Docetaxel-loaded PEGylated liposomes development using factorial design

Josimar O. Eloy[1]
Felipe Tita de Lima[1]
Giovanni L. Raspantini[2]
Juliana M. Marchetti[2]
Marlus Chorilli[1]

[1] São Paulo State University, Rodovia Araraquara Jaú, Km 01, Araraquara-SP, Brazil
[2] University of São Paulo, Avenida do Café – Campus USP, Ribeirão Preto-SP, Brazil

eloy@fcfar.unesp.br

Docetaxel (DTX) is an anticancer drug, but low solubility and bioavailability limit its application. Within this context, liposomes, nanometric lipid vesicular systems, enable encapsulation of drugs, with several advantages, including protection against degradation, sustained drug release and improvement of pharmacokinetics [1]. However, docetaxel-loaded liposomes based on soy phosphatidylcholine (SPC), cholesterol (Chol) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000 (DSPE-PEG(2000)) prepared under high pressure homogeneization have not been reported. Noteworthy, development of formulations by factorial design enable reduction of experiments and determination of optimum variables. For formulation preparation, the hydration of the thin lipid film was employed. Briefly, lipids and drug were solubilized in chloroform in a round-bottom flask and solvent was evaporated under rotary evaporation. Then, the lipid film was hydrated with pH 7.4 PBS buffer at 60°C, followed by homogeneization under high pressure (10000 psi for 20 min). Lipid to drug ratio (10:1, 20:1 and 30:1), Chol:lipid ratio (1:10, 2:10 and 3:10) and DSPE-PEG(2000) mol% (2.5, 5 and 7.5%) were variables studied in order to obtain liposomes with minimal nanometric size and polydispersity (PdI) and maximum encapsulation efficiency, using a Box-Behnken Factorial Design (Minitab Software). Particle size, PdI and zeta potential were evaluated through Dynamic Light Scattering (DLS). Encapsulation efficiency (EE) was assessed using a validated analytical method, based on High Efficiency Liquid Chromatography (HPLC), employing a 250 mm C-18 chromatographic column, mobile phase composed of water:acetonitrile:methanol (35:15:65) at a flow rate of 0.8 ml/min and detection using wavelength at 232 nm. Results showed that particle size, PdI and zeta potential were not statistically dependent on the tested variables. Liposomes had particle size varying from 59.85 ± 0.51 to 144.67 ± 39.49 nm, values suitable for parenteral administration, and PdI ranging from 0.18 ± 0.005 to 0.55 ± 0.013. Zeta potential ranged from -10.7 ± -0.47 to -26.9 ± -0.72 mV, which is acceptable for colloidal stability. Additionally, encapsulation of DTX was adequate, with efficiency varying from 68.56 ± 10.98 to 99.45 ± 2.36%. It was observed a statistically relevant relationship between EE and lipid:drug ratio ($p < 0.05$, using the square method of analysis of variance) and, overall, higher ratios were correlated with better values of encapsulation, as expected (Figure 01). Using the response optimizer tool in the software, the best formulation for maximum encapsulation efficiency should be prepared using lipid:drug ratio at 22.93, Chol:lipid ratio at 0.2 and DSPE mol % at 7.5. In conclusion, only the lipid:drug variable affected DTX encapsulation. Furthermore, the formulation developed herein using factorial design is promising for further physicochemical characterization and evaluation regarding its anticancer properties.

Figure

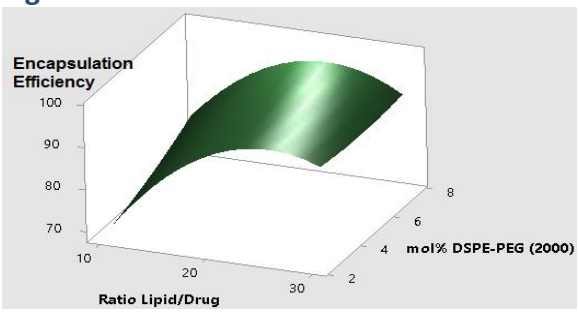

Figure 1. Surface plot of encapsulation efficiency vs lipid/drug ratio and mol% DSPE-PEG(2000)

References

[1] Pereira, S; Egbu, R; Jannati, G; Al-Jamal, W.T., International Journal of Pharmaceutics, 514 (2016) 150-159.