ESRF	**Experiment title:** Refinement of the Structural Study on the Incorporation of Antitumor Agents in Lipid Bilayers	**Experiment number**: SC-1870
Beamline: id10 b	**Date of experiment**: from: 14/09/2005 to: 20/09/2005	**Date of report**: 24/02/2006
Shifts: 18	**Local contact(s)**: Leide CAVALCANTI	*Received at ESRF:*

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Report:

Introduction

We have studied the incorporation of the hydrophobic anti-cancer agent paclitaxel (PXL) into lipid bilayers made by the zwitterionic lipid Dioleoyl phosphatidylcholine (DOPC), and the cationic lipid 1,2-Dioleoyl-3-Trimehylammoniumpropane (DOTAP), and in lipid mixtures with different size of head and tail and/or different net electronic charge. The studied drug has low solubility in aqueous media, and a major problem is to provide it as an injectable formulation. A range of concentration between lipids and PXL was studied in order to better understand the interaction between drug and lipids as drug carriers.

Materials and Methods

The phospholipids were dissolved in organic solvent together with the drug. Then the solvent was evaporated and the drug/lipid film was reconstituted with water and sonicated. The resulting liposome suspensions were deposited on glass slides and stored in an environment of controlled humidity.

Specular reflectivity data were collected at ID10-B (Troika II) ESRF beamline. We worked with uniform filling mode. The X ray beam energy in the experimental hutch was 7.99 keV selected by a diamond monochromator from the first harmonic of three undulator source.

Results and discussion

Figure 1(a) the shows the X-ray reflectivity curves of a lamellar phase for DOTAP/DOPC mixture with several concentrations of PXL. Several Bragg reflections indicate good laminar order of the lipid bilayers. In the presence of the drug the peak positions are shifted with respect to the pure lipid sample. If the fraction of PXL is 5% or higher, Bragg diffraction peaks of the pure drug become visible (indicated by circles). Figure 1(b) shows the evolution of the lamellar spacing as a function of PXL concentration for DOPC, DOTAP/DOPC (50:50) and DOTAP membranes. While the bilayer thickness continuously decreases with increasing fraction of the drug, it increases if the membrane is prepared with DOTAP. For the

DOTAP/DOPC mixture, the layer spacing continuously decreases until a fraction of 4% of paclitaxel, and for higher amounts of the layer spacing returns to higher values.

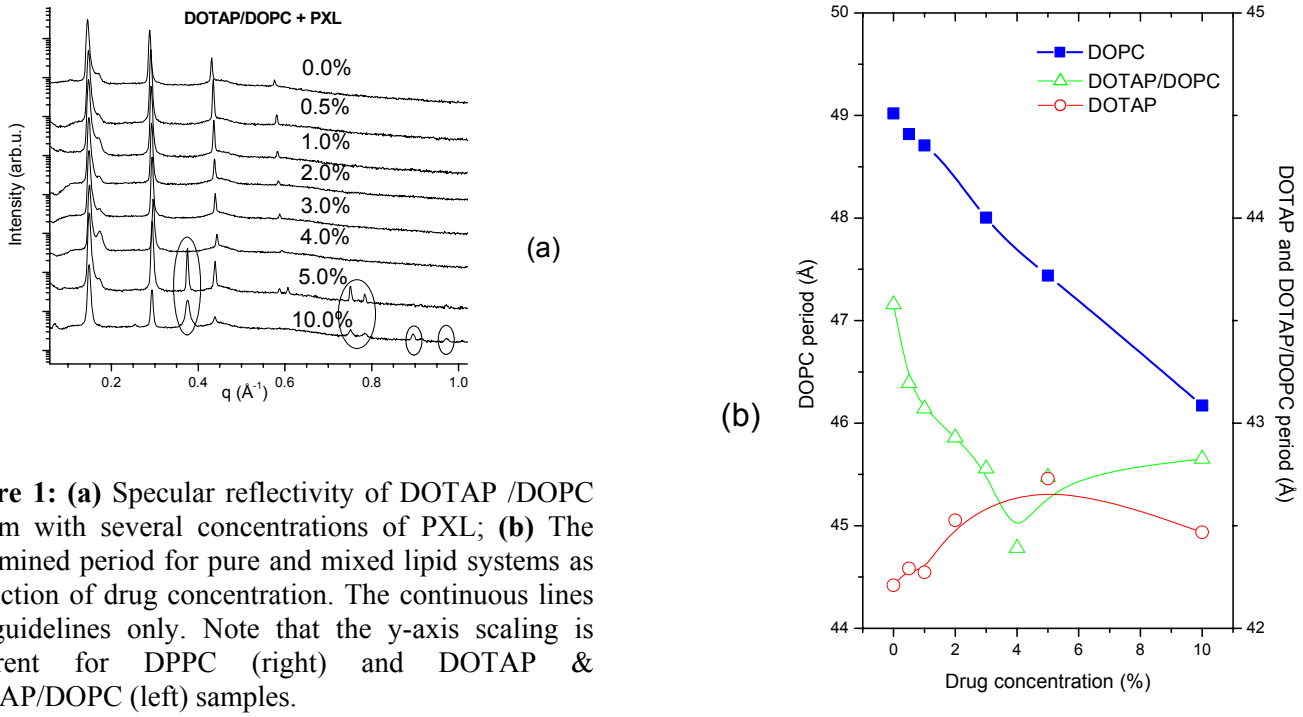

Figure 1: (a) Specular reflectivity of DOTAP /DOPC system with several concentrations of PXL; **(b)** The determined period for pure and mixed lipid systems as a function of drug concentration. The continuous lines are guidelines only. Note that the y-axis scaling is different for DPPC (right) and DOTAP & DOTAP/DOPC (left) samples.

The endpoint of continuous decrease of the DOTAP/DOPC layers pacing coincides with the molar fraction of PXL at which the Bragg peaks of the pure drug become discernible. This suggests, that up to 4% PXL could be inserted in DOTAP/DOPC membrane in a uniform way. Above that value the drug was present in the model membranes also in other states of aggregation. Further data analysis and a detailed investigation of these coherencies using other, independent, methods are ongoing.

The data indicate, that X-ray reflectivity measurements from drug/lipid model membranes can give valuable information about the drug insertion into the lipid matrix. Screening of drug and lipid compositions can be done in a fast and efficient way, and suitable drug/lipid combinations for further development of pharmaceutical carrier systems can be made out.