| ESRF | **Experiment title:** Structural Study on the Incorporation of Antitumor Agents in Lipid Bilayers | **Experiment number**: SC-1299 |
|---|---|---|
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## Report:

**Introduction**

We have studied the incorporation of the hydrophobic anti-cancer agents paclitaxel (PXL) and ellipticine (ELPT) into lipid bilayers made by the zwitterionic lipid dipalmitoylphosphatidylcholine (DPPC), the cationic lipids 1,2-Dimyristoyl-3-Trimehylammoniumpropane (DMTAP) and 1,2-Dioleoyl-3-Trimehylammoniumpropane (DOTAP), and in lipid mixtures with different size of head and tail and/or different net electronic charge. Both drugs have low solubility in aqueous media, and a major problem is to provide them as an injectable formulation. A range of concentration between phospholipids and PXL and, separately, with ELPT was studied in order to better understand the interaction of the system.

**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 on ID10b (Troika02) synchrotron beamline at the ESRF, Grenoble. We were working with 16 bunches mode. The X ray beam energy in the experimental hutch was 8.06 keV selected by a diamond monochromator from the first harmonic of three undulator source. Vertical size of the beam on the sample was 50μm. The typical observed range of scattering vectors was from 0.03 to 0.85 Å-1 for the solid samples.

**Results and discussion**

In Fig. 1 the X-ray reflectivity curves of a lamellar phase for pure DPPC and with 1% and 10% of PXL present are shown. Several Bragg reflections indicate good laminar order of the lipid bilayers. In the presence of the drug the peaks are shifted to higher Q, i.e., the real space repeat distance decreased. The effect with 1% of PXL is larger than that of 10 % PXL. Also, the peak from the 1% measurement appears to be

split. The results are in accordance with the understanding, that, the solubility of PXL in the lipid membrane is rather low, below 1% [1]. Already at 1% the PXL in the lipid membrane is thought to be metastable. At 10% the overloading with PXL is so high that immediate excretion to the equilibrium concentration occurs. With the cationic lipids DMTAP and DOTAP analogous effects were determined. However, the bilayer spacing of the pure lipid was lower and the decrease in bilayer spacing due to the drug was also lower (Table 1). Also for the insertion of ELPT in membranes from DPPC and lipid mixtures a decrease of the bilayer spacing has been determined. In that case, the maximum solubility of 3-4% could be significantly improved if cardiolipin was present as a colipid membrane [2]. The drug-induced reduction of the bilayer spacing by the drug was much lower or even inverted in that case.

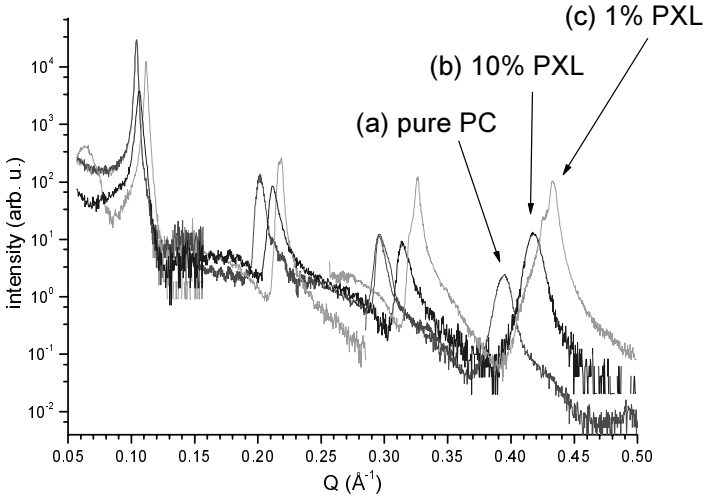


**Figure 1:** Specular reflectivity of (a) pure DPPC and mixed systems with (b) 10% and (b) 1 % of PXL.

| Compound | D spacing (Å) |
|---|---|
| DPPC | 63.7 |
| DPPC + 1% PXL | 58 |
| DPPC + 10 % PXL | 60.1 |
| DMTAP | 48.1 |
| DMTAP + 1 % PXL | 47.2 |
| DPPC + 10 % PXL | 47.7 |
| DOTAP | 46.0 |
| DOTAP + 1 % PXL | 45.2 |
| DOTAP + 10 % PXL | 45.4 |

**Table 1:** d-spacing for different lipid and lipid/drug mixtures

## Summary

The results demonstrate that with the present approach valuable information about the insertion of a hydrophobic drug into a lipid bilayer membrane can be determined. Systematic analysis of the d-spacing as a function of the drug fraction may reveal information on the molecular organization and on the maximum solubility of the drug in the membrane. Further, screening of different lipid compositions with respect to the solubilizing potential for a drug can be done. In subsequent measurements it is planned to investigate the maximum solubility of PXL in membranes from cationic lipid and to check if the solubility can be improved by suitable colipids, such as it was found for ELPT.

## References

[1] Gruber, F., Haas, H., Winter G., A Thorough Investigation of the Thermal Behaviour of Lipid Bilayers in the Presence of Lipophilic Drugs, poster pres. at the Controlled Release Society Meeting Glasgow, 2003
[2] Cavalcanti, L., PhD thesis, Universidade Estadual de Campinas - UNICAMP, SP, Brazil (2001)