AAPS PharmSciTech (2019) 20:122
DOI: 10.1208/s12249-019-1323-1

Research Article

Amphotericin B-Loaded Emulgel: Effect of Chemical Enhancers on the Release Profile and Antileishmanial Activity *In Vitro*

Iluska Martins Pinheiro,[1,2] Ivana Pereira Santos Carvalho,[1] José Alves Terceiro Neto,[1] Gláucia Laís Nunes Lopes,[1] Elvilene de Sousa Coêlho,[1] Enoque Pereira Costa Sobrinho-Júnior,[1] Michel Mualém de Moraes Alves,[1] Fernando Aécio de Amorim Carvalho,[1] and André Luís Menezes Carvalho[1]

Received 13 September 2018; accepted 30 January 2019

Abstract. Cutaneous leishmaniasis is a neglected parasitic disease. Treatment is preferably performed with pentavalent antimony associated or not with amphotericin B (AmB). This study aimed to develop an emulgel with different chemical enhancers of cutaneous release. Initially, AmB emulsions were obtained with the chemical promoters, oleic acid and geraniol and without promoter, then for the evaluation of the formulations, a preliminary stability study was carried out where the formulations were submitted to centrifugation, before and after the freeze-thaw cycle and analyzed appearance, color, pH, spreadability, viscosity, conductivity, droplet size, assay, *in vitro* release study, *in vitro* antileishmania activity in *Leishmania major* promastigotes, and macrophage toxicity in the MTT test. The emulsions were yellowish, with no signs of instability after the centrifugation test. The pH range corresponded to that of the skin, which is 4.6 to 5.8, before and after the freeze-thaw cycle, the formulations had good spreadability and did not present significant viscosity differences before and after the freeze-thaw cycle, presenting a non-Newtonian characteristic. AmB content was within the kinetic model of zero order release, the formulation of 3% AmB and 5% oleic acid (formulation 1) was chosen to proceed with the antileishmania activity test and showed potential activity against the *in vitro* parasite with significant reduction of cytotoxicity on murine macrophages, indicating that the formulation is promising for the treatment of cutaneous leishmaniasis.

KEY WORDS: amphotericin B-loaded; emulgel; antileishmanial activity.

INTRODUCTION

Leishmaniasis is considered a neglected tropical disease in the world, caused by several species of the flagellate protozoan of the genus *Leishmania*. It is endemic and the most severe form can cause disfigurement and death. Pentavalent antimonial compounds are used as first-line treatment of cutaneous leishmaniasis, but are considered toxic. This treatment has many side effects and a high rate of recidivism (20 to 45%). In addition, the development of resistant strains may render the treatment ineffective (1,2).

Topical drug treatment aims to provide high concentrations of drugs at the site of application in order to avoid adverse systemic effects associated with oral or parenteral administration. Topical administration offers the absence of a first pass effect, is painless, and has ease of application. However, topical efficacy is only obtained from potent drugs to an adequate degree of penetration into the skin. The use of chemical enhancers in topical formulations is intended to promote improvement in drug release (3,4).

The clinical efficacy of a topically applied drug depends not only on its pharmacological properties but also on its availability at the site of action. Organogels have been studied successfully as dermal pharmaceutical forms. Soybean lecithin, because of its surfactant properties, is added to the formulations to solubilize lipophilic ingredients and thereby solubilizes lipids within the stratum corneum (5). This study aimed to develop amphotericin B emulsions with different chemical parameters and perform *in vitro* cytotoxicity assays to verify the potential use of the formulations developed. Evaluation of the *in vivo* leishmanicidal activity of amphotericin B emulgel was performed in another study (6).

MATERIALS AND METHODS

Materials

Amphotericin B deoxycholate (amount = 844 μg/mg, VALDEQUÍMICA, São Paulo, Brazil), isopropyl palmitate and isopropyl myristate (EMBRAFARMA, São Paulo,

[1] Federal University of Piauí, Teresina, Piauí, Brazil.
[2] To whom correspondence should be addressed. (e–mail: iluskasmartins@uol.com.br)

Published online: 25 February 2019

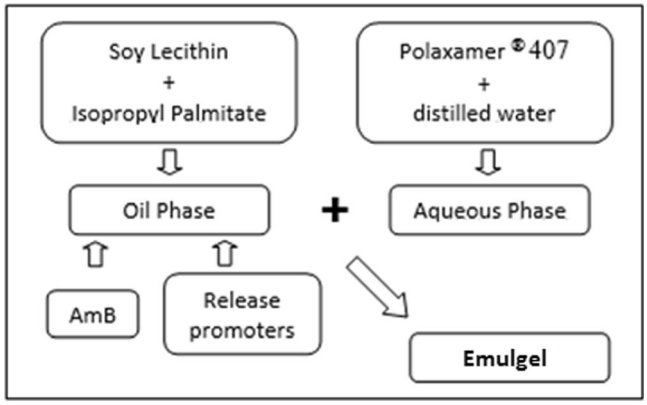

Fig. 1. Emulsion preparation scheme

Brazil), polaxamer 407® (GALENA, São Paulo, Brazil), Schneider's culture medium, RPMI medium (Fetal bovine serum (SFB), MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-dihydro-benzoic acid), and geraniol (SIGMA-ALDRICH) Bromo diphenyltetrazolium, Alamar blue (Resazurin), the antibiotics Penicillin and Streptomycin were purchased from Sigma Chemical (Sigma-Aldrich Brazil).

Formulating Emulgel

Initially, the polaxamer 407 gel was prepared, where the polymer was weighed and placed to swell under refrigeration for 24 h (overnight). To prepare the oil phase, soybean lecithin and isopropyl palmitate or isopropyl myristate were mixed in a 1:1 ratio and reserved. After 24 h of the oily and aqueous phases were prepared separately, the AmB was added in the oil phase, and finally, the addition of the aqueous phase under stirring until the formation of the emulsifier occurred, the chemical promoters were added to the phase oily when applicable. After 24 h of the preparation of the formulations, homogenization and stabilization of the formulations were observed macroscopically (Fig. 1).

Several AmB emulsion formulations were prepared in quantitative and qualitative planning according to Table I.

Preliminary Stability Evaluation (Freeze-Thaw Cycle)

Initially, the samples were submitted to the centrifugation test (3000 rpm/30 min), using 1 g of sample. Then, the preliminary stability of the test samples was evaluated in order to verify the behavior of the formulation by means of extreme variations of temperature, in order to accelerate possible reactions between its components. For this, the six samples were submitted to 6 cycles of 24 h at 45 ± 2°C, and 24 h at − 5 ± 2°C for 12 days and another eight remained at room temperature (25.0 ± 2.0°C). Before and after the cycles, the formulations were analyzed for appearance, color and pH value, spreadability, viscosity, conductivity, AmB content, and droplet size by optical microscopy (7,8).

Appearance, Color, and pH Value. The prepared emulgel formulations were inspected visually for their appearance, color, and pH. The pH of the emulgels was measured by a pH meter, brand Marte, at a working temperature of 25 ± 1°C (9).

Spreadability. The determination of spreadability was performed according to the methodology previously described in the literature (10). A base plate of glass was used in the equipment, positioned on a scale millimeter paper and a light source. The results were expressed as scatterability of the sample as a function of the applied weight, according to the equation below:

$$Ei = \frac{d^2 x \pi}{4}$$

Ei is the spreadability (mm^2) of the sample for a given weight (g), and *d* is the mean diameter (mm).

Viscosity. Viscosity determination was performed using the Quimis digital rotary viscometer, model Q-860M21, using Spindle three (SP = 3) at rotations 6, 12, 30, and 60 rpm (11).

Conductivity. The test was carried out using the conductivity meter, brand Marte, model MB-11, in triplicate (6).

Assay. It was performed in UV-VIS spectrophotometry, brand Shimadzu, model UV1800, in a previously validated method. The wavelength of AmB quantification is 405 nm (12).

Droplet size by optical microscopy. The size and size distribution of emulgel droplets were evaluated by light microscopy using a micrometer ocular lens. At least 100 droplets/sample were considered (13).

Table I. Details and composition of the 6 emulgel formulations

Materials	Uses	Amount in emulgel				
		F1	F2	F3	F4	F5
Soy lecithin + Isopropyl palmitate	Oil phase	30%	30%	30%	30%	–
Soy lecithin + Isopropyl myristate	Oil phase	–	–	–	–	30%
Oleic acid	Release enhancer	5%	–	–	5%	–
Geraniol	Release enhancer	–	5%		–	5%
Amphotericin B	Active ingredient	3%	3%	3%	3%	3%
Polaxamer gel 20% quality sufficient for	Aqueous phase	62%	62%	62%	62%	62%

Drug Release Studies. In vitro release kinetics was performed using Franz diffusion cells in the divisional area of 1.5 cm^2, volume ± 12 mL, and artificial cellulose acetate membranes. The receiver compartment was filled with phosphate buffer pH 7.4 in a system composed of six individual cells bound to a thermostated bath at 37 ± 0.5°C under constant stirring at 100 rpm for a period of 6 h. 400 mg of the formulation was used in the recipient compartment. The collections (3 mL) were performed at the times: 0.5; 1.0; 1.5; 2.0; 4.0; and 6.0 h. The amount of drug released into the recipient chamber was quantified by spectrophotometry using a previously validated analytical method (8).

Analysis of Kinetic Models. The release study data were adjusted to different kinetic models in order to evaluate the model that best adapts to the results obtained. The zero order model was used: $Q = Q0 + K0\ t$; Higuchi's model: $Qt = kH(t)^{0.5}$; and the first order model:ln $Qt = \ln Q0 + K1\ t$, where Q is the amount of drug remaining to be released, $Q0$ is the initial amount of drug, k is the release rate constant, and t is the time.

Statistical Analysis. GraphPad Prism version 5.0 program was used for all statistical analyses. Student's t test was used to compare the means between two formulations, always comparing the samples before and after the freeze-thaw cycle. $P < 0.05$ was considered significant.

Evaluation of Antileishmanial Activity In Vitro

Obtaining Cells and Parasites. The *Leishmania major* strain (MHOM IL/80/Friendlin) was used for evaluation of the antileishmanial activity. The promastigote forms were cultured in supplemented Schneider's medium (10% fetal bovine serum—FBS), 10,000 IU penicillin and 1000 IU streptomycin, incubated in a B.O.D. oven at 26°C. Murine macrophages were collected from the peritoneal cavities of male and female BALB/c mice (4 to 5 weeks of age), after previous (72 h) elicitation by application of 2 mL of 3% thioglycollate intraperitoneally. The experiments were approved by the Committee on Ethics in Animal Experimentation of the Federal University of Piauí on 05/05/2017 through the Opinion/Resolution no. 265/16.

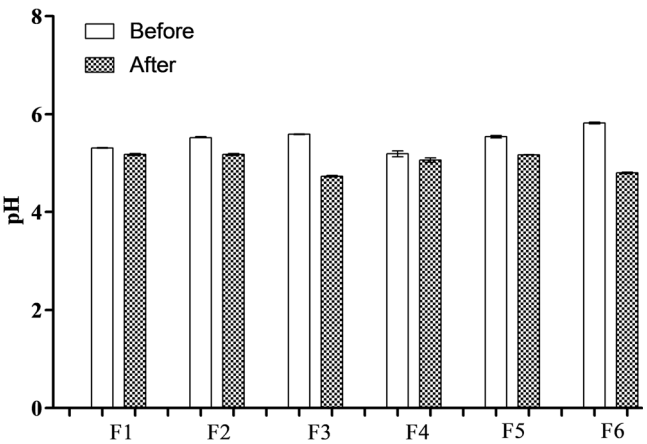

Fig. 2. Graph of the pH determination of the formulations before and after the freeze-thaw cycle

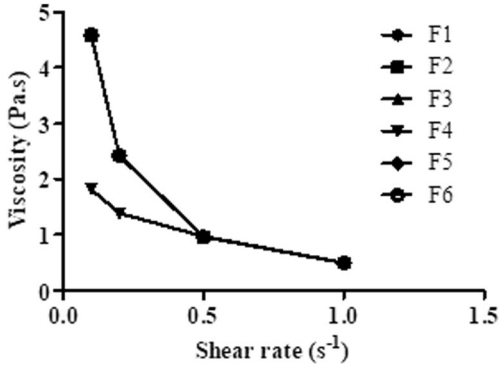

Fig. 3. Graph of the viscosity determination of the formulations before the freeze-thaw cycle

The Antileishmanial Activity of Promastigotes Forms of L. major. The assay was performed with promastigote forms of *L. major* in the log phase of growth. The parasites were seeded in 96-well cell culture plates containing Schneider's medium supplemented in the amount of 1×10^6 *Leishmania*/100 μL of the medium containing the placebo and 3% AmB + oleic acid 5% formulations previously added to the wells in triplicate and serial dilutions were achieved, reaching eight ranges of desired final concentrations (800 to 6.25 μg/mL). The plate was incubated in a biochemical oxygen demand oven (BOD) at 26°C for 48 h, remaining 6 h at the end of this period, 20 μL of 1×10^{-3} mol/L resazurin was added, when incubated again at the board. Plaque reads are performed on a Biotek 31 absorbance plate reader (model ELx800) at wavelength 550 nm, and the results were expressed in terms of inhibition of growth (%) (1,14). The positive control was performed with 2 μg/mL AmB in Schneider's medium and the negative control was equivalent to Schneider's medium alone. In this case, 100% viability was considered for the parasites (1,15).

Determination of Cytotoxicity

Macrophage Cytotoxicity and Selectivity Index (SI). Evaluation of cytotoxicity was performed in 96-well

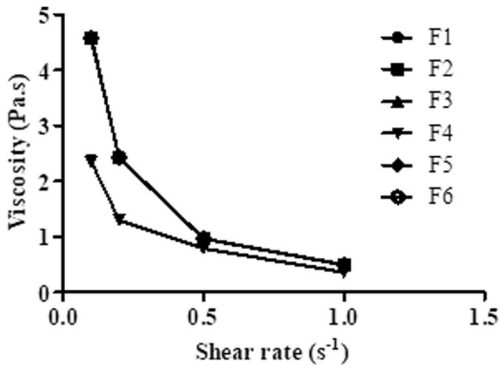

Fig. 4. Graph of the viscosity determination of the formulations after the freeze-thaw cycle

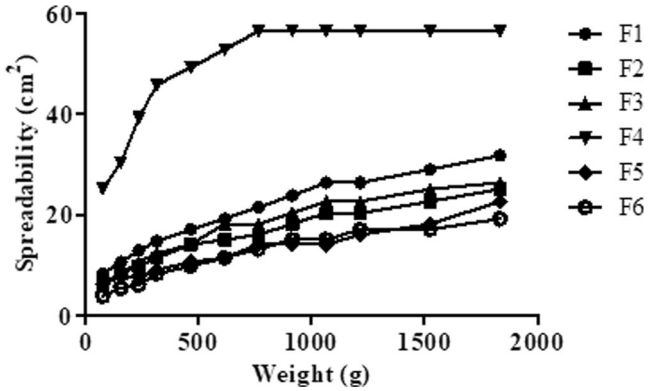

Fig. 5. Graph of the spreadability of the formulations before the freeze-thaw cycle

plates using the MTT assay. 2×10^5 macrophages per well were incubated in 100 μL RPMI 1640 medium (supplemented with 10% FBS, 10,000 IU penicillin, and 1000 IU streptomycin) in a 37-°C incubator and 5% CO2 for 4 h for cell adhesion. After this time, the supernatant was removed for removal of nonadherent cells. The formulation without enhancer and 3% AmB + oleic acid formulations were diluted in supplementing RPMI medium added to the plate containing the macrophages in serial concentrations reaching eight ranges of final concentrations (800 to 6.25 μg/mL) and incubated at 37°C and 5% CO2 for 48 h. After this time, cytotoxicity was assessed by the addition of 10% MTT [5 mg/mL] diluted in 100 μL of supplementing RPMI medium and the plate was incubated again for 4 h at 37°C and 5% CO2. After this time, the supernatant was discarded and the formazan crystals were dissolved by the addition of 100 μL of DMSO. Finally, the absorbance (550 nm) was measured using a Biotek plate reader (ELx800). The selectivity index of each treatment was calculated by dividing the mean cytotoxic concentration (CC50) observed in peritoneal macrophages of BALB/c mice by the mean inhibitory concentration (IC50), calculated for the different forms of parasite presentation (14).

Statistical Analysis. All assays were performed in triplicate in three independent experiments. The mean inhibitory concentration (IC50) and mean cytotoxic concentration (CC50) with a 95% confidence limit were calculated using probit regression from the SPSS 13.0 program. The selectivity index was calculated by dividing the CC50 by the IC50.

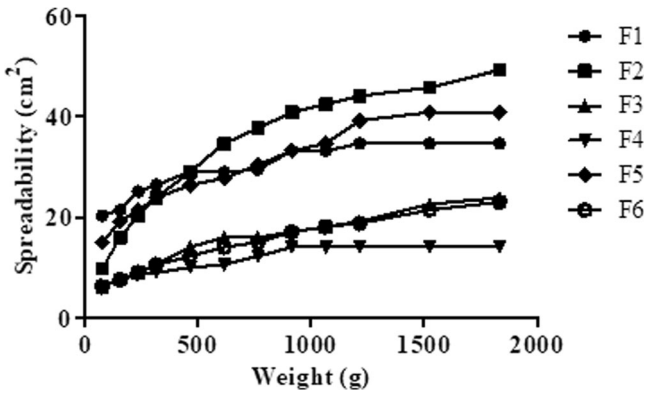

Fig. 6. Graph of the spreadability of the formulations after the freeze-thaw cycle

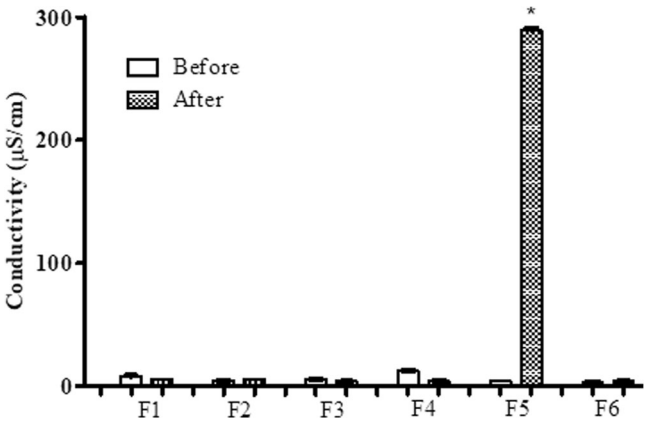

Fig. 7. Graph of the conductivity of the formulations before and after the freeze-thaw cycle. * statistically significant difference for $p < 0.05$, using Student t test, comparing the samples before and after the freeze thaw cycle

Analyses of variance ANOVA followed by the Bonferroni test were performed using the GraphPad Prism version 5.0 program, taking the value of $p < 0.05$ as the maximum level of statistical significance.

RESULTS AND DISCUSSION

Obtaining Emulgel from Amphotericin B

The amphotericin B formulations produced were yellowish, glossy, and viscous sensory typical of an emulgel. Emulgel is an emerging system for topical drug administration; are water-in-oil or oil-in-water emulsions which are filled by mixing a gelling agent. It is a suitable vehicle for hydrophobic drugs; has a high acceptability to patients since it has the advantages of gel and emulsion, such as thixotropic, easily removable, and emollient (15,16).

After 24 h of preparation of the formulations, the macroscopic analysis did not show any alteration of the organoleptic characteristics. All the formulations were homogeneous, of yellow color, and characteristic odor, being sent to the preliminary stability tests.

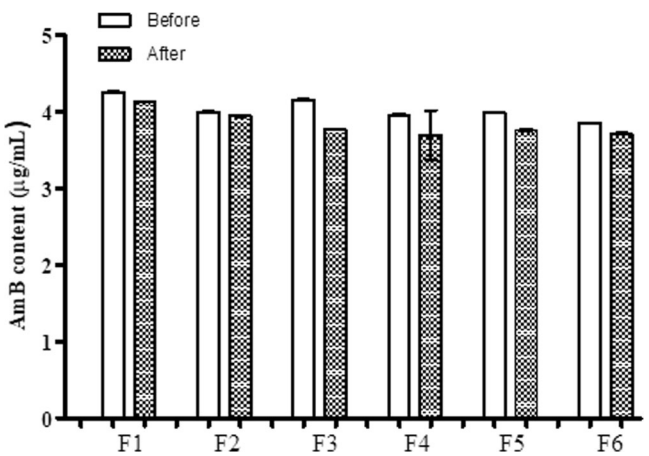

Fig. 8. Graph of the AmB content of the formulations before and after the freeze-thaw cycle

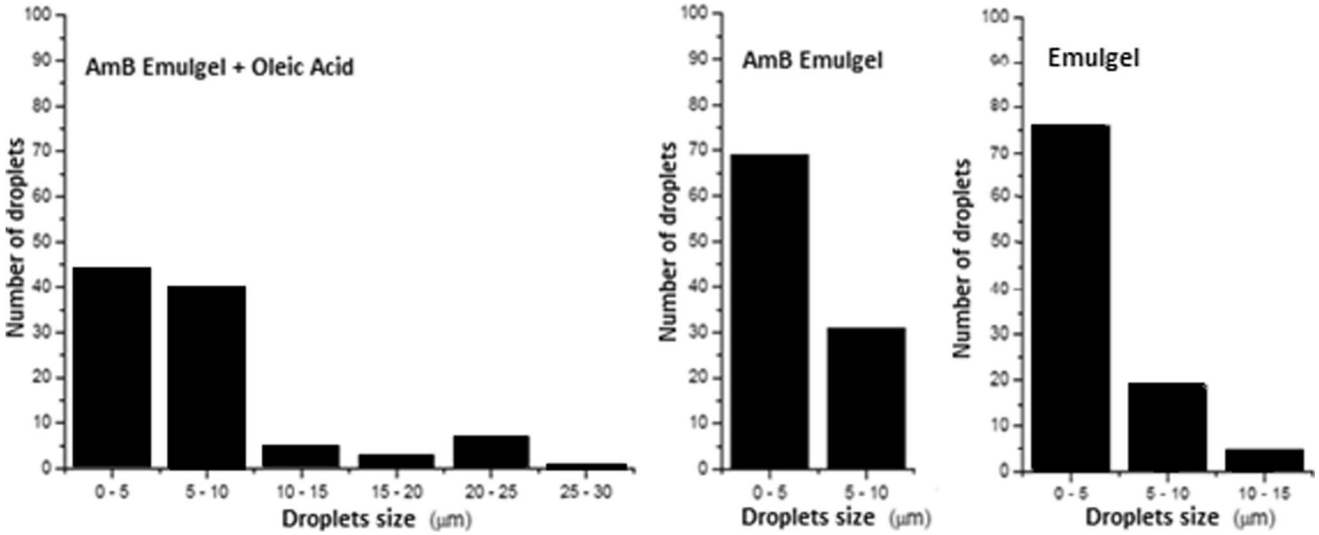

Fig. 9. Droplet size distribution (μm) by number of droplets observed for AmB formulations in the presence or not of chemical enhancer

Preliminary Stability

The stability test represents withdraw: "a crucial and" crucial stage in the development and evaluation of medicinal products since the instability of a pharmaceutical formulation or preparation modifies essential requirements such as quality, safety, and efficacy (17,18). Therefore, the formulations were subjected to extreme temperature and centrifugation conditions in order to accelerate possible processes of instability in the formulation.

The first test performed to evaluate the preliminary stability of the formulations with the exclusive character was the centrifugation. After the emulgel formulations were submitted to centrifugal force, no disruption of the polymer chain of the emulgel was observed, that is, all formulations were approved in the centrifugation test. However, the absence of signs of instability in the emulgel does not ensure its stability, but only indicates that the product could be submitted, without reformulation, to other stress conditions, such as the freeze-thaw cycle (19).

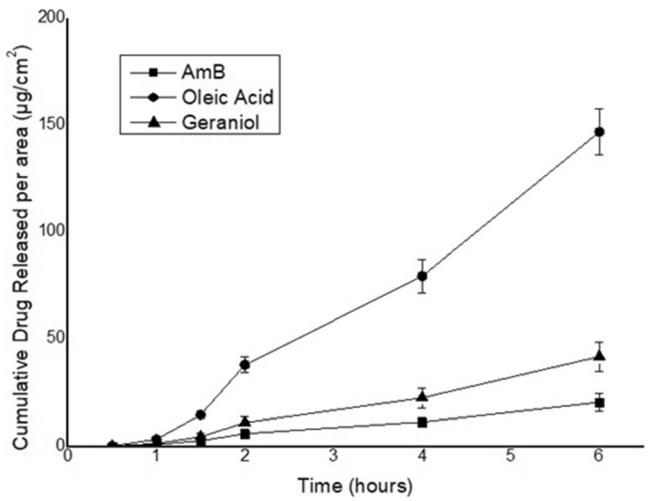

Fig. 10. Graph of the amphotericin B release kinetics with the different chemical enhancers (oleic acid and geraniol)

After the samples were submitted to the freeze-thaw cycle test and at a centrifugal force of 3000 rpm for 30 min, formulations 2, 3, 4, 5, and 6 showed phase separation, that is, the test freeze-thaw cycle caused the desulphurization of the emulgel polymer chain in these formulations.

The pH values obtained by direct potentiometry of the emulgel samples before and after the freeze-thaw cycle were plotted in Fig. 3. There was no significant variation of pH in formulation 1, which indicates an initial compatibility of the components of the formulation (Fig. 1). The pH of the skin is slightly acidic and may range from 4.6 to 5.8 (15,20,21). All formulations presented pH values compatible with the pH of the skin, proving to be promising for topical use (Fig. 2).

The viscosity of the formulation is another factor that affects the release of the drug since it can reduce the rate of diffusion thereof, *i.e.*, There is an inverse proportionality between the viscosity and the release rate. The higher the viscosity, the lower the diffusion of the drug (22). According to Figs. 3 and 4, we observe that the viscosities of the formulations reduce as the speed of rotation of the equipment increases. This is characteristic of non-Newtonian systems (23).

In order for a body to flow, a force called shear force must be exerted on it, which will generate a deformation gradient. This is dependent on the internal resistance of the substance. According to viscosity, we can divide the fluids into two classes (according to their properties of fluids and deformation): Newtonian and non-Newtonian. For Newtonian fluids, the viscosity value will be constant regardless of the shear velocity (or stress). As for non-Newtonian fluids, viscosity varies, as it depends on several factors such as substance structure and resting time (24).

Semisolid preparations undergo strong influences on scattering and adhesion in the skin, extrusion of the packages, and release of active principles (25). The spreadability test determines changes in the area of product coverage and may facilitate or hinder the application of the product (26). The spreadability tests performed showed that the relationship between the areas and the limit stress of the prepared emulgel were statistically the same. This confirms the results observed in the viscosity tests.

Table II. Kinetic parameters of the release profile of the Amb for different chemical enhancers in different kinetic models, zero order, Higuchi, and first order and cumulative drug released in 6 h (Q6h)

Chemical enhancer	Zero order			Higuchi			First order		
	J	r^2	Kl	J	r^2	Kl	J	r^2	kl
AmB without enhancer	3.65	0.989	3.0×10^{-4}	13.86	0.953	1.2×10^{-2}	17.01	0.856	1.4×10^{-3}
Oleic Acid	27.26	0.987	2.2×10^{-3}	103.60	0.943	8.6×10^{-3}	127.18	0.838	1.1×10^{-2}
Geraniol	7.71	0.988	6.4×10^{-4}	29.30	0.944	2.4×10^{-3}	35.95	0.840	3.0×10^{-3}

Q_{6h} cumulative drug release in 6 h, r^2 determination coefficient, J flow (μg/cm^2/h)

When looking at Figs. 5 and 6, it is evident that in formulation 4, there is a greater change in the occupied area, showing its instability. The data concerning the spreadability are important, because if the formulation produced has a better surface area occupied regardless of the applied force, it can be used more pleasantly at the time of its application on the injured skin and in smaller quantity, leading to a reduction of the consumption of this preparation with the same therapeutic efficacy (19).

Only formulation 5 presented a statistically significant difference in the conductivity analysis after freeze-thaw cycle for $p < 0.05$. A large variation in conductivity may be related to loss of formulation stability (Fig. 7).

According to Fig. 8, it is observed that, on the content, there was a statistically significant difference only for formulation 4 after the freeze-thaw cycle, considering $p < 0.05$.

In fact, phase separation in this formulation was visible after the freeze-thaw cycle and even after shaking with a glass stick and then weighing the sample to perform analysis of the content, it was still heterogeneous macroscopically.

To analyze droplet size and distribution, the most stable formulation of the study was chosen. According to Fig. 9, it is observed that the formulations had a number of droplets distributed basically in two sizes of droplets (0 to 5 μm and 5 to10 μm). Photomicroscopic evaluations showed the presence of spherical globules. The characterization of the droplet diameter has been used in the investigation of the physical stability. Instability phenomena of emulsified systems such as aggregation, flocculation, and coalescence can be monitored through the evolution of the mean diameter as well as the droplet size distribution (19).

In fact, the droplet size is one of the important parameters in the evaluation of the physical stability of the formulation, since the phenomena like flocculation and coalescence can be monitored by the evolution of the average diameter as well as the distribution of this. The incorporation of drugs can influence the droplet diameter and distribution (20,27,28).

The use of *in vitro* drug release assays has assumed increasing importance as it allows the control of the production and the finished product and validates changes made in the product, after commercialization, as well as modifications introduced during the processes of increase of the scale of production. The release studies are also of fundamental importance for determining the behavior of the drug in relation to the vehicle and the system in which it is incorporated, allowing to verify even the existence of the interaction between the active and the components of the formulation (29).

Among the tests carried out, in the kinetics of *in vitro* release by means of Franz cells, it was observed that the formulation containing oleic acid at 5% presented the highest AmB release being stable after the freeze-thaw cycle (Fig. 10).

Terpenes are a popular choice for chemical enhancers in transdermal drug delivery studies. The effect of a specific terpene on the skin depends on its exact physicochemical properties, in particular, its lipophilicity. In general, smaller terpenes with non-polar groups are better skin chemical enhancers such as oleic acid (30). Oleic acid is a chemical enhancer because it reversibly induces a temporary increase in skin permeability. Studies report their action from the 1% concentration (31). Considering the analyzed parameters, it was observed that the formulations have different release profiles. It can be observed in Table II that all the formulations presented a better adaptation to the mathematical model of order zero. This model can be used to describe the diffusion of the drug from various types of

Table III. Antileishmania activity, cytotoxic effects on mammalian cells, and calculated selectivity index values for Pbc, AmB, AmB 3% with oleic acid 5%

Substances	Promastigotes CI_{50}μg/mL	Macrophages CC_{50}μg/mL	Selectivity index IS
Pcb	> 800	> 800	ND
Amb3% oleic acid 5%	17,164	735,041	42,822
AmB	1742[a]	8750[a]	5022[a]

ND not determined
[a] ALVES *et al.*, 2017

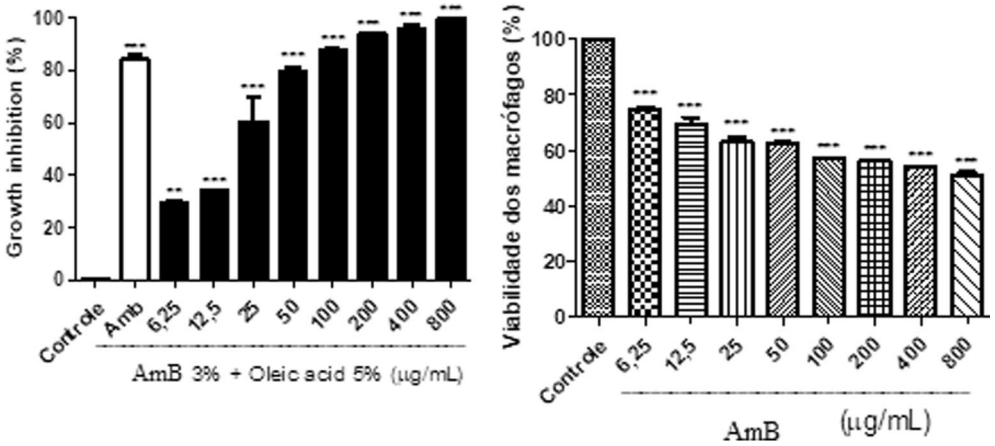

Fig. 11. Antileishmania and cytotoxic activity of the formulation AmB 3% oleic acid 5% on promastigotes and *L. major* forms. The parasites (1×10^6) were exposed to different concentrations of the substances. AmBc was used as a positive control at the concentration of 2 µg/mL. Macrophages were plated (2×10^5) and exposed to serial concentrations of the formulation. One-way ANOVA was performed, followed by the Bonferroni multiple comparison post-test, assuming the * $p < 0.05$ *vs.* control; ** $p < 0.01$ *vs.* control; *** $p < 0.001$ *vs.* control. The results represent the mean ± SEM of the experiments performed in triplicate

modified release dosage forms, as in the case of some transdermal systems. Pharmaceutical dosage forms following this profile release the same amount of drug per unit of time and are the ideal method of drug release in order to achieve a prolonged pharmacological action (32).

Thixotropic products tend to have a longer shelf life because during storage they have a constant viscosity, which makes it difficult to separate the constituents of the formulation. In addition, they deform during application, become more fluid facilitating the spreading and recovering the viscosity at the moment of the application (33). Formulation 1, which contains oleic acid as a chemical enhancer, presented with characteristics indicating better physicochemical stability.

Taking into account the *in vivo* studies performed, the use of oleic acid as a chemical promoter improved the therapeutic response, corroborating with the results of the kinetic study of AmB release (6).

After the physical-chemical tests and the *in vitro* release study, the formulation that presented the best results was the 3% AmB formulation with 5% oleic acid, where the oil phase was composed of soybean lecithin and isopropyl palmitate (formulation 1). *In vitro* antileishmania activity tests proceeded with this formulation.

Antileishmanial Activity on Promastigote Forms of *L. major*

The formulation containing AmB 3% and oleic acid 5% inhibited the growth of promastigotes of *L. major* in a concentration-dependent manner. 100% inhibition of the concentration of 800 µg/mL was observed after 48 h of incubation, resulting in an IC 50 value of 17.164 µg/mL. Positive control AmB at 2 µg/mL inhibited about 84% of parasite growth with IC50 value of 1.742 µg/mL (1).

According to Table III, in evaluating the cytotoxicity on peritoneal macrophages of BALB/c mice, the formulation of 3% AmB and 5% Oleic acid significantly reduced the toxicity compared to AmB. In this study, we obtained a value of CC50 for the formulation of AmB 3% oleic acid 5% 735.041 µg/mL, while the value of CC50 for AmB is 8.750 µg/mL (1).

The selectivity index was evaluated, our formulation was about eight times more selective for the parasites than for the macrophages, whereas the selectivity of AmB is only 5.022. One of the major drawbacks of treatment of the disease with AmB is the side effects that this drug produces (34,35) and one of the reasons for these effects is the lack of drug selectivity. In this study, a topical formulation based on 3% AmB and 5% oleic acid, which was able to reduce the cytotoxicity of the conventional drug and yet with potential activity against the parasite (Fig. 11), was developed.

CONCLUSION

It is concluded that amphotericin B emulgel presents better physicochemical stability when associated with oleic acid as a release enhancer, was better adapted to a zero order kinetic model, indicating a controlled release profile, where it is independent of concentration and presents potential activity against the parasite *in vitro* with a significant reduction of cytotoxicity on murine macrophages, indicating that the formulation is promising as a new therapeutic alternative for cutaneous leishmaniasis.

ACKNOWLEDGMENTS

The authors are grateful to the Leishmania Laboratory of the Center for Research in Medicinal Plants and the Pharmacy-School of the UFPI.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest No potential conflict of interest was reported by the authors.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Alves MMM, Brito LM, Souza AC, Queiroz BCSH, Carvalho TP, Batista JF, *et al.* Gallic and ellagic acids: two natural immunomodulator compounds solve infection of macrophages by Leishmania major. Naunyn-Schmiedeberg's Arch Pharmacol. 2017;390:893–903. https://doi.org/10.1007/s00210-017-1387-y.
2. Mendonça DVC, Lage LMR, Lage DP, Chavez-Fumagalli MA, Ludolf F, Roatt BM, *et al.* Polaxamer 407 (Pluronic® F127) - based polymeric micelles for amphotericin B: in the vitro biological activity, toxicity and in vivo therapeutic efficacy against murine tegumentary leishmaniasis. Exp Parasitol. 2016;169:34–42. https://doi.org/10.1016/j.exppara.2016.07.005.
3. Perez AP, Altube MJ, Schilrreff P, Apezteguia G, Celes FS, Zacchino S, *et al.* Topical amphotericin B in ultradeformable liposomes: formulation, skin penetration study, antifungal and antileishmanial activity in vitro. Colloids Surfaces B Biointerfaces. 2015;139:190–8. https://doi.org/10.1016/j.colsurfb.2015.12.003.
4. Goh CF, Lane ME. Formulation of diclofenac for dermal delivery. Int J Pharm. 2014;473:607–16. https://doi.org/10.1016/j.ijpharm.2014.07.052.
5. Zhang Q, Song Y, Page SW, Garg S. Evaluation of transdermal drug permeation as modulated by lipoderm and pluronic lecithin organogel. J Pharm Sci. 2017;107(2):587–94. https://doi.org/10.1016/j.xphs.2017.09.008.
6. Pinheiro IM, Carvalho IP, Carvalho CES, Brito LM, Silva ABS, Conde Júnior AM, *et al.* Evaluation of the in vivo leishmanicidal activity of amphotericin B emulgel: an alternative for the treatment of skin leishmaniasis. Exp Parasitol. 2016;164:49–55. https://doi.org/10.1016/j.exppara.2016.02.010.
7. Parsaee S, Sarbolouki MN, Parnianpour M. In-vitro release of diclofenac diethylammonium from lipid-based formulations. Int J Pharm. 2002;241:185–90. https://doi.org/10.1016/S0378-5173(02)00238-7.
8. Jain A, Deveda P, Vyas N, Chuhan J, Khambete H, Jain S. Development of antifungal emulsion-based gel for topical fungal infection(s). Int J Pharm Res Devp. 2011;2:18–22.
9. Suresh, *et al.* Development and in vitro characterization of piroxicam loaded emulgel for topical delivery. Ijppr. Human, v. 2, n. 3, 2015. ISSN:2349–7203.
10. Knorst MT. Desenvolvimento tecnológico de uma forma farmacêutica plástica contendo extrato concentrado deAchyrocline satureoides (lam.)Compositae-marcela. Porto Alegre, 1991. 228p. [Dissertação de Mestrado -Faculdade de Farmácia - Universidade Federal do Rio Grande do Sul].
11. Antunes AEC, Liserre AM, Faria EV, Yotsuyanagi K, Lerayer ALS. Analise descritiva quantitativa de buttermilk probiotico. Alim Nutr. 2012;23(4):619–30.
12. Coêlho ES, Lopes GLN, Pinheiro IM, Holanda JNP, Alves MMM, Nogueira NC, et al. Emulgel based on amphotericin B and bacuri butter (Platonia insignis Mart.) for the treatment of cutaneous Leishmaniasis: characterization and in vitro assays. Drug Dev Ind Pharm. 2018;44:1–11. https://doi.org/10.1080/03639045.2018.1492610.
13. Perioli L, Pagano C, Mazzitelli S, Rossi C, Nastruzzi C. Rheological and functional characterization of new antiinflammatory delivery systems designed for buccal administration. Int J Pharm. 2008;356(1):19–28. https://doi.org/10.1016/j.ijpharm.2007.12.027.
14. Valadares DG, Duarte MC, Oliveira JS, Chávez-Fumagalli MA, Martins VT, Costa LE, et al. Leishmanicidal activity of the Agaricus blazei Murill in different Leishmania species. Parasitol Int. 2011;60:357–63. https://doi.org/10.1016/j.parint.2011.06.001.
15. Oliveira LGC, Brito LM, Alves MMM, Amorim LV, Sobrinho-Júnior EPC, Carvalho CES, et al. In vitro effects of the neolignan 2,3-dihydrobenzofuran against leishmania amazonensis. Basic Clin Pharmacol Toxicol. 2017;120(1):52–8. https://doi.org/10.1111/bcpt.12639.
16. Ajazuddin AA, Gupta S, Patel RJ, Giri TK, Tripathi DK. Recent expansions in an emergent novel drug delivery technology: emulgel. J Control Release. 2013;171(2):122–32. https://doi.org/10.1016/j.jconrel.2013.06.030.
17. Sigh P, Sharma G, Balla R, Gill NS. Emulgel: an emerging technique for topical drug delivery system. IJRAPR. 2015;5(1):1–8 ISSN: 2230–9306.
18. Khunt DM, Mishra AD, Shah DR. Formulation design & development of piroxicam emulgel. Int J Pharmtech Res. 2012;4:1332–44 ISSN: 0974-4304.
19. Krongrawa W, Limmatvapirat S, Pongnimitprasert N, Meetam P, Limmatvapirat C. Formulation and evaluation of gels containing coconut kernel extract for topical application. Asian J Pharm Sci 2018; Accepted Manuscript,494. https://doi.org/10.1016/j.ajps.2018.01.005.
20. Varma VNSK, Maheshwari PV, Navya M, Reddy SC, Shivakumar HG, Gowda DV. Calcipotriol delivery into the skin as emulgel for effective permeation. Saudi Pharm J. 2014;22(6):591–9. https://doi.org/10.1016/j.jsps.2014.02.007.
21. Gonçalves GMS, Campos PMBGM. Aplicação de métodos de biofísica no estudo de eficácia de produtos desmocosméticos. Braz J Pharm Sci. 2009;45:1–10. https://doi.org/10.1590/S1984-82502009000100002.
22. Baibhay J, Gurpreet S, Rana AC, Seema S. Development and characterization of clarithromycin emulgel for topical delivery. Int J Drug Dev Res. 2012;4:310–23.
23. Yener G, Dal O, Üner M. Effect of vehicles on release of meloxicam from various topical formulations. Open Drug Deliv J. 2009;3:19–23. https://doi.org/10.2174/1874126600903010019.
24. Yassin GE. Formulation and evaluation of optimized clotrimazole emulgel formulations. Br J Pharm Res. 2014;4(9):1014–30. https://doi.org/10.9734/BJPR/2014/8495.
25. Lahoud MH, Campos R. Aspectos teóricos relacionados à reologia farmacêutica. Visão Acadêmica. 2010;11:65–73. https://doi.org/10.5380/acd.v11i1.21356.
26. Silva KER, Alves LDS, Soares MFR, Passos RCS, Faria AR, Rolim Neto PJ. Modelos de avaliação da estabilidade de fármacos e medicamentos para a indústria farmacêutica. Revista de Ciências Farmacêuticas Básica e Aplicada.2009; 30:129–135. ISSN:1808–4532.
27. Cordeiro MSF, Costa JKB, Lima CG, Campelo Junior JDC, Melo AFM. Desenvolvimento tecnológico e avaliação de estabilidade de gel dermatológico a partir do óleo essencial de gengibre (Zingiber officinalleRoscoe). Rev Bra Farm. 2013;94:148–53.
28. Lin Y, Tsai M, Fang Y, Huang Y, Wu P. Microemulsion formulation design and evaluation for the hydrofobic compound: catechin topical applications. Colloids Surf B: Biointerfaces2018;161: 121–128. https://doi.org/10.1016/j.colsurfb.2017.10.015.
29. Mendonça CC, Silva ICL, Rodrigues KA, Campos MAL, Medeiros MCM, Casteli VC *et al.* Emulsões O/A contendo Cetoconazol 2, 0%: avaliação da estabilidade acelerada e estudos de liberação *in vitro*. Rev. Ciênc. Farm. Básica Apl. 2009;30(1):35-46
30. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via the synergistic action of chemicals. Biochim Biophys Acta. 2009;1788(11):2362–73.
31. Meenaksh D. Emulgel: a novel approach to topical drug delivery. Int J Pharma Bio Sci. 2013;4:847–56. https://doi.org/10.1016/j.bbamem.2009.08.015.
32. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;13:123–33. https://doi.org/10.1016/S0928-0987(01)00095-1.
33. Loch CR, De Marco MG, Schwedersky MB, Haas SE. Avaliação físico-química e determinação do comportamento reológico de emulsões de cetoconazol 2% comercializados em farmácias magistrais no município de Erechim/RS. Rev Bras Farm. 2011;92:299–305.
34. Alizadeh BH, et al. Leishmanicidal evaluation of novel synthetic chromenes. Arch Pharm (Weinheim). 2008;341:787–93. https://doi.org/10.1002/ardp.200800128.
35. Carvalho NB, Prates FVO, Silva RC, Dourado MEF, Amorim CF, Machado PRL, et al. In vitro immunomodulatory activity of a transition-state analog inhibitor of human purine nucleoside phosphorylase in cutaneous leishmaniasis. J Immunol Res. 2017;2017:1–6. https://doi.org/10.1155/2017/3062892.