P-055 **Microemulsion systems to carrier amphotericin B for ocular use**

Egito EST. [1,2*#] Silveira WLL. [1,2] Silva-Filho MA.[1] Ferreira LF. [1] Brasil FC [1] Damasceno BPLD. [3]
[1] LASID, UFRN, Natal-RN, Brazil [2] PPGCSA, UFRN, Natal-RN, Brazil [3] UEPB, Campina Grande-PB, Brazil. * Supervisor # socrates@ufrnet.br

INTRODUCTION AND OBJECTIVES

Ocular fungal infections are an important ophthalmologic problem causing significant ocular morbidity (Carrasco 2011). The pharmacological approach of management of these infections involves administration of antifungals agents, including amphotericin B (AmB) in its micellar form, Fungizon®. However the owing physiologic constraints of the eye and the low concentration of this drug achieved by the optical route define insufficient bioavailability (Gratieri 2010, Mazouri 2001, Thomas 2003).

A microemulsion (ME) is a system that contains water and oil coexisting in thermodynamic equillibrium due to the presence of a surfactant film at the oil-in-water interface. They are clear, stable, transparent and isotropic systems and currently has aroused the interest of pharmaceutical scientists because of their capacity to be used for ocular applications (Pestana 2008, Vandamme 2002).

The aim of this work was to develop and characterize a ME system in order to use it as carrier for AmB in topical applications in the eye.

MATERIALS AND METHODS

The ME formulation was prepared from a pseudo-ternary phase diagram procedure and had, as water phase, a phosphate buffer pH 7.4 solution and Lipoid® S100 and, as oil phase, Miglyol®812N and Tween®80 (Vandamme 2002). Both water and oil phases were magnetically stirred until complete homogenization. The ME was achieved by addition of the water phase in the oil phase followed by sonication process and ultrasound bath. The following parameters were then evaluated: macroscopic appearance, pH, refractive index (RI) and transmittance rate, conductivity, droplet size and polidispersity index, viscosity and rheology.

The drug was incorporated into the system at a concentration of 1.5 mg/mL. Briefly, AmB was dissolved (15 mg/mL) in NaOH solution (1 N) and added, under magnetic stirring to the ME. The pH was adjusted to 7.5-8.0 with 1 N HCl solution. The drug content and the entrapment efficiency for the ME were verified by a validated spectrophotometric analysis.

RESULTS AND DISCUSSION

The ME formulation presented in Table 1 was chosen based on its homogeneity aspect, transparent appearance and absence of precipitates. Such characteristics are typical for a Winsor IV system and, therefore, could be used as a drug delivery system for ophthalmic use (Winsor 1948).

Table 1. Composition of the ME system

Components	ME ($\%_{w/w}$)
Miglyol® 812 N	11
Lipoid® S100	6.3
Tween® 80	14.7
Phosphate buffer pH 7.4 solution	68

Table 2. Physcochemical characteristics

Parameters	Mean ± SD
pH	7.40 ± 0.038
Rheology	Newtonian
Viscosity (mPas)	48.28 ± 0.36
Droplet size (nm) X_{90}	13.96 ± 0.49
Polidispersity index	0.241 ± 0.003
Refractive index	1.378 ± 0.001
Transmittance rate (%)	96.47 ± 1.92
Conductivity (µS)	722.33 ± 5.13

The pH value of the ME was physiological and predicts the maximum comfort and absence of eye irritation when this preparation will be instilled in the eye (Fialho 2004, Hasse 1997).

This system presented a viscosity value that allows sterile filtration and can still increase the ocular residence time without the occurrence of discomfort, blurred vision, or foreign body sensation with the cornea surface. Afterwards, it has flown with a Newtonian behavior, which ensures that blinking should not have an effect on the viscosity (Fialho 2004, Hasse 1997).

The small droplet size of the ME system, at the range of nanometers, is due to the interaction between the co-surfactant molecules with the surfactant film which decrease the radius of the curvature of the microdroplets, producing transparent systems. It showed the spherical shape and uniform droplet size of the ME as shown by the TEM analysis (Figure 1).

The refractive index of the developed system was similar to the refractive index of water (1.333). In addition, the

developed system showed a transmittance rate of 96 %. These parameters data are a proof of transparency and allows the use of this system by ocular applications (Fialho 2004, Hasse 1997).

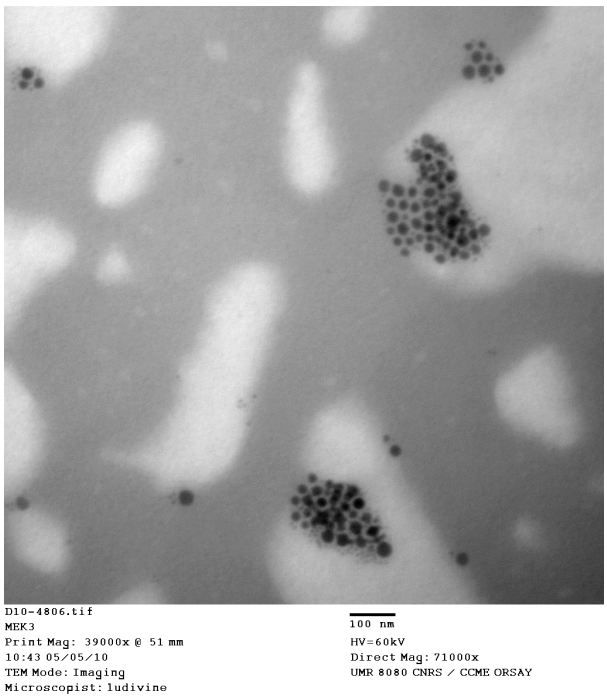

Figure 1. TEM picture of the ME

The high conductivity of the system is characteristic of thermodynamically stable oil in water MEs. This type of ME is preferred for use in the eye because of the droplet structure can often be retained during the dilution process by the lachrymal fluid (Fialho 2004, Hasse 1997).

UV-visible procedure used to determine the final amount of the drug incorporated into the ME demonstrated to be sensitive and adequate for the AmB quantification into ME (Figure 2).

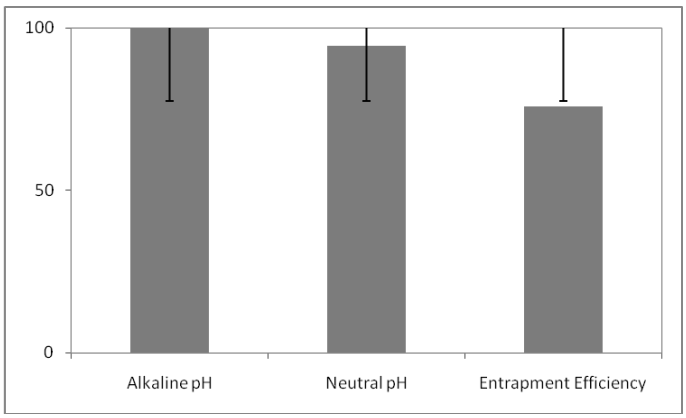

Figure 2. Quantification of AmB

Initially, the AmB was totally solubilized (100 %) into the system favored by the alkaline pH values. However, at pH 7.5-8.0, some coacervates (crystals) were observed and this dosage decrease to 94.42 ± 12.89 % when compared with the nominal AmB content (1.5 mg/mL). The average entrapment efficiency of AmB in the ME formulation was 75.92 ± 3.89 % and this result demonstrates that the drug was partitioned into the oil and aqueous phase. Therefore, this method favored the incorporation of the AmB into the ME droplets.

CONCLUSION

From the results, the ME system containing Lipoid® S100/Tween® 80 as surfactants, Mygliol® 812N, as oil phase and phosphate buffer, as aqueous phase, in the presented proportion, seen to be valuable delivery system in terms of easy manufacturing and high compatibility with the requiremets of the ocular route as demonstrated with the physicochemical results, which make it very appropriate for ocular applications to incorporate lipophilic drugs.

The ME system was able to carry the AmB and the data showed that the incorporation method was important to help on the entrapment process of this drug into this lipidic structure. Therefore, It can be used as one promising system to be applied by topical ocular route to control ocular fungal infections.

REFERENCES

- Carrasco MA. et al. (2011) *Treatment of severe fungal keratitis with subconjunctival amphotericin B*. Cornea 30 608-611
- Gratieri T. et al. (2010) *Current efforts and the potential of nanomedicine in treating fungal keratitis*. Expert Review of Ophthalmology 5(3) 365-384
- Manzouri B. et al. (2001) *Pharmacotherapy of fungal eye infections*. Expert Opinion on Pharmacotherapy 2 1849-1857
- Thomas PA. (2003) *Current perspectives on ophthalmic Mycoses*. Clinical Microbiology Reviews 16(4) 730-797
- Pestana KC. et al. (2008) *Oil-in-water lecithin-based microemulsions as a potential delivery system for amphotericin B*. Colloids and Surface B: Biointerfaces 66 253-259
- Vandamme TF. (2002) *Microemulsions as ocular drug delivery systems: Recent developments and future challenges*. Progress in Retinal and Eye Research 21 15-34
- Winsor PA. (1948) *Hidrotropy, solibilization and related emulsification processes. 1 to 4*. Transactions Faraday Society 44 376-398
- Fialho SL. et al. (2004) *New vehicle based on a microemulsion for topical ocular administration of dexamethasone*. Clinical and Experimental Ophthalmology 32 626-632
- Hasse A. et al. (1997) *Development and characterization of microemsulsions of ocular applications*. European Journal of Pharmaceutics and Biopharmaceutics 43 179-183