P-082	**Experimental settings for the production of amphotericin B-loaded PLA nanoparticles**	

Silva, A.E., Silva, K.C.H., Silva, K.G.H. and Egito, E.S.T. [*#]
[1]LASID, UFRN, Natal-RN, Brazil
* Supervisor # socrates@ufrnet.br

INTRODUCTION AND OBJECTIVES

Nanotechnology has been largely used for different applications in several industry fields, such as textile, paper, electronics and medical therapeutics (Kumari 2010). Polymeric nanoparticles have attracted much attention as drug delivery systems due to their particular interaction with cells, such as macrophages, besides the ability of improving bioavailability and biodistribution of drugs at specific tissues and organs (Aggarwal 2009).

Leishmaniasis is an endemic disease to many countries caused by protozoans of the genus *Leishmania*, which are obligate intracellular parasites. They cause serious infection in the phagolysosome of host macrophages (Briones 2008). Several drugs are available for the treatment of leishmaniasis. However, they have limited use due to their high toxicity or resistance (Maltezou 2010).

Amphotericin B (AmB) is a polyene antibiotic with a broad spectrum antifungal activity. Moreover, AmB presents excellent activity against leishmaniasis due to its ability of accumulating in phagocytic cells (Briones 2008). However, frequent dose-dependent adverse effects, such as nephrotoxicity and hypokalemia, are limiting factors for its use (Maltezou 2010). Thus, nanocarriers for AmB are promising tools to reduce its toxicity and improve its macrophage targeting.

The aim of this work was to produce polymeric nanoparticles based on poly(lactic) acid (PLA) containing AmB by nanoprecipitation and evaluate the influence of some experimental settings on the production of the nanoparticles, such as surfactants and sonication process.

MATERIALS AND METHODS

Production of AmB-loaded PLA nanoparticles
PLA nanoparticles containing AmB were produced by nanoprecipitation based on solvent displacement process (Espuelas 1997). Briefly, 125 mg of PLA and 1 mg of AmB were dissolved under magnetic stirring and sonication, in 20 mL of acetone and ethanol (1:1 v/v) as the cosolvent. The organic phase was adjusted to pH 4 with HC1 in order to promote AmB solubilization. Afterwards, it was poured into 40 ml of water containing 125 mg of Pluronic™ F68 and F108, separately, under sonication in order to prepare two formulations: NANOF68 and NANOF108, respectively. Other two formulations using the same surfactants were also produced without being subject to sonication (PARTF68 and PARTF108, respectively). Finally, the organic solvents were evaporated under reduced pressure at 58°C.

Particle size, polydispersity index and pH
The mean particle size and polydispersity index (PI) were evaluated by dynamic light scattering using a particle analyzer (Delsa™ Nano C, Beckman Coulter, USA) at 25ºC.

The nanoparticles were evaluated regarding the pH value of the dispersion medium immediately after their production and after storage for 80 days at 4ºC.

RESULTS AND DISCUSSION

According to the scanning electronic microscopy (SEM) analysis, the particles that were not subject to sonication presented rough surfaces and a very irregular size distribution with a large mean diameter (Figure 1).

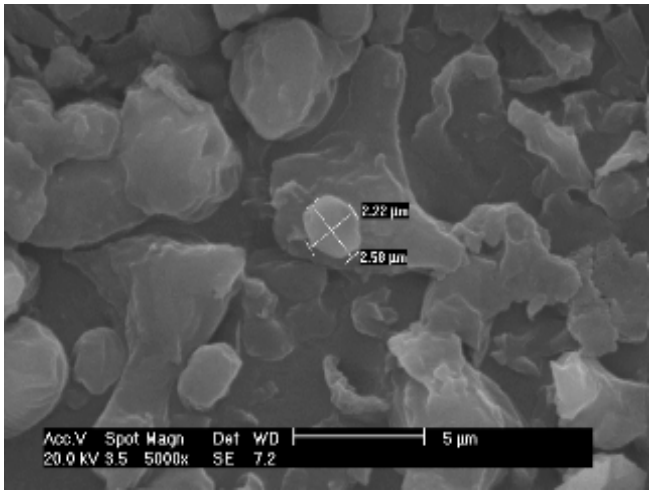

Figure 1: SEM images of PARTF68.

On the other hand, the particles which were subject to sonication (NANOF68 and NANOF108) presented a mean diameter size of 229.7 ± 4.7 and 211.7 ± 1.6 nm, respectively. PI values for NANOF68 and NANOF108 were found to be 0.474 and 0.144, respectively.

The results clearly indicate that the sonication step when performed after the water-in-oil emulsion step has a huge influence on the particle size. In fact, the sonication step was a crucial factor for the production of suitable AmB-loaded PLA nanoparticles, since the experimental set-up with no sonication produced polymeric particles with neither nanometer size nor suitable morphology.

By the end of the production process, the samples presented as suspensions at aqueous medium. The pH

values found immediately after the production and after 80 days are summarized at the Table 1.

Table 1 : pH values of particle suspensions immediately after their production and after storage for 80 days

	Day 0	Day 80
PARTF68	3.95	3.71
NANOF68	3.77	4.26
NANOF108	3.20	3.00

The stability of pharmaceutical preparations may be predicted by variations in their pH value. For nanoparticles prepared by nanoprecipitation, their value fall within the range of 3.0 and 7.5 (Mora-Huertas 2010). As shown in Table 1, our formulations are in accordance with that principle. Besides, no significant variations could be observed in the pH and visual appearance during the 80 days of storage at 4ºC. This fact may be an evidence of good stability of such preparations.

The influence of two different surfactants (Pluronic™ F68 and F108) on the particles size was also evaluated. Although the nature of the surfactant is an essential factors for the nanoparticle size (Mora-Huertas 2010), no significant difference in the mean size of our formulations was observed.

CONCLUSIONS

Nanoprecipitation followed by sonication is a suitable methodology for producing AmB-loaded PLA nanoparticles using both Pluronic™ F68 and F108 as surfactants. Therefore, this is a promising way to produce drug nanocarriers. However, further physicochemical and biological studies need to be performed.

REFERENCES

- Kumari A., et al. (2010). *Biodegradable polymeric nanoparticles based drug delivery systems*. Colloids and Surfaces B: Biointerfaces. (1). 1-18.
- Aggarwal P., et al. (2009). *Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy.* Advanced Drug Delivery Reviews. (6). 428-437.
- Briones E., et al. (2008). *Delivery systems to increase the selectivity of antibiotics in phagocytic cells.* Journal of Controlled Release. (3). 210-227.
- Maltezou H. C. (2010). *Drug resistance in visceral leishmaniasis.* Journal of Biomedicine and Biotechnology. (p.617521). 1-8.
- Espuelas M. S., et al. (1997). *Poly(ε-caprolacton) nanospheres as an alternative way to reduce amphotericin B toxicity.* International Journal of Pharmaceutics. (1). 19-27.
- Mora-Huertas C. E., et al. (2010). *Polymer-based nanocapsules for drug delivery.* International Journal of Pharmaceutics. (1-2). 113-142.