Preliminary design of freeze-dried microemulsion containing Amphotericin B

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INTRODUCTION AND OBJCTIVE

Amphotericin B (AmB) is an antibiotic widely prescribed in the treatment involving systemic fungal infections. However, side effects are involved with its use, as the nephrotoxic action, leading to the choice for a less toxic formulation of the drug. In order to improve the pharmacotherapy safety, new pharmaceutical forms have been studied as a drug delivery system for the AmB (Darole 2008).

Microemulsions (MEs) are comprised of oil, water and surfactants, being characterized as isotropic, clear and thermodynamically stable systems with a small droplet size (Date 2008). To improve the system microbiological stability, the water content may be removed by freeze-drying (FD) technique (Fissore 2011). This industrial process removes water from frozen samples using sublimation under vacuum (Abdelwahed 2006).

During the FD process, some mechanical stresses are produced, especially during the freezing stage in which the contact of the crystallized water with the droplet surface might destabilize the system. Therefore, the addition of sugars in formulations as cryoprotectants is used to prevent such damage (Abdelwahed 2006).

A pathway to evaluate the capacity of drug entrapment by the ME system after the FD process, is through the use of UV-spectrophotometric method. This analytical procedure is characterized by its accurate and simple technique to estimate the drug content in pharmaceuticals products (Moreno 2000).

The aim of this work was to analyze if the FD process influences on the drug content (DC), pH, conductivity and droplet size (DS) of the ME system, comparing formulations with different concentrations of Maltose (MT) as cryoprotectant.

MATERIALS AND METHODS

Materials

The Miglyol 812® used as oil phase was obtained from CONDEA Chemie GMBH (Hamburg, Germany). Lipoid S100® used as surfactant was purchased from LIPOID GMBH (Ludwigshafen, Germany), Tween 80® used as surfactant was obtained from Sigma Aldrich Inc (St. Louis, USA) and the MT was acquired from Sigma Aldrich Inc (St. Louis, USA). The $NaHPO_4$ and the Na_2HPO_4 used to produce the phosphate buffer pH 7.4 were purchased from Vetec Química Fina Ltda (Rio de Janeiro, Brazil).

Methods

The ME presented 68 % of phosphate buffer pH 7.4, 14.7 % of Tween 80®, 6.3 % of Lipoid S100® and 11 % of Miglyol 812®. After weighed the components, the sample was mixed under magnetic stirring following by three cycles of sonication for 1.5 min and ultrasonic bath for 3 min.

The AmB content of 1.5 mg/mL was incorporated in the ME using the solutions of NaOH and HCl 1N, preceded by the MT addition on concentrations at 5 % and 12.5 %. The electrical conductivity of these systems were analyzed by DM-32 conductivity (Digicrom Analytical, Campo Grande, SP, Brazil) and the pH was measured by a PG-2000 pHmeter (GEHAKA, Morumbi, SP, Brazil) at 25 ± 2 °C. The samples were frozen at -80 °C for 24 h before the FD process.

The droplet size distribution analyses were carried out by Dynamic Light Scattering (DLS) using a ZetaPlus (Holtsville, NY, USA). To perform such analysis, the MEs were previously diluted with water at the ratio of 1:20. The DC of the samples was measured by UV-spectrophotometer (Biochrom Libra S32, USA) at the wavelength of 405 nm. The MEs were diluted using Dimethyl Sulfoxide and Methanol as solvents on the proportions of 1:9 and then 1:20 in Methanol.

All the tests were performed in triplicate, before and after the FD process. The results were analyzed by paired t-test using the GraphPad software.

RESULTS AND DISCUSSION

The systems were clear and showed a dark yellow color after the incorporation of AmB. The formation of a powder cake for both formulations was observed after the FD process. However, the aspect of the samples containing 12.5 % of MTB was better than the samples with 5 %, which presented less reconstitution time that could be explained by the optimum cryoprotectant concentration to the system stability (Abdelwahed 2006).

The freeze-dried ME containing 5 % and 12.5 % of the cryoprotectant, except for the conductivity of the ME with 12.5 % of MT, showed significant changes

in the pH and conductivity values in comparison to the non- freeze-dried samples, as it could be observed in Tables 1 and 2. This is possibly explained by the stress at the freezing stage caused by the effect of phosphate buffer crystallization (Tang 2004). It is important to note that the samples values of pH and conductivity remained inside the physiologic range, as well as in the characteristic charge measurement of an oil-in-water (o/w) system.

Table 1 : Characterization of ME with AmB containing 5 % of MT before and after FD process.

	Conductivity (µS)	Drug Content (mol/L)	pH	Size (nm)
Before freeze-drying	1582.55*	1.975 x 10^{-3}	7.59*	54.1*
After freeze-drying	1639.40*	1.942 x 10^{-3}	7.40*	32.3*

$*p < 0.05$ compared with the same sample before FD.

Table 2 : Characterization of ME with AmB containing 12.5 % of MT before and after FD process.

	Conductivity (µS)	Drug Content (mol/L)	pH	Size (nm)
Before freeze-drying	1358.45	1.788 x 10^{-3}	7.54*	51.6*
After freeze-drying	1358.20	1.902 x 10^{-3}	7.32*	36.4*

$*p < 0.05$ compared with the same sample before FD.

However, the systems DS had a considerable decrease after the reconstitution stage for both samples with 5 % and 12.5 % of MT concentrations. The possible explanation about the DS reduction after the FD process is based on the co-surfactant role of MT, as described in the literature with the Mannitol. These cryoprotectants have a polyalcohol chains that allow it to be located at the o/w interface of the ME, leading to a higher stabilization of the system (Moreno M. A. 2001).

Furthermore, based on the non-significant statistical results, the initial DC was preserved in both formulations with different amounts of MT after FD. This fact is probably due to the higher protective action against the mechanical stress caused by the ice crystals on the droplets surface of the MT at this range of concentration. The formation of hydrogen bonds with the polar groups of the system surface serves as water substitute after the FD process, increasing the stability of the system (Abdelwahed 2006).Another explanation for the improvement of stability is based on the possibility of occur a MT binding with the AmB-lecithin, forming a stable complex (Moreno M. A. 2001).

CONCLUSIONS

The FD process of a ME system containing AmB can be achieved by using cryoprotectants. The results revealed that the formulation containing 12.5 % of MT presented a powder cake with the best aspect and shorter reconstitution time, proving its feasibility. This preliminary work can be an important step for future studies on FD of emulsion based drug delivery systems.

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