OPTIMIZATION AND CHARACTERIZATION OF POLY (LACTIC-CO-GLYCOLIC ACID) NANOPARTICLES LOADED WITH AN HIV-1 INHIBITOR SYNTHETIC PEPTIDE

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Introduction: It has been reported previously that patients coinfected with HIV and GB virus C (GBV-C) have a prolonged survival. Recently, it has been shown that the GBV-C envelope glycoproteins are enabling to interfere with HIV-1 fusion and entry (1). We report herein the synthesis of a peptide inhibitor of HIV-1 derived form the envelope E1 protein of GBV-C virus and the preparation, optimization and ***in vitro*** characterization of poly (lactic-co-glycolic acid) PLGA-Nanoparticles (NPs) encapsulating this peptide sequence. **Methods:** The peptide was synthesized by the **Fmoc** solid phase peptide synthesis method (2), purified by High Performance Liquid Chromatography (HPLC) and characterized by mass spectrometry (MALDI-Tof). Peptide-loaded PLGA NPs were prepared by a double emulsion/solvent evaporation technique (3). After selecting the factors that influenced physicochemical properties of the peptide-NPs, a three factors, five-level central composite rotatable design 2^3 + star was applied to optimize the formulation. The factors selected were the volume of the inner aqueous phase, the concentration of PLGA and the concentration of peptide. The main interactions and effect of these independent variables were studied on particle size, polydispersity index (PDI), and entrapment efficiency (EE). The selected standard conditions were set as 0.8% (w/v) of PLGA; 0.025% (w/v) of peptide; 2.5% (w/v) of Poly (vinyl alcohol) PVA; 0.5 mL of inner aqueous phase; 30 seconds of primary emulsion and 90 seconds of secondary emulsion. The mean size (Z) and PDI were determined by photon correlation spectroscopy and the EE was assessed determining the non-entrapped peptide by HPLC. The destabilization process of the formulation selected was evaluated using a Turbiscan Lab Expert ®. **Results:** The experimental responses of a total of 16 formulations resulted in a mean size nanoparticle diameter ranging from 209 to 471 nm, with polydispersity index from 0.06 to 0.4 and EE of peptide values ranging from 13% to 84%. The formulation with 0.86 % (w/v) of PLGA; 0.025% (w/v) of peptide; 2.5% (w/v) of PVA; 0.5 mL of the inner aqueous phase, was found suitable for obtaining a high entrapment efficiency (84%) with an adequate average size of 262 nm and unimodal size distribution. The figure 1 shows the three-dimensional response surface of diagram corresponding to effects of peptide and polymer on the entrapment efficiency of peptide-NPs. As illustrated, the highest E.E was obtained with peptide concentration around of 0.025 %(w/v) and polymer concentration around 0.86 %(w/v), thus being these parameters considered relevant in the encapsulation process. Turbiscan data showed that the Peptide-NPs formulation selected has a good stability during more than 72 hours (Figure 2). **Conclusion:** The results obtained suggested that the double emulsion/solvent evaporation is a suitable method for peptides' entrapment. Besides, the factorial design is a valuable tool to provide screening trials useful to select an optimized formulation with a minimum number of experiments.

References

1. Mohr, EL, Stapleton, JT. GB virus type C interactions with HIV: the role of envelope glycoproteins. J. Virol. Hepatol. 2009; 16: 757-768

2. Fmoc solid phase peptide synthesis. A practical approach. W.C. Chan and P.D. Whire Eds. Oxford University Press Inc. New York, 2000.

3. Zhang JX, Zhu KJ. An improvement of double emulsion technique for preparing bovine serum albumin-loaded PLGA microspheres. J Microencapsul. 2004; 21 (7):775-85.

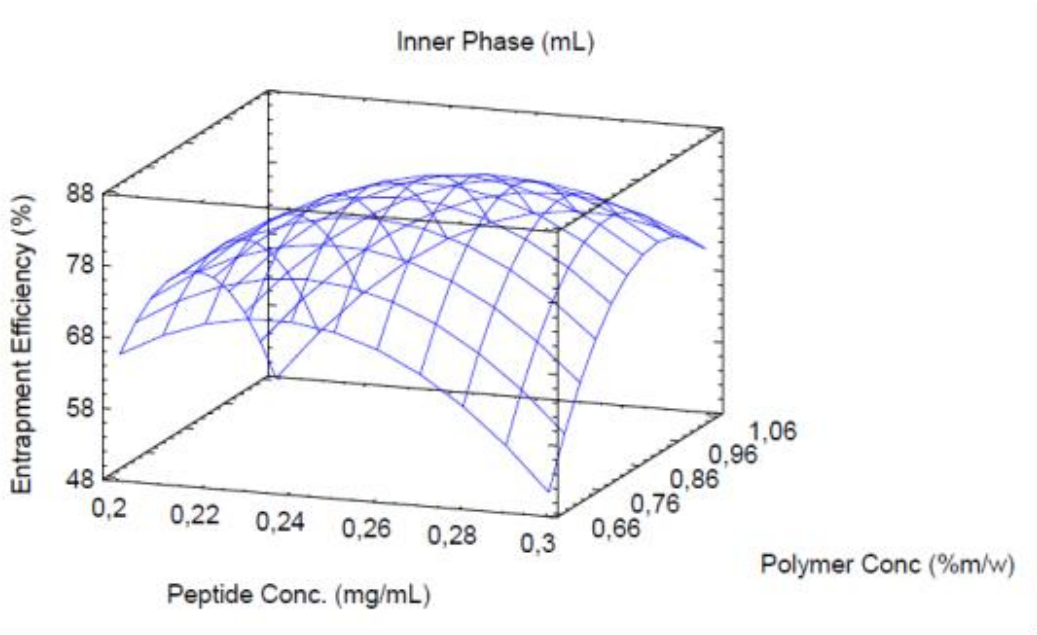

Figure 1. Surface response diagram of entrapment efficiency.

(a) (b)

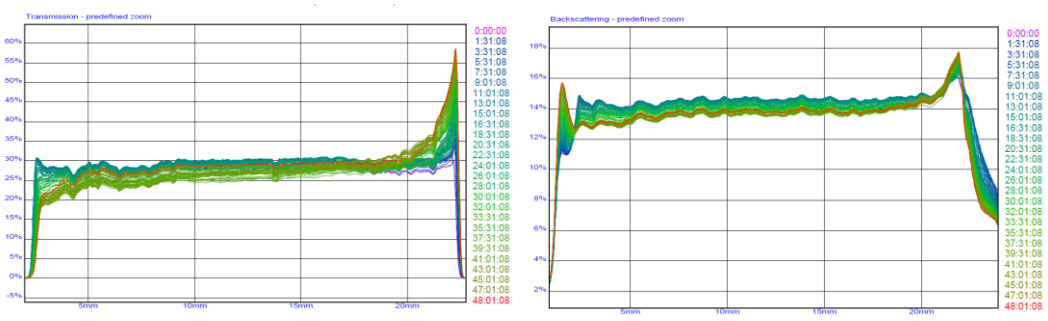

Figure 2. Turbiscan profiles for a Peptide-NPs sample. (a). Transmission level (%) versus high cell (mm); (b) Backscattering (%) versus high cell (mm).