A review of current treatments strategies based on paromomycin for leishmaniasis

A.P.S. Matos[a,b,c,**], A.L. Viçosa[d], M.I. Ré[b], E. Ricci-Júnior[c], C. Holandino[a]

[a] *Laboratório Multidisciplinar de Ciências Farmacêuticas, Departamento de Fármacos e Medicamentos, Faculdade de Farmácia, Universidade Federal Do Rio de Janeiro, Cidade Universitária, Rio de Janeiro, RJ, Brazil*
[b] *Toulouse University, CNRS, Rapsodee Research Center, IMT Mines Albi, Jarlard Campus, F-81013, Albi 09, France*
[c] *Laboratório de Desenvolvimento Galênico - LADEG, Departamento de Fármacos e Medicamentos, Faculdade de Farmácia, Universidade Federal Do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*
[d] *Laboratório de Farmacotécnica Experimental, Instituto de Tecnologia Em Fármacos – Farmanguinhos, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil*

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A B S T R A C T

Leishmaniasis is a neglected disease caused by protozoan parasites of the *Leishmania* genus, which affects many people in several countries. This disease has three major clinical forms: cutaneous, mucocutaneous and visceral. The current treatments consist of an intravenous, intralesional or intramuscular administration of pentavalent antimonials, but other drugs can be used, among them, amphotericin B, pentamidine, paromomycin and miltefosine. However, these therapies have many side effects. Hence, there is an increase of studies searching for new formulations using different technologies and different routes of administration for leishmaniasis treatment. Paromomycin sulfate (PM) is an aminoglycoside antibiotic, belonging to class III of biopharmaceutical classification system, used intravenously and topically with leishmanicidal activity. This review will provide a general overview of PM current leishmaniasis treatments and new PM formulations. Treatments using PM are available in ointments or creams for topical administration and PM solution for intramuscular administration. The topical treatment with PM presents low efficacy, probably related to low drug permeability across the skin. To improve PM permeability and efficacy, researchers are establishing micro and nanotechnologies. However, further studies are still required to investigate more physicochemical properties and *in vitro/in vivo* parameters.

1. Introduction

Leishmaniasis is a parasitic infectious disease caused by approximately 20 species of protozoan of the *Leishmania* genus and transmitted by female phlebotomine sandflies [1–4]. This disease belongs to the group of neglected diseases and is one of the major health problems in the world [4,5], more specifically in 98 countries [6,7], with 12 million sick people and 2 million new cases reported annually [5–9]. An estimate of 26,000 to 65,000 deaths occur each year [10]. Furthermore, cases of Leishmania and HIV (human immunodeficiency virus) co-infection are increasing and have been described in 35 countries [3,5,7,11].

Leishmaniasis parasites have a digenetic life cycle with two morphological forms: promastigotes, form flagellated in the digestive organs of sand fly vector and amastigotes, form no flagellated in the phagolysosome of mammalian host macrophages [3,4,7,12–15].

This disease is manifested in three major clinical forms: cutaneous, mucocutaneous and visceral [3,4,8,11,12,16]. The severity and form of clinical manifestations depend on the infecting parasite species, site of inoculum, the number of parasites inoculated and host immunity response [17–19].

Cutaneous leishmaniasis (CL) is the most common type of this disease through the appearance of skin lesions developing at the area of sandfly bite [7,13,18], mostly ulcers and maybe leave life-long scars and serious disability [10,15]. The CL lesions usually appear on the face, neck, arms, and legs [7,8]. For cutaneous leishmaniasis, the species of *Leishmania* most common are *Leishmania major*, *Leishmania tropica*, *Leishmania mexicana*, *Leishmania braziliensis* and *Leishmania panamensis* [2,4,13,18]. CL is endemic in more than 70 countries, in which 90% of the cases occur in seven countries (Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia and Syria) [2–4,6].

Mucocutaneous leishmaniasis (MCL) is characterized by hematogenous or lymphatic dissemination of parasites from cutaneous lesion [2,7] and caused by *Leishmania amazonensis*, *Leishmania braziliensis*,

* Corresponding author. Laboratório Multidisciplinar de Ciências Farmacêuticas, Departamento de Fármacos e Medicamentos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Cidade Universitária, Rio de Janeiro, RJ, Brazil.
E-mail address: anapaulasmatos@ufrj.br (A.P.S. Matos).

Table 1
Current treatments of leishmaniasis (adapted from Bezerra de Menezes et al., 2015; Lindoso et al., 2012; Zulfiqar et al., 2017).

Drugs	Administration route	Dosage	Advantages	Disadvantages	Resistance
Amphotericin B deoxycholate	Intravenous	0.75–1 mg/kg/day (15 or 20 days daily or alternately)	Primary resistance is unknown	Need slow intravenous infusion, toxicity, unstable in high temperatures	Laboratory strains
Liposomal amphotericin B	Intravenous	3–5 mg/kg single dose or 10–30 mg/kg total dose	High effective and low toxicity	Need slow intravenous infusion, high cost, unstable in high temperatures	Not documented
Miltefosine	Oral	100–150 mg/day for 28 days	Effective and safe	Cost, poor patient compliance, cannot be used in pregnant patients	Laboratory strains, some cases reported in India
Paromomycin	Intramuscular, intravenous or topic	15 mg/day for 21 days or 20 mg/kg for 17 days	Low cost	Efficacy varies between and within regions	Laboratory strains
Pentamidine	Intramuscular	3 mg/kg/day every other day for 4 injections	Short treatment	Efficacy varies between *Leishmania* species	Not documented
Pentavalent antimonials	Intramuscular, intravenous or intralesional	20 mg/kg/day for 28–30 days	Easily availability and low cost	Length treatment, painful injection and toxicity	Common
Sitamaquine[a]	Oral	2 mg/kg/day for 21 days	Effective	Toxicity	Not documented

[a] . **Sitamaquine is in phase II study for leishmaniasis treatment.**

Leishmania panamensis, *Leishmania guyanensis*, *Leishmania major, Leishmania infantum* and *Leishmania tropica* [2,4,8]. MCL is manifested by nasal inflammation followed by nasal cartilage infiltration and destruction of nasal septum and can cause partial or total destruction of nose, mouth and throat mucous membrane [2,7,10,13,15]. MCL presents more than 90% of cases in four countries: Bolivia, Brazil, Ethiopia and Peru. Nevertheless, there is no reported number of cases over the year and by countries of this type of leishmaniasis [20].

Visceral leishmaniasis (VL), also known as kala-azar, is the most severe manifestation of leishmaniasis, in which the parasites infected vital organs [3,16]. VL is manifested by hepatosplenomegaly, prolonged fever and pancytopenia [3,8] and, if untreated, can be fatal [3,8,10,15,16]. *Leishmania infantum* and *Leishmania donovani* are the Leishmania species responsible by VL [13,16]. Visceral leishmaniasis is endemic in 65 countries and more than 90% of cases occur in five countries (Bangladesh, Brazil, India, Nepal and Sudan) [3,4,6,7,16].

Table 1 gathers active pharmaceutical ingredients (APIs) in current use for the treatment of leishmaniasis.

The first-line drugs used to leishmaniasis treatment is an intravenous, intralesional or intramuscular administration of pentavalent antimonials as meglumine antimoniate and sodium stibogluconate (SSG) [7,9,14,15,19]. The mechanism of action of these compounds is still not properly understood, but the drugs can inhibit glycolysis step of metabolism and fatty acid oxidation of the parasite [4,7,10,21] and pentavalent form is reduced to trivalent form [15]. The most frequent side effects of these drugs are myalgia, arthralgia, anorexia and leukopenia. Furthermore, pentavalent antimonials can be cardiotoxic, nephrotoxic and hepatotoxic, which limited these drugs use in pregnant and elderly people [21–24].

The second-choice treatment is an intravenous administration of amphotericin B, a polyenic antibiotic with high leishmanicidal activity. This drug binds to the ergosterol molecules present in the cytoplasmic membrane of parasites increasing membrane permeability and ion influx [4,15,22,23,25]. There are four commercial formulations available of this drug: amphotericin B deoxycholate, liposomal amphotericin B, cholesterol dispersion of amphotericin B and lipid complex of amphotericin B [21–23]. Amphotericin B deoxycholate causes more side effects including fever, headache, nausea, vomiting, tremors and hypotension. Moreover, all amphotericin B formulations still present nephrotoxicity and cardiotoxicity and are restricted to the hospital environment. Liposomal and lipid-base formulations present lower toxicity but are more expensive than amphotericin B deoxycholate [21–24].

Pentamidine is an aromatic diamidine, which has been marketed in the form of two salts: isethionate (di-b-hydroxyethane sulfonate) and mesylate (di-b-hydroxymethyl sulfonate) and are administered by intravenous or intramuscular routes [4,22,23]. The mechanism of action may be related to a decrease of mitochondrial membrane potential by drug accumulation in the mitochondria [4,22,23]. This drug can induce different side effects such as hypoglycemia, hyperglycemia, nephrotoxicity and hepatotoxicity. Furthermore, pentamidine can cause insulin-dependent diabetes [21–23].

Miltefosine is, originally, an anticancer drug (hexadecylphosphocholine), which shows interesting results in leishmaniasis treatment and has been considered an advancement in the research of new treatment of this disease specially because can be administered orally [4,24,25]. The miltefosine mechanism of action is based on drug intracellular accumulation in parasites through transporters [4,22,23]. This drug shows several side effects such as vomiting, diarrhea, toxicity in the gastrointestinal tract. Furthermore, it presents a teratogenic effect and is prohibited for pregnant women [21–24]. As other drugs used to treat leishmaniasis, miltefosine is already triggering resistance in parasites [4,24]. Since 2017 is on the market one product containing miltefosine (Milteforan®) for canine visceral leishmaniasis in Brazil [26].

Sitamaquine is an aminoquinoline developed for visceral

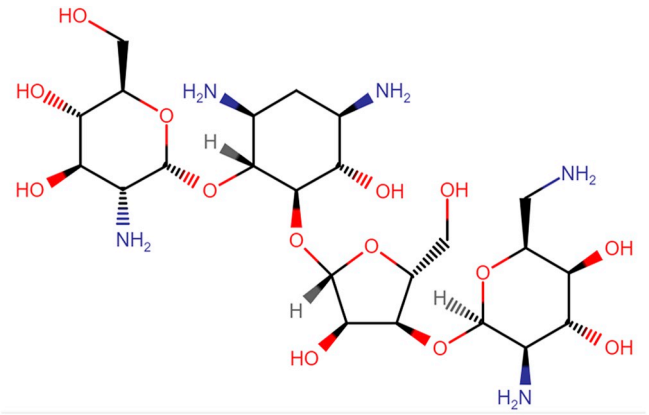


Fig. 1. Chemical structure of PM

leishmaniasis treatment. Its mechanism of action affects the mobility, morphology and growth of the protozoans, through electrostatic interaction between polar ionic groups of the phospholipids present in the parasite membrane and positive charge of the drug [4,27]. It is a new medicine, which is currently in phase II clinical trials in India and Kenya, presenting promising results for oral use [27]. The worst problem with this drug is the poor knowledge of the toxicity of its metabolic [4].

Imidazoles (ketoconazole) and triazoles (fluconazole, itraconazole) are antifungal compounds with the same mechanism of action. These drugs act binding to the ergosterol molecules in the cytoplasmic membrane of parasites increasing membrane permeability. Triazoles are less toxic, interfere less with human synthesis of cholesterol and are slower metabolized than imidazoles [17,22,23]. These compounds can be administered orally and present lower toxic side effects than pentavalent antimonials [17].

Paromomycin is an aminoglycoside antibiotic, discovered in the 1960s, with leishmanicidal activity [4,22,23], but more effective against cutaneous leishmaniasis [4,28]. The mechanism of action of this compound is not well known. Different routes can administer this drug (intramuscular, intravenous and topical). The most frequent side effects of this drug are ototoxicity, local pain (injectable paromomycin) [4,22,23], erythema, vesicles and skin irritation [29].

Heat therapy or Thermotherapy has already been described in the literature as an alternative treatment of cutaneous leishmaniasis [30]. There are several methods and several devices to provide localized heat therapy. One of these devices, ThermoMed® was approved by Food Drug and Administration (FDA) for CL treatment [30]. In general, Thermotherapy is applied locally for 30 s, heat of 50 °C, in one to four weekly sessions and with local anesthesia [29,31]. There are some studies that compared the efficacy of thermotherapy with meglumine antimoniate and showed efficacy similar to pentavalent antimonials with safety results [29,32–34]. However, this therapy cannot be applied in cutaneous lesions near mucosa or in lesions, which compromise mucosa [34]. Furthermore, hyperpigmentation and secondary infections are side effects currently reported for this therapy [29].

There are also other local therapies for cutaneous leishmaniasis treatment as cryotherapy, phototherapy and CO_2 laser. Cryotherapy consists of liquid nitrogen applied to the lesion for 15–20 s twice or three times per session and repeated weekly until healing [31,35]. This therapy acts destroying infected tissues and showed effectiveness specially combined with meglumine antimoniate [29,35]. The side effects of this therapy include erythema, hypo- or hyperpigmentation secondary infections and burning [29].

CO_2 laser is a technique that used a source of continuous CO_2 laser in lesions with pulse width between 0.5 and 5 s, power around 30–100 W and it is applied in one or a few sessions [29,36]. This therapy promotes the thermolysis of infected tissues. Moreover, it presents efficacy with few side effects such as hyperpigmentation, hypertrophic scarring and persistent redness. However, more studies are required to evaluate the efficacy in different Leishmania species [29].

Photodynamic therapy (PDT) acts by a production of reactive oxygen species (ROS) using a photosensitizer molecule in the presence of low-intensity visible light to promote cell death [29,37–39]. This therapy is non-invasive, does not affect intact skin around the lesions [37,40]. Porphyrins precursors, specially 5-aminolevulinic acid (ALA) or methyl-aminolevulinic acid (MAL), and phthalocyanines are often used as photosensitizers with visible red light (633 nm) application [29,38,40]. PDT requires special medical equipment and repeatable applications [40]. The side effects of this therapy include pruritus, mild burning sensation, redness and hypo or hyperpigmentation [29,40].

The current treatments using all mentioned APIs have several problems, such as severe side effects, low patient adhesion, high cost and parasite resistance [4,5,13,19–21], which have even motivated the development of novel drugs to enhance leishmaniasis treatment [22,41] or more effective and safer therapies [14].

Based on these problems, Paromomycin is the focus of this review because this API has been studied for topical administration, an interesting route for local treatment, especially for CL treatment. Furthermore, formulation strategies containing this API alone or in combined therapy have been proposed to address its poor efficacy for VL and CL treatments. Therefore, this review aims to provide a general overview of Paromomycin current leishmaniasis treatments (ointments, creams, injectable formulations) and new paromomycin formulations using diverse types of drug delivery systems (microparticles, solid lipid nanoparticles, liposomes among others).

2. Paromomycin sulfate (PM)

PM (Fig. 1) is an aminoglycoside antibiotic [3,4,11,14,42,43] produced by a fermentation of *Streptomyces rimosus* [11,42,43] with effective against a broad spectrum of bacteria and protozoa, including leishmaniasis [11,25,42,43]. This drug is commercially available in its salt form (sulfate) and has empirical formula $C_{23}H_{45}N_5O_{14}$ xH_2SO_4, molecular weight (M_w) 615.6 g of drug base or M_w 896.86 g for the salt form [44–46]. PM is soluble in water and insoluble in organic solvents. It presents high hygroscopic and chemical degradation in high temperatures (> 200 °C) [47,48]. Furthermore, PM belongs to class III (high solubility and low permeability) of the Biopharmaceutical Classification System (BCS) [47] and it has poor oral absorption [42,44].

The mechanism of PM is not completely understood, but inhibition of translocation and recycling of ribosome subunits [4,11,14,15,43,49–54] as well as modification in mitochondrial membrane potential [4,14,15,41,49,51–53] are probably responsible for protein synthesis inhibition and mitochondrial effects, respectively as illustrated in Fig. 2.

3. Current paromomycin treatments

The first report of PM formulation against leishmaniasis was the development of an ointment, which contained 15% of PM and 12% of methylbenzethonium chloride for cutaneous leishmaniasis [55]. This ointment used in a topical treatment twice a day for six or more days showed efficacy against mice and humans infected with *L. major* in Israel [55,56]. Krause and Kroeger, in 1994, analyzed this same ointment against *L. panamensis* and obtained an efficacy around 70% in Ecuador [57]. In 1997, Ozgoztasi and Bavdar evaluated the effectiveness of 15% of PM and 12% of methylbenzethonium chloride ointment in comparison with oral treatment with ketoconazole against *L. major* and observed a complete cure in 37.5% of patients treated with PM ointment while ketoconazole presented no effect [58]. Arana and coworkers, in 2001, performed a double-blind study with PM ointment (15% of PM and 12% of methylbenzethonium chloride) used twice a day for 20 days in Guatemala against *L. mexicana* and *L. braziliensis*. The

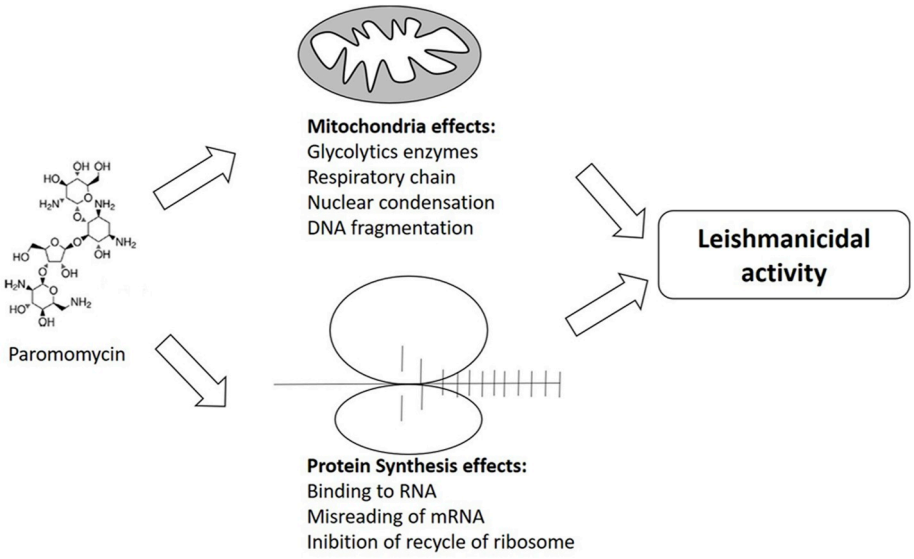


Fig. 2. Possible mechanism of PM leishmanicidal activity.

authors verified that PM ointment had more efficacy than placebo [59]. Asilian and Davami, in 2006, compared the efficacy of 15% of PM and 12% of methylbenzethonium chloride ointment with photodynamic therapy in patients infected by *L. major* in Iran and verified that PDT was more effective than PM ointment [60]. Nowadays, it is available a commercial formulation only in the Israel market as Leishcutan®. This formulation presents local toxicity, which was described as local irritancy associated with the use of methylbenzethonium chloride [16,61,62].

Another study, developed in England, involved the development of an ointment with 15% PM and 10% urea. This formulation presented lesser toxicity than ointment with 15% PM and 12% of methylbenzethonium. However, further studies are required to confirm the efficacy of this preparation (15% PM + 10% urea) [63]. In Honduras, Neva and colleagues, in 1997, analyzed the efficacy of this same ointment against *L. mexicana* and *L. infatum* and detected the inefficacy of this formulation in lesions treatment, suggesting the PM, probably, had no efficacy against these leishmania species [64]. In Iran, Asilian and colleagues, in 2003, evaluated the clinical and parasitological effectiveness of the ointment with 15% PM and 10% urea against *L. major* and detected an efficacy increase proportional to the treatment time [65]. Another group, in 2005, evaluated the same ointment (15% PM and 10% urea), in Iran, and obtained no effect of this PM ointment in cutaneous leishmaniasis treatment [66]. In 2004, Armijos and co-workers compared the effectiveness of intramuscular meglumine antimoniate with topical treatments containing PM (ointment with 15% of PM and 12% of methylbenzethonium chloride and ointment with 15% PM and 10% of urea) in Ecuador against New World Leishmania species. They observed that topical treatment was safe but presented lower efficacy than meglumine antimoniate and no differences between the two topical PM ointments were observed. However, they encouraged the use of PM formulations as viable alternatives for people without access to antimonials treatment [67]. Faghihi and Tavakoli-kia, in 2003, compared, in Iran, the effectiveness of 15% PM and 10% of urea ointment with intralesional meglumine antimoniate against *L. major* and observed lower efficacy of PM formulation in comparison with antimoniate [68]. In 2005, Shazad and colleagues compared two different treatments (15% PM and 10% of urea ointment and intralesional meglumine antimoniate) against *L. major* and verified no differences in the percentage of cure, suggesting PM ointment was safe and effective as intralesional meglumine antimoniate [69].

In 1999, a cream containing 15% of PM and 0.5% gentamicin was developed [62]. The effectiveness of this formulation was evaluated against four Leishmania species (*L. major*, *L. mexicana*, *L. amazonensis* and *L. panamensis*) and compared with others PM formulations (PM alone, 15% of PM and 12% of methylbenzethonium chloride ointment and ointment with 15% PM and 10% of urea). The research group observed that 15% of PM and 0.5% of gentamicin had more efficacy than the other PM formulations, especially against New World Leishmania strains, and apparently low toxicity [62]. Another group, in 2009 and 2013, compared the effectiveness of 15% of PM and 0.5% of gentamicin cream and 15% of PM cream against *L. major* and verified no differences of efficacy between these two creams [61,70]. One study held in Peru and Panama, in 2013, evaluated the efficacy, safety and pharmacokinetics parameters of 15% of PM and 0.5% gentamicin cream and compared to 15% of PM cream. In both formulations, the same amount of PM had systemic absorption [71]. In Brazil, since 2005, one gel formulation containing 10% PM for topical use has been developed in laboratory scale, with good results against mice infected with *L. amazonensis* and infected hamsters with *L. braziliensis* [72]. From 2013 up to now, the scale-up of the production of this formulation is under development in partnership with a governmental brazilian pharmaceutical industry (Instituto de Tecnologia em Fármacos – Farmanguinhos/Fiocruz) and Drug for Neglected Diseases Institute (DNDi) [73].

Soto and co-workers, in 1998, evaluated the combination of topical 15% of PM and 12% of methylbenzethonium chloride with injectable antimoniate and discovered that PM did not increase the effectiveness of meglumine antimoniate against *L. panamensis* [74]. El-On and co-workers, in 2007, analyzed a combined treatment with 15% of PM and 12% of methylbenzethonium chloride ointment with imiquimod against *L. major*. There was no difference between treatments with PM alone in infected mice BALB/c and with a combination of PM and imiquimod [75].

In 2006, PM was approved for the treatment of visceral leishmaniasis in India. Sundar and colleagues, in 2007, evaluated the efficacy of intramuscular PM for VL in India and discovered that PM had the same efficacy of amphotericin B administered intravenously [76]. In Bangladesh, another study of intramuscular PM, in 2015, in phase IIIb of the human clinical trial, showed an efficacy around 94% considered an effective and safe treatment for visceral leishmaniasis in that country [77]. Parenteral PM has been used for VL and one study in Kenya showed 79% of patients cured by this formulation, while only 50% of patients were cured in another study in Colombia with a higher dose of PM for CL treatment. These studies suggested that injectable PM present more effective results against VL than CL [78]. Musa and colleagues, in

Table 2
Current PM formulations for leishmaniasis treatment in the World.

Treatment	Dosage	Effectiveness	Country	Reference
Topical – Cutaneous leishmaniasis				
Parenteral – Visceral leishmaniasis				
PM	15 mg/kg/day for 19 days	79%	Kenya	(Berman, 1997)
PM	22.5 mg/kg/day for 14 days	50%	Colombia	(Berman, 1997)
Intramuscular – Visceral leishmaniasis				
PM	11 mg/kg/day for 21 days	95%	India	(Sundar et al., 2007)
PM	11 mg/kg/day for 21 days	94.2%	Bangladesh	(Jamil et al., 2015)
PM combined with parenteral or intramuscular SSG or PM monotherapy	15 mg/kg/day of PM + 20 mg/kg/day of SSG for 17 days or 20 mg/kg/day of PM for 21 days	91% for PM + SSG, 84% for PM monotherapy	East Africa	(Musa et al., 2012)
PM combined with parenteral or intramuscular SSG	15 mg/kg/day of PM + 20 mg/kg/day of SSG for 17 days	95.1%	East Africa	(Kimutai et al., 2017)
PM combined with parenteral Ambisome or PM combined with oral Miltefosine	5 mg/kg single dose of Ambisome + 15 mg/kg/day of PM for 10 days or 15 mg/kg/day of PM for 10 days +2.5 mg/kg/day of Miltefosine for 10 days	99.4% for PM + Ambisome, 97.9% for PM + Miltefosine	Bangladesh	(Rahman et al., 2017)
Intramuscular – Post-kala-azar Dermal leishmaniasis				
PM combined with intramuscular SSG	15 mg/kg/day of PM + 20 mg/kg/day of SSG for 17 days	97.4%	South Sudan	(Abongomera et al., 2016)

2012, performed a multi-center phase III study in 4 East Africa countries (Ethiopia, Kenya, Sudan and Uganda) to evaluate the efficacy and safety of PM intramuscular treatment and combined PM intramuscular and SSG intramuscular or intravenous treatment and the results were compared with SSG intramuscular or intravenous monotherapy. Monotherapy with SSG intramuscular or intravenous was more effective than monotherapy with PM intramuscular after six months post-treatment. However, there was no difference in effectiveness between combined therapy with antimoniate therapy, suggesting that the combined treatment (PM and antimoniate) could be used for VL therapy in East Africa [79].

In 2010, the World Health Organization (WHO) recommended a new visceral leishmaniasis treatment in East Africa. This treatment consists of a combined therapy containing a pentavalent antimonial (SSG) and PM intramuscular [80]. One study held in South Sudan, in 2016, investigated and compared the effectiveness of SSG monotherapy and combined therapy (SSG + PM) for severe post-kala-azar dermal leishmaniasis. In this study, combined treatment was more effective than monotherapy, suggesting that combined treatment is an interesting option for post-kala-azar dermal leishmaniasis treatment [81].

Kimutai and co-workers, in 2017, performed a pharmacovigilance study of a treatment using intravenous or intramuscular SSG combined with intramuscular PM in Eastern Africa for visceral leishmaniasis treatment. They monitored 3126 patients infected with *L. donovani* and treated with combined therapy. In this research, 95.1% of patients treated presented initial cure and, only 0.3% had non-response to the treatment. Thus, the authors suggest that WHO recommendation for SSG + PM combined therapy is safe, effective and adequate as first-line VL treatment in East Africa [82].

There are some surveys under development using combined therapy. One study in phase III of the human clinical trial, in Bangladesh, evaluated the safety and efficacy of combined regimen for VL treatment. They compared the monotherapy (liposomal amphotericin B – Ambisome®) current treatment with combined therapy as Ambisome® with miltefosine (single dose of liposomal amphotericin B + seven days of miltefosine), Ambisome® with PM (single dose of liposomal amphotericin B + ten days of PM) and PM with miltefosine (ten days with both treatment). After six-month post-treatment, high cure rates were obtained for all therapies (98.1% for monotherapy, 99.4% for Ambisome® + PM, 94.4% for Ambisome® + miltefosine and 97.9% for PM + miltefosine). In accordance with these results, the authors proposed the use of a combined regimen as an option for VL treatment in that region [83].

In 2017, Hendrickx and co-workers evaluated *in vitro* and *in vivo* responses of combined treatment (PM + miltefosine) against *L. infantum*. The *in vivo* responses in hamster model showed more efficacy of the combined therapy than monotherapy Moreover, no resistance was detected when combined treatment was applied. Therefore, the authors recommended this therapy as an option for VL treatment [84]. Another study published in 2017 investigated *in vitro/in vivo* effectiveness of combined therapy (chloroquine + amphotericin B, chloroquine + miltefosine and chloroquine + PM) against two species of cutaneous leishmaniasis, *L. major* (Old World specie) and *L. mexicana* (New World specie). *In vitro* assays, involving combined treatment using chloroquine and amphotericin B promoted an increase of toxicity in host cells, while treatment using chloroquine + miltefosine presented an enhancement of the antileishmanial activity of miltefosine against *L. major* and no changes in efficacy was observed against *L. mexicana*. Furthermore, chloroquine + PM showed an increase of antileishmanial activity against two Leishmania species. *In vivo* assays were carried out only with chloroquine + PM treatment. PM alone and chloroquine + PM promoted a reduction in lesion size of BALB/c infected with *L. major*, while no significant reduction was found in mice infected with *L. mexicana*. In relation to parasite load assay, they obtained a decrease in the number of parasites detected in mice skin, infected with *L. major* and treated with PM alone and chloroquine + PM. However, parasite burdens had similar results in all groups tested (placebo, PM alone, chloroquine alone and chloroquine + PM) in *L. mexicana* infected mice. The authors suggested that combined treatment (PM + chloroquine) provided limited effectiveness compared to PM monotherapy [85]. Schwartz and co-workers, in 2018, published a study comparing the efficacy of PM alone cream, human antibody alone (anti-TNFα) cream and cream containing PM + antibody (anti-TNFα) to control the local inflammatory response in BALB/c mice infected with *L. major* [86]. The authors observed that the topical application of antibody alone had no effect in the parasite load and the lesion size still increased. However, mice treated with PM alone and combination therapy (PM + anti-TNFα) showed a similar reduction in parasite load and reduction in lesion size. Combination therapy (PM + anti-TNFα) promoted a significant decrease in neutrophilic infiltrate, which mediated the downregulation of TNF_{α}, interleukins (1β and 17) and CCL3 leading to smaller lesions than PM alone treatment. Nevertheless, further studies are required to explore the administration of antibodies and combination therapy (PM + anti-TNFα). Table 2 presents an overview of the current PM formulations for leishmaniasis treatment in the world tested in humans.

According to Table 2, PM intramuscular has the best results of efficacy, but this treatment was only practiced in two regions (India-Bangladesh and East Africa). PM ointment and PM cream presented variable efficacy, which can be related to Leishmania specie treated and

Table 3
Clinical studies involving PM for leishmaniasis treatment (adapted from Clinical Trials, 2019).

Title of study	Treatment	Type of leishmaniasis	Country	Status*
An open label randomized study to access the safety and efficacy of short course paromomycin	PM	Visceral	India	Completed
Paromomycin for individuals with uncomplicated cutaneous leishmaniasis	PM	Cutaneous	United States	Available
An effectiveness study of paromomycin IM injection	PM	Visceral	Bangladesh	Completed
Pharmacokinetics, Safety, and Efficacy Trial of Walter Reed (WR) 279,396 (Paromomycin + Gentamicin Topical Cream) and Paromomycin Topical Cream	WR 279,396 (15% PM + 0.5% gentamicin) topical cream and 15% PM topical cream	Cutaneous	Panama	Completed
Safety, Efficacy, and Pharmacokinetics (PK) of Topical Paromomycin/Gentamicin Cream	WR 279,396 (15% PM + 0.5% gentamicin) topical cream	Cutaneous	United States	Terminated
Safety, Efficacy and Pharmacokinetics (PK) Study of WR 279,396 Versus Paromomycin	WR 279,396 (15% PM + 0.5% gentamicin) topical cream and 15% PM topical cream	Cutaneous	Peru	Completed
Safety and efficacy study of paromomycin	PM and Amphotericin B	Visceral	India	Completed
Phase 3 Study of WR 279,396 and Paromomycin Alone	WR 279,396 (15% PM + 0.5% gentamicin) topical cream and 15% PM topical cream	Cutaneous	Panama	Completed
Phase 3 Study to Evaluate WR 279,396 vs. Paromomycin Alone	WR 279,396 (15% PM + 0.5% gentamicin) topical cream; 15% PM topical cream and cream without drug	Cutaneous	Tunisia	Completed
Expand Access/Assess Safety and Efficacy of Paromomycin intramuscular (IM) Injection	PM	Visceral	India	Completed
Efficacy/Safety of Sodium Stibogluconate (SSG) Versus Paromomycin (PM) and SSG/PM Combination	PM; SSG and combination PM + SSG	Visceral	Ethiopia and Kenya	Completed
Miltefosine/Paromomycin Phase III Trial	PM; miltefosine; SSG	Visceral	Ethiopia and Kenya	Recruiting
Topical paromomycin	PM; pentamidine isethionate; placebo	Cutaneous	Bolivia	Completed
Phase III, Study of Three Short Course Combo (Ambisome®, Miltefosine, Paromomycin) Compared with AmBisome®	Liposomal amphotericin B; liposomal amphotericin B + miltefosine; liposomal amphotericin B + PM; miltefosine + PM	Visceral	Bangladesh	Completed
Cosmetic outcome of leishmaniasis scar after WR279,396 application	WR 279,396 (15% PM + 0.5% gentamicin) topical cream	Cutaneous leishmaniasis scar	Tunisia	Completed
WR 279,396	WR 279,396 (15% PM+ 0.5% gentamicin) topical cream	Cutaneous	France	Completed
Efficacy of Topical Liposomal Form of Drugs	Liposomal meglumine antimoniate (Glucantime); liposomal PM; placebo	Cutaneous	Iran	Completed
WR 279,396 open label treatment protocol	WR 279,396 (15% PM + 0.5% gentamicin) topical cream	Cutaneous	Tunisia	Terminated
Combination therapy in India	Amphotericin B deoxycholate; liposomal amphotericin B + miltefosine; liposomal amphotericin B + PM; miltefosine + PM	Visceral	India	Completed
Safety and Efficacy Study to Evaluate Different Combination Treatment Regimens	Amphotericin B deoxycholate; Ambisome + miltefosine; Ambisome + PM; miltefosine + PM	Visceral	India	Completed
A Study to Evaluate the Efficacy and Tolerance of WR279,396 for Old World Leishmaniasis	WR 279,396 (15% PM + 0.5% gentamicin) topical cream with Tegaderm dressing; WR 279,396 (15% PM + 0.5% gentamicin) topical cream with Gauze and Tape dressing	Cutaneous	Tunisia	Completed
Topical treatment with WR 279,396: A phase 2 study in the Old World	WR 279,396 (15% PM + 0.5% gentamicin) topical cream and placebo	Cutaneous	France and Tunisia	Completed
Short Course regimens for treatment of Post-kala-azar dermal leishmaniasis (PKDL)	PM, Ambisome and Miltefosine	Post-kala-azar dermal	Sudan	Recruiting
Topical treatment of recalcitrant ulcerative Old World Leishmaniasis with WR 279,396	WR 279,396 (15% PM + 0.5% gentamicin) topical cream	Cutaneous	United States	Terminated
Oral Miltefosine plus Paromomycin in American Cutaneous Leishmaniasis	Miltefosine; PM; Miltefosine + PM	Cutaneous	Bolivia	Not yet recruiting

WR 279,396 – Walter Reed (WR) US Army Medical Research Patent for the development of a cream containing 15% PM and 0.5% gentamicin.
* Legend Status: Not yet recruiting: The study has not started recruiting participants; Recruiting: The study is currently recruiting participants; Available: Expanded access is currently available for this intervention, and patients who are not participants in the clinical study may be able to gain access to the drug, biologic, or medical device being studied; Terminated: The study has stopped early and will not start again. Participants are no longer being examined or treated; Completed: The study has ended normally (that is, the participant's last visit has occurred).

the chosen period or frequency of treatment. Furthermore, PM + urea showed lower efficacy than other topical formulations (PM + methylbenzethonium chloride and PM + gentamicin).

There is no evidence, which supports topical treatment containing PM against New World Leishmania species [72], due to these formulations present lesser efficacy than parenteral meglumine antimoniate [87]. This is probably the reason why PM formulations have not yet been approved for leishmaniasis treatment in Brazil [88]. Moreover, PM current topical formulations present low permeability because this drug belongs to class III of BCS. Although these problems, there are some clinical studies under development or already achieved using PM formulations. Table 3 presented the clinical studies published in Clinical Trials.gov site using keywords as "paromomycin" and "leishmaniasis".

In accordance with Table 3, it is possible to observe that Tunisia and India are the countries with more clinical trials involving PM formulations. In addition, the PM formulation more evaluated is 15% PM + 0.5% gentamicin cream for topical cutaneous leishmaniasis treatment, which could mean an increase of researchers' interest in developing topical formulations for this disease treatment.

Furthermore, the parasite resistance of PM current formulations has been reported and it is related to the decrease of PM uptake by Leishmania [48,89]. The parasite resistance, the few studies of PM efficacy in New World, the few numbers of clinical studies, the toxicity of formulations presented on the market and the poor permeability of this drug are motivating the development of new PM formulations for leishmaniasis treatment.

Fig. 3 shows the number of publications between 1997 and 2018, obtained in database "Web of Science", using keywords as "paromomycin + Leishmaniasis" and "paromomycin + Leishmaniasis + drug delivery systems (DDS)". These data revealed that studies on PM drug delivery systems for leishmaniasis were not numerous before 2009. However, a growing interest in this topic has been observed from 2010 and more specifically within the last three years. This review will focus on the new formulations using DDS containing PM as monotherapy and in combined therapy developed in the last years.

4. Recent advancements in formulation strategies for leishmaniasis

The recent advancements in formulation strategies for leishmaniasis treatment using PM (microspheres, liposomes, solid lipid nanoparticles - SLN, gels, polymeric films, emulsions) are presented in Table 4. They will be discussed firstly for VL and then for CL leishmaniasis treatments.

4.1. *Visceral leishmaniasis*

Microspheres and liposomes are the two types of DDS proposed for the treatment of VL.

4.1.1. *Microspheres*

Microspheres can be defined as solid spherical particles ranging in size from 1 to 1000 μm. They can be produced from different materials as polymers (natural or synthetic), wax and proteins as carrier matrices for drug delivery (Fig. 4) [90]. Microspheres can be produced by different processes such as spray drying, emulsification-solvent evaporation, polymerization and coacervation among others [91,92]. Generally, they have advantages compared to conventional dosage pharmaceutical formulations like lower toxicity, improved bioavailability, increased stability and extended drug delivery in a specific site of action [93].

In 2011, Khan and Kumar [48] proposed a formulation based on protein microspheres containing PM for visceral leishmaniasis treatment and parenteral use. This formulation aimed to overcome the limitations of most PM formulations (low efficacy, high doses required or local toxicity). The choice of albumin as a drug carrier is due to its biodegradability, nontoxicity and low cost. Moreover, albumin is approved by FDA for clinical use. Spray drying was the process used to produce these PM-loaded albumin microspheres. It is a rapid, well established, reproducible and one-step process of converting a liquid formulation to a powder. The liquid formulation, sometimes a solution, an emulsion or a suspension, is sprayed through a nozzle into a chamber that simultaneously has hot gas being blown into it. As the liquid droplets are released through the nozzle and enter in contact with the

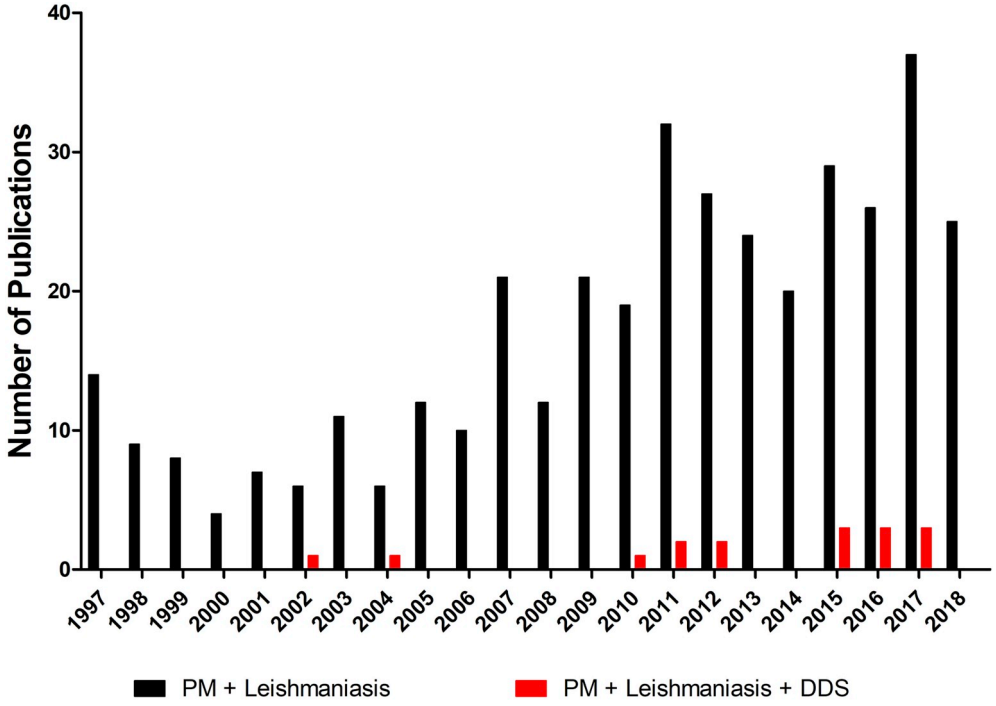


Fig. 3. Number of Publication of Paromomycin (PM) among 1997–2018 obtained from database 'Web of Science' using keywords as "paromomycin + Leishmaniasis" and "paromomycin + Leishmaniasis + drug delivery systems (DDS)".

Table 4
Recent advancements in formulations strategies for PM.

Formulation strategies	Material	Encapsulation method	Type of leishmaniasis/Specie/ Use	References
Microspheres	Albumin	Spray drying	Visceral/*L. donovani*/Parenteral	(Khan et al., 2013b, 2013a; Khan and Kumar, 2011)
Liposomes	Cholesterol, dicetyl phosphate, triglycerol monostearate, hexaglycerol distearate, decaethylene glycol mono n-hexadecyl ether or hexa glycol mono n-hexadecyl ether	Fusion method	Visceral/*L. donovani*/Parenteral	(Williams et al., 1998)
Liposomes	Phosphatidylcholine and stearylamine	Conventional solvent evaporation method	Visceral/*L. donovani*/Parenteral	(Banerjee et al., 2011)
Liposomes	Phosphatidylcholine, polyethyleneglycol, dimiristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, dimiristoyl phosphatidylglycerol, diestaroyl phosphatidylethanolamine, dipalmitoyl phosphatidylglycerol, stearylamine	Dehydration -rehydration method	Visceral/*L. infantum*/Parenteral	(Gaspar et al., 2015)
Liposomes	Soybean phosphatidylcholine with or without cholesterol	Solvent evaporation method and reverse-phase evaporation method	Cutaneous/No leishmanicidal activity analyzed/Topical	(Ferreira et al., 2004)
Liposomes	Soybean phosphatidylcholine, cholesterol, propyleneglycol, vitamin E, propylparaben, methylparaben	Fusion method	Cutaneous/*L. major*/Topical	(Jaafari et al., 2009)
Liposomes	Soybean phosphatidylcholine with or without cholesterol, propyleneglycol, vitamin E, propylparaben, methylparaben	Reverse-phase evaporation method	Cutaneous/*L. major*/Topical	(Carneiro et al., 2010)
Liposomes	Soybean phosphatidylcholine, cholesterol, sodium cholate, propylparaben, methylparaben, propyleneglycol, vitamin E	Fusion method	Cutaneous/*L. major*/Topical	(Bavarsad et al., 2012)
Liposomes	Cholesterol, phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, miltefosine	Freeze-drying double emulsion method	Cutaneous/*L. major*/Topical	(Momeni et al., 2013)
Liposomes	Soybean phosphatidylcholine, cholesterol, sodium cholate, propylparaben, methylparaben, propyleneglycol, vitamin E	Fusion method	Cutaneous/No leishmanicidal activity analyzed/Topical	(Kalantari et al., 2014)
Solid lipid nanoparticles	Stearic acid or cetyl palmitate, Tween 80, Span 85, sodium sulfate	Microemulsion method or solvent difusion method	Cutaneous/No leishmanicidal activity analyzed/Topical	(Ghadiri et al., 2012, 2011)
Solid lipid nanoparticles	Stearic acid, Tween 80	Modified high shear homogenization microemulsion method	Cutaneous/*L. major* and *L. tropica*/Topical	(M. Heidari-Kharaji et al., 2016; Maryam Heidari-Kharaji et al., 2016; Kharaji et al., 2015)
Hydrophilic gel	Hydroxyethylcellulose	Fusion method	Cutaneous/*L. brazilensis* and *L. amazonensis*/Topical	(Gonçalves et al., 2005)
Hydrophilic gel	Hydroxyethylcellulose and methylbenzethonium chloride	Fusion method	Cutaneous/*L. braziliensis*/Topical	(Santos et al., 2008)
Nanogel	Poloxamer 407	Cold method	Cutaneous/*L. major* and *L. infantum*/Topical	(Brugués et al., 2015)
Hydrophilic gel	Hydroxyethylcellulose, propyleneglycol, methylparaben	Fusion method	Cutaneous/*L. major* and *L. amazonensis*/Topical	(Mussi et al., 2007)
Hydrophilic gel	Hydroxyethylcellulose, propyleneglycol, methylparaben	Fusion method	Cutaneous/*L. major* and *L. amazonensis*/Topical	(Aguiar et al., 2010, 2009)
Hydrophilic gel	Hydroxyethylcellulose, propyleneglycol, methylparaben	Fusion method	Cutaneous/*L. braziliensis*/Topical	(de Morais-Teixeira et al., 2015)
Hydrogel	Chitosan, 2- hydroxipropyl β-cyclodextrin, β-glycerolphosphate, transcutol	Mixture and homogenization	Cutaneous/*L. major*/Topical	(Schwartz et al., 2014)
Polymeric film	Hydroxyethylcellulose, hydroxypropylmethylcellulose, gentamicin	Fusion method	Cutaneous/*L. major*/Topical	(Tolouei et al., 2011)
Oil/Water emulsion	Silicone based polymeric lipophilic surfactant, polyalkylene oxide block copolymer, paraffin oil	Emulsification by homogenization	Cutaneous/No leishmanicidal activity analyzed/Topical	(Gomes et al., 2010)

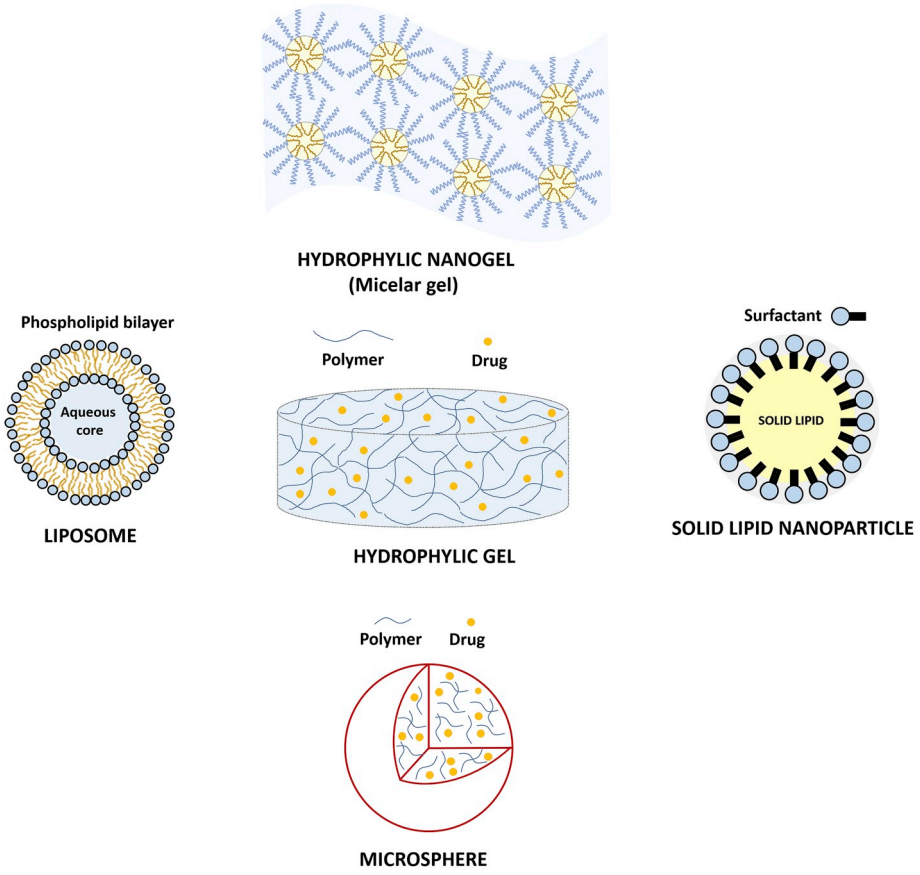


Fig. 4. Different types of drug delivery systems.

hot gas, the solvent content of each droplet is removed by evaporation, thus turning it from liquid to powder form [91,92,94]. The authors prepared three different concentrations (2%, 5% and 8% w/v) of a total solid content with drug and albumin (1:9 w/w ratio) and analyzed the physicochemical properties and *in vitro* release of the microspheres. PM was not thermally degraded during the spray drying process. The PM-loaded albumin microspheres ranged between 2 μm and 4 μm in size, being suitable for macrophage uptake [48].

In 2013, Khan and colleagues described two different studies involving these PM-loaded albumin microspheres [51,95]. Firstly, they evaluated the pharmacokinetic parameters of the microspheres administered intravenously in male rats, developed and validated a new bioanalytical method for quantifying PM until 40 ng/mL using a derivatization method (PM lacks strong chromophore). The PM-loaded albumin microspheres presented no nephrotoxicity when compared to PM intramuscular injection [51]. Khan and co-workers then evaluated the efficacy and stability of the PM-loaded albumin microspheres and observed a significant increased efficacy of this formulation compared to PM solution and a good stability in all temperature and humidity conditions tested, from which they considered that it could be a good choice for VL PM treatment against *L. donovani* [95]. However, the critical point of this formulation is the administration route (intravenous), which is difficult for patient adhesion. Furthermore, more *in vitro/in vivo* studies are required including evaluate antileishmanial activity against *L. infantum* (New World Leishmaniasis specie).

4.1.2. Liposomes

Liposomes are bilayer phospholipid spherical vesicles (Fig. 4) that are already present in the market [14,96,97]. They are great candidates for the intracellular delivery system and antileishmanial drug delivery. Liposomal formulations generally present lower toxicity and higher efficacy than conventional formulations for leishmaniasis treatment [96,97]. Furthermore, they are biocompatible, biodegradable and, as drug delivery systems are able to increase drug efficacy and stability [41].

Liposomes are classified as multilamellar vesicles (MLV) and unilamellar vesicles (large unilamellar vesicles – LUV or small unilamellar vesicles – SUV). They can be prepared by different methods as mechanical dispersion (sonication, micro-emulsification, fusion, membrane extrusion among others), solvent dispersion (reverse-phase evaporation, solvent injection) and detergent removal (detergent dialysis) [98,99].

The arrival on the market of the first liposome formulation with amphotericin b for leishmaniasis treatment (amphotericin b liposomal – Ambisome®) motivated the increased number of researches on liposome formulations for other leishmanicidal drugs. Following this path, Williams and co-workers, in 1998, developed a liposome formulation with PM to treat visceral leishmaniasis [100]. They used a different molar ratio of surfactants, cholesterol and dicetyl phosphate melted and glucose solutions. The formulations with hexa or decaethylene glycol mono n-hexadecyl ether showed good stability and PM entrapment. Liposomes prepared with hexaethylene presented higher size (500 nm) and a maximum PM load of 20 μg/mL, whereas decaethylene glycol led to lower liposomes (200 nm) with higher drug content (between 20 μg/mL– 40 μg/mL). *In vitro* and *in vivo* responses against *L. donovani* of PM liposomes were evaluated and compared to free PM. PM liposomes prepared with decaethylene glycol mono n-hexadecyl ether promoted higher parasite suppression in the liver. However, in the spleen and the bone marrow, PM liposomes presented suppression results similar to the control group [100].

Other group working with liposomes [49] have studied cationic phosphatidylcholine with stearylamine liposomes containing PM for intravenous single-dose treatment. They evaluated the *in vitro* and *in vivo* behavior of this formulation. The liposomes were prepared by the

conventional solvent evaporation method with 10% of entrapment of efficacy of PM. *In vitro* analyses against *L. donovani* revealed a higher efficacy of PM liposomes, needing a lower dose than free PM to give the same effect. Moreover, mice treated with PM liposomes presented a higher reduction of parasite burden, with no *in vivo* toxicity. The authors also evaluated the ability to induce protective immunity of this formulation. The PM liposomes promoted an immunomodulatory effect on $CD4^+$ and CD8 + T cells for gamma interferon production and down-regulated interleukin-10. This effect could represent longer protection and higher efficacy than Ambisome® [49].

Another study in 2015 analyzed the efficacy of PM associated with liposomes, for parenteral administration, in infected murine models with *L. infantum* [101]. Six formulations of PM liposomes were developed varying the lipid composition by dehydration-rehydration method (lipids dissolved in chloroform, dried and hydrated with PM aqueous solution). It was observed that the inclusion of polyethylene glycol (PEG) in lipid composition or stearylamine reduced 30% of the entrapment efficiency in comparison with formulations prepared without PEG and stearylamine. This finding was attributed to the increase of zeta potential, which affects drug/lipid interactions. In the stability study, they analyzed four stability conditions (PM liposomes in buffer suspension at 4 °C, PM liposomes in inactivated fetal bovine serum at 37 °C, lyophilized and lyophilized with cryoprotector trehalose) and discovered that the best one was the PM liposome lyophilized with trehalose. They obtained PM liposomes with size lower than 120 nm. *In vitro* studies were developed with three formulations. One formulation, with negative charge surface, presented more than 90% internalization levels, which indicates that negative charge and rigidity of the liposome membrane promoted the cells uptake. In the biodistribution study, the PM liposomes showed preferential targeting in the liver, lung and spleen, while free PM presented a fast elimination from the bloodstream. Furthermore, the PM liposomes showed efficacy against *L. infantum* and no renal toxicity, suggesting that these formulations are an interesting option for visceral leishmaniasis treatment [101].

4.2. Cutaneous leishmaniasis

Liposomes, SLN, and hydrogels are the three types of DDS more investigated for the treatment of CL.

4.2.1. Liposomes

Liposomes (Fig. 4), as described before, are bilayer phospholipid vesicles and one of the drug delivery systems also studied for CL treatment with PM. Ferreira and co-workers, in 2004, evaluated the skin permeation of two types of PM liposomes for topical administration [102]. They prepared large multilamellar vesicles (MLV) by solvent evaporation method (conventional method) and large unilamellar vesicles (LUV) by reverse-phase evaporation method (method with high aqueous space and able to encapsulate aqueous material) using only soybean phosphatidylcholine or a mixture of soybean phosphatidylcholine and cholesterol as a lipid MLV presented a low PM encapsulation of around 7.5% compared to LUV. Moreover, PM encapsulation in LUV depended on the lipid composition, varying from 27.2% when prepared from a mixture of lipids (soybean phosphatidylcholine and cholesterol) to 41.9%, when prepared only with soybean phosphatidylcholine. Thus, the skin permeation study was carried out only with PM-LUV. In the permeation test, PM-LUV presented low permeation across intact skin (around 1.5% permeated). Although the permeation of PM was higher across stripped skin than across intact skin, PM-LUV permeated lower than blank-LUV or PM solution after 10 h. The explanation was that PM liposomes, when used in stripped skin, promoted a drug-controlled release. More studies are required to investigate PM skin permeation, but this formulation could be an alternative for leishmaniasis treatment [102].

Other researchers also developed PM liposomes for topical use. In 2009, Jaafari and co-workers [103] prepared two formulations of PM liposomes by fusion method, varying the percentage of the drug (10% and 15%). The fusion method is a simple, solvent-free process, leading to an ideal viscosity for direct application in the skin. The two PM liposomes presented comparable particle sizes (less than 500 nm) and encapsulation efficiencies (around 60%). They evaluated the *in vitro* and *in vivo* efficacy of the PM liposomes. *In vitro* studies against *L. major* showed that PM liposomes were three to four times more effective than PM solution. Furthermore, *in vivo* studies with BALB/c mice infected with *L. major* demonstrated that mice treated with liposomal formulation presented a significant reduction of lesion size after one week of treatment. After 12 weeks, PM liposomes showed a reduction in parasite burden, while blank liposomes had an increase of parasite burden. No significant differences were detected between the formulations prepared with different PM loads (10 or 15%). Based on these results, liposomes could be an interesting carrier for PM, however, further biodistribution and immunomodulatory studies are required [103]. Another study with PM liposomes was conducted in 2010 using the reverse-phase evaporation method [104]. The authors prepared two LUV (PM with soybean phosphatidylcholine and PM with a mixture of soybean phosphatidylcholine and cholesterol) and investigated skin permeation in BALB/c mice infected with *L. major*. The surface charges of liposomes were modified after loading PM (drug promoted a decrease of negative charges), probably due to an interaction between PM and the external monolayer of liposomes. Permeation test through intact skin of mice showed low levels of permeated drug (1.9% of PM solution and 4.8% of PM liposomes prepared with soybean phosphatidylcholine and cholesterol), which increased with PM liposomes prepared with soybean phosphatidylcholine (7.2%). This formulation also presented the highest level of permeation (14.6 $\mu g/cm^2$) across stripped skin. The lower values of permeation observed with PM liposomes prepared with cholesterol can be explained by the cholesterol presence that increases the rigidity of vesicles decreasing skin permeation of the drug. In *in vivo* assay, the treatment held with PM liposome gel was significantly better than treatment with free PM gel. This study also verified that liposomes promoted controlled drug release and increased the topical delivery properties of this drug [104].

In 2012, Bavarsad and co-workers prepared, by fusion method following a previous study [103], a new class of liposomes called transfersome [105]. Transfersomes are elastic vesicles formed by bilayer phospholipid with edge activator, which promotes bilayers deformability. This deformability allows drug permeation across intact skin when applied in non-occlusive conditions [105]. These authors evaluated the *in vitro* and *in vivo* effects of this formulation. Eighteen formulations were prepared, varying the percentage of lipids and the percentage of ethanol using factorial design. All the formulations presented more than 50% of drug entrapment, but formulations containing 2% of sodium cholate were most stable. Nine PM transfersomes (formulations with 6% of sodium cholate and/or with 10% of ethanol) were excluded from the study due to their instability. *In vitro* studies against *L. major* showed that transfersomes formulations were more effective than PM cream or solution. Skin permeation investigated with four transfersomes (those with best *in vitro* properties and more stable vesicles) showed that these formulations retained more than 60% PM, while PM cream retained only 13%. PM transfersomes reduced lesion sizes and promoted lower parasite burden *in vivo* studies, without complete cure 12 weeks post-infection. No differences were observed between the four PM transfersomes in these studies. In conclusion, PM transfersomes produced with 2% of sodium cholate, with or without ethanol, could be an alternative option for cutaneous leishmaniasis treatment [105].

In 2013, Momeni and co-workers [97] prepared liposomes by a method based on the freeze-drying of double emulsions, which is reported to be a method with high encapsulation efficiency for hydrophilic or hydrophobic drugs and being able to produce sterile small unilamellar liposomes. Liposomes containing only PM were not generated by this method, because phospholipid and PM produced

agglomerated particles, which could require further studies to control agglomeration. The authors then decided to associate another API (miltefosine) to PM and generate a combined therapy. PM-miltefosine liposomes were prepared with cholesterol, resulting in an encapsulation efficiency lower than 40%, considered too low for *in vivo* assay. In addition, there was not PM liposome produced by the same method for comparison between mono and combined therapy [97].

Kalantari and co-workers, in 2014, investigated the possible nephrotoxicity and hepatotoxicity of PM liposomes using male Wistar rats model [106]. They prepared PM liposomes by fusion method following one previous study [103]. The toxicity of formulation was evaluated in three periods of treatment (10, 20 and 30 days). No differences in the liver and kidney weight index were detected between the group receiving PM liposomes topically twice a day and the control group. Also, the histopathological characteristics after the treatment provided that PM liposomes could be toxicity if used in long-term treatment. In fact, after 30 days, the liver showed reversible swelling cells, whereas the kidney presented a mild renal tubular necrosis. These changes were not observed after 10 or 20 days of treatment with PM liposomes, suggesting that PM liposomes could be used as a short treatment without promoting nephrotoxicity and hepatotoxicity [106].

4.2.2. Solid lipid nanoparticles (SLN)

SLN (Fig. 4) are a class of nanoparticles, which have a lipid matrix core stabilized by surfactants with a size lower than 1000 nm, solid at room temperature. SLN show some advantages as low toxicity, improve drugs solubility and bioavailability, large surface area and controlled drug release [107]. Furthermore, these nanoparticles can be used as a carrier for hydrophilic or hydrophobic drugs [96,107] and have been studied as delivery system for hydrophilic and hydrophobic drugs combined, especially for cancer treatment [108]. This system can be suitable for several routes, such as pulmonary, ocular, intranasal, subcutaneous, dermal, rectal intravenous and oral [96,107–109]. The use of physiological lipids like triglycerides and cholesterol promotes more compatibility and safety of SLN formulations. Moreover, SLN have potential use in epidermal application with controlled release, lower skin irritation and active protection [53,96]. SLN can be produced by various methods, among them, emulsification solvent evaporation, high-pressure homogenization, hot homogenization, solvent diffusion, hot microemulsion dilution [110,111].

Concerning PM formulations, a work about PM loaded-SLN was published in 2011 [109]. SLN were prepared by two different methods: microemulsion (aqueous solution with PM dispersed in melted lipids under agitation and immediately dispersed in cold water) and solvent diffusion (aqueous phase with PM was heated, added in organic phase containing solvent and lipids, homogenized and dispersed in cold water). The authors applied an experimental design to evaluate the estimated effects of three physicochemical properties (entrapment efficiency, particle size and polydispersity index) of these systems. The best PM-SLN system was prepared by microemulsion with 39% of PM entrapment efficiency. It provided a prolonged release profile over 24 h with a 64% PM release. Its thermal behavior was characterized [109].

In 2012, the same group of authors extended the study of PM loaded-SLN, improving the formulation using a statistical experimental design and two parameters (particle size and entrapment efficiency) [28]. Some characteristics were predicted as the amount of drug, percentage of surfactant, ratio lipid/drug, method to prepare the SLN and lipid composition. After prediction, the PM-SLN formulation (90 mg of PM, 0.75% of surfactant, stearic acid as lipid and a ratio of 1:4 drug/lipid) was prepared by microemulsion and the product obtained presented the following characteristics: 42% of PM entrapment efficiency, prolonged release during 24 h with 64% PM release [28].

In 2015, Kharaji and co-workers [53] evaluated the *in vitro* efficacy of PM-SLN against *L. tropica* and *L. major* based on the optimal formulation described in 2012 [28]. Four formulations of PM-SLN were prepared by a modified high shear homogenization microemulsion technique varying the percentage of PM-SLN (oil phase) in the microemulsion and the particle size. Moreover, *in vitro* cytotoxicity against human monocyte (THP1) and promastigotes and amastigotes of *L. major* and *L. tropica* response were evaluated. The results showed that free drug and blank SLN presented non-toxic effect, while all formulations PM-SLN showed cytotoxicity in THP1 cells. However, PM-SLN obtained with higher particle size (PM-SLN 15% 980 nm and PM-SLN 15% 1500 nm) were very toxicity against monocyte and excluded from the other assays. The two formulations with lower particle size (PM-SLN 15% 120 nm and PM-SLN 12.5% 240 nm) showed cytotoxicity only when used in high concentrations (> 6000 μg/mL), suggesting that PM-SLN toxicity is size-dependent. In the case of effective against promastigotes and amastigotes of *L. tropica* and *L. major*, the blank solid lipid nanoparticle presented no effect and the two formulations PM-SLN showed more efficacy than pure drug. When comparing *in vitro* results obtained of PM-SLN formulations, PM-SLN 15% 120 nm exhibited more efficacy and lower toxicity than PM-SLN 12.5% 240 nm. The fluorescence microscopy confirms that PM-SLN formulations had lower infection levels than free drug and Blank-SLN. Therefore, PM-SLN promoted an increase in the effectiveness of PM and could be an interesting alternative for leishmaniasis treatment [53].

The same group of authors (Kharaji et al.), in 2016, analyzed *in vivo* efficacy of PM-SLN, developed in their previous study [53], against BALB/c mice model infected with *L. tropica* and *L. major* [112,113]. The first study was conducted to evaluate *in vivo* response against *Leishmania tropica* using the most effective PM-SLN formulation (PM-SLN 15% 120 nm) [113]. In that work, they observed that PM-SLN has no toxicity after 1 week administered in healthy mice. Moreover, BALB/c mice were infected with *L. tropica* and treated with two different administrations of PM-SLN (intramuscular and intralesional). Spleens and lymph nodes were removed and submitted to parasite burden, parasite load quantification and cytokine measurement. PM-SLN administered intramuscular and intralesional presented lower levels of parasite burden and parasite load when compared to parasite levels in blank-SLN, free PM and mice without treatment. Furthermore, amphotericin B, PM-SLN intramuscular and intralesional showed higher responses of IFN-γ secretion and nitric oxide production, suggesting that PM-SLN is effective and improve PM efficacy against leishmaniasis. These results support that solid lipid nanoparticle is a new and promising alternative for cutaneous leishmaniasis treatment [113].

A subsequent study of the same group evaluated *in vivo* response against *L. major* using the PM-SLN formulation with the best results *in vitro* [112]. The lymph nodes of BALB/c mice infected with *L. major* were removed and submitted to parasite burden and cytokine measurement. Results showed that mice treated by the intramuscular route with PM-SLN formulations had lower levels of parasite burden than mice treated with amphotericin B, suggesting that PM-SLN and amphotericin B work similarly in parasite inhibition. Additionally, PM-SLN and amphotericin B presented higher levels of cytokine productions (IFN-γ and interleukin 4) and higher nitric oxide levels when compared to mice groups without treatment, with blank-SLN or free PM treatment. These results reinforced the previous findings [106] that PM-SLN can improve PM efficacy in leishmaniasis treatment [112].

4.2.3. Gels

Hydrophilic gels (Fig. 4), also known as hydrogels, are cross-linked polymer networks dispersed in water medium produced by one or more monomers reaction [114,115]. They can present some interesting properties as *stimuli* sensitivity (physical or chemical) and aqueous swelling, and due to these properties, they have been studied for several applications. Hydrogels can be classified on several ways according to the synthesis route (homopolymers, copolymers or multipolymers), physical structure (amorphous, crystalline, semi-crystalline or hydrocolloids), ionic charge (anionic, cationic, amphiphilic or neutral), size (macrogels, microgels or nanogels) and bonds nature (chemical or physical). They can be produced by physical *stimuli* (temperature,

electric field, magnetic field, light, pressure or sound) or chemical *stimuli* (pH, ionic strength, molecular species or solvent composition) [114,115].

Nanogels are small hydrogel particle size (between 10 and 100 nm) with high structural stability in which drugs could be uploaded in a spontaneous process. Nanogels multifunctionality can be related to copolymers combination using hydrophilic and hydrophobic polymers. This system has gained attention in recent years [43].

Gonçalves and co-workers, in 2005, developed a 10% PM hydroxyethylcellulose gel [72]. According to previous studies of this research group, a hydrophilic formulation containing PM showed slightly greater leishmanicidal response than hydrophobic formulation as an ointment. Furthermore, the procedure to obtain this type of formulation is easy, rapid and less expensive than other techniques. For this reason, the PM gel activity was evaluated against infected BALB/c mice or hamsters with *L. amazonensis* or *L. braziliensis*, respectively. In the treatment of infected mice with *L. amazonensis*, PM gel promoted the reduction of lesion sizes compared to placebo and higher activity in comparison with meglumine antimoniate. However, in infected hamsters with *L. braziliensis*, PM gel and meglumine antimoniate presented similar effects, suggesting that PM gel could be an alternative treatment for topical cutaneous leishmaniasis [72]. In 2008, following these *in vivo* results, Santos and co-workers [116] studied the efficacy of a gel containing 10% PM with 0.1% methylbenzethonium chloride in 15 patients infected with *L. braziliensis*. These patients presented, at least, one contraindication of meglumine antimoniate treatment. After 20 days of treatment, 21.4% of patients were cured, 50% of patients had some improvement and 28.6% had treatment failure. The patients not cured were submitted to another treatment period with PM gel or no treatment. In the groups treated with 2 times of PM gel, 62.5% of patients were healed. This work presented one problem related to patient's adhesion because most patients prolonged the time of treatment by themselves. Although this problem with the duration of treatment, this PM gel showed interesting results with low toxicity. Therefore, the authors suggested novel studies involving this treatment and new clinical studies using combined therapy with PM gel and meglumine antimoniate or other drugs for leishmaniasis treatment [116].

In 2015, Brugués and co-workers investigated physicochemical properties, permeability, *in vivo* tolerance and antileishmanial activity against *L. infantum* and *L. major* of a new controlled transdermal release PM nanogel [43]. The nanogel was prepared using 20% of Poloxamer 407 (P407) and 5% of PM. P407 is a thermoreversible copolymer that promotes, improves or control drug skin permeation with curative properties. The nanogel with 5% of PM was prepared and the physicochemical properties were evaluated and compared to 5% PM ointment prepared by the group. The P407 nanogel with 5% of PM had a lower particle size (around 9 nm) and almost neutral charge (−0.49 mV) in comparison with nanogel without the drug. In the rheological study, a more rigid system was formed when the temperature was increased. The nanogel presented considerable viscosity and partial rigidity with better spreadability than PM ointment. A stability test was conducted with three different temperatures (−20 °C, 4 °C, 25 °C). No significant changes in their pH values and rheological profile have been noted during storage under all conditions. The nanogel retained more than 98% of PM load after 10 days. However, after 30 days, differences could be observed on the PM retention in the formulations, the nanogel stored at the lower temperature presenting higher PM load compared to the formulation stored at room temperature. The PM nanogel presented a faster drug release compared to PM ointment, with 36.9% of PM released against 1.2% of PM released from ointment after 6 h of experiment. Moreover, in the permeation study, the PM ointment had more retention values than hydrogel, attributed to the occlusive nature of the ointment formulation. *In vivo* tolerance study showed similar behavior between nanogel and untreated area, while ointment promoted hydration in the skin. In relation to cytotoxicity assay, the PM gel presented lower toxicity than PM solution in VERO cells and higher toxicity than PM solution in RAW cells. The antileishmanial test showed PM nanogel with lower IC_{50} values than free drug, suggesting more activity against *L. infantum* and *L. major* of PM nanogel than the free drug. All these results indicated that PM nanogel could also represent a promising formulation to use in leishmaniasis treatment [43].

Another type of CL treatment based on hydroxyethylcellulose gel loaded with PM was proposed in the literature [117]. The effectiveness of this treatment was compared to treatments performed with topical or oral formulations of fluconazole with well-known leishmanicidal activity against BALB/c mice infected with *L. major* or *L. amazonensis*. A gradual decrease of the lesion size until complete healing was observed in mice treated with PM hydroxyethylcellulose gel, whereas fluconazole formulations did not reduce the lesion size. Furthermore, PM hydroxyethylcellulose gel presented high efficacy against *L. major* and *L. amazonensis* and could be a low-cost alternative for cutaneous leishmaniasis treatment [117].

Hydrogel in combined therapy is another strategy used in CL treatment. Combined therapy is an interesting alternative in CL treatment, which allows a decrease of treatment time, improvement of efficacy and prevention of possible parasite resistance. Several examples can be mentioned. Among them, combined therapy of 10% of topical PM hydroxyethylcellulose gel and oral miltefosine was tested in BALB/c mice infected with *L. major* [118]. In that therapy, the first step was the choice of the miltefosine dose (25 mg/kg/day), which was done through a dose-effect test. PM alone and combined treatment presented a reduction of parasite burden lesion in comparison with miltefosine alone and placebo. However, in relation to systemic efficacy, the combination PM and miltefosine reduced more parasite burden in the spleen when compared to monotherapies. A PM gel half-life of around 2 h in plasma collected of infected mice, suggest a systemic absorption of the topical application. The combined therapy using topical PM and oral miltefosine represents a potential option for CL treatment [118].

The same group, in 2010, evaluated the effectiveness of this combined therapy in infected BALB/c mice with *L. amazonensis* [119]. They investigated the efficacy of the combined therapy with three different doses of miltefosine and verified that the highest dose analyzed (25 mg/kg/day) promoted a reduction of 100% in the parasite burden, whereas the intermediary dose (10 mg/kg/day) promoted a reduction around 93%, and the lowest dose (5 mg/kg/day) showed an insignificant reduction to the control group. The combined treatment (PM gel + miltefosine 10 mg/kg/day) presented the lowest level of parasite burden in the lesion and in the spleen compared to monotherapies. These results were very similar to those obtained in their previous study [118]. This combined therapy had a higher effect against *L. amazonensis*, being another interesting option for New World leishmaniasis treatment [119].

Further to these studies [118,119], binary combinations of three formulations (oral miltefosine, intramuscular meglumine antimoniate, topical PM hydroxyethylcellulose gel) were taken to treat infected golden hamsters with *L. braziliensis,* in comparison to monotherapies of each formulation [120]. It was verified that only combined treatment of PM gel and intramuscular meglumine antimoniate presented a significant lesion size reduction when compared with all monotherapies. Furthermore, monotherapy using meglumine antimoniate showed a difference in the reduction of viable parasites in comparison with control. All combined treatment using PM promoted a large reduction of viable parasites compared to monotherapies, suggesting that combined treatment had more efficacy in infected hamsters [120].

Still in the hydrogel category, a chitosan-cyclodextrin hydrogel formulation was formulated for the topical treatment of CL [121]. This hydrogel was loaded with PM or with diselenide, a novel selenium compound with pharmaceutical properties including leishmanicidal activity showed in the previous study [122]. For *in vitro* permeation assay, they tested and compared the permeation diffusion of both formulations in pig ear skin. PM presented a better diffusion across the skin (intact and stripped) in comparison to diselenide, leading to the

conclusion that diselenide chitosan hydrogel did not have an antileishmanial activity, contrarily to PM-loaded chitosan-cyclodextrin hydrogel [121].

4.2.4. Other formulations

Besides liposomes, SLN and hydrogels, other formulations have been developed like polymeric films or drug modifications.

Polymeric films are an interesting option for topical and transdermal application [123]. These formulations are prepared by addition of the drug in solution containing polymer and, when these formulations are applied on the skin, produce a film. The polymer can improve the drug release due to its acts as drug reservoir [123,124]. There are several polymers that can be used to produce polymeric films, such as hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC), hydroxypropyl cellulose (HPC), poly vinylpyrrolidone (PVP), poly vinyl alcohol (PVA), among others [125]. Furthermore, these systems can be produced by solvent casting, hot melt extrusion and inkjet printing methods [125].

Polymeric films constituted of HPMC and ethylcellulose and containing 15% of PM and 0.5% of gentamicin were proposed [126]. The association (PM + gentamicin) was previously used in a cream formulation with clinical acceptable results [70]. The films were produced by direct compression and solvent casting methods. The formulations were evaluated *in vitro* against *L. major* and *in vivo* in infected BALB/c mice. *In vitro* assay showed an increase of viable parasites in placebo and control groups, while PM films promoted a decrease in the number of viable parasites. The group of infected mice treated with PM film had 80% of cure, with the remaining 20% presenting reduction of the lesion size after 28 days of treatment. These findings suggest that polymeric films applied topically can be useful for a gradual release of PM aiming CL treatment. It is however clear that more studies should be conducted to confirm the efficacy of these formulations [126].

Another investigation related to PM topical delivery was related to the formulation vehicle (emulsion or ointment). The authors prepared three different formulations [127]: 1% PM water/oil/water (W/O/W) emulsion, 1% PM oil/water (O/W) emulsion and 1% PM ointment. The *in vitro* release profile and skin permeation of these formulations were evaluated. The W/O emulsion showed a higher PM release (51.7% after 5 h). All formulations presented no permeation across intact (normal) skin. However, PM absorption from O/W emulsion (around 87%) through stripped (without *stratum corneum* barrier) skin was 5–6 times higher than that observed for the other two formulations. Therefore, the authors proposed O/W emulsion containing PM as the best alternative to improve the topical delivery of this drug [127].

Switching to another strategy, in 2011, Nogueira and co-workers [128] developed PM ion pairing to enhance skin permeation of this drug. Ion pairing is a chemical reaction based on the addition of opposite charges together in a solution to form a new structure (salts). This strategy was used to enhance the hydrophobicity of PM and, hence, the drug permeability across the skin. The authors selected four different acids (lactic, butyric, benzoic and cinnamic) for PM salts synthesis. After the synthesis, the salts were submitted to thermal analysis, solubility studies and *in vitro* permeation skin. Thermal analysis showed a decomposition peak less than 220 °C and an endothermic event around 50-70 °C related to water evaporation for all PM salts and PM base. However, PM base decomposes at a higher temperature than PM salts. The PM salts presented an increase of hydrophobicity and PM benzoate was the most hydrophobic molecule. PM base and PM salts have no permeation across intact skin. PM butyrate had the highest value of permeation across stripped skin, suggesting that this salt can favor PM skin permeation, which could improve topical CL treatment using this drug [128].

5. Combined treatment using PM and other antileishmanial drug

Combined treatment for leishmaniasis is an interesting option that has been studied and described in the literature. The treatment using two different pathways to simultaneous local and systemic effects, especially for CL treatment, shows several advantages (lower side effects, high efficacy) in comparison with monotherapy. One of the most studied drugs for combined treatment is PM. Some studies that investigate the combination of PM with other antileishmanial drugs as antimonials, AmB, miltefosine, among others.

Since 2010, intramuscular administration of PM combined with intravenous or intramuscular SSG is one of the first-line treatments for VL in Eastern Asia approved by WHO [82]. Moreover, intramuscular PM combined with oral miltefosine has been approved and used as an alternative recommended treatment in India and Bangladesh for VL treatment [83].

Besides, topical PM combined with oral miltefosine is the combination more investigated to improve CL treatment [118,120]. This combination has been showing good results, as improve efficacy, through the effect of these two drugs. Although PM and miltefosine have a different mechanism of action (PM acts in ribosome subunits and mitochondrial membrane while miltefosine promotes drug intracellular accumulation in parasites), it is not yet known if these drugs promote synergic effect. It is already known that PM shows a short half-life while miltefosine presents a large half-life [118].

Despite the increase in works involving combined therapy, studies focus in evaluate possible synergistic effects of drugs used in this type of therapy must be carried out. Besides, studies involving the encapsulation of two antileishmanial drugs, especially using PM as one of the drugs, in only one system/formulation may be a viable option to improve combined therapy, being important for the development of research in this field.

6. Futures of leishmaniasis treatment using PM

The development of treatments for visceral and cutaneous leishmaniasis using PM is in progress, following mainly two lines. One focuses on conventional formulations like ointments or creams for topical administration and PM solution for intramuscular administration, the other explores micro and nanotechnologies (drug delivery systems).

From the latter approach, combined therapies against leishmaniasis appeared as a novel type of therapy improving efficacy, decreasing treatment time and decreasing the possibilities of parasite resistance. Treating leishmaniasis with PM faces several difficulties since such treatments are toxic and with variable efficacy. It is desirable to develop delivery systems (DDSs) that maintain the antileishmanial activity of PM but reduce its toxicity and improve the efficacy. The intracellular pathways followed by DDS are still poorly controlled. To optimize therapeutic action in VL leishmaniasis, DDS must deliver the drug in the same spatial compartment as the target. To that aim, phagocyted DDS must be directed to phagolysosomes where the amastigotes reside. Moreover, DDS act as a source of sustained release. The therapeutic effect of hydrosoluble drugs such as PM can be enhanced if achieving sustained release from the cytoplasm to the phagolysosomal vacuole membrane and amastigotes membrane.

It has been demonstrated that DDSs like protein microspheres and liposomes can be internalized by macrophages and deliver PM allowing VL treatment. Microspheres can be produced with different materials and with different process technologies, however, some physical and physicochemical characteristics such as particle size and PM load efficiency need to be adjusted to avoid low PM loading efficiency and prevent burst effect (premature drug release). Liposomes are microparticulate lipoidal vesicles, which are under extensive investigation as drug carriers for improving the PM delivery. Two important advantages of liposomes are biocompatibility and biodegradability, which are due to lipid characteristics. Due to new developments in liposome technology, several liposome-based drug formulations are currently in a clinical trial, and recently some of them have been approved for clinical use. Formulation of PM in liposomes has provided an opportunity to

allow leishmaniasis treatments. The preparation of liposomes results in different properties for these systems. The benefits and limitations of liposome as PM carrier critically depend and based on physicochemical and colloidal characteristics such as size, composition, loading efficiency and stability, as well as their biological interaction with the cell membranes.

The topical CL treatment with PM presents low efficacy mainly related to low drug permeability across the skin due to PM molecule character (very hydrophilic and high molecular weight). To improve PM permeability and efficacy, emulsions, hydrogels and nanostructures like liposomes and SLN have been developed. Liposome is the most DDS studied for novel PM formulations because its structure is similar to skin membrane composition (bilayer phospholipids), which allow an increase of PM permeation across intact skin when compared to conventional formulations. Moreover, in stripped skin (condition of CL lesions), liposomes promote the sustained release. The other advantage of PM liposomes is the small size of vesicles that contribute to improving PM skin permeation. The disadvantage of PM liposomes studies is, the majority of them, evaluated skin permeation in a mouse skin model that is difficult to extrapolate to human skin behavior. SLN have the same advantages as small size and bilayer phospholipids composition, which can improve PM permeation. However, this DDS requires skin permeation studies to evaluate PM-SLN behavior across the skin, which still not yet showed in literature. Hydrophilic gels have been studied using different polymers (hydroxyethylcellulose, poloxamer, chitosan) containing PM and have some advantages as easy preparation, low cost and solvent-free. Moreover, it was observed higher PM permeation across damage skin in comparison with hydrophobic vehicles (ointments, creams). Nonetheless, these formulations still present low PM permeation through intact skin. PM hydroxyethylcellulose gel was the only formulation, which has submitted to clinical studies in a short group of patients infected with *L. braziliensis* and had interesting results. PM poloxamer gel had a sustained PM release profile and presented the most complete study of physicochemical properties of novel PM DDS formulation. There are also studies with PM emulsion and PM polymeric films, which require improvement in formulation and skin permeation analysis, respectively. Another possibility to improve PM skin permeation was to promote a change in the PM chemical molecule in order to make it more hydrophobic. Nevertheless, PM with this molecular change continues with low intact skin permeation due to high molecular weight. Based on this scenario, liposomes and hydrophilic gel (specially poloxamer gel) are promising alternatives to PM topical formulations for CL treatment. Furthermore, fluconazole and novel compound diselenide showed no effect against CL, when administered in topical formulations.

It can be observed that PM efficacy administered topically in the CL treatment depends on the choice of the vehicle (ointment, cream, gel) and/or type of carrier (microparticles, nanoparticles, liposome, etc.) in addition to treatment protocol (number of applications, days of treatment). Moreover, the majority of the studies used *L. major* as a Leishmania specie model to evaluate antileishmanial activity. However, it is important to investigate other Leishmania species, including New World species.

Thus, the development of PM DDS and the creation of treatment protocol are essential to achieve success for CL and VL treatments using this drug. Moreover, further studies are still required to investigate more physicochemical properties and *in vitro/in vivo* parameters of the novel formulations, including also clinical trials of the most promising formulations. In comparison with conventional formulations, these formulations have higher costs. However, the increase of efficiency and reduction of undesired side effects can, in turn, result in further economic investments. The formulations have to be adjusted with regard to their production costs permitting a satisfactory cost-benefit ratio.

There are also alternatives therapies as combined therapy and heat therapy. The heat therapy has been investigated as a complement of antimonials treatment or used alone. Maybe PM formulations combined with heat therapy can be another option. Combined therapy using two antileishmanial drugs has been evaluated. This therapy has some advantages as increase efficacy, decrease parasite resistance, decrease treatment time. Moreover, combined therapy using topical PM and oral miltefosine is the most studied for CL treatment. This combination presents promising results with easy administration (patients can be administered themselves), lower side effects and attractive cost-benefit ratio, Therefore, the search continues for a low-cost, low-tox, thermal-stable, easy-to-use, smart delivery formulations, using PM for effectively treat leishmaniasis. Even for the treatment of an old disease, the challenge of finding new drugs and formulations still persists in order to guarantee the control of this disease that is still a great problem of global public health.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] T.T.H. Pham, G. Barratt, J.P. Michel, P.M. Loiseau, M. Saint-Pierre-Chazalet, Interactions of antileishmanial drugs with monolayers of lipids used in the development of amphotericin B-miltefosine-loaded nanocochleates, Colloids Surf. B Biointerfaces 106 (2013) 224–233, https://doi.org/10.1016/j.colsurfb.2013.01.041.

[2] R. Reithinger, J.-C. Dujardin, H. Louzir, C. Pirmez, B. Alexander, S. Brooker, Cutaneous leishmaniasis, Lancet Infect. Dis. 7 (2007) 581–596.

[3] T.S. Tiuman, A.O. Santos, T. Ueda-Nakamura, B.P.D. Filho, C.V. Nakamura, Recent advances in leishmaniasis treatment, Int. J. Infect. Dis. 15 (2011), https://doi.org/10.1016/j.ijid.2011.03.021.

[4] N. Singh, M. Kumar, R.K. Singh, Leishmaniasis: current status of available drugs and new potential drug targets, Asian Pac. J. Trop. Med. 5 (2012) 485–497, https://doi.org/10.1016/S1995-7645(12)60084-4.

[5] D.C. Soares, C.G. Pereira, M.Â.A. Meireles, E.M. Saraiva, Leishmanicidal activity of a supercritical fluid fraction obtained from Tabernaemontana catharinensis, Parasitol. Int. 56 (2007) 135–139, https://doi.org/10.1016/j.parint.2007.01.004.

[6] J. Alvar, I.D. Vélez, C. Bern, M. Herrero, P. Desjeux, J. Cano, J. Jannin, M. de Boer, Leishmaniasis worldwide and global estimates of its incidence, PloS One 7 (2012), https://doi.org/10.1371/journal.pone.0035671.

[7] D. Pace, Leishmaniasis, J. Infect. 69 (2014), https://doi.org/10.1016/j.jinf.2014.07.016 S10–S18.

[8] A. Dutta, G. Mandal, C. Mandal, M. Chatterjee, In vitro antileishmanial activity of Aloe vera leaf exudate: a potential herbal therapy in leishmaniasis, Glycoconj. J. 24 (2007) 81–86, https://doi.org/10.1007/s10719-006-9014-z.

[9] P. Mansueto, A. Seidita, G. Vitale, A. Cascio, Leishmaniasis in travelers: a literature review, Trav. Med. Infect. Dis. 12 (2014) 563–581, https://doi.org/10.1016/j.tmaid.2014.09.007.

[10] World Health Organization, Leishmaniasis, https://www.who.int/en/news-room/fact-sheets/detail/leishmaniasis, (2019) accessed April 26, 2019.

[11] S. Sundar, J. Chakravarty, Paromomycin in the treatment of leishmaniasis, Expet Opin. Invest. Drugs 17 (2008) 787–794, https://doi.org/10.1517/13543784.17.5.787.

[12] V. Saranavan, P. Das, Recent progress in drug targets and inhibitors towards combating leishmaniasis, Acta Trop. 17 (2018), https://doi.org/10.1016/j.actatropica.2018.02.010.

[13] B. Zulfiqar, T.B. Shelper, V.M. Avery, Leishmaniasis drug discovery: recent progress and challenges in assay development, Drug Discov. Today 22 (2017) 1516–1531, https://doi.org/10.1016/j.drudis.2017.06.004.

[14] A. de Souza, D.S.S. Marins, S.L. Mathias, L.M. Monteiro, M.N. Yukuyama, C.B. Scarim, R. Löbenberg, N.A. Bou-Chacra, Promising nanotherapy in treating leishmaniasis, Int. J. Pharm. 547 (2018) 421–431, https://doi.org/10.1016/j.ijpharm.2018.06.018.

[15] S. Kapil, P.K. Singh, O. Silakari, An update on small molecule strategies targeting leishmaniasis, Eur. J. Med. Chem. 157 (2018) 339–367, https://doi.org/10.1016/j.ejmech.2018.08.012.

[16] J.H. No, Visceral leishmaniasis: revisiting current treatments and approaches for future discoveries, Acta Trop. 155 (2016) 113–123, https://doi.org/10.1016/j.actatropica.2015.12.016.

[17] P. Mitropoulos, P. Konidas, M. Durkin-Konidas, New World cutaneous

leishmaniasis: updated review of current and future diagnosis and treatment, J. Am. Acad. Dermatol. 63 (2010) 309–322, https://doi.org/10.1016/j.jaad.2009.06.088.
[18] G. Carneiro, M.G. Aguiar, A.P. Fernandes, L.A.M. Ferreira, Drug delivery systems for the topical treatment of cutaneous leishmaniasis, Expet Opin. Drug Deliv. 9 (2012) 1083–1097, https://doi.org/10.1517/17425247.2012.701204.
[19] S. Sundar, J. Chakravarty, An update on pharmacotherapy for Leishmaniasis, Expet Opin. Pharmacother. 16 (2015) 237–252, https://doi.org/10.1517/14656566.2015.973850.
[20] World Health Organization, Leishmaniasis - Situations and Trends, (2019) https://www.who.int/gho/neglected_diseases/leishmaniasis/en/ accessed April 26, 2019.
[21] J. Bezerra de Menezes, C. Sampaio Guedes, A. Oliveira Petersen, D. Mothé Fraga, P. Sampaio Veras, Advances in development of new treatment for leishmaniasis, BioMed Res. Int. (2015) 1–11, https://doi.org/10.1155/2015/815023.
[22] J.A.L. Lindoso, J.M.L. Costa, I.T. Queiroz, H. Goto, Review of the current treatments for leishmaniases, Res. Rep. Trop. Med. 3 (2012) 69–77, https://doi.org/10.2147/rrtm.s24764.
[23] H. Goto, J.A.L. Lindoso, Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis, Expert Rev. Anti Infect. Ther. 8 (2010) 419–433, https://doi.org/10.1586/eri.10.19.
[24] M. Akbari, A. Oryan, G. Hatam, Application of nanotechnology in treatment of leishmaniasis: a Review, Acta Trop. 172 (2017) 86–90, https://doi.org/10.1016/j.actatropica.2017.04.029.
[25] M.Z. Handler, P.A. Patel, R. Kapila, Y. Al-Qubati, R.A. Schwartz, Cutaneous and mucocutaneous leishmaniasis: differential diagnosis, diagnosis, histopathology, and management, J. Am. Acad. Dermatol. 73 (2015) 911–926, https://doi.org/10.1016/j.jaad.2014.09.014.
[26] V.M. Ribeiro, The use of Milteforan® in the treatment of canine visceral leishmaniasis in Brazil, Rev. V&Z Em Minas 131 (2016) 44–46.
[27] L. Imbert, S. Cojean, D. Libong, P. Chaminade, P.M. Loiseau, Sitamaquine-resistance in Leishmania donovani affects drug accumulation and lipid metabolism, Biomed. Pharmacother. 68 (2014) 893–897, https://doi.org/10.1016/j.biopha.2014.08.009.
[28] M. Ghadiri, S. Fatemi, A. Vatanara, D. Doroud, A.R. Najafabadi, M. Darabi, A.A. Rahimi, Loading hydrophilic drug in solid lipid media as nanoparticles: stastitical modeling of entrapment efficiency and particle size, Int. J. Pharm. 424 (2012) 128–137, https://doi.org/10.1016/j.ijpharm.2011.12.037.
[29] P.W. Nassif, T.F.P. De Mello, T.R. Navasconi, C.A. Mota, I.G. Demarchi, S.M.A. Aristides, M.V.C. Lonardoni, J.J.V. Teixeira, T.G.V. Silveira, Safety and efficacy of current alternatives in the topical treatment of cutaneous leishmaniasis: a systematic review, Parasitology 144 (2017) 995–1004, https://doi.org/10.1017/S0031182017000385.
[30] I.M.F. Lobo, M.B.P. Soares, T.M. Correia, L.A.R. de Freitas, M.I. Oliveira, M. Nakatani, E. Netto, R. Badaro, J.R. David, Heat therapy for cutaneous leishmaniasis elicits a systemic cytokine response similar to that of antimonial (Glucantime) therapy, Trans. R. Soc. Trop. Med. Hyg. 100 (2006) 642–649, https://doi.org/10.1016/j.trstmh.2005.08.011.
[31] N.E. Aronson, C.A. Joya, Cutaneous leishmaniasis: updates in diagnosis and management, Infect. Dis. Clin. 33 (2019) 101–117, https://doi.org/10.1016/j.idc.2018.10.004.
[32] N.E. Aronson, G.W. Wortmann, W.R. Byrne, R.S. Howard, W.B. Bernstein, M.A. Marovich, M.E. Polhemus, I.-K. Yoon, K.A. Hummer, R.A. Gasser, C.N. Oster, P.M. Benson, A randomized controlled trial of local heat therapy versus intravenous sodium stibogluconate for the treatment of cutaneous Leishmania major infection, PLoS Neglected Trop. Dis. 4 (2010) e628, https://doi.org/10.1371/journal.pntd.0000628.
[33] J.A. Cardona-Arias, L. López-Carvajal, M.P. Tamayo-Plata, I.D. Vélez, Comprehensive economic evaluation of thermotherapy for the treatment of cutaneous leishmaniasis in Colombia, BMC Publ. Health 18 (2018) 1, https://doi.org/10.1186/s12889-018-5060-2.
[34] L. Lopez, M. Robayo, M. Vargas, I.D. Vélez, Thermotherapy. An Alternative for the Treatment of American Cutaneous Leishmaniasis vol. 13, (2012), pp. 1–7. Trials http://www.trialsjournal.com/content/13/1/58%5Cnhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=2012541296.
[35] P. Layegh, F. Pezeshkpoor, A.H. Soruri, P. Naviafar, T. Moghiman, Efficacy of cryotherapy versus intralesional meglumine antimoniate (glucantime) for treatment of cutaneous leishmaniasis in children, Am. J. Trop. Med. Hyg. 80 (2009) 172–175, https://doi.org/10.4269/ajtmh.2009.80.172.
[36] A. Asilian, A. Sharif, G. Faghihi, S. Enshaeieh, F. Shariati, A.H. Siadat, Evaluation of CO2 laser efficacy in the treatment of cutaneous leishmaniasis, Int. J. Dermatol. 43 (2004) 736–738, https://doi.org/10.1111/j.1365-4632.2004.02349.x.
[37] S. de Oliveira, E.J. da Ordem Trahamane, J. Monteiro, G.P. Santos, P. Crugeira, F. Sampaio, C. Oliveira, M.B. Neto, A. Pinheiro, Leishmanicidal effect of antiparasitic photodynamic therapy—ApPDT on infected macrophages, Laser Med. Sci. 32 (2017) 1959–1964, https://doi.org/10.1007/s10103-017-2292-9.
[38] E. Ricci-Junior, L.B. de Oliveira de Siqueira, R.A.S. Rodrigues, F. Sancenón, R. Martínez-Máñez, J.A. de Moraes, R. Santos-Oliveira, Nanocarriers as photherapeutic drug delivery system: appraisal of three different nanosystems in an in vivo and in vitro exploratory study, Photodiagnosis Photodyn, Ther 21 (2018) 43–49, https://doi.org/10.1016/j.pdpdt.2017.11.003.
[39] L.B. de Oliveira de Siqueira, V. da Silva Cardoso, I.A. Rodrigues, A.L. Vazquez-Villa, E.P. dos Santos, B. da Costa Leal Ribeiro Guimarães, C. dos Santos Cerqueira Coutinho, A.B. Vermelho, E. Ricci-Júnior, Development and evaluation of zinc phthalocyanine nanoemulsions for use in photodynamic therapy for Leishmania spp . Nanotechnology 28 (6) (2017) 065101, , https://doi.org/10.1088/1361-6528/28/6/065101.
[40] E.M. Van Der Snoek, D.J. Robinson, J.J. Van Hellemond, H.A.M. Neumann, A review of photodynamic therapy in cutaneous leishmaniasis, J. Eur. Acad. Dermatol. Venereol. 22 (2008) 918–922, https://doi.org/10.1111/j.1468-3083.2008.02805.x.
[41] L. De Almeida, A.T. Fujimura, M.L. Del Cistia, B. Fonseca-Santos, K.B. Imamura, P.A.M. Michels, M. Chorilli, M.A.S. Graminha, Nanotechnological strategies for treatment of leishmaniasis-a review, J. Biomed. Nanotechnol. 13 (2017) 117–133, https://doi.org/10.1166/jbn.2017.2349.
[42] A. Pujol-Brugués, A.C. Calpena-Campmany, C. Riera-Lizandra, L. Halbaut-Bellowa, B. Clares-Naveros, Development of a liquid chromatographic method for the quantification of paromomycin. Application to in vitro release and ex vivo permeation studies, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 133 (2014) 657–662, https://doi.org/10.1016/j.saa.2014.06.017.
[43] A.P. Brugués, B.C. Naveros, A.C. Calpena Campmany, P.H. Pastor, R.F. Saladrigas, C.R. Lizandra, Developing cutaneous applications of paromomycin entrapped in stimuli-sensitive block copolymer nanogel dispersions, Nanomedicine 10 (2015) 227–240, https://doi.org/10.2217/nnm.14.102.
[44] R.N. Davidson, M. den Boer, K. Ritmeijer, Paromomycin, Trans. R. Soc. Trop. Med. Hyg. 103 (2009) 653–660, https://doi.org/10.1016/j.trstmh.2008.09.008.
[45] United States Pharmacopeia, Paromomycin Sulfate, 35th ed., (2012).
[46] United States National Library of Medicine, Paromomycin ChemIDplus, https://chem.nlm.nih.gov/chemidplus/rn/7542-37-2, (2019) accessed April 26, 2019.
[47] The International Pharmacopoeia - Eighth Edition - World Health Organization, Paromomycin Sulfate (Paromomycini Sulfas), (2018) http://apps.who.int/phint/pdf/b/6.1.261.Paromomycin-sulfate-(Paromomycini-sulfas).pdf accessed April 26, 2019.
[48] W. Khan, N. Kumar, Drug targeting to macrophages using paromomycin-loaded albumin microspheres for treatment of visceral leishmaniasis: an in vitro evaluation, J. Drug Target. 19 (2011) 239–250, https://doi.org/10.3109/1061186X.2010.492524.
[49] A. Banerjee, M. De, N. Ali, Combination therapy with paromomycin-associated stearylamine-bearing liposomes cures experimental visceral leishmaniasis through Th1-biased immunomodulation, antimicrob, Agents Chemother 55 (2011) 1661–1670, https://doi.org/10.1128/aac.00524-10.
[50] L. De Almeida, B. Fonseca-santos, P. Michels, L. De Almeida, A.T. Fujimura, M. Lucia, D. Cistia, B. Fonseca-santos, K.B. Imamura, P.A.M. Michels, M. Chorilli, M.A.S. Graminha, Nanotechnological Strategies for Treatment of Leishmaniasis — A Review Nanotechnological Strategies for Treatment of Leishmaniasis — A Review, (2017), https://doi.org/10.1166/jbn.2017.2349.
[51] W. Khan, S.S. Sharma, N. Kumar, Bioanalytical method development, pharmacokinetics, and toxicity studies of paromomycin and paromomycin loaded in albumin microspheres, Drug Test. Anal. 5 (2013) 453–460, https://doi.org/10.1002/dta.339.
[52] J. Chakravarty, S. Sundar, Drug resistance in leishmaniasis, J. Global Infect. Dis. 2 (2010) 167–176, https://doi.org/10.4103/0974-777X.62887.
[53] M.H. Kharaji, D. Doroud, T. Taheri, S. Rafati, Drug targeting to macrophages with solid lipid nanoparticles harboring paromomycin: an in vitro evaluation against L. Major and L. Tropica, AAPS PharmSciTech 17 (2015) 1110–1119, https://doi.org/10.1208/s12249-015-0439-1.
[54] A. Khatami, A. Firooz, F. Gorouhi, Y. Dowlati, Treatment of acute Old World cutaneous leishmaniasis: a systematic review of the randomized controlled trials, J. Am. Acad. Dermatol. 57 (2007) 335, https://doi.org/10.1016/j.jaad.2007.01.016 e1-335.e29.
[55] J. El-On, G.P. Jacobs, E. Witztum, C.L. Greenblatt, Development of topical treatment for cutaneous leishmaniasis caused by Leishmania major in experimental animals, Antimicrob, Agents Chemother 26 (1984) 745–751, https://doi.org/10.1128/AAC.26.5.745.
[56] J. El-on, S. Halevy, M.H. Grunwald, L. Weinrauch, M.B. Sheva, Topical treatment of Old World cutaneous leishmaniasis caused by Leishmania major : a double-blind control study, J. Am. Acad. Dermatol. 27 (1992) 227–231, https://doi.org/10.1016/0190-9622(92)70175-F.
[57] G. Krause, A. Kroeger, Topical treatment of American cutaneous leishmaniasis with paramomycin and methylbenzethonium chloride: a clinical study under field conditions in Ecuador, Trans. R. Soc. Trop. Med. Hyg. 88 (1994) 92–94, https://doi.org/10.1016/0035-9203(94)90517-7.
[58] O. Ozgoztasi, I. Baydar, A randomized clinical trial of topical paromomycin versus oral ketoconazole for treating cutaneous leishmaniasis in Turkey, Int. J. Dermatol. 36 (1997) 61–63, https://doi.org/10.1046/j.1365-4362.1997.00022.x.
[59] B.A. Arana, C.E. Mendoza, N.R. Rizzo, A. Kroeger, Randomized, controlled, double-blind trial of topical treatment of cutaneous leishmaniasis with paromomycin plus methylbenzethonium chloride ointment in Guatemala, Am. J. Trop. Med. Hyg. 65 (2001) 466–470, https://doi.org/10.4269/ajtmh.2001.65.466.
[60] A. Asilian, M. Davami, Comparison between the efficacy of photodynamic therapy and topical paromomycin in the treatment of old world cutaneous leishmaniasis: a placebo-controlled, randomized clinical trial, Clin. Exp. Dermatol. 31 (2006) 634–637, https://doi.org/10.1111/j.1365-2230.2006.02182.x.
[61] A. Ben Salah, N. Ben Messaoud, E. Guedri, A. Zaatour, N. Ben Alaya, J. Bettaieb, A. Gharbi, N. Belhadj Hamida, A. Boukthir, S. Chlif, K. Abdelhamid, Z. El Ahmadi, H. Louzir, M. Mokni, G. Morizot, P. Buffet, P.L. Smith, K.M. Kopydlowski, M. Kreishman-Deitrick, K.S. Smith, C.J. Nielsen, D.R. Ullman, J.A. Norwood, G.D. Thorne, W.F. McCarthy, R.C. Adams, R.M. Rice, D. Tang, J. Berman, J. Ransom, A.J. Magill, M. Grogl, Topical paromomycin with or without gentamicin for cutaneous leishmaniasis, N. Engl. J. Med. 368 (2013) 524–532, https://doi.org/10.1056/nejmoa1202657.

[62] M. Grogl, B.G. Schuster, W.Y. Ellis, J.D. Berman, Successful topical treatment of murine cutaneous leishmaniasis with a combination of paromomycin (aminosidine) and gentamicin, J. Parasitol. 85 (1999) 354–359, https://doi.org/10.2307/3285646.

[63] A.D.M. Bryceson, A.H. Moody, A. Murphy, Treatment of 'old world' cutaneous leishmaniasis with aminosidine ointment: results of an open study in london, Trans. R. Soc. Trop. Med. Hyg. 88 (1994) 226–228, https://doi.org/10.1016/0035-9203(94)90308-5.

[64] F.A. Neva, C. Ponce, E. Ponce, R. Kreutzer, F. Moudabber, P. Olliaro, Non-ulcerative cutaneous leishmaniasis in Honduras fails to respond to topical paromomycin, Trans. R. Soc. Trop. Med. Hyg. 91 (1997) 473–475.

[65] A. Asilian, T. Jalayer, M. Nilforooshzadeh, R.L. Ghassemi, R. Peto, S. Wayling, P. Olliaro, F. Modabber, Treatment of cutaneous leishmaniasis with aminosidine (paromomycin) ointment: double-blind, randomized trial in the Islamic Republic of Iran, Bull. World Health Organ. 81 (2003) 353–359.

[66] F. Iraji, A. Sadeghinia, Efficacy of paromomycin ointment in the treatment of cutaneous leishmaniasis: results of a double-blind, randomized trial in Isfahan, Iran, Ann. Trop. Med. Parasitol. 99 (2005) 3–9, https://doi.org/10.1179/136485905x16372.

[67] R.X. Armijos, M.M. Weigel, M. Calvopiña, M. Mancheno, R. Rodriguez, Comparison of the effectiveness of two topical paromomycin treatments versus meglumine antimoniate for New World cutaneous leishmaniasis, Acta Trop. 91 (2004) 153–160, https://doi.org/10.1016/j.actatropica.2004.03.009.

[68] G. Faghihi, R. Tavakoli-kia, Treatment of cutaneous leishmaniasis with either topical paromomycin or intralesional meglumine antimoniate, Clin. Exp. Dermatol. 28 (2003) 13–16, https://doi.org/10.1046/j.1365-2230.2003.01169.x.

[69] B. Shazad, B. Abbaszadeh, A. Khamesipour, Comparison of topical paromomycin sulfate (twice/day) with intralesional meglumine antimoniate for the treatment of cutaneous leishmaniasis caused by L. major, Eur. J. Dermatol. 15 (2005) 85–87.

[70] A. Ben Salah, P.A. Buffet, G. Morizot, N. Ben Massoud, A. Zâatour, N. Ben Alaya, N.B.H. Hamida, Z. El Ahmadi, M.T. Downs, P.L. Smith, K. Dellagi, M. Grögl, WR279,396, a third generation aminoglycoside ointment for the treatment of Leishmania major cutaneous Leishmaniasis: a phase 2, randomized, double blind, placebo controlled study, PLoS Neglected Trop. Dis. 3 (2009) e432, https://doi.org/10.1371/journal.pntd.0000432.

[71] W.R. Ravis, A. Llanos-Cuentas, N. Sosa, M. Kreishman-Deitrick, K.M. Kopydlowski, C. Nielsen, K.S. Smith, P.L. Smith, J.H. Ransom, Y. Lin, M. Grogl, Pharmacokinetics and absorption of paromomycin and gentamicin from topical creams used to treat cutaneous leishmaniasis, Antimicrob. Agents Chemother. 57 (2013) 4809–4815, https://doi.org/10.1128/aac.00628-13.

[72] G.S. Gonçalves, A.P. Fernandes, R.C.C. Souza, J.E. Cardoso, F. De Oliveira-Silva, F.C. Maciel, A. Rabello, L.A.M. Ferreira, Activity of a paromomycin hydrophilic formulation for topical treatment of infections by Leishmania (Leishmania) amazonensis and Leishmania (Viannia) braziliensis, Acta Trop. 93 (2005) 161–167, https://doi.org/10.1016/j.actatropica.2004.10.007.

[73] Isaúde net, Gel produzido pela Fiocruz pode revolucionar tratamento da leishmaniose » Isaúde, (2013) http://www.isaude.net/pt-BR/noticia/33843/ciencia-e-tecnologia/gel-produzido-pela-fiocruz-pode-revolucionar-tratamento-da-leishmaniose accessed April 28, 2019.

[74] J. Soto, P. Fuya, R. Herrera, J. Berman, Topical paromomycin/methylbenzethonium chloride plus parenteral meglumine antimonate as treatment of American cutaneous leishmaniasis: controlled study, Clin. Infect. Dis. 26 (1998) 56–58, https://doi.org/10.1086/520249.

[75] J. El-On, E. Bazarsky, R. Sneir, Leishmania major: in vitro and in vivo anti-leishmanial activity of paromomycin ointment (Leshcutan) combined with the immunomodulator Imiquimod, Exp. Parasitol. 116 (2007) 156–162, https://doi.org/10.1016/j.exppara.2006.12.004.

[76] S. Sundar, T.K. Jha, C.P. Thakur, P.K. Sinha, S.K. Bhattacharya, Injectable Paromomycin for visceral leishmaniasis in India, N. Engl. J. Med. 356 (2007) 2571–2581, https://doi.org/10.1056/NEJMoa0904327.

[77] K.M. Jamil, R. Haque, R. Rahman, M.A. Faiz, A.T.M.R.H. Bhuiyan, A. Kumar, S.M. Hassan, H. Kelly, P. Dhalaria, S. Kochhar, P. Desjeux, M.A.A. Bhuiyan, M.M. Khan, R.S. Ghosh, Effectiveness study of paromomycin IM injection (PMIM) for the treatment of visceral leishmaniasis (VL) in Bangladesh, PLoS Neglected Trop. Dis. 9 (2015) 1–11, https://doi.org/10.1371/journal.pntd.0004118.

[78] J.D. Berman, Human Leishmaniasis : clinical, diagnostic , and chemotherapeutic developments in the last 10 years, Clin. Infect. Dis. 24 (1997) 684–703.

[79] A. Musa, E. Khalil, A. Hailu, J. Olobo, M. Balasegaram, R. Omollo, T. Edwards, J. Rashid, J. Mbui, B. Musa, A.A. Abuzaid, O. Ahmed, A. Fadlalla, A. El-Hassan, M. Mueller, G. Mucee, S. Njoroge, V. Manduku, G. Mutuma, L. Apadet, H. Lodenyo, D. Mutea, G. Kirigi, S. Yifru, G. Mengistu, Z. Hurissa, W. Hailu, T. Weldegebreal, H. Tafes, Y. Mekonnen, E. Makonnen, S. Ndegwa, P. Sagaki, R. Kimutai, J. Kesusu, R. Owiti, S. Ellis, M. Wasunna, Sodium stibogluconate (SSG) & paromomycin combination compared to SSG for visceral leishmaniasis in east africa: a randomised controlled trial, PLoS Neglected Trop. Dis. 6 (2012) e1674, , https://doi.org/10.1371/journal.pntd.0001674.

[80] World Health Organization, Control of the Leishmaniases - WHO Technical Report Series 949, (2010) https://apps.who.int/iris/bitstream/handle/10665/44412/WHO_TRS_949_eng.pdf;jsessionid=A7218CC35C8295AEFF2801689F3D33FB?sequence=1 accessed April 28, 2019.

[81] C. Abongomera, F. Gatluak, J. Buyze, K. Ritmeijer, A comparison of the effectiveness of sodium stibogluconate monotherapy to sodium stibogluconate and paromomycin combination for the treatment of severe post kala azar dermal leishmaniasis in south Sudan - a retrospective cohort study, PloS One 11 (2016) 1–12, https://doi.org/10.1371/journal.pone.0163047 e0163047.

[82] R. Kimutai, A.M. Musa, S. Njoroge, R. Omollo, F. Alves, A. Hailu, E.A.G. Khalil, E. Diro, P. Soipei, B. Musa, K. Salman, K. Ritmeijer, F. Chappuis, J. Rashid, R. Mohammed, A. Jameneh, E. Makonnen, J. Olobo, L. Okello, P. Sagaki, N. Strub, S. Ellis, J. Alvar, M. Balasegaram, E. Alirol, M. Wasunna, Safety and effectiveness of sodium stibogluconate and paromomycin combination for the treatment of visceral leishmaniasis in eastern africa: results from a pharmacovigilance programme, Clin. Drug Invest. 37 (2017) 259–272, https://doi.org/10.1007/s40261-016-0481-0.

[83] R. Rahman, V. Goyal, R. Haque, K. Jamil, A. Faiz, R. Samad, S. Ellis, M. Balasegaram, M. den Boer, S. Rijal, N. Strub-Wourgaft, F. Alves, J. Alvar, B. Sharma, Safety and efficacy of short course combination regimens with AmBisome, miltefosine and paromomycin for the treatment of visceral leishmaniasis (VL) in Bangladesh, PLoS Neglected Trop. Dis. 11 (2017) 1–15, https://doi.org/10.1371/journal.pntd.0005635 e0005635.

[84] S. Hendrickx, M. Van den Kerkhof, D. Mabille, P. Cos, P. Delputte, L. Maes, G. Caljon, Combined treatment of miltefosine and paromomycin delays the onset of experimental drug resistance in Leishmania infantum, PLoS Neglected Trop. Dis. 11 (2017) 1–10, https://doi.org/10.1371/journal.pntd.0005620 e0005620.

[85] G.-J. Wijnant, K. Van Bocxlaer, V. Yardley, S. Murdan, S.L. Croft, Efficacy of paromomycin-chloroquine combination therapy in experimental cutaneous leishmaniasis, Antimicrob. Agents Chemother. 61 (2017) 1–8 e00358-17.

[86] J. Schwartz, E. Moreno, A. Calvo, L. Blanco, C. Fernández-Rubio, C. Sanmartín, P. Nguewa, J.M. Irache, E. Larrea, S. Espuelas, Combination of paromomycin plus human anti-TNF-α antibodies to control the local inflammatory response in BALB/mice with cutaneous leishmaniasis lesions, J. Dermatol. Sci. 92 (2018) 78–88, https://doi.org/10.1016/j.jdermsci.2018.07.005.

[87] D.H. Kim, H.J. Chung, J. Bleys, R.F. Ghohestani, Is paromomycin an effective and safe treatment against cutaneous leishmaniasis? A meta-analysis of 14 randomized controlled trials, PLoS Neglected Trop. Dis. 3 (2009) 1–10, https://doi.org/10.1371/journal.pntd.0000381 e381.

[88] Drugs for Neglected Diseases initiative (DNDi), Diseases & Projects - about Leishmaniasis - Current Treatments, (2019) https://www.dndi.org/diseases-projects/leishmaniasis/leish-current-treatments/ accessed May 1, 2019.

[89] A. Jhingran, B. Chawla, S. Saxena, M.P. Barrett, R. Madhubala, Paromomycin: uptake and resistance in Leishmania donovani, Mol. Biochem. Parasitol. 164 (2009) 111–117, https://doi.org/10.1016/j.molbiopara.2008.12.007.

[90] V. Maravajhala, N. Dasari, A. Sepuri, S. Joginapalli, Design and evaluation of niacin microspheres, Indian J. Pharmaceut. Sci. 71 (2010) 663–669, https://doi.org/10.4103/0250-474x.59549.

[91] C. Tomaro-Duchesneau, S. Saha, M. Malhotra, I. Kahouli, S. Prakash, Microencapsulation for the terapeutic delivery of drugs, live mammalian and bacterial cells, and other biopharmaceutics: current status and future directions, J. Pharm. (Lahore) (2013) 1–19, https://doi.org/10.1155/2013/103527 103527.

[92] M.N. Singh, K.S.Y. Hemant, M. Ram, H.G. Shivakumar, Microencapsulation: a promising technique for controlled drug delivery, Res. Pharm. Sci. Sci. 5 (2010) 65–77.

[93] T.K. Giri, C. Choudhary, Ajazuddin, A. Alexander, H. Badwaik, D.K. Tripathi, Prospects of pharmaceuticals and biopharmaceuticals loaded microparticles prepared by double emulsion technique for controlled delivery, Saudi Pharmaceut. J. 21 (2013) 125–141, https://doi.org/10.1016/j.jsps.2012.05.009.

[94] B.B. Patel, J.K. Patel, S. Chakraborty, D. Shukla, Revealing facts behind spray dried solid dispersion technology used for solubility enhancement, Saudi Pharmaceut. J. 23 (2015) 352–365, https://doi.org/10.1016/j.jsps.2013.12.013.

[95] W. Khan, R. Kumar, S. Singh, S.K. Arora, N. Kumar, Paromomycin-loaded albumin microspheres: efficacy and stability studies, Drug Test. Anal. 5 (2013) 468–473, https://doi.org/10.1002/dta.389.

[96] G.A. Islan, M. Durán, M.L. Cacicedo, G. Nakazato, R.K.T. Kobayashi, D.S.T. Martinez, G.R. Castro, N. Durán, Nanopharmaceuticals as a solution to neglected diseases: is it possible? Acta Trop. 170 (2017) 16–42, https://doi.org/10.1016/j.actatropica.2017.02.019.

[97] A. Momeni, M. Rasoolian, A. Momeni, A. Navaei, S. Emami, Z. Shaker, M. Mohebali, A. Khoshdel, Development of liposomes loaded with anti-leishmanial drugs for the treatment of cutaneous leishmaniasis, J. Liposome Res. 23 (2013) 134–144, https://doi.org/10.3109/08982104.2012.762519.

[98] A. Akbarzadeh, R. Rezaei-sadabady, S. Davaran, S.W. Joo, N. Zarghami, Y. Hanifehpour, M. Samiel, M. Kouhi, K. Nejati-Koshki, Liposome : classification , preparation , and applications, Nanoscale Res. Lett. 8 (2013) 1, https://doi.org/10.1186/1556-276X-8-102.

[99] A. Laouini, C. Jaafar-Maalej, I. Limayen-Blouza, S. Sfar, C. Charcosset, H. Fessi, Preparation, characterization and applications of liposomes: state of the art, J. Colloid Sci. Biotechnol. 1 (2012) 147–168, https://doi.org/10.1166/jcsb.2012.1020.

[100] D. Williams, A.B. Mullen, A.J. Baillie, K.C. Carter, Comparison of the efficacy of free and non-ionic-surfactant vesicular formulations of paromomycin in a murine model of visceral leishmaniasis, J. Pharm. Pharmacol. 50 (1998) 1351–1356, https://doi.org/10.1111/j.2042-7158.1998.tb03358.x.

[101] M.M. Gaspar, S. Calado, J. Pereira, H. Ferronha, I. Correia, H. Castro, A.M. Tomás, M.E.M. Cruz, Targeted delivery of paromomycin in murine infectious diseases through association to nano lipid systems, Nanomed. Nanotechnol. Biol. Med. 11 (2015) 1851–1860, https://doi.org/10.1016/j.nano.2015.06.008.

[102] L.S. Ferreira, G.A. Ramaldes, E.A. Nunan, L.A.M. Ferreira, In vitro skin permeation and retention of paromomycin from liposomes for topical treatment of the cutaneous leishmaniasis, Drug Dev. Ind. Pharm. 30 (2004) 289–296, https://doi.org/10.1081/DDC-120030423.

[103] M.R. Jaafari, N. Bavarsad, B.S.F. Bazzaz, A. Samiei, D. Soroush, S. Ghorbani, M.M.L. Heravi, A. Khamesipour, Effect of topical liposomes containing paromomycin sulfate in the course of Leishmania major infection in susceptible

BALB/c mice, Antimicrob. Agents Chemother. 53 (2009) 2259–2265, https://doi.org/10.1128/AAC.01319-08.
[104] G. Carneiro, D.C.M. Santos, M.C. Oliveira, A.P. Fernandes, L.S. Ferreira, G.A. Ramaldes, E.A. Nunan, L.A.M. Ferreira, Topical delivery and in vivo antileishmanial activity of paromomycin-loaded liposomes for treatment of cutaneous leishmaniasis, J. Liposome Res. 20 (2010) 16–23, https://doi.org/10.3109/08982100903015025.
[105] N. Bavarsad, B.S. Fazly Bazzaz, A. Khamesipour, M.R. Jaafari, Colloidal, in vitro and in vivo anti-leishmanial properties of transfersomes containing paromomycin sulfate in susceptible BALB/c mice, Acta Trop. 124 (2012) 33–41, https://doi.org/10.1016/j.actatropica.2012.06.004.
[106] H. Kalantari, A.A. Hemmati, N. Bavarsad, A. Rezaie, S. Ahmadi, Effect of topical nanoliposomes of paromomycin on rats liver and kidney, Jundishapur J. Nat. Pharm. Prod. 9 (2014) e17565http://www.ncbi.nlm.nih.gov/pubmed/25625051%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4302405.
[107] V. Mishra, K.K. Bansal, A. Verma, N. Yadav, S. Thakur, K. Sudhakar, J.M. Rosenholm, Solid lipid nanoparticles: emerging colloidal nano drug delivery systems, Pharmaceutics 10 (2018) 1–21, https://doi.org/10.3390/pharmaceutics10040191.
[108] M. Geszke-Moritz, M. Moritz, Solid lipid nanoparticles as attractive drug vehicles: composition, properties and therapeutic strategies, Mater. Sci. Eng. C 68 (2016) 982–994, https://doi.org/10.1016/j.msec.2016.05.119.
[109] M. Ghadiri, A. Vatanara, D. Doroud, A.R. Najafabadi, Paromomycin loaded solid lipid nanoparticles: characterization of production parameters, Biotechnol. Bioproc. Eng. 16 (2011) 617–623, https://doi.org/10.1007/s12257-010-0331-5.
[110] M. Kotmakçı, H. Akbaba, G. Erel, G. Ertan, G. Kantarcı, Improved method for solid lipid nanoparticle preparation based on hot microemulsions: preparation, characterization, cytotoxicity, and hemocompatibility evaluation, AAPS PharmSciTech 18 (2016) 1355–1365, https://doi.org/10.1208/s12249-016-0606-z.
[111] K. Manjunath, J.S. Reddy, V. Venkateswarlu, Solid lipid nanoparticles as drug delivery systems, methods find, Exp. Clin. Pharmacol. 27 (2005) 1–20, https://doi.org/10.1358/mf.2005.27.2.876286.
[112] M. Heidari-Kharaji, T. Taheri, D. Doroud, S. Habibzadeh, A. Badirzadeh, S. Rafati, Enhanced paromomycin efficacy by solid lipid nanoparticle formulation against Leishmania in mice model, Parasite Immunol. 38 (2016) 599–608, https://doi.org/10.1111/pim.12340.
[113] M. Heidari-Kharaji, T. Taheri, D. Doroud, S. Habibzadeh, S. Rafati, Solid lipid nanoparticle loaded with paromomycin: in vivo efficacy against Leishmania tropica infection in BALB/c mice model, Appl. Microbiol. Biotechnol. 100 (2016) 7051–7060, https://doi.org/10.1007/s00253-016-7422-y.
[114] M. Mahinroosta, Z. Jomeh Farsangi, A. Allahverdi, Z. Shakoori, Hydrogels as intelligent materials: a brief review of synthesis, properties and applications, Mater. Today Chem. 8 (2018) 42–55, https://doi.org/10.1016/j.mtchem.2018.02.004.
[115] E.M. Ahmed, Hydrogel : preparation , characterization , and applications : a review, J. Adv. Res. 6 (2015) 105–121, https://doi.org/10.1016/j.jare.2013.07.006.
[116] A.M. dos Santos, E.F. Noronha, L.A.M. Ferreira, C.O. Carranza-Tamayo, E. Cupolillo, G.A.S. Romero, Effect of a hydrophilic formulation of topical paromomycin on cutaneous leishmaniasis among patients with contraindications for treatment with pentavalent antimonials, Rev. Soc. Bras. Med. Trop. 41 (2008) 444–448.
[117] S.V. Mussi, A.P. Fernandes, L.A.M. Ferreira, Comparative study of the efficacy of formulations containing fluconazole or paromomycin for topical treatment of infections by Leishmania (Leishmania) major and Leishmania (Leishmania) amazonensis, Parasitol. Res. 100 (2007) 1221–1226, https://doi.org/10.1007/s00436-006-0394-6.
[118] M.G. Aguiar, D.L. Silva, F.A. Nunan, E.A. Nunan, A.P. Fernandes, L.A.M. Ferreira, Combined topical paromomycin and oral miltefosine treatment of mice experimentally infected with Leishmania (Leishmania) major leads to reduction in both lesion size and systemic parasite burdens, J. Antimicrob. Chemother. 64 (2009) 1234–1240, https://doi.org/10.1093/jac/dkp365.
[119] M.G. Aguiar, A.M.M. Pereira, A.P. Fernandes, L.A.M. Ferreira, Reductions in skin and systemic parasite burdens as a combined effect of topical paromomycin and oral miltefosine treatment of mice experimentally infected with Leishmania (Leishmania) amazonensis, Antimicrob, Agents Chemother 54 (2010) 4699–4704, https://doi.org/10.1128/AAC.00809-10.
[120] E. de Morais-Teixeira, M.G. Aguiar, B. Soares de Souza Lima, L.A.M. Ferreira, A. Rabello, Combined suboptimal schedules of topical paromomycin, meglumine antimoniate and miltefosine to treat experimental infection caused by Leishmania (Viannia) braziliensis, J. Antimicrob. Chemother. 70 (2015) 3283–3290, https://doi.org/10.1093/jac/dkv254.
[121] J. Schwartz, E. Moreno, C. Fernández, I. Navarro-Blasco, P.A. Nguewa, J.A. Palop, J.M. Irache, C. Sanmartín, S. Espuelas, Topical treatment of L. major infected BALB/c mice with a novel diselenide chitosan hydrogel formulation, Eur. J. Pharmaceut. Sci. 62 (2014) 309–316, https://doi.org/10.1016/j.ejps.2014.06.010.
[122] D. Plano, Y. Baquedano, D. Moreno-Mateos, M. Font, A. Jiménez-Ruiz, J.A. Palop, C. Sanmartín, Selenocyanates and diselenides: a new class of potent antileishmanial agents, Eur. J. Med. Chem. 46 (2011) 3315–3323, https://doi.org/10.1016/j.ejmech.2011.04.054.
[123] K. Kathe, H. Kathpalia, Film forming systems for topical and transdermal drug delivery, Asian J. Pharm. Sci. 12 (2017) 487–497, https://doi.org/10.1016/j.ajps.2017.07.004.
[124] K. Frederiksen, R.H. Guy, K. Petersson, Formulation considerations in the design of topical, polymeric film-forming systems for sustained drug delivery to the skin, Eur. J. Pharm. Biopharm. 91 (2015) 9–15, https://doi.org/10.1016/j.ejpb.2015.01.002.
[125] S. Karki, H. Kim, S.J. Na, D. Shin, K. Jo, J. Lee, Thin films as an emerging platform for drug delivery, Asian J. Pharm. Sci. 11 (2016) 559–574, https://doi.org/10.1016/j.ajps.2016.05.004.
[126] S. Tolouei, S.J. Hasheminia, M. Narimani, A. Khamesipour, M.A. Shatalebi, S.H. Hejazi, Leishmanicidal activity of films containing paromomycin and gentamicin sulfate both in vitro and in vivo, Iran, J. Parasitol. 6 (2011) 60–65.
[127] S.F. de O. Gomes, E. de A. Nunan, L.A.M. Ferreira, Influence of the formulation type (o/w, w/o/w emulsions and ointment) on the topical delivery of paromomycin, Rev. Bras. Ciencias Farm. 40 (2010) 345–352, https://doi.org/10.1590/s1516-93322004000300010.
[128] I.R.L. Nogueira, G. Carneiro, M.I. Yoshida, R.B. De Oliveira, L.A.M. Ferreira, Preparation, characterization, and topical delivery of paromomycin ion pairing, Drug Dev. Ind. Pharm. 37 (2011) 1083–1089, https://doi.org/10.3109/03639045.2011.559660.