fluoroquinolone and one injectable second-line anti-TB drug plus isoniazid and rifampicin (D´Ambrosio et al. 2015; Yuen et al., 2015).

The following strategies were established to develop novel anti-TB weapons:

First, the priority was focused on the synthesis and discovery of new drug candidates for patients associated with complex pathologies such as TB coinfection in HIV positive patients, which are now at different levels of clinical trials (Table 1).

The second strategy was based on the new discoveries in physiology combined with molecular biology, which are provide strong candidates such as small interfering RNAs (siRNAs) for the treatment of different resilient pathologies including TB (Merkel et al., 2014). The fundamentals of the siRNAs mechanism are based on the inhibition of posttranscriptional gene expression in the target cells by RNA interference (RNAi), activating the degradation of mRNA and consequently, cell apoptosis. The mechanism of RNAi starts by delivering small interfering RNA/RNAs, usually of 21-26 synthetic oligonucleotides length, inside the microbial cells, or alternatively long double-stranded RNAs, and also plasmid DNA containing short hairpin RNAs that are processed by Dicer (nuclease) biocatalyst into siRNA. *In vitro* and *in vivo* experiments in mice using siRNA anti-TB showed promising therapeutic results (Dhiman et al., 2008; Rosas-Taraco et al., 2011). The *in vitro* studies using siRNA performed in human monocyte-derived macrophages infected with two different strains of *M. tuberculosis* targeted the expression of the antiapoptotic bfl-1/A1protein. The inhibition of bfl-1/A1protein synthesis in both Mtb strains by the siRNA induces the apoptotic cascade in infected macrophages, without any detectable effect on uninfected cells (Dhiman et al., 2008). Alternatively, siRNA molecules were used *in vivo* to enhance the immune response to chronic infection produced by Mtb in mice. The main idea was to activate the expression of the TGFb1 protein by inhibiting the immunosuppressive cytokine TGFb1 using siRNA, and consequently Th1 is enhanced and macrophage activation occurs concomitantly with the immune response in mice. Another advantage of this strategy is the increased expression of unspecific antimicrobial molecules such as NO and iNOS by the depletion of TGFb1 expression, which cause lethal effects on microbial invaders (Rosas-Taraco et al., 2011). Besides the promising siRNA strategies on anti-TB therapies, the main challenge remains in the selection of proper RNAi and the strategies for molecular delivery to the target cells (Lam et al. 2012, Merkel et al., 2014).

The third alternative of anti-TB therapies is based on the administration of drug contained in specific nanodevices, which target infected cells only, using proper amount of the drug under the therapeutic windows, lowering drug concentration in the patient, reducing undesirable side effects, and avoiding Mtb resistance mechanisms. However, the task is complex since anti-TB therapies usually require extended treatment because of low antibiotic permeability through the membranes, antibiotic instability under physiological conditions, and limited drug administration due to its high toxicity. Strategies of molecular delivery, based on passive and active mechanisms developed in the carriers

using nanontechnological approaches, are now in progress. There are many nanodevices reported in the literature but they can mainly be grouped into the following:

- Micro- and nanoparticles synthesized using natural and synthetic polymers such as polylactic acid, acrylate derivatives, collagen, alginic acid, and mixtures of them.
- Lipidic nanostructures such as archeosomes, solid lipid nanoparticles, nanostructured particles, liposomes, and micelles. The systems are configured by combining several types of lipids such as stearic, oleic, linoleic acids, phosphatidylcholine-cholesterol/ phosphatidylcholine and cholesterol, or synthetic molecules such as Spams (®), and non-lipidic molecules (surfactants, proteins, etc.).
- Hybrid systems developed using coacervates o molecular solutions, such as caprolactone-co-glycolide, chitosan-PLA, wheat germ agglutinin, and PGLA.

Drug delivery nanodevices were recently reviewed (Kaur and Singh, 2014; Mehanna et al., 2014).

3.7. Filiarasis and onchocerciasis

Filariasis is caused by parasitic worms called filariae. Filariae are microscopic roundworms that dwell in the blood and tissues of humans. The most important filarial diseases for humans are lymphatic filariases, in which the adult worms are found in the lymphatic system. Lymphatic / Lymphatic filariasis is sometimes also referred to as elephantiasis (Chandy et al., 2011; Yadav and Srivastava, 2014).

Ivermectin and suramin are extremely potent semisynthetic derivatives of the antinematodal principle obtained from *Streptomyces avermitilis*. In 2010, Turner and coworkers proved the microfilaricidal activity of doxycycline against *Onchocerca volvulus* (*O.volvulus*) in an area of *Loa loa* co-endemicity, which is one of the filaria nematodes (Turner et al., 2010). Mebendazole and flubendazole irreversibly block the uptake of exogenous glucose by nematodes, producing glycogen depletion and low generation of ATP required for survival. Due to these effects, the parasites die, or are slowly immobilized and gradually eliminated from the gut. Human blood levels are not affected. Diethylcarbmazine (DEC) has a highly selective effect on the microfilariae. Concomitant administration of corticosteroids with DEC is now considered the most appropiatre treatment in order to minimize the allergic manifestations secondary to the disintegration of microfilariae, particularly in *O. volvulus* and *L. loa* infections.

Onchocerciasis is caused by infection with the filarial nematode *Onchocerca volvulus* and can result in eye or skin lesions. The parasite is transmitted to humans by black flies of the genus *Simulium*. In 2007, WHO (2015b) estimated 37 million persons were infected with onchocerciasisin in endemic countries (30 in Africa, six in the Americas, and one in the Arabian Peninsula). The strategy of the Onchocerciasis Elimination Program of the Americas (OEPA) is to support national programs in

the six endemic countries to provide twice-yearly mass drug administration (MDA) of ivermectin to ≥85% of the eligible population at risk (Cruz-Ortiz et al., 2012).

As mentioned before, filiarasis is a tropical disease caused by filarial worms, *Wuchereria bancrofti, Brugia malayi or B. timori.*The infection can be treated with drugs such as diethyl-carbamazine citrate (DEC), albendazole, tetracycline, ivermectin, and suramin. About 98% of the infected populations are living in African and South-East Asia regions. Onchocerciasis, commonly known as "river blindness", is caused by the parasitic worm *Onchocerca volvulus*. It is transmitted to humans through exposure to repeated bites of infected blackflies of the genus *Similium*.

WHO states that currently, more than 99% of infected people live in African countries; the disease also exists in some foci in Latin America and Yemen (WHO, 2015b). Today, it is treated with invermectin as the main strategy to eliminate onchocerciasis in Africa (Hoerauf, 2008).

In some cases of fungal and parasitic skin infections, nanotechnology appears to iniciate an attempt to improve existing treatments (Leslie et al., 1993). Since the 1990´s, many nanostructures have been investigated to improve the treatment of neglected diseases.

DEC encapsulated in liposomal forms was effective in the elimination of filarial parasite infected by *Brugia malayi* from systemic circulation up to 60 days postinfection in animals (Owais et al. 2003). In addition, the coadministration of liposomal formulation of DEC with tuftsin (immunomodulator), which is a suppressor of the microfilarial stage of the parasite after 90 days posttreatment, was also effective against the adult parasite.

To treat infection by *Wolbachia* bacteria symbiont to filarial parasite, Bajpai et al. (2005) reported the use of tetracycline in a liposome formulation. The liposome loaded with tetracycline was found to be more effective when compared to the free form of the drug, eliminating tetracycline toxicity. The relevance of using this type of liposome formulation was demonstrated in the response, as compared with free forms of treatment of human lymphatic filariid *Brugia malayi* infection in rodent host *Mastomys coucha*. The liposomal formulation increased the time of the drug release up to 48 h, which decrease *A. aegypti* the number of administrations and lowered the possibility of toxic effects. When it was combined with liposome-encapsulated antibiotics, it enhanced microfiliaricidal activity, but was not so efficient in adulticidal activity (Dangi et al., 2010).

To improve targeting to lymphatics and to increase the retention time of DEC, Kartick (2014) encapsulated this drug in SLNs. All the parameters studied improved and their entrapment efficiencies were significant (~70%). DEC release time from SLNs was around 150 min. A study in *Sprague Dawley* rats showed an approximate 5-fold increase in the amount of DEC that reached lymphatics in the case of SLN encapsulation, compared with the free form of DEC. This high DEC concentration led to the elimination of microfilariae from the lymphatics.

To extend the release of drugs Moghimi et al. (1994) formulated a polystyrene system as nanospheres administered by subcutaneous injection in Wistar female rats to verify drainage and passageway across the tissue lymph interface in dermal lymphatic capillaries in the rat footpads. The authors

observed a rapid drainage of the polymer-coated nanospheres from interdermal tissue when compared to uncoated nanospheres, minimized interactions between macrospheres (when coated, their size increases) and amorphous ground substances, faciliting the movement of nanospheres from the interstitium towards the lymphatic system.

Another study developed nanosphere for lymph node targeting qualities by Hawley et al. (1997) developed nanospheres with good lymph node targeting characteristics for subcutaneous injection in rats, using poly(lactide)-poly(ethylene glycol) (PLA:PEG), polystyrene (PS) and poly(lactide-co-glycolide) (PLGA) nanospheres and copolymers. The surface-modified nanospheres were very useful for targeting the treatments of lymph nodes.

Rao et al. (2010) studied nanoparticles prepared by nanoprecipitacion. The nanoparticles formed by conjugation were PLGA-PMA (1-pyrenemethylamine): PLA-PEG (PP) and PLGA-PMA:PLGA-COOH (PC) in different proprortions and sizes. All rats received nanoparticles containing polymerin differents lymph regions (nodes of interest). Nanoparticles were administered by subcutaneous injection in rats and they were monitored and evaluated by various techniques (*in vivo* study). The *in vitro* study was just to evaluate the degradation of nanoparticles. Lymphatic absorption and node retention of PP nanoparticles appeared to be inversely related to size and hydrophobicity. On the contrary, these processes with PC nanoparticles were directly proportional to the anionic charge on the particles. The best PP particle size for *in vivo* lymphatic uptake and retention in a rat model was 50 nm. PPs were adequate for sustained release and delivery into the lymphatics for prevention and/or treatment of oligometastases.

Ali et al. (2013) encapsulated ivermectin at a 3:1 ratio of poly (lactic-co-glycolic acid) (PLGA) nanoparticles with polyvinylalcohol (PVA) (nano-IVM) as surfactant by nanoprecipitacion or emulsion-solvent evaporation methods, to test against human lymphatic filariid *Brugia malayi* in rodent host *Mastomys coucha*. Finally *in vivo* studies showed that a combination of entrapped IVM (nano-IVM) with DEC had an enhanced microfilaricidal effectiveness and slightly better macrofilaricidal effectiveness than any of the single formulations or free drug combinations.

Subcutaneously administered ultrafine PLGA nanoparticles containing doxycycline hydrochloride against lymphatic filarial parasites have been recently reported (Singh et al., 2016).

The biodegradable polyanhydride nanoparticle-based platform for the codelivery of the antibiotic doxycycline with the antiparasitic drug, ivermectin, to reduce microfilarial burden and rapidly kill adult worms was recently published (Binnebose et al., 2015).

A study was carried out to compare the administration route and the enhancement of uptake in the lymphatic system using emulsion nanoparticles of standard DEC drug for filariasis (Karajgi and Vyas, 1994). It was found that intraperitoneal injection (ip) was better than intravenose administration (iv) in which lymphatic uptake was not observed. The authors suggested that a nanoparticle-in-oil emulsion system exhibited excellent potential as a lymphotropic carrier system.

Ravichandran (2010) tested a new nanosuspension tablet (nanonization by high-pressure homogenization) of albendazol in rats, which showed a bioavailability 2 to 3 times larger than that of the existing drugs in the market.

To evaluate the larvicidal effectivity, a study against filariasis vector, *Culex quinquefasciatus* (*C. quinquefasciatus*), was conducted using silver nanoparticles synthesized by *Eclipta porstata* extract (Rajakumar and Rahumam, 2011). The results showed that biogenic silver nanoparticles presented a good activity against larvae of *C. quinquefasciatus,* (LC_{50}= 4.56 mg/L).

Dhanasekaran and Thangaraj (2013) obtained larvicidal results with biogenic nanoparticles (from extract of *Agaricus bisporus*) against the mosquito vector of lymphatic filariasis (*Culex sp.)*. The larvacidal activities were analyzed by standart procedures recommended by WHO. Every 3 h, for 24 h, mortality was assessed to determine acute toxicity. Particularly, 5.0 mg/L biogenic nanoparticles made of *Agaricus bisporus* kill 100% of *Culex larvae*. Very recently, Murugan et al. (2016d), Govindarajan et al. (2016) and Adesuji et al. (2016) showed the effect of biogenic silver nanoparticles on the filiarasis vector *Culex quinquefascius,* among othersh. Unfortunately, in all these cases, the silver chloride nanoparticles appeared complexed with silver nanoparticles, and not only silver nanoparticles were acting on the vectors as reported.

3.8. Toxoplasmosis

Toxoplasmosis infection by the protozoan parasite *Toxoplasma gondii* has a worldwide distribution. This parasite belongs to the coccidian subclass, *phylum Apicomplexa*, and the three parasitic stages (tachyzoite, cyst, and oocyst) have been completely characterized. Toxoplasmosis encephalitis (TE) in Toxoplasma-seropositive AIDS patients must be managed with trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis. If patients cannot tolerate TMP-SMX, they must use dapsone-pyrimethamine plus folinic acid. A twice weekly treatment with pyrimethamine-sulfadoxine has proved to be effective, but skin toxicity limits its use. Atovaquone with or without pyrimethamine-folinic acid has also been considered, since it is efficiently absorbed in the digestive tract. Toxoplasma-seronegative persons must be checked annually for IgG antibody to Toxoplasma to determine whether they have seroconverted with the risk of TE (Robert-Gangneux and Dardé, 2012).

In the area of nanotechnology, many new drugs have been reported for therapeutic purposes of toxoplasmosis. The common treatment of toxoplasmosis usually reported by the Centers for Disease Control and Prevention (CDC, USA) is a combination of drugs such as pyrimethamine and sulfadiazine, plus folinic acid.

In the 1990s, liposomal carriers were important to initiate a new form of combating the protozoans. Tachibana et al. (1990) used stearylamine-bearing liposomes (SA/PC) against *Toxoplasma gondii* (RH strain) in the tachyzoite phase, using *in vivo* and *in vitro* assays. They were injected in female mice to assess their toxicity. Each group received 1, 5 or 10 mg/mL of 30 mol% SA/PC-liposomes after *T. gondii* strain infection. They found that *in vitro* viable activity of SA/PC liposomes gradually dropped

with the decrease in liposome concentration. *In vivo* results showed that SA/PC-liposomes had preventive and curative effects.

Another study involving liposomes and toxoplasmosis was conducted by Elsaid et al. (1999, 2001). They studied antigens incorporated in liposomes against *T. gondii* in mice. ELISA antibody level, after immunization, was higher in all mice immunized with *T. gondii* antigen, but the differences between groups were not statistically significant. However, immunization with purified 32-kDa antigen of tachyzoite (L/pTAg*)* or total tachyzoite and/or bradyzoite antigen encapsulated in liposomes enhanced protective immunity (humoral and cellular), probably contributing to the limitation of the systemic spread of *T. gondii* and reducing the vertical transmission of toxoplasmosis.

Recently, El-Zawawy et al. (2015) tested the effectiveness of triclosan (TS) and triclosan-loaded liposomes (liposomal-TS) against a virulent strain of *T. gondii* in Swiss albino mice. The infection was intraperitoneal and the treatment was oral. After treatment, both TS and liposomes-TS induced significant reduction of parasite (tachyzoite) burden; however, the latter was more efficient. Other parameters such as mouse mortality and viability, morphology alteration and infectivity of tachyzoites from infected mice in comparison with noninfected mouse controls showed the same profile. The authors concluded that when TS was loaded in liposomal structures the release phase was longer, prolonging the TS action in peritoneal fluid and in the liver.

As noted above, other authors decided to test a standard drug such as pyrimethamine (PYR) in the treatment of toxoplasmosis after modification using nanotechnology. Pissinate et al. (2014) compared SU-PYR (surfactant prepared) and PYR-loaded lipide-core nanocapsules (PYR-LNC), using poly (Ɛ-caprolactone) (PCL), against *T. gondii*. They used LLC-MK2 (kidney, Rhesus monkey, *Macaca mulata*) strain in an *in vitro* assay. *In vivo* studies using peritoneal injections were carried out in mice.

Comparative formulations were prepared just with LNC (lipid-core nanocapsules). Body weight gain was used as the parameter to confirm the toxicity of formulations after 10 days. The LLC-MK2 cells showed the same tolerance with PYR-LNC and PYR solution, but proliferation was affected with TC50 values of 6.0 μM for both formulations. After 8 days, the untreated mice and LNC group died. The group treated with PYR-LNC showed better results than that treated with SU-PYR. After 60 days, the mice treated with 10 mg SU-PYR (10%), 7.5 mg PYR-LNC (15%), and 10 mg PYR-LNC (30%) were alive.

To improve the effect of atovaquone, which previously demonstrated good results in *in vitro* responses against *T. gondii*, Scholer et al. (2001) prepared atovaquone nanosuspension (ANS) for *in vitro* and *in vivo* assays. The *in vitro* test after 48 h of treatment showed a decrease in infection capacity of the parasite in the presence of both formulations (SLAs and drug-free), and growth was almost inhibited at concentrations above 1.0 μg/mL. With regard to cytotoxicity, viability was high (~80%) even up to 3.0 μg/mL and remained stable from 1.0 μg/mL. All animals received sulfadiazine for 3 weeks and then started with the ANSs and free atovaquone. A survival rate of 88% was obtained during the treatment

with therapeutic effects (10 mg/kg body weight/day). These results indicated a good direction to the treatment against *T. gondii*.

Rhodamine B-labeled polystyrene latex particles were coated with polybutyl cyanoacrylate under physical inclusion of two different new drugs against toxoplasmosis. Pentamidine-loaded as well as pentamidine-free core-shell model drug carriers were added to cultured human macrophages (J774-A1 cells), infected by *T. gondii*, following an infection protocol. As the main result, the drug-free references in the two series of core-shell model drug carriers achieved ca. 85% of the observed maximum effectiveness of toxoplasmosis therapy. These data correlated well with an immunestimulating effect on the human macrophages, caused by the cell uptake of colloidal substrate, foreign to the body (Leyke et al., 2013).

The data that unloaded pentamidine nanoparticles had the same effect as penramidine-loaded nanoparticles offer the opportunity to use the nanoparticles directly for treatment. Perhaps, part of the effect of atovaquone crystal nanoparticles in a *Toxoplasma*-mouse model should be attributed to the effect of nanoparticles (Scholer et al., 2001), rather than to the direct effect of the drug against the parasite.

One of the clinical manifestations of infection caused by *T. gondii* is toxoplasmic encephalitis (TE). It could be lethal if the patient does not receive treatment. The most effective treatment causes hematological disturbances and allergic reactions. To eliminate these effects, atovaquone was prepared as nanoscale suspensions (ANSs) capped with poloxamer 188 (P188) and sodium dodecyl sulfate (SDS) (SDS-coated ANS) (Shubar et al., 2011). The authors compared ANSs to commercial atovaquone (Wellvone) in murine models. They observed an increase in oral bioavailability and decrease of inflammatory signs in the brain of animals treated with SDS-coated ANS.

Gaafar et al. (2014) conducted a study involving two types of nanoparticle, silver nanoparticles (Ag NPs) and chitosan nanoparticles (CS NPs). After animal infections (male Swiss albino mice), three different treatments were tested (CS NPs, Ag NPs, and their combination) and compared with two controls (healthy animals and infected animals without treatment). Toxicity was determined by the concentration of Ag in different tissues (intestine, liver, kidney, brain, and lungs). A low parasite count resulted in the group that received CS NPs/Ag NPs (100 μg/mL/100 μg/mL) after 4 days of infection. The best result found was that the concentrations of Ag particles in the main tissues stayed within the safe range of 9 μg/g body weight, with the highest level being detected in the liver followed by intestine, kidney, lungs, and brain.

3.9. Dengue virus and others viruses

Dengue virus infection (DVI) causes different kinds of illness ranging from asymptomatic infection to a flu-like mild undifferentiated fever with high fever over the 40°C (Benelli and Mehlhorn, 2016; Durán et al., 2016a). Other diseases can mimic DVI, such as influenza, measles, typhoid, leptospirosis

or any nonspecific viral syndrome. A definitive diagnosis of DF therefore can only be made by specific laboratory test (Mungrue, 2014).

DVI infection is the wildest mosquito borne infection affecting 2.5 billion people in tropical and subtropical regions of the world. DVI is a member of Flaviviridae family and is transmitted to humans by infected female Aedes genus, especially *Aedes aegypti* and/or *Aedes albopictus* (Beatty et al., 2010).

The only treatment against dengue is preventive and supportive care. Several research studies have been conducted to use siRNA against DVI, and many of them have provided results of effectively using siRNA against DVI replication (Idrees and Ashfag, 2013). Also, molecules such as iminosugars, such as deoxynojirimycin (DNJ) and its N-alkylated derivatives, demonstrate antiviral activity against DVI by targeting host cellular factors required for viral morphogenesis (Sayce et al., 2010). The flavonoids fisetin and quercetin and baicalein also exhibit anti-dengue virus activities (Zandi et al., 2012). New approaches in DV and ZV treatments now focus on the use of nanoparticles combined with natural and more effective molecules as efficient antiviral systems (Duran et al., 2016).

Current challenges in the treatment of Dengue and Zika viruses focus on the development of effective antivirals based on nanoparticles (Durán et al., 2016a) (**Figure 6**). In addition, there are some discussions about the perspectives of nanodevices in other viral illnesses, especially in those which do not have an established cure or new alternatives in treatment are under exploration (HIV, HBV, etc.).
During the last decades, eradication of virus infections has been a challenge in the medical field, since the problem is not only the spread of viruses but also their ability to evolve by mutations in the genetic material, which makes them a real nightmare in health.

Particularly, the dengue virus possesses four distinct, but closely related, serotypes (named DEN-1, DEN-2, DEN-3 and DEN-4), which makes targeting difficult. The virus attack may cause a spectrum of illnesses from the more moderate, such as the asymptomatic state or flu-like mild undifferentiated fever, to the more severe such as the famous dengue fever with hemorrhagic problems. If subsequent infections by other serotypes are produced, the risk of developing severe dengue increases (Mungrue, 2014). Currently, there are no approved vaccines or antiviral agents against the dengue virus, which opens a gate for the design of new strategies to effectively combat the infection, and nanotechnology appears as a new feasible alternative. A promising vaccine has been developed and tested, but only a partial and heterogeneous efficacy was found. In addition, it is suspected to be responsible for adverse side effects (López-Gatell et al., 2016).
Nowadays, the only treatment against dengue is preventive and supportive care. For these reasons, the development of new rapid and specific diagnostic systems is a useful tool for prematurely detecting the infection at its initial stages. Some nanodiagnostic tools are based on nanomaterials such as liposomes, nanopores and nanowires, which are coupled with conventional methods such as fluorescence, potentiometry and voltammetry. Most of them include enzyme-linked immunosorbent assays and reverse transcriptase polymerase chain reaction. Although these systems give rapid

diagnostics and use inexpensive materials, they are currently unavailable for practical use in clinical practice (Peh et al., 2010). An effective biosensor was developed by the use of an optical membrane-based DNA/RNA hybridization system using liposome amplification, which allows the detection of generic and serotype-specific synthetic dengue genomic sequences (for serotypes 1−4) in blood samples of patients. The method uses a biotinylated DNA capture probe immobilized on a streptavidin-coated polyethersulfone membrane, in addition to a generic DNA reporter probe coupled to the outer surface of dye encapsulating liposomes. When the sample of amplified ARN passes through the capture zone of the membrane strip, the quantification of reporter probe-tagged liposomes is proportional to the number of RNA molecules present in the sample, and the signal is detected by electrochemiluminescence. While the nonspecific RNA molecule does not generate a signal, a generic sequence added to the dengue RNA during the amplification step is hybridized and the signal is turned on. This biosensor showed an excellent correlation in detection, is portable, inexpensive, and very easy to use (Baeumner et al., 2002).

Another diagnostic approach proposes the use of a silicon nanowire (SiNW)-based sensor for the detection of products from DEN-2 genes after reverse-transcription-polymerase chain reaction (RT-PCR) by the hybridization with a specific peptide nucleic acid (PNA) covalently attached onto the SiNW surface. The positive reaction was verified by measuring the change in resistance of the SiNW before and after hybridization (Zhang et al., 2010).

In this scenario, research groups have proposed the green-synthesis of silver nanoparticles (AgNP) as novel and effective tools against the different dengue serotypes. The use of natural extracts of plants as reducing and stabilizing agents for nanoparticle preparation provides interesting properties.

More recently, gold nanoparticles have been used for highly sensitive and rapid detection of dengue infection. In one study, a diagnostic device was developed for serum detection of antigens from a nonstructural protein (NS1) responsible for dengue virus pathogenicity by an immunoassay with nanogold particles conjugated to antibodies from NS1 (Hussain et al., 2014). Jahanshahi et al. (2014) developed a new method based on surface plasmon resonance (SPR) for detection of anti-dengue virus in human serum in less than 10 min. The DEN-1, DEN-2, DEN-3, and DEN-4 serotypes were immobilized onto the biochip surface that consists of a gold coating on glass accompanied by nanoparticles and the immobilized antigens. Another study proposed the detection of dengue-specific immunoglobulins (IgGs) in salivary fluids by stacking flow immunoassay, in which the liquid conjugate was composed of G protein conjugated to 40 nm gold nanoparticles. The importance of IgGs detection lies in distinguishing between primary and secondary dengue infection, since IgGs are only present in second episodes (Zhang et al., 2015).

The presented nanodiagnostic systems provide an insight into the mechanisms and bases of detection and how to increase the sensitivity in the shortest time, which is feasibly extrapolable to detection of other viral pathologies.

Once viral infection is established, new alternatives in treatment are required and need to be explored. Several research studies have been conducted on the use of small interference RNA (siRNA) as a next generation in the treatment of dengue infection, since the siRNA could effectively inhibit the virus replication (Idrees and Ashfag, 2013).

However, some studies showed that antivirals could be an interesting trend in the coming future. Iminosugars, such as deoxynojirimycin (DNJ) and its *N*-alkylated derivatives, have demonstrated antiviral activity against DVI by targeting host cellular factors required for viral morphogenesis (Sayce et al., 2010). Flavonoids such as fisetin, quercetin and baicalein also exhibited anti-dengue virus activities, particularly against DEN-2 serotype, by a mechanism that is probably related to inhibition of RNA polymerase (Zandi et al., 2011, 2012).

Nevertheless, this kind of compound commonly showed a low bioavailability, especially for therapeutic uses, so several strategies to overcome this limitation are focused on the use of a wide diversity of nanocarriers including liposomes, solid lipid nanoparticles, nanoemulsions, nanocrystals or polymeric nanoparticles, among others (Teles et al., 2005).

Liposomal formulations have shown interesting properties in terms of the effective dose of antiviral, decreasing the toxic concentrations and enhancing the retention time of the drug at blood circulation (due to PEGylation). Experiments *in vivo* with a lethal antibody-dependent enhancement mouse model of dengue pathogenesis demonstrated that delivery of iminosugars (all deoxynojirimycin derivatives) via polyunsaturated ER-targeting liposomes (PERLs) increases the survival rate and prevents the viral accumulation in organs and serum. In comparison with free administration of iminosugars, liposomes only need a 3-log10 less dose to achieve the survival of the animals. In addition, the formulation exhibited a greater *in vitro* potency against dengue virus by inhibiting both the number of infected cells and the release of infectious viral particles from primary human monocyte-derived macrophages (Miller et al., 2012).

Alternatively, AgNP synthesized using the Moringa oleifera seed extracts showed *in vitro* antiviral activity against DEN-2 infecting Vero cells. Also, those nanoparticles were highly effective against the dengue vector *A. aegypti* (Sujitha et al., 2015). In a similar work, biosynthesized AgNPs using the aqueous extract of *Bruguiera cylindrica* leaves were tested against *A. aegypti* at low doses to reduce larval and pupal population of *A. aegypti* (Murugan et al., 2015). Also, the use of alga-mediated synthesis of metal nanoparticles becomes an interesting tool to fight the dengue virus and its vector *A. aegypti*, showing a reduced cytotoxicity in mammalian cells (Murugan et al., 2016). Other approaches suggested the use of carbohydrate polymers for the preparation of silver nanoparticles with bioactive properties similar to the commercially available AgNPs (Saha et al., 2016). However, despite the *in vitro* effectiveness of mosquitocidal nanoparticles prepared with natural products at laboratory scale, more studies are needed about their possible nontarget effects against mosquito's natural predators and other aquatic organisms (Benelli 2016). Regarding the Zika virus, eco-friendly silver nanoparticles were also employed. A low-cost technology based on extracts of *Hugonia mystax* showed toxic effects

against *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus* larvae, while a reduced toxicity was observed for aquatic biocontrols of *Gambusia affinis, Diplonychus indicus, and Anisops bouvieri* (Govindarajan et al., 2016a). Similar recent studies have focused on the biofabrication of Quisqualis indica-AgNPs as an eco-friendly tool against Zika virus, malaria and filariasis vectors (Govindarajan et al., 2016b). Also, polydispersed silver nanocrystals using *Malva sylvestris* are an ecological alternative to kill the mosquito larvae (Govindarajan et al., 2016c).

Interestingly, the extrapolation of the techniques to treat other related viruses was compared. The ability of PERLs to produce a cholesterol-lowering effect impacts positively on the inhibition of virus replication, and antiviral activities were observed not only for dengue virus, but also for hepatitis B virus (HBV), HCV, and HIV-1 (De Mareuil et al., 2002; Pollock et al., 2010). Clayton et al. (2009) designed pegylated liposomes with the surface covered by targeting ligands derived from the Fab' fragment of HIV-gp120-directed monoclonal antibody F105, and evaluated the encapsulation of a novel HIV-1 protease inhibitor. They demonstrated that immunoliposomes were effectively targeted and internalized into HIV-1-infected cells, delivering and accumulating the protease inhibitor into cytoplasm. These results highlight the potentiality of the liposomal carriers as vehicles for antivirals and the possibility to transport other cytotoxic drugs for a wide array of viruses.

Other studies have considered the development of solid lipid nanoparticles and nanostructured lipidic carriers as possible matrices for the encapsulation of antivirals against dengue virus. Baicalein, a well-known flavonoid with antiviral activity, was encapsulated in SLN at 60.73% by a solvent injection method and showed a controlled release profile (Liu et al., 2009). In a similar way, baicalein was incorporated into 100 nm tocol NLCs for intravenous administration, detecting higher plasmatic levels and a longer half-life than administration of free antiviral (Tsai et al., 2012). SLN also improved the poor orally availability of lopinavir (antiviral for HIV) by encapsulation in glyceryl behenate based SLN for delivery targeted to intestinal lymphatic vessels (Alex et al., 2011).

The preparation of nanoemulsions is an attractive model to incorporate essential oils for the control of vector-borne diseases. Ghosh et al. (2013) proved that these formulations mixed with essential oils derived from plants have a dose and time dependence for killing the mosquito larva of *Aedes aegypti*. Another group found similar results with a nanoemulsion containing *Rosmarinus officinalis* essential oil (250 ppm concentration) and evaluated its larvicidal activity against *A. aegypti* larvae, observing mortality levels of 80 ±10% and 90 ±10% after 24 h and 48 h, respectively (Duarte et al., 2015).

Several polymers such as poly(lactide-co-glycolide) (PLGA), poly(D,L-lactic acid) (PLA), polyglycolide, polycaprolactone (PCL), poly(d,l-lactide), chitosan, and PLGA–polyethylene glycol (PEG) have been developed for passive and ligand-targeted delivery of therapeutic drugs (Prabhu et al., 2015; Jhaveri and Torchilin, 2015). In particular, it was observed that carriers for antiviral drugs exert the antiviral activity of the encapsulated molecules, indicating that direct interaction between the nanoparticles and the virus may inhibit the viral attachment to cells (Lembo and Cavalli, 2010). A tetravalent dengue system composed of bovine serum albumin NPs was evaluated in a murine model.

The BSA-NPs could absorb the four serotypes of dengue in their inactive form and after administration; an induction of anti-DENV IgG antibodies was occurred (Silva et al., 2012). Other nanoparticles composed of chitosan/Mycobacterium bovis Bacillus Calmette-Guerin cell wall components were able to encapsulate a novel dengue nanovaccine (produced by UV inactivation of DENV-2) and showed interesting immunogenic properties in a Swiss albino mouse model by generation of humoral and cellular immune responses (Hunsawonga et al., 2015). Polymeric nanoparticles of PLA and methacrylic acid copolymers were designed to entrap the peptidomimetic compound CGP 57813, a potent inhibitor of an HIV-type protease. Due to its high lipophilicity, it showed a poor biodisponibility. While PLA NPs showed an increase of at least two times in the area under the plasma concentration-time curve after intravenous injection in mice, no sufficient plasmatic levels were detected after oral delivery, and only the methacrylic acid copolymer NPs provided reasonable values (Leroux, et al., 1995). However, in other studies PLA NPs demonstrated to be feasible drug delivery carriers to enhance the tissue uptake and the targeting of other macromolecules with anti-HIV-1 activity (Ham et al., 2009). Also, PLGA NPs have shown interesting applications as vaccine-delivery vehicles for the treatment of virus (Demento et al., 2010).

Polyhexylcyanoacrylate nanoparticles were loaded with saquinavir (another HIV protease inhibitor) or zalcitabine (a nucleoside analog) and tested at *in vitro* conditions in primary human monocytes/macrophages. A dose-dependent reduction of HIV type 1 antigen production was observed for both types of NPS (Bender et al., 1996). Hexylcyanoacrylate nanoparticles were developed as a colloidal azidothymidine (AZT) carrier for specific targeting of the antiviral drug to reticuloendothelial cells by oral delivery in HIV patients (Löbenberg et al., 1997). By modifying this kind of NP by coating with polysorbate 80, it was possible to change the body distribution of AZT after intravenous injection of rats, with a higher concentration of the drug at brain levels. Although the drug was attached to the NPs, its efficacy was not reduced (Löbenberg et al., 1998). AZT was also encapsulated in polybutylcyanoacrylate (PBCA) and methylmethacrylate–sulfopropylmethacrylate (MMA–SPM) nanoparticles, and the study of their permeability across the blood–brain barrier elucidated an enhancement in their permeability with a reduction in NP size (Kuo et al., 2006). Furthermore, AZT loaded poly-(isohexylcyanoacrylate) nanospheres displayed promising features to target the antiviral to the epithelium of intestine and gut-associated lymphoid tissues, which are the main reservoirs of HIV in the GI tract (Dembri et al., 2001). The preparation of pH sensitive NPs was an interesting alternative to improve the bioavailability of a poorly water-soluble HIV-1 protease inhibitor. In this sense, NPs made of the poly(methacrylic acid-co-ethylacrylate) copolymer Eudragit®L100-55 were orally delivered to Beagle dogs and led to an increase in the plasmatic concentrations due to a selective release of the antiviral close to its absorption site (De Jaeghere et al., 2000). Shah et al. (2006) demonstrated that poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) NPs were a feasible vehicle for the intracellular delivery of saquinavir. The PEO-PCL NPs exhibited spherical shape and uniform size distribution around 200 nm. Their uptake by THP-1

human monocyte/macrophage (Mo/Mac) cell line indicated a significantly higher incorporation of the drug in comparison with aqueous solution.

It is important to mention that the surface of the described NPs can be tailored in order to modify the biodistribution of the antivirals, for targeting specific organs and tissues or, with more precision, delivering the drug at a particular kind of cell by attachment of ligands that recognize the receptor from cell surface (Gunaseelan et al., 2010).

Although few reports are currently found in the literature, the first products are appearing on the market and a new tendency is indicating that nanocrystals could be a possible delivery system to improve the bioavailability of antiviral drugs (Shegokar and Müller, 2010). For example, pure quercetin, a flavonoid with anti-dengue virus activity, showed a limited *in vivo* efficacy because of its low solubility and reduced absorption at intestine level. However, synthesis of four types of cocrystals led to an improvement in physicochemical and pharmacokinetic characteristics in comparison with quercetin alone (Smith et al., 2011). Other reports described the use of cellulose nanocrystals (CNCs) as viral inhibitors. A first approach was carried out with unmodified CNCs derived from tunicates in a single model bacteriophage, and a decrease in phage infection of host *E. coli* was observed (Serizawa et al., 2013). In a next step, the modification of CNCs by surface attachment of multivalent displays of tyrosine sulfate mimetic ligands resulted in inhibition of the alphavirus infectivity in Vero cells. Considering these results and the chemical structure of other known polyanionic inhibitors, the potential use of CNCs for inhibition of other viruses, such as (HIV) and herpes simplex viruses, should be explored (Zoppe et al., 2014). A recent work also reported the production of nanocrystals of a reverse transciptase inhibitor from HIV-1 virus (CSIC) by a three-phase NP engineering technology for intravaginal delivery (Gong et al., 2014).

Finally, in the last decade new research papers have shown the potentiality of metal NPs for the treatment of virus infections (Durán et al., 2016a). The interaction of silver and gold NPs with proteins is under exploration, and the mechanism of antiviral activity is not totally established (Bhattacharya et al., 2008; Durán et al., 2016b). However, it was observed that gold NPs conjugated to fragments of the HIV inhibitor TAK-779 (named SDC-1721) showed an excellent antiviral effect, while only free SDC-1721 had no activity. This means that gold NPs are able to convert inactive molecules into potent antivirals (Bowman et al., 2008). On the other hand, silver NPs have also shown interesting properties by interacting with HIV virus and therefore preventing the binding to host cells, as long as they are in the range of 1-10 nm in size (Elechiguerra et al., 2005). The green synthesis of metal NPs has been proposed as an alternative to chemical methods, particularly through the use of fungi, since the benign environment and renewable source of fungi act as a reducing agent for the synthesis of metal NPs (Durán et al., 2010b, 2015). These kinds of NPs have exhibited a tested efficacy against mosquito larvae (Salunkhe et al., 2011; Soni and Prakash, 2012abcd; 2013). In addition, leaf extracts from plants have been utilized for silver NP production and become an eco-friendly alternative for adulticidal activity against filariasis, malaria, and dengue vector mosquitoes (Suganya et al., 2013; Veerakumar et

al., 2014). Sujitha et al. (2015), Murugan et al. (2015b, 2016c) used biogenic silver NPs against DEN2 and its mosquito vector *Aedes aegypti.*

FINAL REMARKS

Since there are very few recently published reviews focusing on leishmaniasis, malaria, schistosomiasis, trypanosomiasis, tuberculosis, onchocerciasis, leprosy, filiarasis, and degue virus, the present review pointed out the most relevant advances in these areas. Despite the short time of nanotechnology in the public scenario, it has brought novel tools for therapeutic purposes of several diseases, and it is going to have a high impact on the treatment of ND. Nanocarrier development has demonstrated promising results in the therapy of many of these diseases with less toxicity, enhanced efficacy and improved bioavailability of drugs, as well as prolonged drug release with a limited number of doses. Particularly, the development of green technologies for the synthesis of nanodevices and the use of natural compounds with biocide activities are going to be a future trend for the development of novel and more efficient ND therapeutics by reducing patient complaints and improving their quality of life. Finally, this review deals with the current status of nanobiotechnology in the treatment of neglected diseases and describes many possible tools to explore new procedures in the treatment of a wide range of diseases, mainly neglected ones. One important aspect is the concern not only about neglected diseases, but also about the elimination of disease vectors. Therefore, it is clear that it is possible to extensively use nanotechnology to manage these diseases that are ignored by the goverments and the pharma and chemical industries.

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Figures

Fig. 1. Chronological cartoon of relevant TB and anti-TB therapies. Updated from Lalloo and Ambaram, 2010.

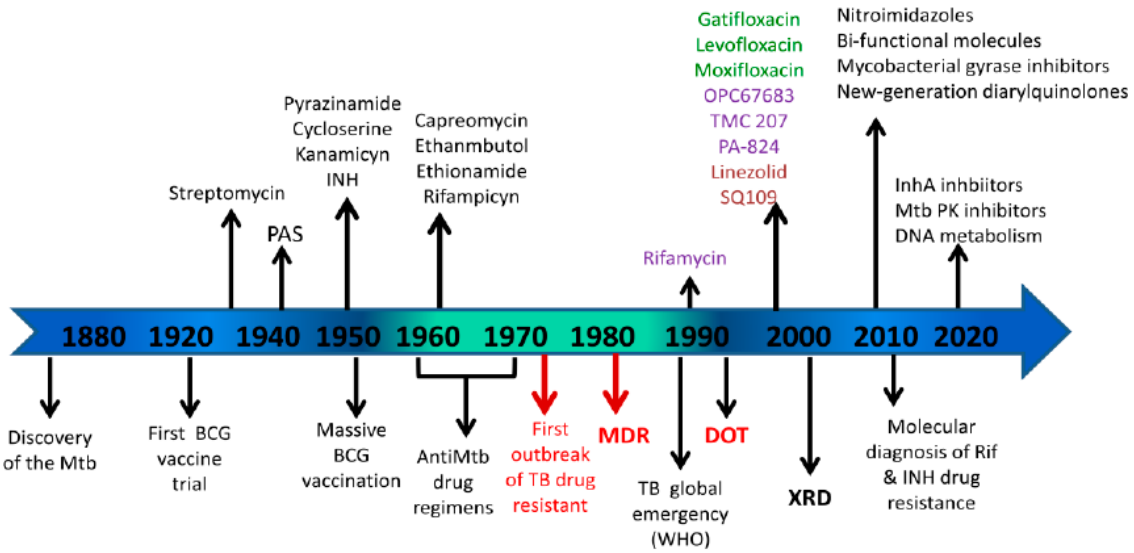


Fig. 2. Nanostructures used in nanobiotechnology.

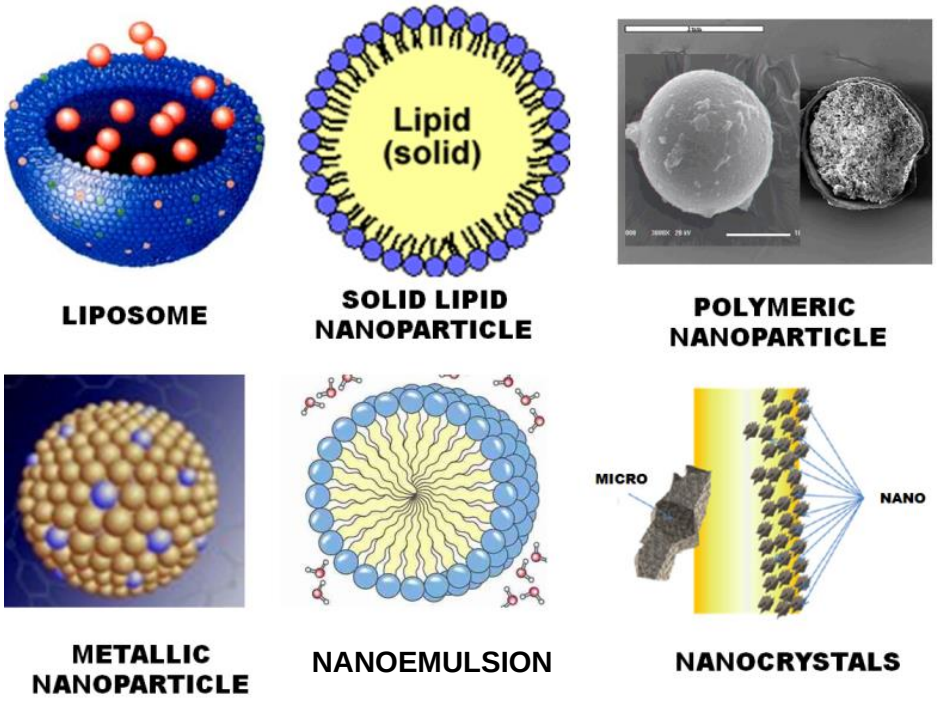


Fig. 3. Solid Lipid Nanoparticles (SLN) for treatment of infections. A) TEM image of SLN prepared by ultra-sonication method (unpublished result). B) Controlled release profile of levofloxacin form SLN and NLC. Reproduced from Islan et al. (2016) with permission.

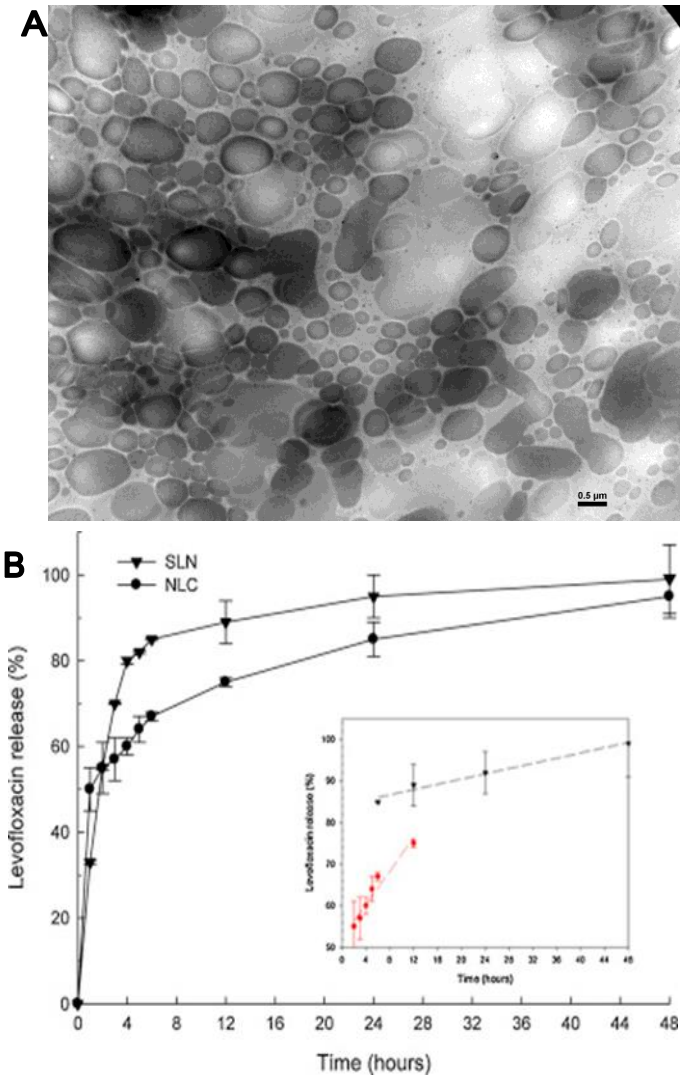


Fig. 4. Nanoparticles designed to specifically bind the malaria parasite using ligands for attachment to host cells, and thus blocking the invasion. A) Parasite (blue, DAPI) with NPs (red, hydrophilic sulforhodamine B dye) bounded on surface and recorded with a super-resolution 3D structured illumination microscopy (scale bar 1 micron). B) TEM image of an ultrathin portion of untreated parasite. C) TEM images of parasite treated with NPs that specifically bind on the surface (scale bar 500 nm). It could be observed the nucleus (nu), the rhoptries (rh) and the dense granules (dg) of the merozoites (Najer et al., 2014). Adapted from Najer et al., (2014) with permission.

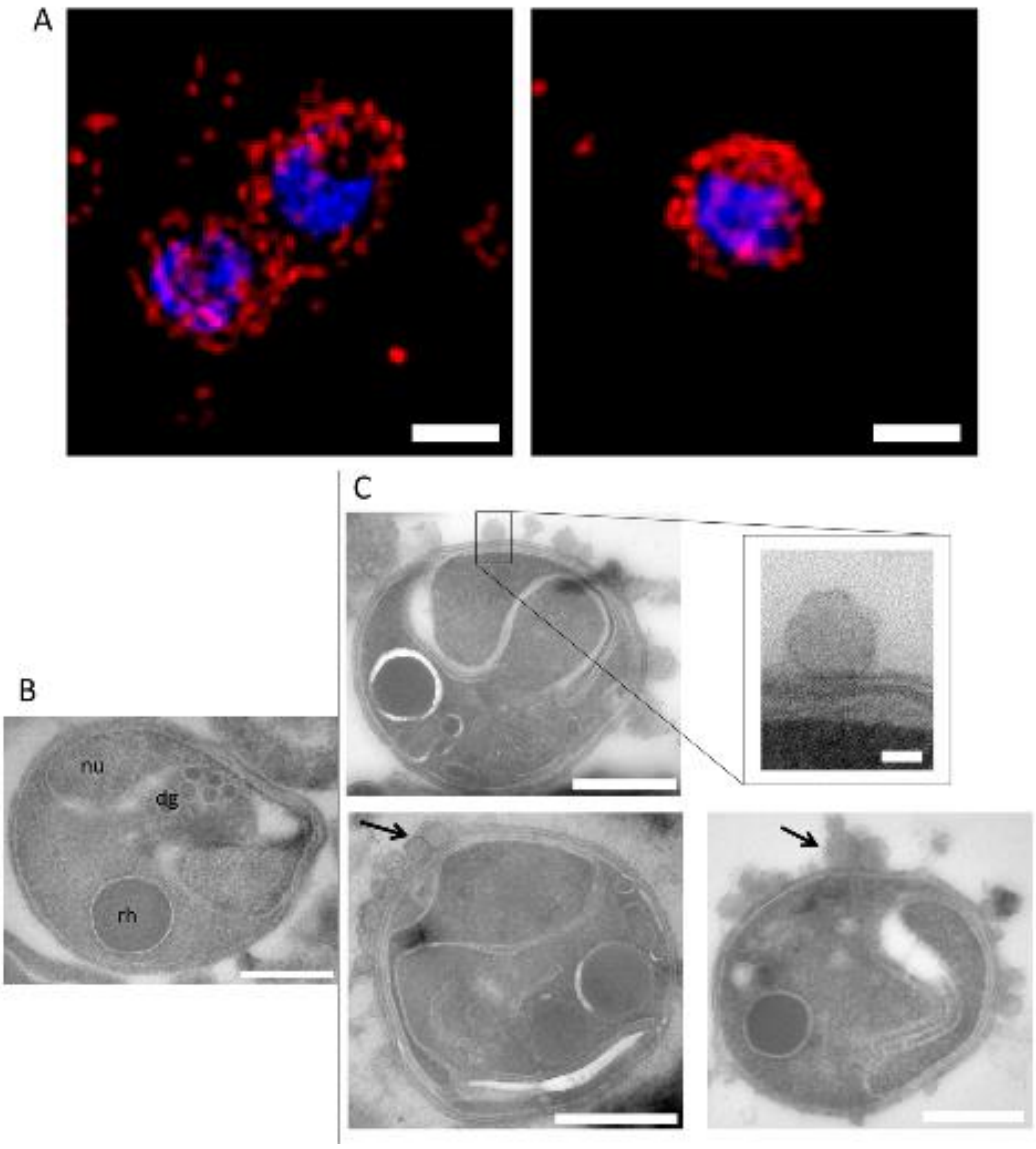


Fig. 5. *In vivo* efficacy studies of β-lapachone (β-LP) loaded chitosan NPs in Leishmaniasis treatment. **A)** Lesion size in *L. major* infected BALB/c mice after 21 days of treatment with topical β-LP loaded NP (20 mg/kg day) is reduced in comparison with untreated control (***$P< 0.001$). **B)** Photograph showing a control mouse (left) and a mouse treated with β-LP NP (right). **C-F)** Parasite burden comparison after 21 days treatment in skin **(C)**, liver **(D)**, spleen **(E)** and lymph node **(F)**. No reduction in the parasite burdens are observed compared to the control group. Reproduced from Moreno et al. (2015) with permission.

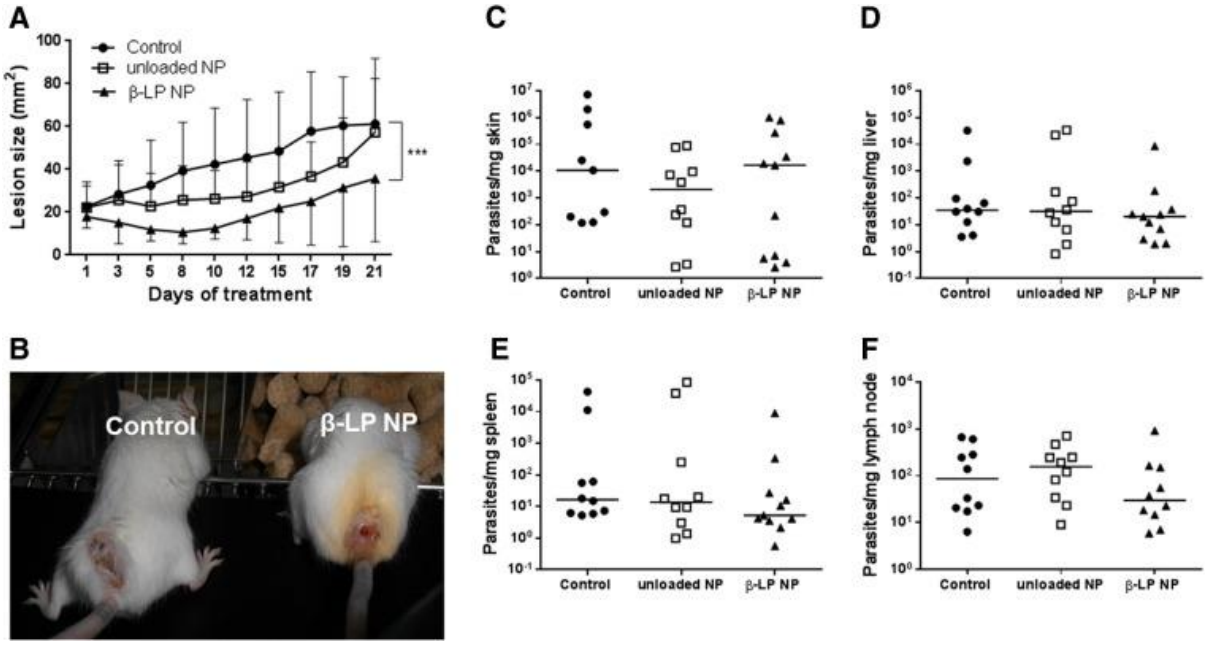
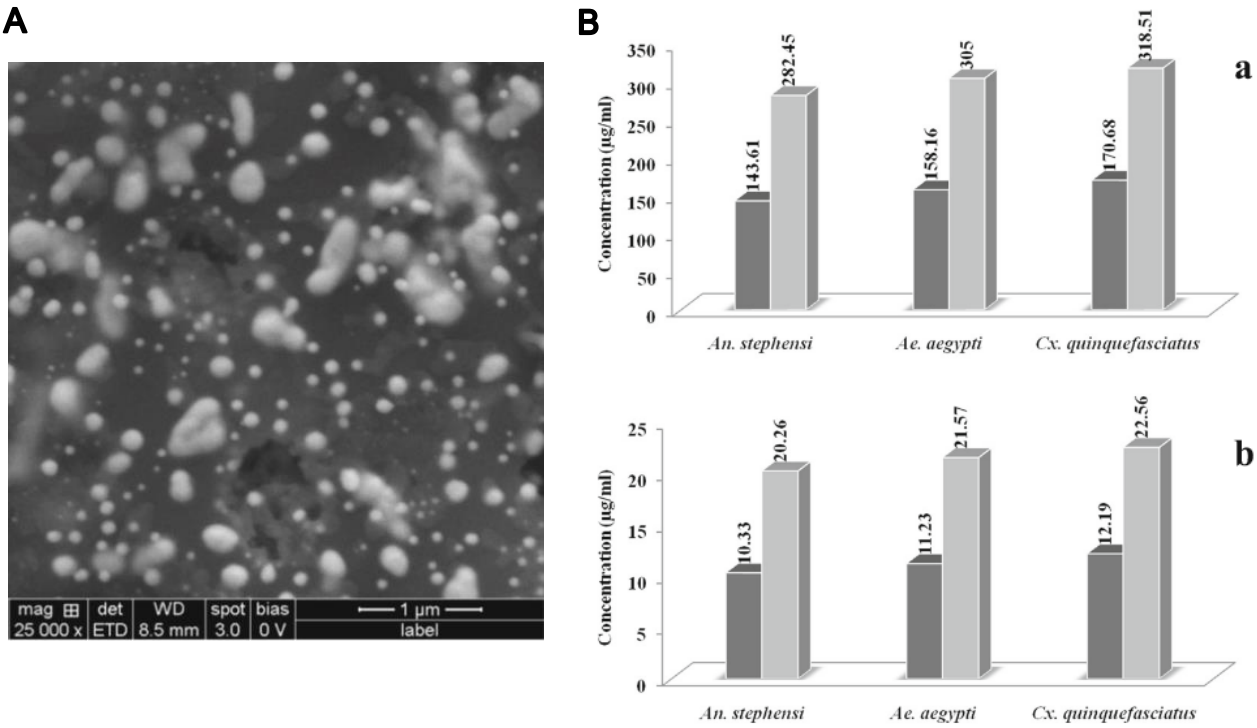


Fig. 6. Polydispersed silver nanoparticles *using Malva sylvestris* extracts. A) Scanning electron microscopy (SEM) images of nanoparticles at ×25,000. B) Larvicidal activity of aqueous leaf extract (a) and silver nanoparticles (b) against the mosquito vectors *Anopheles stephensi*, *Aedes aegypti*, and *Culex Quinquefasciatus*. Lethal concentration: LC_{50} (dark grey) and LC_{90} (soft grey). Reproduced from Govindarajan et al. (2016c) with permission.



Tables

Table 1.

Recent developments on novel antituberculosis drugs (modified from Lallo and Ambaram, 2010).

Drug		Targets	Clinical phase
Class	Name		
Diarylquinoline	Bedaquiline TMC207	ATP synthase	IIb
Ethylenediamine	SQ109	Cell wall (mycolic acid)	I
Fluoroquinolones	Gatifloxacin Levofloxacin Moxifloxacin	DNA gyrase	III
Iminophenazine	Clofazimine	DNA synthesis and enhance phospholipase A2	In the market
Nitroimidazole	Delanamid PA-824 TBA354	Cell wall (blocking mycolic acid synthesis and release intracellular nitric oxide	IIa
Rifamycins	Rifabutin Rifampicin Rifapentine	RNA polymerase	IIb

Table 2

Marketed drugs for the treatment of malaria.

Drug		Targets
Class	Name	
Aminoalcohols	Halofantrine Lumefantrine	Complex formation with hemin. Inhibition of nucleic acid and protein synthesis
4-Aminoquinolines	Chloroquine Amodiaquine	inhibition of hematin formation (heme polymerase)
8-Aminoquinolines	Primaquine Tafenoquine	Radical formation with NADPH
Biguanides	Proguanil (Chloroguanide)	Antifolate
Alcoholquinolines	Mefloquine Quinine Quinidine	Polymerization inhibition of haeme to hemozin
Diaminopyrimidines	Pyrimethamine	Inhibition of hematin formation (heme polymerase)
Naphthoquinone	Atovaquone	Anti-mitochorndial activity (electron transport)
Naphthyridine	Pyronaridine	Inhibits -hematin formation
Sesquiterpine lactones	Artermisinin Arteether Artemether Artesunate	Interference with parasite transport proteins, disruption of parasite mitochondrial function
Sulfonamides - sulfones	Dapsone Sulfadoxine Sulfamethopyrazine	Inhibition of dihydropteroate synthase

Table 3

Lipidic based nanocarriers for treatment of neglected diseases.

Device	Encapsulated drug	Properties	Illness	References
Liposomes	Amphotericin B	- Displayed a significant reduction in treatment toxicity. - I*n vitro* trypanocidal activity.	Leishmaniasis; Trypasomiasis	Balasegaram et al., 2012; Papagiannaros et al., 2005; Cencig et al., 2011.
	Anthelmintic, tartar emetic, praziquantel	- Antiparasitic activities after injected subcutaneously. - Decrease the amounts of eggs and worms of schistosomes by oral delivery.	Schistosomiasis	Melo et al., 2003; Mourao et al., 2005; Freeza et al., 2013.
	Artemether, arteether, artesunate, chloroquine, artemether/lumefantrine, sulphadoxine/pyrimethamine, chlorproguanil/dapsone, mefloquine, atovaquone/proguanil, primaquine, artemisinin	- Protect drugs from degradation. - Controlled release and fewer adverse effects. - Increase the blood-circulation time.	Malaria	Date et al., 2007; Owais et al., 1995; Isacchi et al., 2011.
	Clofazimine	- Enhanced antibacterial activity against *M. leprae*.	Leprosy	Patel et al., 1999; Patel and Misra, 1999.
	Diethylcarbamazine citrate, tetracycline	- Effective in the elimination of filarial parasites from systemic circulation. - Increased the time of drug release.	Filiarasis	Owais et al., 2003; Bajpai et al., 2005.
	Etanidazole	- Decrease the parasitemia levels of *T. cruzi* in infected mice after endovenous administration.	Trypasomiasis	Salomon, 2011.
	Iminosugars	- Enhance the antiviral activity and drug circulation in blood.	Dengue	Miller et al., 2012.
	Rifampicin, p*y*razinamide, rifabutin	- High concentration of the drug in the target organ (*e.g.*, lungs). - Higher antimicrobial activity.	Tuberculosis	Kaur IP and Singh 2014; Kaur M. et al, 2014.
	Tearylamine, antigens against *T. Gondii*, triclosan	- Preventive and curative effects. - Longer release phase, prolonging the drug action in peritoneal fluid and in the liver.	Toxoplasmosis	Tachibana et al., 1990; Elsaid et al., 1999, 2001; El-Zawawy et al., 2015.

Lipidic NPs (SLN, NLC and LCN)	Amphotericin B, oryzalin	- Increaase 40 times the AmB bioavailability compared with free drug. - Decrease its toxicity.	Leishmaniasis	Jung et al., 2009; Lopes et al., 2014.
	Artemether in NLCs.	- Reduce the parasite burden instantly.	Malaria	Parashar et al., 2014.
	Diethylcarbamazine citrate	- Elimination of microfilariae from the lymphatics.	Filiarasis	Kartick, 2014.
	5-Hydroxy-3-methyl-5-phenyl-pyrazoline-1-(S-benzyl dithiocarbazate) in SLNs.	- Show *in vitro* and *in vivo* trypanocidal activity.	Trypanosomiasis	Carneiro et al., 2014.
	Praziquantel in SLNs	- Increase the oral praziquantel bioavailability in rats. - Decrease toxicity in HepG2 cells when compared to free prazinquantel.	Schistosomiasis	Mishra et al. 2014; Yang et al., 2009; Souza et al., 2014.
	Pyrimethamine in LNC	- Increase the survival rate of infected animals.	Toxoplasmosis	Pissinate et al., 2014.
	Rifampicin, isoniazid, pyrazinamide	- Exhibit a better therapeutic efficacy in Guinea pigs.	Tuberculosis	Kaur IP and Singh, 2014; Kaur M. et al, 2014.

Abbreviations: SLN, solid lipid nanoparticles; NLC, nanostructured lipid carriers; LCN, lipid-core nanocapsules.

Table 4

Examples of nanocarriers based on polymers and natural macromolecules developed for neglected disease therapies.

Polymer/device	Encapsulated drug	Properties	Illness	References
Chitosan NPs	Amphotericin B, β-lapachone	- Effective in reducing the infection in animal models infected with *L. amazonensis.* - Decrease toxicity of free drug. - Important drug accumulation in the dermis and permeation through the skin.	Leishmaniasis	Ribeiro et al., 2015.; Moreno et al., 2015.
	Chloroquine	- Effective against *P. berghei* infection in Swiss mice model.	Malaria	Tripathy et al., 2012, 2013.
	Isoniazid, rifampicin	- Efficacy against mycobacterium. - Sustain deliver of the drugs.	Tuberculosis	Garg et al., 2016
	Mycobacterium bovis Bacillus Calmette-Guerin cell wall components.	- Dengue nanovaccine.	Dengue	Hunsawong et al., 2015.
	Pentamidine (NPs coated with nanobodies)	- Diminish the minimal effective therapeutic dose (*in vivo*). - Increasing its efficacy. - Diminish toxicity. - Overcome drug resistance mechanisms.	Trypasomiasis	Unciti-Broceta et al., 2015.
Gelatin NPs	Amphotericin B	- High accumulation of AmB in liver and spleen. - Enhance anti-leishmanial activity.	Leishmaniasis	Khatik et al., 2014.
PLGA NPs	β-Aescin, artemisinin	- Reduction in cytotoxicity. - Maintenance of anti-leishmanial efficacy.	Leishmaniasis	Van de Ven, 2011; Want et al., 2015.
	Ivermectin	- Enhanced microfilaricidal effectiveness. - Slightly better macrofilaricidal effectiveness.	Filiarasis	Ali et al., 2014.
	Prazinquantel	- Localized effect on intestinal membranes. - Prolonged period of time. - Increase water solubility and chemical stability.	Schistosomiasis	Mainardes and Evangelista, 2005; Fonseca et., 2013; Mainardes et al., 2006.

	Rifampicin, isoniazid, pyrazinamide	- Strong therapeutic efficacy. - High encapsulation efficiency. - Prolonged plasma drug levels. - Greater dispersibility.	Tuberculosis	Kaur and Singh, 2014; Kaur M. et al., 2014.
	Bis-Triazole DO870	- Produce a significant cure of mice infected with *T. cruzi*.	Trypanosomiasis	Urbina et al., 2001; Molina et al., 2001.
Other biodegradable NPs	Allopurinol, grandisin, violacein, bis-triazole DO870, and megazol	- Show great *in vitro* trypanocidal activity.	Trypanosomiasis	Gonzalez-Martin et al., 2000; Duran et al., 2001; Molina et al., 2001; Stecanella et al., 2013; Lima and Albuquerque, 2012.

Abbreviation: PLGA, Poly Lactic-co-Glycolic Acid.

Table 5

Nanoemulsions, nanosuspensions and nanocrystals in treatment of Neglected Diseases.

Encapsulated drug	Properties	Illness	References
Semisynthetic derivatives of artemisinin, artemether and arteether	- Enhance treatment for uncomplicated *P. falciparum* malaria. - Improve drug solubility and its bioavailability.	Malaria	Dwivedi et al., 2015.
2-(butylamino)-1-phenyl-1-ethanethiosulfuric acid (BphEA)	- Higher schistosomicidal activity compared to free BphEA,	Schistosomiasis	Araujo et al., 2007.
Essential oils of andiroba (*Carapa guaianensis*) and aroeira (*Schinus molle*)	- High *in vitro* efficiency against *T. evansi.*	Trypanosomiasis	Baldissera et al., 2013.
Dapsone (4,4'-diaminodiphenylsulfone)	- Enhanced bacteriostatic action against *M. leprae.* - Improve the clinical use by increasing the bioavailability.	Leprosy	Santos et al., 2012; Borges et al., 2013, Monteiro et al., 2012.
Ramipril	- Improve the bioavailability.	Tuberculosis	Kaur and Singh, 2014; Kaur et al., 2014.
Diethylcarbamazine citrate	- Exhibited great potential as a lymphotropic vehicle system.	Filiarasis	Karajgi and Vyas, 1994.
Atovaquone capped with poloxamer 188 (P188) and sodium dodecyl sulfate (SDS)	- Increase the oral bioavailability and decrease the inflammatory signs in brain of animals. - Decrease the infection capacity of parasite.	Toxoplasmosis	Shubar et al., 2011; Scholer et al., 2001.
Essential oils (from *Rosmarinus officinalis*	- kill the mosquito larva of *Aedes aegypti.*	Dengue	Duarte et al., 2015.
Hemozoin (NC)	- Kill the malaria parasite in theranostic applications.	Malaria	Hleb and Lapotko, 2014.
Clofazimine (NC)	- Reduce the bacterial load in lungs.	Tuberculosis	Kaur and Singh 2014; Kaur et al, 2014.
Quercetin (NC)	- Enhance the anti-dengue virus activity of the drug by increasing bioavailability	Dengue	Smith et al., 2011.
Silver (NC)	- Kill the mosquito larvae	Zika	Govindarajan et al., 2016c.

Abbreviation: NC, nanocrystals.

Table 6

Metal nanoparticles for the treatment of Neglected diseases.

Carrier	Properties	Illness	References
Biologically generated silver NPs	- Show *in vitro* antiviral activity against DEN-2 infecting vero cells. - Effective against the dengue vector *A. aegypti.*	Dengue	Sujitha et al., 2015; Murugan et al., 2015 and 2016.
	- Exhibit good activity against larvae of *C. quinquefasciatus.* - Larvicidal activity against the mosquito vector of lymphatic filariasis (*Culex sp.).*	Filariasis	Rajakumar and Rahumam, 2011; Dhanasekaran and Thangaraj, 2013.
	- Are administered by subcutaneous intralesional route in experiments against *Leishmania amazonensis* promastigotes. - Demonstrate to be 4 times more effective than chemically generated AgNPs. - Showed the same effectiveness than 300-fold higher doses of amphotericin B. - Are more effective than 3-fold higher doses of Chem-AgNP.	Leishmaniasis	Rossi-Bergmann et al., 2012.
	- Molluscicidal activity with low toxicity to other aquatic organisms.	Schistosomiasis	Yang et al., 2011; Guang et al., 2013.
	- Toxic effects against *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus* larvae. - Eco-friendly.	Zika	Govindarajan et al., 2016a,b.
Chemically synthesized silver NPs	- Reduce the parasite counts with Ag levels in the accepted range of toxicity.	Toxoplasmosis	Gaafar et al., 2014.
Gold and silver NPs	- Bound tightly to the arginine substrate that selectively inhibit an arginine kinase present in the microorganism but absent in humans. - Become candidates for trypanocidal drug studies.	Trypasomiasis	Miranda et al., 2006; Adeyemi and Whiteley, 2014.

Abbreviation: NPs, nanoparticles.