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Amphiphilic association systems for Amphotericin B delivery

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Abstract

The present study describes the production and characterization of amphiphilic association systems for Amphotericin B (AMB). In particular, three different classes of microemulsions and different monoglyceride–water systems were produced. Formulations were characterized for macroscopic aspect, pH, rheology, mean size and size distribution, both in the absence and in the presence of AMB.

AMB solubility was investigated in the different formulations by HPLC studies. The formulations increased AMB solubility up to 20-fold with respect to the single oil and aqueous phases employed for microemulsion production.

AMB diffusion studies from two microemulsions taken as models were performed in a Franz cell system using a nylon membrane.

The physical and chemical stability of AMB-containing amphiphilic association systems were investigated for three months after production. For physical stability studies both the macroscopic aspect, droplet mean size and dimensional distribution were analysed. For chemical stability studies, the AMB content of the formulations was quantified by HPLC analysis. Microemulsions and monoglyceride–water systems were free from phase separation for up to three months and in some cases the AMB content was unchanged even after three months.

Keywords: Amphotericin B; Microemulsions; Monoglyceride–water systems Franz cell

1. Introduction

Candida albicans is a ubiquitous fungus normally found on the skin, in the stomach, colon, vagina, rectum, mouth and throat. *Candida* causes health problems only when there is an overgrowth in one of these areas of the body. When this organism proliferates, it may produce symptomatic infections of the mouth, intestines, vagina, or skin (Rex et al., 2000). Among people abusing intravenous drugs, *Candida* infections can lead to heart valve inflammation. Vaginitis caused by *Candida* (commonly called trush) often afflicts women on birth control pills or antibiotics (Sobel et al., 1998). Trush is a common early symptom of HIV disease, usually occurring in the mouth and/or vagina. It can become a serious problem if it is left untreated. People with AIDS can also develop trush deeper within their bodies in windpipe, oesophagus or lungs (Sobel et al., 2001).

Amphotericin B (AMB) is a broad spectrum antifungal agent mainly used for the treatment of invasive fungal infections (Sperry et al., 1998). Due to its low solubility, formulation of this polyene antibiotic until now had been realized by means of a mixed-micellar dispersion with sodium deoxycholate (Fungizone®, Bristol-Myers Squibb, USA) often causing serious

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side effects, or by different lipid-based pharmaceutical formulations, involving highly cost complex manufacturing methods (Lopez-Berestein, 1987; Guo and Working, 1993; Janoff et al., 1993; Bekersky et al., 1999; Andres et al., 2001). The development of new pharmaceutical formulations for the topical administration of AMB might therefore be desirable (Moreno et al., 2001).

Microemulsions are isotropic liquid systems thermodynamically transparent homogeneous systems comprising a polar phase, an organic phase (non-miscible with the first one), surfactant and co-surfactant. Their formation is spontaneous. These characteristics are related to the specific microstructure of these systems, which comprise microdroplets of dispersed phase (15–200 nm), in a continuous phase. Microemulsions can be defined as dynamic structures in which the interface is spontaneously and continuously fluctuating (Lam and Schrecter, 1987; Kreilgaard et al., 2000). As a function of components ratio, microemulsion can be constituted by normal, reverse swollen micelles, or by a bicontinuous structure.

One of the most interesting microemulsion features is the solubilization power. Microemulsions in fact are able to solubilize hydrophilic, lipophilic and amphiphilic drugs (Cortesi et al., 1997; Kreilgaard et al., 2000). It has been demonstrated that microemulsions-based formulations enable to increase transdermal delivery of drugs with respect to conventional vehicles, as a function of microemulsion constituents (Bonina et al., 1995; Dreher et al., 1997; Schmalfuss et al., 1997).

Another interesting drug delivery system is typified by water insoluble swelling lipids, such as monoglycerides. In the presence of water, monoglycerides can form various mesophases such as reverse micellar, lamellar, hexagonal and cubic phases, as a function of water content and/or temperature (Shah et al., 2001). In particular, the cubic phase is constituted of curved three-dimensional bicontinuous bilayers, separating two congruent networks of water channels (D'Antona et al., 2000). Due to their peculiar structure, cubic phases are able to control the release of drugs with different size and solubility (Burrows et al., 1994). Nevertheless, the extremely high viscosity of the cubic phase limits its use to specific administration ways, such as the periodontal, the mucousal and the vaginal routes (Shah et al., 2001). In order to solve this drawback, an alternative is represented by the use of low viscous liquid crystal phases, precursor to the cubic one, easy to handle at room temperature and to administer (Engström et al., 1992). After in vivo administration, monoglyceride–water systems are able to (a) adhere to mucosa due to their bioadhesive properties, (b) swell in contact with biologic fluids undergoing a transition to the cubic phase, and consequently (c) increase their viscosity, generating a stiff delivery system able to control drug release.

Goal of the present study was to develop formulations stable and easy to prepare for the topical administration of AMB. In particular, the present paper describes (a) the production of different microemulsions and monoglyceride–water systems especially designed for the administration of lipophilic drugs; (b) the characterization of the formulations in term of macroscopical appearance, pH, dimensional distribution and viscosity; (c) the determination of AMB content; (d) the evaluation of AMB diffusion coefficients by Franz diffusion cell; and (e) the chemical and physical stability studies until three months from production of formulations.

2. Materials and methods

2.1. Materials

Tween 80, Span 80, Isopropylpalmitate and mineral oil were purchased from Fluka Chemical (Riedel-de Haen, Switzerland). Isopropylmiristate, Plurol Isostearique® (isostearic acid ester of polyglycerol, containing 30–35% of diglycerol, 20–25% of triglycerol, 15–20% of tetraglycerol and 10% of pentaglycerol and higher oligomers), Labrasol® (a mixture consisting of 30% mono-, di- and triglycerides of C8 and C10 fatty acids, 50% of mono- and diesters of poly(ethylene glycol) (PEG 400) and 20% of free PEG 400), Transcutol P® (diethylene glycol monoethyl ether), Solubilizant γ 2420® (a mixture consisting of polyoxyethylene octylphenyl ether and polyoxyethylene sorbitan monolaurate (20 E.O.)) and Isostearyl Isostearate were purchased from Gattefossè (Lyon, France). Isopropanol and Amphotericin B were from Sigma Chemical Company (St. Louis, USA). Soy phosphatidyl choline, Phospholipon® 90 was purchased from Nattermann Phospholipid GmbH

(Cologne, Germany). Distilled glyceryl monooleate, Myverol 18-99® was from Quest International (Lindtseedijk, Holland).

The same batch of the microemulsion components was used in all experiments. Solvents were of HPLC grade and all other chemicals were of analytical grade.

2.2. Production of microemulsions

Three different classes of microemulsions were produced using different compositions of biocompatible ingredients.

All microemulsions were produced with double-distilled water in order to avoid surface-active impurities.

Twelve different microemulsions were obtained varying the ratios between surfactant/cosurfactant and the amount of oil phase.

Production of microemulsions was performed spontaneously by admixing appropriate quantities of the component and drug with gentle mixing at room temperature.

AMB loaded microemulsions were protected from the light by storing in dark-brown bottles wrapped with aluminium foil.

2.3. Production of monoglyceride–water systems

Monoglyceride–water systems were produced by adding different amounts of water (namely 0, 0.1, 0.25, 0.5, 1, 1.5 and 2%, w/w) to melted monoglycerides at 42 °C. When a uniform mixture was formed under stirring, the containers were sealed, to avoid water evaporation, and placed in an oven for 24 h. In the case of AMB containing formulations, after production drug was added to the systems and maintained under stirring for 72 h.

2.4. Characterization of formulations

Microemulsions and monoglyceride–water systems were characterized in terms of macroscopic aspect, lack of birefringence, pH, viscosity and droplets dimensional distribution both in placebo and in drug containing formulations.

Organoleptic properties and transparency were optically evaluated.

Isotropy was evaluated by an inverted Nikon optical microscope equipped with a device for observing birefrangent structures composed of a 90° revolving polarizer.

pH measurements were performed by a pH meter Senton 1001 (Integrated Sensor Technology, New York, USA).

Rheology analyses were performed by a rheometer SR200 (Rheometrics, Possum Town, New York, USA) at room temperature.

Dimensional distributions were determined by Zetasizer 3000 PCS (Malvern Instr., Malvern, England) equipped with a 5 mW helium neon laser with a wavelength output of 633 nm. Glassware was cleaned of dust by washing with detergent and rinsing twice with water for injections. Measurements were made at 25 °C at an angle of 90°. Data were interpreted using the Contin software (Malvern Instr., Malvern, England). All analyses were done in triplicate.

2.5. Analysis of Amphotericin B content

Solubility of AMB in microemulsions, in monoglyceride–water systems and in the single aqueous and organic phases was determined by saturating each formulation with an excess of AMB. The obtained mixtures were maintained under stirring at room temperature for 72 h.

AMB content in the produced formulations was evaluated by extraction of drug with centrifugation cycles (15 min at 6000 rpm) followed by RP-HPLC chromatographic analysis.

A Hypersil BDS-ultrasphere C18 column (25 cm × 0.46 cm) stainless steel packed with 5 mm particles was eluted at room temperature with a mobile phase consisting of a mixture of sodium acetate 0.1 M, pH 4/acetonitrile, 60:40 v/v, at a flow rate of 1.2 ml/min. UV-Vis detector was set at 405 nm. A 20 μl sample of receptor phase was injected into the liquid chromatograph and quantified by an AMB standard of known concentration. Analyses were conducted in triplicate, mean and standard deviations values were calculated.

2.6. Drug diffusion studies

AMB diffusion kinetics were determined by a Franz diffusion cell (1 cm diameter orifice, 0.78 cm^2 area) assembled with a Nylon membrane (pores 0.45 μm). The receptor phase was a mixture of phosphate buffer 60 mM pH 7.4 and methanol (80:20, v/v). The upper

part of the chamber was sealed to avoid evaporation. The receptor phase was stirred by means of a constantly spinning bar magnet and thermostated at 37 °C.

One millilitre of AMB organic solution or 1 g of the form to be analysed were placed into the donor cell compartment and tamped down on the membrane, previously moistened with the receptor phase. At predetermined time intervals comprised between 1 and 8 h, samples (0.15 ml) of receptor phase solution were withdrawn and the AMB concentration in the receptor phase was measured using HPLC. Each removed sample was replaced with an equal volume of simple receptor phase.

The calculated AMB concentrations were plotted as a function of time and the diffusion coefficients were computed from the linear portion of the accumulation curve, and expressed both as experimentally observed fluxes (J_o) and as normalized fluxes J_n ($J_n = J_o/C$, where C is the AMB concentration in the analysed form, expressed in mg/ml). All the obtained permeation rates were determined six to eight times in independent experiments and the mean values ± standard deviations were calculated.

2.7. Stability studies

Physical and chemical stability studies were conducted in triplicate at 0, 1, 2 and 3 months from formulations production.

Physical stability studies were performed analysing macroscopic aspect (phase separation, turbidity and macroscopic viscosity) under visual inspection and droplets dimensional distribution by PCS.

Chemical stability was evaluated on drug loaded formulations, stored at 4 °C, determining AMB content by HPLC analyses.

Log (AMB residual content, %) was plotted against time and the slopes (m) were calculated by linear regression.

The slopes (m) were then substituted into the following equation for the determination of k values:

$$k = m \times 2.303 \quad (1)$$

Shelf life values (the time for 10% loss, t_{90}) were then calculated by the following equation:

$$t_{90} = 0.105/k \quad (2)$$

as reported by Wells (1988).

3. Results and discussion

3.1. Production of microemulsions

The preformulatory study enabled to obtain 12 true microemulsions in term of homogeneity, transparency and optical isotropy.

In particular, three different classes of formulations based on different constituents were produced.

The first class was based on the use of the couple Labrasol and Plurol as surfactant and cosurfactant. The second class was characterized by the presence of lecithin as surfactant ("LEC" microemulsions), while the third class was based on the use of the couple Tween 80-Span 80 ("TS" microemulsion). In addition, the first class was divided in two subcategories, namely "LPI" (Table 1) and "TL" (Table 2).

For "LPI" formulations, the use of different ratios of Labrasol/Plurol (1:1, 2:1 and 3:1, w/w) led to the production of water in oil microemulsions. A Labrasol/Plurol ratio 2.5:1 w/w and an increase of the water

Table 1
Composition of LPI microemulsions

Formulation	Labrasol (S) (%)	Plurol[a] (Co) (%)	Isosiso[b] (O) (%)	Water (W) (%)	S/Co (w/w)	Me type
LPI1	33	13	12	42	2.5:1	O/W
LPI2	35	35	10	20	1:1	W/O
LPI3	47	23	10	20	2:1	W/O
LPI4	53	17	10	20	3:1	W/O
LPI5	42	21	26	11	2:1	W/O

S: surfactant; Co: cosurfactant; O: oil phase; W: water phase; S/Co: surfactant/cosurfactant; Me type: microemulsion type.
[a] Plurol Isostearique.
[b] Isostearyl Isostearate.

Table 2
Composition of TL microemulsion

Form.	Labrasol (S) (%)	Plurol[a] (Co) (%)	Isosiso[b] (O) (%)	M.oil[c] (O) (%)	T.P[d] (T) (%)	Sol.γ[e] (T) (%)	Water (W) (%)	S/Co (w/w)	Me type
TL	12	9	1	2	20	10	46	4.6:1	O/W

Form.: formulation; S: surfactant; Co: cosurfactant; O: oil phase; W: water phase; S/Co: surfactant/cosurfactant; Me type: microemulsion type.
[a] Plurol Isostearique.
[b] Isostearyl Isostearate.
[c] Mineral oil.
[d] Transcutol P.
[e] Solubilizant γ 2420.

Table 3
Composition of LEC microemulsions

Formulation	Lecithin (S) (%)	Isopropanol (Co) (%)	IPM[a] (O) (%)	Water (W) (%)	S/Co (w/w)	Me type
LEC1	25	25	25	25	1:1	W/O
LEC2	25	25	45	5	1:1	W/O
LEC3	13	12	65	10	1:1	W/O
LEC4	18	7	65	10	3:1	W/O

S: surfactant; Co: cosurfactant; O: oil phase; W: water phase; S/Co: surfactant/cosurfactant; Me type: microemulsion type.
[a] Isopropylmiristate.

amount (from 20 to 42%, w/w) enabled to obtain an oil in water microemulsion.

In the case of "LEC" microemulsions, whose compositions are reported in Table 3, the use of lecithin as surfactant, isopropanol as cosurfactant (1:1 or 3:1, w/w) and Isopropylpalmitate as oil phase allowed the production of 4 w/o microemulsions. Moreover, the use of the couple Tween 80 and Span 80 (surfactants) together with isopropanol (cosurfactant) (3:1 or 4:1, w/w) and Isopropylpalmitate as oil phase (50 or 84%, w/w) led to the production of 2 w/o microemulsions (Table 4). Unfortunately, TS2 microemulsion displayed phase separation after 6 days from production.

Production of monoglyceride–water systems, performed by simple addition of different amounts of water to melted monoglycerides, gave rise to reverse micellar systems easy to handle. These systems are able to swell in water and to form the stiff cubic phase co-existing with excess water.

3.2. Characterization of formulations

In order to characterize microemulsions and monoglyceride–water systems, pH, viscosity values and droplet size distributions were determined both for placebo and AMB containing forms. Tables 5 and 6 summarized the obtained results. As reported in Table 5, in general in the case of micoemulsions, the addition of AMB led to a slight increase in pH values, on the contrary in the case of monoglyceride–water

Table 4
Composition of TS microemulsions

Form.	Tween 80 (S) (%)	Span 80 (S′) (%)	Isopropanol (Co) (%)	Isosiso[a] (O) (%)	Water (W) (%)	S/Co (w/w)	Me type
TS1	23	7	9	50	10	3:1	W/O
TS2	9	3	3.2	84	1	4:1	W/O

Form.: formulation; S, S′: surfactants; Co: cosurfactant; O: oil phase; W: water phase; S/Co: surfactants (S + S′)/cosurfactant; Me type: microemulsion type.
[a] Isostearyl Isostearate.

Table 5
Characterization of AMB amphiphilic association systems

Formulation	pH	pH AMB[a]	Viscosity (cP)	Viscosity (cP) AMB[a]
LPI1	4.0 ± 0.02	5.5 ± 0.01	108.61 ± 1.5	61.37 ± 1.2
LPI2	4.6 ± 0.03	4.9 ± 0.02	140.56 ± 1.6	90.50 ± 1.3
LPI3	4.8 ± 0.01	5.2 ± 0.03	141.84 ± 4.7	63.25 ± 1.2
LPI4	5.0 ± 0.03	5.5 ± 0.03	145.89 ± 1.5	95.28 ± 1.1
LPI5	4.9 ± 0.01	5.1 ± 0.03	267.29 ± 1.8	126.50 ± 1.8
TL	3.8 ± 0.01	4.3 ± 0.01	68.23 ± 1.3	n.d.
LEC1	4.8 ± 0.02	4.7 ± 0.02	n.d.	n.d.
LEC2	4.2 ± 0.02	4.3 ± 0.02	n.d.	n.d.
LEC3	3.7 ± 0.01	3.9 ± 0.01	n.d.	n.d.
LEC4	3.5 ± 0.01	3.7 ± 0.01	n.d.	n.d.
TS1	5.3 ± 0.03	5.0 ± 0.02	n.d.	41.58 ± 1.1
MYV1	3.5 ± 0.01	3.0 ± 0.01	40.36 ± 1.9	98.00 ± 1.8
MYV2	3.8 ± 0.01	2.9 ± 0.01	97.71 ± 1.8	178.10 ± 1.8
MYV3	3.6 ± 0.02	2.4 ± 0.01	365.77 ± 1.9	173.80 ± 1.4
MYV4	3.9 ± 0.01	1.8 ± 0.01	380.00 ± 2.0	171.40 ± 1.6
MYV5	3.5 ± 0.03	2.4 ± 0.01	400.00 ± 1.8	125.90 ± 1.2
MYV6	3.5 ± 0.01	2.8 ± 0.01	417.35 ± 2.1	131.00 ± 2.1
MYV7	3.3 ± 0.02	2.7 ± 0.02	550 ± 2.0	118.03 ± 1.1

n.d.: not determinable. Data represent the average of three independent experiments ± S.D.
[a] Values determined for AMB-containing microemulsions.

systems pH values tend to decrease in the presence of AMB. The pH values of microemulsions were always comprised of between 3.5 and 5.5, both for placebo and for AMB-containing formulations. These results suggest administering the formulations either percutaneously or on the vaginal mucosa for the treatment of candidiasis.

As a general rule, the presence of AMB causes a decrease of viscosity values, both for microemulsions and for monoglyceride–water systems (Table 5). In the case of monoglyceride–water systems, as expected, viscosity is a function of the water content. Both placebo- and AMB-containing amphiphilic association systems exhibit Newtonian rheological behaviour (data not shown).

Concerning mean size of droplets, determined by PCS and expressed as *Z* Average, the presence of AMB does not show a significant effect, both in the case of

Table 6
Dimensional characterization of AMB amphiphilic association systems

Microemulsion	Droplet mean size (nm)[a]	Droplet mean size (nm)[a]; AMB[b]
LPI1	44.6 ± 0.8	31.5 ± 0.2
LPI2	34.2 ± 1.8	30.1 ± 1.2
LPI3	30.8 ± 2.2	29.6 ± 1.3
LPI4	30.4 ± 1.9	30.5 ± 2.2
LPI5	35.7 ± 1.5	38.3 ± 1.2
TL	22.9 ± 1.1	31.0 ± 0.8
LEC2	28.0 ± 1.2	30.0 ± 2.3
LEC3	25.5 ± 1.3	26.0 ± 1.4
LEC4	33.0 ± 1.4	29.8 ± 1.2
TS1	5.3 ± 0.2	5.2 ± 0.4
MYV1	12.3 ± 0.1	2.6 ± 0.2

Data represent the average of three independent experiments ± S.D.
[a] *Z* Average values determined by PCS analysis.
[b] Column reports droplet mean size of AMB-containing microemulsions.

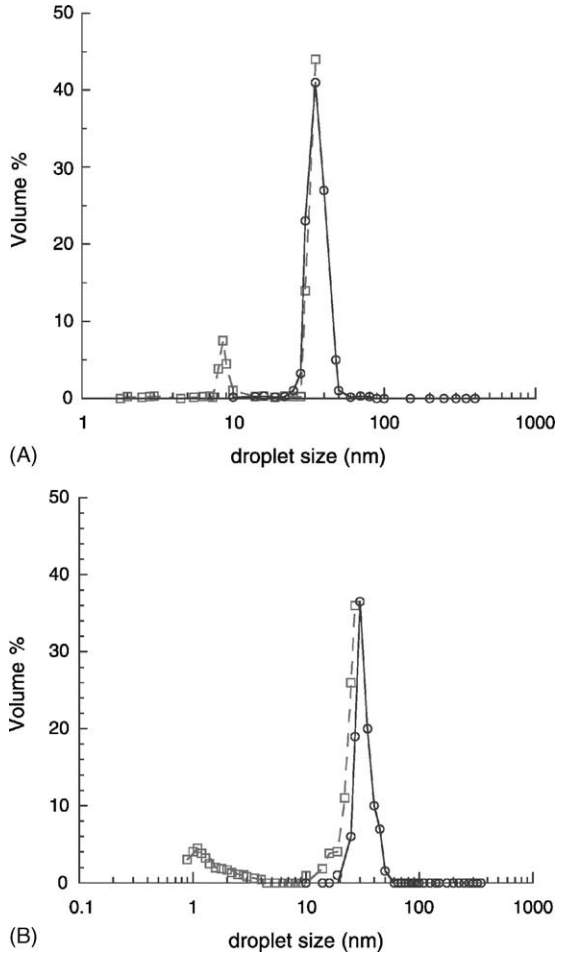

Fig. 1. Dimensional distributions of LPI5 (panel A) and LEC2 (panel B) microemulsions produced in the absence (□) or in the presence (○) of AMB and expressed as percentage of volume, as determined by PCS.

microemulsions and of the pure monoglyceride system MYV1 (Table 6). It is to be underlined that in the case of monoglyceride–water systems, it was impossible to perform PCS measurements, due to the sample viscosity.

Fig. 1 shows the size distributions of LPI5 and LEC2 microemulsions taken as examples. As it can be noted, the presence of AMB changes the dimensional distribution of microemulsions, from a bi-modal (in the case of placebo formulations) to a mono-modal droplet size distribution. This behaviour could be attributed to the amphiphilic properties of the AMB molecule that could act as a co-surfactant located at the interface between the two phases, thus stabilizing the microemulsion disperse system.

3.3. Amphotericin B content

In order to evaluate the solubilizing power of the produced formulations, the AMB solubility was evaluated both in water and in the oil phases employed for microemulsions production. Table 7 reports AMB solubility in neat aqueous and oil phases, microemulsions and monoglyceride–water systems, as determined by HPLC studies.

Comparison of the measured solubility demonstrated that LPI, LEC, TL and TS microemulsions either monoglyceride–water systems can increase AMB solubility with respect to water, Isopropylpalmitate and Isostearyl Isostearate.

In particular, with regard to LPI1 microemulsions, AMB solubility is 8.6-fold higher with respect to that displayed by the single oil phase and 20.6-fold higher than that in water. TL is scarcely able to solubilize AMB, anyway AMB solubility is 1.4-fold higher with respect to that displayed by the single oil phase and 3.2-fold higher than that in water. In the case of LEC4, AMB solubility is 19.46-fold higher with respect to that displayed by the oil phase and 27.5-fold higher than that in water.

TS1 microemulsion enables to increase AMB solubility up to 14.7-fold with respect to water and 6.1-fold with respect to the oil phases.

Concerning monoglyceride–water systems, it should be noted that solubility power with respect to AMB is a function of the water content, ranging from 21.49 μg/ml for pure monoglycerides (MYV1), to 110.86 mg/ml for the systems containing 2% w/w of water (MYV7). MYV7 enabled to increase AMB solubility by 13-fold with respect to water and 5.1-fold with respect to pure monoglycerides.

3.4. Drug diffusion studies

AMB diffusion studies were performed by an in vitro system based on Franz cell and a nylon membrane. In order to have the same thermodynamic activity, microemulsions were saturated in AMB. Fig. 2 reports comparative kinetics of AMB as DMSO solution or incorporated in LPI4 and LEC4 microemulsions taken as models. As reported in

Table 7
AMB solubility in different solvents

Vehicle	AMB content (μg/ml)	AMB increasing content ratio with respect to water	AMB increasing content ratio with respect to oil
Water	8.40 ± 0.1	–	–
Isopropylmiristate	11.89 ± 0.5	–	–
Isopropylpalmitate	19.83 ± 0.2	–	–
Isostearyl Isostearate	20.13 ± 0.4	–	–
LPI1	173.24 ± 0.8	20.62	8.6
LPI2	8.40 ± 0.1	6.03	2.51
LPI3	8.40 ± 0.1	11.4	4.75
LPI4	8.40 ± 0.1	13.27	5.54
LPI5	8.40 ± 0.1	12.26	5.11
TL	8.40 ± 0.1	3.24	1.35
LEC1	30.64 ± 0.2	3.64	2.57
LEC2	88.78 ± 0.3	10.56	7.46
LEC3	80.99 ± 0.3	9.64	6.81
LEC4	231.46 ± 2.3	27.55	19.46
TS1	123.79 ± 1.1	14.73	6.14
MYV1	21.49 ± 0.1	2.55	–
MYV2	53.88 ± 0.2	6.4	2.5
MYV3	69.5 ± 0.1	8.27	3.23
MYV4	73.81 ± 0.2	8.78	3.43
MYV5	79.49 ± 0.5	9.46	3.69
MYV6	110.80 ± 0.9	13.19	5.15
MYV7	110.86 ± 0.1	13.19	5.15

Data represent the average of three independent experiments.

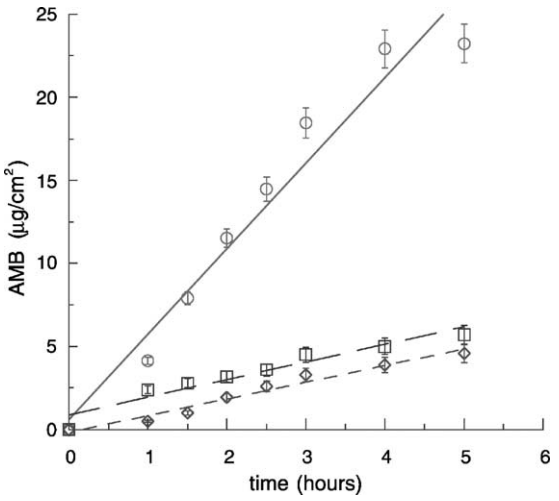

Fig. 2. In vitro diffusion kinetics of AMB as DMSO solution (○) or incorporated in LPI4 (□) and LEC2 (◇) microemulsions. Diffusion studies were performed by an in vitro system based on a Franz cell and a nylon membrane. Data represent the mean of six independent experiments ± S.D.

Table 8
In vitro diffusion coefficients of AMB incorporated in different vehicles

Vehicle	J_s (μg/cm^2 × h)	C (mg/ml)	J_n (cm/h × 10^3)	Log J_n
DMSO sol	5.15	2.00	2.57	0.41
LPI4	1.05	0.11	9.50	0.97
LEC4	2.61	0.23	11.39	1.05

The determinations were performed in a Franz cell system. The reported results represent the average of six independent experiments.

Table 8, diffusion coefficients (J_n) of AMB incorporated in LPI4 and LEC4 are about four-fold higher with respect to the J_n of AMB in organic solution.

3.5. Stability studies

Physical stability studies were performed on formulations stored at room temperature evaluating (a) macroscopic aspect (phase separation, changes in colour or drug precipitation) and (b) droplet mean size.

With regard to macroscopic aspect, LEC1 and LEC3 have shown phase separation after one month

from production, whereas in the case of LEC2, phase separation was detectable only after two months from production.

On the other hand, both LPI (1, 2, 3, 4 and 5) TL, LEC4, TS1 microemulsions and MYV1–MYV7 monoglyceride–water systems were free from phase separation phenomena for at least three months. During this period, changes in colour, creaming or drug precipitation were not detectable.

The stability of droplet size plays an important role in optimizing amphiphilic association systems (Aboofazeli et al., 2000), PCS can be a powerful

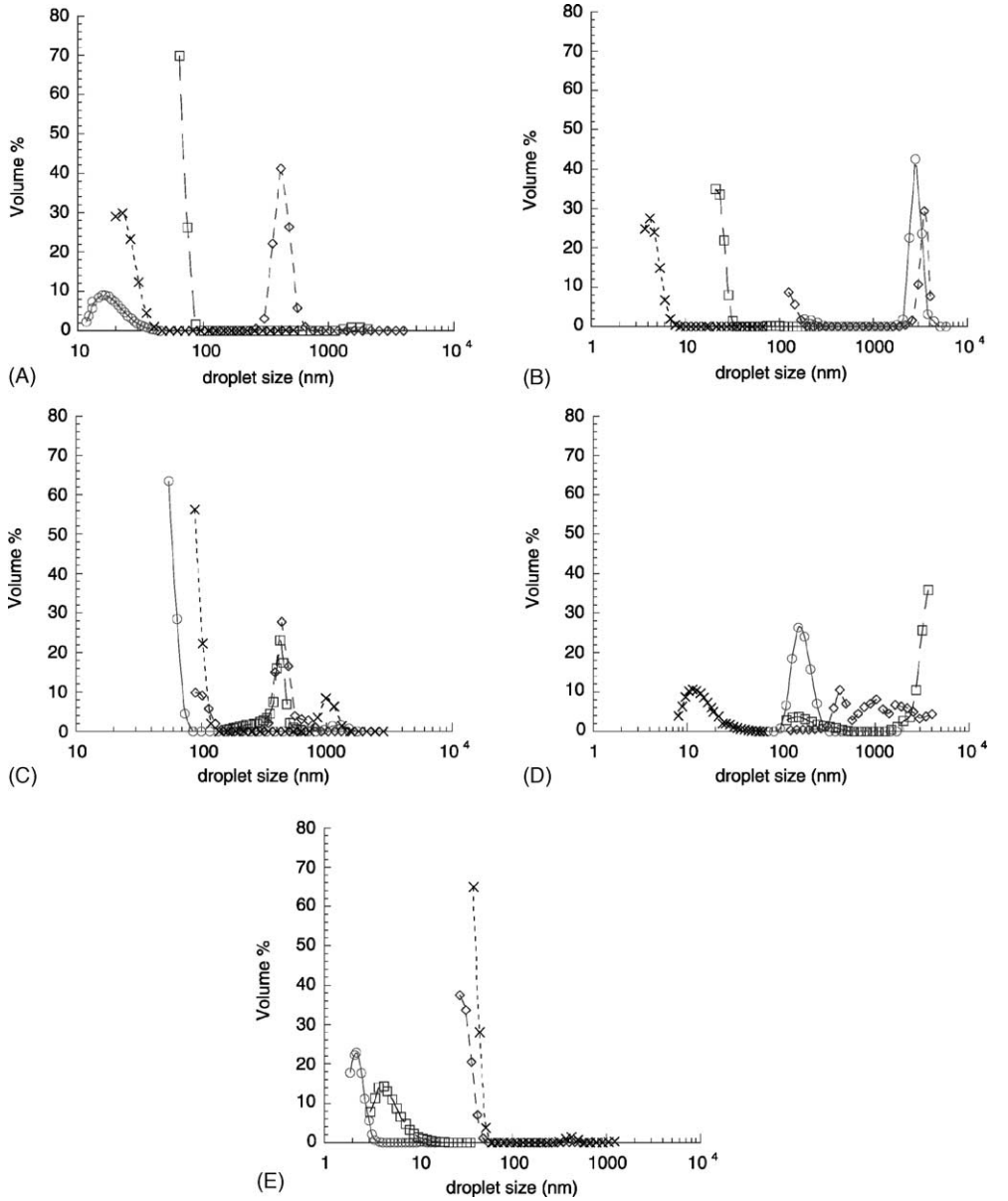

Fig. 3. Dimensional distributions of LPI1 (panel A), LPI4 (panel B), TL (panel C), LEC4 (panel D) and TS1 (panel E) microemulsions. Measurements were performed by PCS at time 0 (○), and after one month (□), two months (◇) or three months (×) from production. Data represents the mean of four independent determinations.

Table 9
AMB content in different microemulsions as a function of time

Microemulsion	AMB (μg/ml)	Residual AMB (%)[a]		
	0 month	1 month	2 months	3 months
LPI1	173.24 ± 1.8	58.0 ± 3.2	33.2 ± 1.8	17.5 ± 1.4
LPI4	111.54 ± 1.1	109.8 ± 1.6	81.7 ± 2.9	33.5 ± 1.5
TL	27.28 ± 0.1	109.8 ± 1.5	105.0 ± 2.2	95.3 ± 3.4
LEC4	231.46 ± 2.3	112.2 ± 0.8	104.71 ± 1.7	100.0 ± 2.4
TS1	123.79 ± 1.1	79.1 ± 2.3	66.4 ± 1.3	53.8 ± 2.0
MYV7	110.86 ± 0.1	72.0 ± 4.2	40.4 ± 3.7	22.1 ± 2.5

Data represent the average of three independent experiments ± S.D.
[a] As a function of initial AMB content.

technique in determining the droplet size distribution as a function of time. Mean size of microemulsion droplets showed a general increase after one month from production followed by a decrease or a stabilization to the initial values after three months from production (Fig. 3). This behaviour can be associated to the dynamic microstructure of the microemulsions in which the interface is spontaneously and continuously fluctuating, possibly leading to a final stabilization of the association system.

Chemical stability studies were performed on selected AMB loaded formulations stored at 4 °C, namely LPI1 and 4, TL, LEC4, TS1 microemulsions and MYV7 as monoglyceride–water system. Table 9 reports AMB content in the different formulations as a function of time, expressed as percentage of initial drug content. Surprisingly, it was found that in the case of LPI4, TL and LEC4 microemulsions, AMB content was increased up to 112% after one month from production, with respect to drug content determined at time 0. This behaviour was attributed to the microstructure of the formulation, where surfactant and cosurfactant are in a continuous dynamic equilibrium, possibly able to promote solubilization of undissolved drug. After three months from production, AMB content was at least 17.5% with respect to initial drug content in all tested formulations. In particular, in the case of LEC4, AMB content was unchanged.

Shelf life stability was calculated plotting Log (AMB residual content, %) against time, obtaining first order kinetics (Fig. 4). The slopes (m), calculated by linear regression, were substituted into Eq. (1). Shelf life values (t_{90}) were then calculated and reported in Table 10.

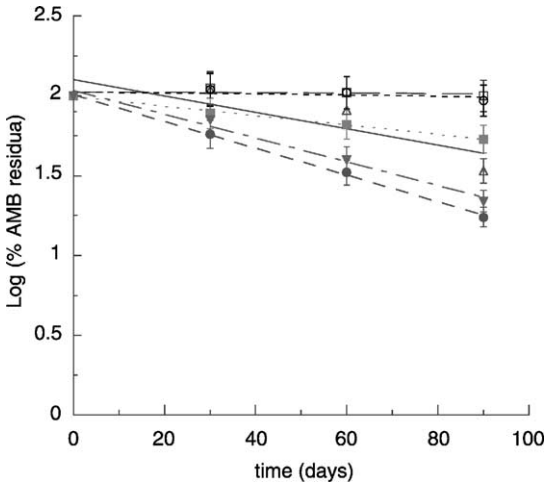

Fig. 4. Variation of AMB residual content in LPI4 (◆), LPI1 (●), LEC2 (□), TL (○), TS1 (■) and MYV5 (▼) as a function of time. Data represent the mean of four independent determinations performed by HPLC.

Table 10
AMB stability in different microemulsions

Microemulsion	K	t_{90} (days)[a]
LPI1	19.00×10^{-3}	5.52
LPI4	12.00×10^{-3}	8.75
TL	0.82×10^{-3}	128.00
LEC4	0.23×10^{-3}	455.92
TS1	6.70×10^{-3}	15.67
MYV7	17.00×10^{-3}	6.17

The reported results represent the average of three independent experiments.
[a] Time at which the drug concentration has lost 10%.

It was found that TL microemulsion could maintain 90% of AMB stability for almost four months. In the case of LEC4 microemulsion, t_{90} was longer than one year (455.92 days). LPI4 displayed the shortest t_{90} (5.52 days).

In a paper by Moreno and colleagues, it was found that lyophilization was a necessary process in order to overcome the stabilization problem of a lecithin-based microemulsion for the AMB vehiculation (Moreno et al., 2001). The authors claimed precipitation of drug after one week and phase separation after two weeks from microemulsion preparation. It is to be underlined that in the present study LEC microemulsions were stable both physically and chemically, probably because of the use of high purity soybean phosphatydylcholine (93 ± 3%, w/w).

4. Conclusion

The characterization of AMB containing microemulsions and monoglyceride–water systems here described demonstrated that in the case of microemulsions in general the addition of AMB led to a slight increase in pH values, while in the case of monoglyceride–water systems pH values slightly decrease in the presence of AMB. As a general rule, the presence of AMB causes reduction in viscosity values. The presence of AMB does not seem to significantly affect mean dimension of droplets but changes the dimensional distribution of microemulsions, from a bi-modal (in the case of placebo formulations) to a mono-modal droplet size distribution. This change in dimensional distribution probably depends on the amphiphilic character of the AMB molecule that can stabilize the microemulsion disperse system.

Comparison of the measured solubility demonstrated that microemulsions and monoglyceride–water system can increase AMB solubility with respect to the sole aqueous and oil phases. In particular, in the case of LEC4, AMB solubility is 19.4-fold higher than that in water. The in vitro Franz cell system associated to Nylon membrane enabled to perform preformulatory comparative study of AMB diffusion from different microemulsions.

Concerning physical stability, both LPI, TL, LEC4, TS1 microemulsions and monoglyceride–water systems were free from phase separation phenomena for almost three months. Mean size of microemulsion droplets showed a general increase after one month from production followed by a stabilization to the initial values after three months from production. Shelf life stability evaluation demonstrated that LEC4 microemulsion could maintain 90% of AMB stability for more than one year.

Based on the above reported considerations, the present study indicates that microemulsions and monoglyceride–water system can be proposed as alternative formulations for the topical delivery of AMB for instance in the case of vaginal or skin mycosis such as candidiasis.

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