REPORT

Solubility of amphotericin B in water-lecithin-dispersions and lecithin-based submicron emulsions

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Abstract: The aim of this work was to evaluate water-lecithin-dispersions (WLDs) as carriers for Amphotericin B (AmB) and to compare the drug solubility in WLDs and O/W lecithin-based submicron emulsions (SMEs) in order to evaluate the influence of lecithin content on the dosage form solubilization of the active compound. WLDs and different SMEs with either 1.2 or 2.4% of lecithin were prepared. WLD with 2.4% lecithin show a 10-fold increase in solubilization of AmB compared with 1.2% lecithin WLD. SMEs with 1.2% lecithin show an increase of over 400 times in solubilization compared with WLD containing the same concentration of lecithin, whereas SMEs with 2.4% lecithin show an increase of over 40 times compared with the corresponding WLD. Drug solubilization in SMEs with 2.4% lecithin is not significantly greater than in those containing 1.2% lecithin. The content of surfactant Brij 97® had a significant influence on drug solubilization in SMEs ($P<0.05$). Results indicate that indicate that SMEs are proper systems to solubilize AmB. It can be assumed that solubilization is due to the formulation microstructure and not to the separate components themselves.

Keywords: lecithin, dispersions, submicron emulsion, Amphotericin B

INTRODUCTION

Water-lecithin-dispersions (WLDs) were studied as potential drug carriers by Sznitowska *et al.* (2002) Lecithin is a widely used excipient due to its excellent bio-compatibility and WLDs are easy preparation systems obtained by dispersing lecithin in water or in an isotonic aqueous solution with means of extensive mixing at 40-60°C in order to obtain good hydration of lecithin. Neither special manufacturing procedure, nor additional lipids and surfactants are used (Sznitowska *et al.*, 2002).

Submicron emulsions (SMEs) and nanoemulsions can be defined as fine emulsions with mean droplet diameters ranging from 50 to 1000 nm. They are made from GRAS surfactants ("Generally recognized as safe" by US Food and Drug Administration), and can be easily produced in large quantities by shear methods (Shah *et al.*, 2010; Ebrahim *et al.*, 2008). Much attention has been given recently to the use of lipid SMEs in drug delivery because of their ability to incorporate drugs with poor solubility within the oil phase, which could provide a high drug loading efficiency without the need for potentially toxic excipients. Furthermore, emulsions prepared by homogenization are preferable for industrial-scale methods because it is a cost-effective and technically simple approach and it offers a more physically stable and safer product than products obtained by solvent mixture. Lipid emulsions can enhance the solubilization, stabilize the incorporated drugs and also may have sustained-release and targeting effects (Peng *et al.*, 2010; Sznitowska *et al.*, 2001; Akkar *et al.*, 2004). Micro-emulsions and SMEs have been greatly investigated in the last years for their common use as vehicles for topical administration (Fang *et al.*, 2004; Pappinen and Urtti, 2006; Azeem *et al.*, 2009).

Leishmaniasis is an endemic disease in more than 70 countries caused by parasites of the genus *Leishmania* spp. *Cutaneous leishmaniasis* is the most prevalent clinical form of the disease. There is a need for topical dosage forms for the treatment of this pathology as monotherapy or associated with oral therapies (WHO Report. 118th reunion). Amphotericin B (AmB), a polyene-type antifungal with antileishmanial activity, is one of the few drugs available for the treatment of this disease but parenteral delivery of AmB is associated with several disadvantages such as hemolysis and nephrotoxicity (Lemke *et al.*, 2005). Currently, the drug is commercially available as mixed micellar formulation, liposomes and lipid complexes. Although liposomes and lipid complexes have succeeded in reducing the adverse-effects of AmB, the cost of these formulation restrict their clinical utility. On the contrary, mixed micellar formulation is cost-effective but could not improve the tolerability of the AmB (Dupont *et al.*, 2002; Date *et al.*,

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Table 1: Composition of oil -in-water lecithin-based submicron emulsions; IPM (isopropyl myristate)

Components	%								
	SME1	SME2	SME3	SME4	SME5	SME6	SME7	SME8	SME9
Phosfolipon 90G®	1.2	1.2	1.2	1.2	1.2	1.2	2.4	2.4	2.4
Brij 97®	20	20	20	25	25	25	25	25	25
IPM	2.8	3.0	3.2	2.8	3.0	3.2	2.8	3.0	3.2
Distilled water qs	100	100	100	100	100	100	100	100	100

2008). Hence, it would be useful to count with a low-cost topical formulation of AmB to avoid the toxic effects caused by its parenteral administration.

Previous reports have mentioned the use of soybean lecithin (20% w/w phosphatidilcholine content) for AmB microemulsions (Moreno *et al.*, 2003). Based on bibliography, in the present work WLDs and oil-in-water lecithin-based SMEs with lecithin of 90% w/w phosphatidilcholine content were prepared. The main objective of the experiments was to evaluate WLDs as easily prepared carriers for AmB and secondly to compare WLDs and SMEs drug loading to evaluate the influence of lecithin content of these dosages forms on AmB solubilization, with no adjustment of pH condition. These results are the basis for future research for design, preparation and characterization of other biocompatible lipid-based formulations, therefore a preliminary stability evaluation was carried out.

MATERIALS AND METHODS

Materiales

Amphotericin B was a kind gift from Unifarma SA, Argentina. Lecithin (Phospholipon 90G®) was purchased from Lipoid, Germany; Polyoxyethylene (10) oleyl ether (BRIJ 97®) was kindly supplied by Croda LA; isopropyl miristate (IPM) and all the other reagents were of pharmaceutical grade.

Methods

Excess of AmB in IPM and Brij 97® was shaken at room temperature for 72 hs. After that time the mixtures were filtered through 0.45µm PTFE filter (Millipore). In order to determine solubility of the drug in the excipients an aliquot of the filtrate was diluted with methanol and drug concentration was determined by UV spectrophotometry (UV-VIS Spectrophotometer Shimadzu UV-260) at 406 nm, using a calibration curve of AmB in methanol covering the range 0.5-10 µg/ml (correlation factor 0.9995-1.000) using methanol as blank. Assays were done in triplicate.

WLDs were obtained by adding lecithin to distilled water and stirring at 60°C with a magnetic stirrer followed by a high shear mixer (Ultra Turrax® T25 Basic, IKA LABORTECHNIK, Germany). Oil-in-water lecithin-based SMEs were prepared by dispersing lecithin in distilled water at 60°C with a magnetic stirrer. Then the corresponding weights of IPM and Brij 97® were mixed and added to the still warm aqueous lecithin dispersion while stirring with a high shear mixer at 16000 rpm for 2 minutes. The mixtures were allowed to stabilize for a few minutes until clear and transparent isotropic mixtures were observed. Centrifugation test was performed for 20 min at 3,500 rpm to evaluate whether the systems separated. AmB was added in excess to SMEs and WLDs and shaken for 24 h at room temperature protected from light. The formulations were filtered and the solubilized drug was evaluated as mentioned above.

The droplet size distribution and average droplet size of SMEs were measured using a NanoZetasizer -zs (Malvern Instrument, Malvern, UK). No dilution of the samples was made for the test. Stability studies of loaded SMEs, including drug concentration, refractive index (Officine Galileo Refractometer), and observation of macroscopic aspect were carried out for a month at room temperature and at 4ºC.

STATISTICAL ANALYSIS

Data were analyzed statistically by unpaired T-student Test, two-tailed using GraphPad InStat (level of significance for $p<0.05$).

RESULTS

The maximum concentration of surfactant was set at 25%. The selected formulations are shown in table 1, systems with 2.4% lecithin and 20% Brij 97® failed to form SMEs according to the adopted criteria. WLD with 2.4% lecithin showed a 10-fold increase in solubilization of AmB compared with 1.2% lecithin WLD (4.47 and 0.44 µg/mL, respectively); pH of WLDs was 6.3 and 4.9 for 1.2% and 2.4 % respectively. Solubility of AmB in the other components of the SMEs was very low (< 0.5 µg/mL and 8.02µg/mL for IPM and Brij 97®, respectively). Drug loaded in SMEs is shown in Fig. 1. The resulting pH of SMEs was 3.23-4.00. In that condition, SMEs with 1.2% lecithin showed an increase over 40 times in solubilization compared with WLD containing the same concentration of lecithin, whereas SMEs with 2.4% lecithin showed an increase over 400 times. However, solubilization capacity of SMEs with 2.4% lecithin was not significantly greater than that of SMEs containing

Table 2: Droplet size of blank and loaded submicron emulsions (SME and SMA, respectively), after 1 month of storage at room temperature

Formulation	Zave (d.nm)	SD	PDI	SD	Formulation	Zave (d.nm)	SD	PDI	SD
SME1	7.145	0.010	0.236	0.047	SMA1	6.939	0.307	0.226	0.038
SME2	7.261	0.122	0.258	0.013	SMA2	7.480	0.114	0.280	0.009
SME3	7.433	0.090	0.215	0.012	SMA3	7.796	0.311	0.263	0.025
SME4	6.271	0.190	0.210	0.011	SMA4	5.886	0.132	0.599	0.172
SME5	6.143	0.783	0.285	0.072	SMA5	6.700	0.132	0.300	0.009
SME6	7.874	1.383	0.293	0.037	SMA6	7.306	0.542	0.323	0.017
SME7	6.576	0.195	0.370	0.037	SMA7	7.064	0.394	0.505	0.043
SME8	6.278	1.046	0.371	0.007	SMA8	13.672	1.919	0.409	0.014
SME9	6.025	0.854	0.409	0.014	SMA9	15.663	8.932	0.410	0.029

1.2% lecithin, considering same proportions of the other components (unpaired t-Student Test, $P<0.05$). Solubilization by SME containing the highest proportion of oil phase (2.4% lecithin and 3.2% IPM) was not significantly different than SME containing the lowest proportion of oil phase (1.2% lecithin and 2.8% IPM), both containing 25% of surfactant (unpaired t-Student Test, $P<0.05$). Solubilization potentials of SMEs containing 25% of Brij 97® were significantly greater than those of SMEs containing 20% of the surfactant, considering same proportions of the other components (unpaired t-Student Test, $P<0.05$).

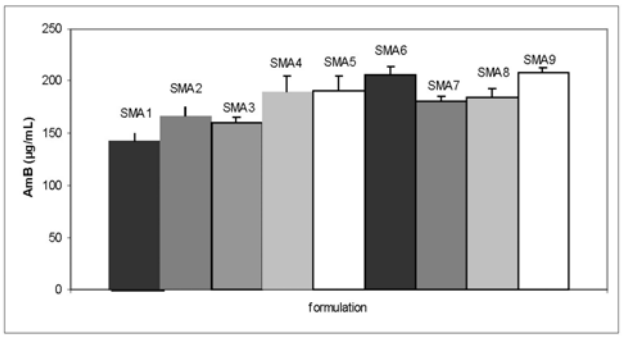

Fig. 1: Submicron emulsions drug loading. AmB: Amphotericin B.

Fig. 2 shows drug content in loaded SMEs (SMA) after a month of storage both at room temperature and 4ºC; in all cases drug content remained more stable at 4ºC. SMA1, SMA4 and SMA5 were the ones that retained 90% for at least 15 days without visible changes in the dosage form. Both blank and loaded formulations refractive indexes were: water < MEs < glycerin (Water 1.3338/ glycerin 1.4672) at time of preparation and after a month. Droplet size by intensity (Zave and PDI) is reported in table 2. SMA8 and SMA9 had larger droplet size and greater dispersion; they were the first to become unstable and more viscous. The systems remained macroscopically stable during the study except for SMA6, SMA8 and SMA9 which became more viscous after a month in the fridge.

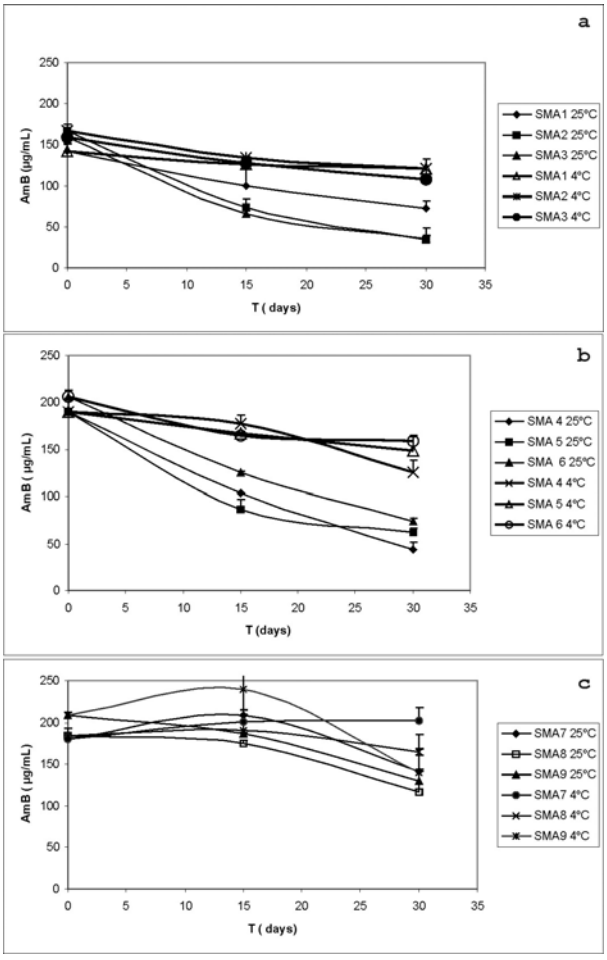

Fig. 2: Stability of submicron emulsions loaded with Amphotericin B (SMA; AmB).
Ratio lecithin/Brij97®: **a**: 1.2/20, **b**: 1.2/25 and **c:** 2.4/25

UV spectra of AmB in distilled water and methanol, and spectrum of diluted SMA1 in distilled water are shown in fig. 3.

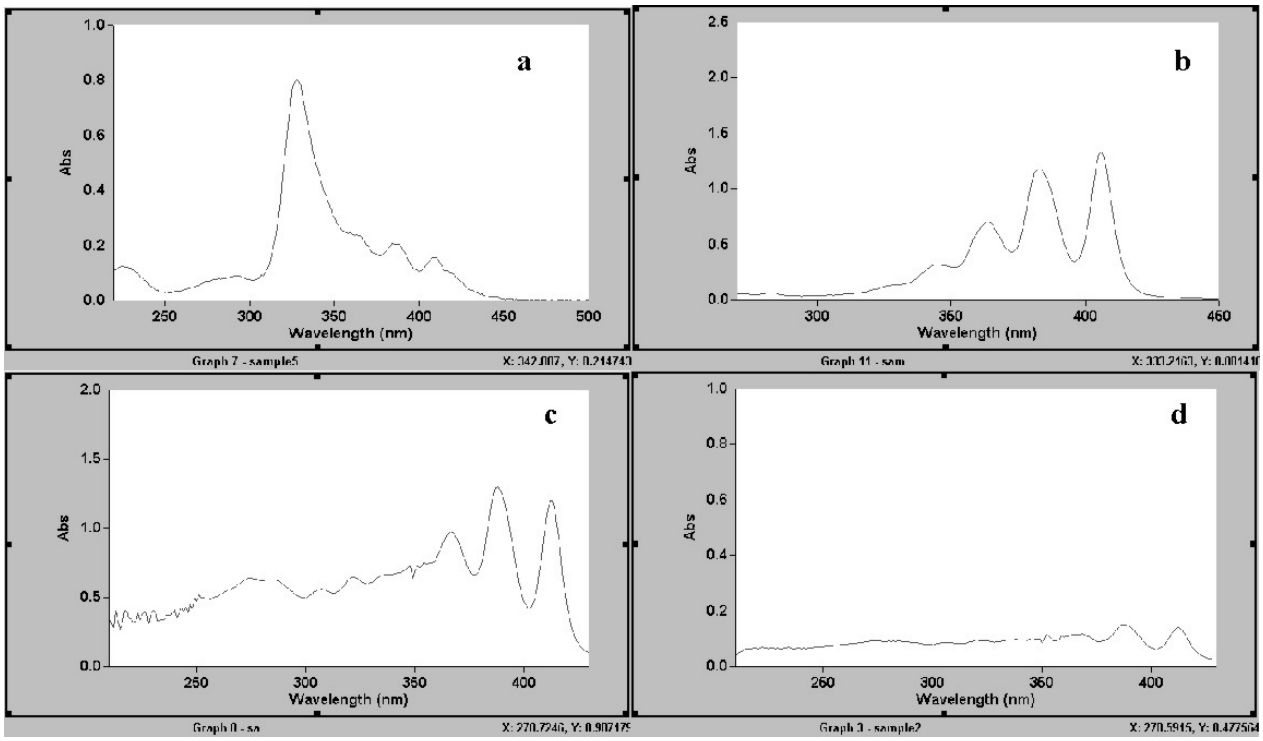

Fig. 3: **a** Amphotericin B 10 µg /ml in distilled water (drug self-aggregated), **b** Amphotericin B 10 µg/ml en Methanol **c** SMA1 1/10 in distilled water and **d** SMA1 second dilution 1/10 in distilled water

DISCUSSION

In order to compare, WLD 1.2 and 2.4% and different SMEs with either 1.2 or 2.4 % of lecithin were prepared. WLDs showed no major improvement in drug solubility when compared with drug solubility in water and there were no differences in the solubilization potential of SMEs related to lecithin concentrations assayed, showing that lecithin has no relevant potential to solubilize AmB. It can be assumed that enhanced solubilization in SMEs is due to the formulation microstructure and not to the separate components because drug solubility in these formulations is greater than just additive solubility given by the components. Moreover drug loading increased with the main surfactant content, which is supposed to enlarge the interface area where AmB is expected to be located because of its amphiphilic structure (Pestana *et al.*,2008). It has been reported the solubilization capacity of the high interface area of these type of systems (Azeem *et al.*, 2009).

Additionally, UV spectra showed that the drug was not aggregated in SME even after dilution with water, which is an important fact as major adverse effects of the drug are related to the aggregate state (Yu *et al.*, 1998; Vandermeulen *et al.*, 2006).

In conclusion WLDs showed to have poor solubilization capacity for the drug under study. Results indicate that SMEs are proper systems to solubilize AmB. Further studies are required to evaluate these SMEs as potential compounding topical formulation.

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