

## Preparation of stable nanoliposomes co-encapsulating doxorubicin and fluoxetine to overcome MDR: Effect of lipid composition and PEG coating

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**Background and Aims:** Fluoxetine (FLX), which belongs to selective serotonin reuptake inhibitors family, is an effective agent in reversing P-glycoprotein mediated multidrug resistance (MDR). Unlike the first-generation chemosensitizers such as verapamil and cyclosporine, FLX exerts its ability to chemosensitize MDR cells at low doses, well below its human safety range. Recent studies have shown that co-encapsulation of an anticancer drug and a MDR modulator can increase effectiveness and decrease adverse effects of the drug. However, an unfavorable interaction of FLX with hydrophobic membranes and cholesterol has been reported. Therefore, we aimed to prepare and characterize an optimized stable co-encapsulated liposomal formulation including FLX and doxorubicin (DOX) by investigating different lipid compositions and presence of PEG.

**Methods:** After preparation of empty liposomes by thin layer film hydration method and extrusion, DOX and FLX were loaded employing remote drug-loading method with pH gradient using sulfate ammonium. In attempt to increase the encapsulation efficiency (EE) of drugs, improve drug release rate and the stability, the influence of various parameters such as type of lipid (DSPC, DPPC, DMPC and Egg PC), percentage of cholesterol and presence of PEG were evaluated.

**Results:** The lipid composition of DSPC:DSPE-PEG2000:CHOL (70:5:25 %mol) was selected as the optimum formulation with an average diameter of  $89.92 \pm 0.2$  nm. The EE for DOX and FLX were about 99% and 72%; respectively. The drug release profiles in PBS at 37 °C up to 48 hours for DOX and FLX were recorded as approximately 24% and 4%. The prepared nanoliposomes showed significant stability when stored at least for fifteen days at 4°C and 25°C.

**Conclusions:** Co-encapsulation of DOX and FLX presents a promising anticancer formulation regarding EE, stability, particle size and release rate and should be explored further in drug resistance cell lines and animal tumor xenograft models.

**Keywords:** Liposome; Fluoxetine; Doxorubicin; Co-encapsulation; P-glycoprotein