ESRF	**Experiment title:** Lipid-DNA-Metal complexes for gene delivery:X-ray diffraction study of PEGylated lamellar complexes.	**Experiment number**: SC 1831
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Report:

Gene therapy requires efficient systems for the delivery of DNA and other biological macromolecule into cells. The major constraint in this field is to devise ideal 'vehicles' to target appropiate cells and achieve physiological levels of the desired gene production. With the aim of providing new biological materials acting as efficient nonviral gene delivery agents, over the last few years we have carried out a systematic study of the interactions among DNA, neutral liposomes (L) and metal cations (Me^{2+}) in triple L- DNA-Me^{2+} complexes [1-3]. The goal of the present research is the development of vectors that lead to a significant increase of the transfection efficiency. The inclusion of a biocompatible polymer shell is essential in developing a synthetic gene delivery system that can successfully evade the immune system for systemic *in-vivo* applications. The functionalization of the neutral lipids with PEG should enhance the binding of the carrier to the DNA molecules.

To this purpose, we have carried out a series of x-ray diffraction experiments focused to study the nano-scale structure of ternary mixtures of polyethyleneglycol(PEG)-lipids conjugated, DNA (from calf thymus) and divalent cations (among the most common in biological cells) and their suptamolecular assembly. The final goal is to gain information on the relationships between structure and physico-chemical properties of the component materials.

The results obtained from a preliminar elaboration of the experimental data seems to be quite promising. In particular, fig.1-A shows the small-angle x-ray diffraction patterns of the DOPE- PEG(350)/DOPE mixture, at different concentrations of the PEG(350)-lipid. The set of periodically spaced sharp peaks originates from an inverted hexagonal structure *(H_{II})*, whose structural elements are infinitely-long rigid rods, all identical and crystallographically equivalent, regularly packed in a 2D hexagonal lattice. The cylinders are filled by water and are dispersed in the continuous medium constituted by hydrocarbon chains whereas the polar groups are located at the water-hydrocarbon chain interface. The addition of PEG(350)-lipid leads to a regular increase of the 2D hexagonal lattice spacing from a = 73 Å to a = 76 Å. At 15% of PEG(350)-lipid the hexagonal phase disappears.

Remarkably, the addiction of $CaCl_2$ to the mixtures of DOPE- PEG(350)/DOPE over 15% of the PEG-lipid induces a cubic phase (Figure 1-B). The Bragg peaks associated to the cubic lattice are spaced in the ratios $\sqrt{2}$: $\sqrt{3}$: $\sqrt{4}$: $\sqrt{6}$: $\sqrt{8}$: $\sqrt{9}$: $\sqrt{10}$, corresponding to the Q^{224} phase with the space group *Pn3m* with lattice parameter a=157 Å .

Figure 1-C shows the diffraction patterns of the [DOPE-PEG(350)/DOPE]-DNA-Ca^{2+} complexes at molar ratio 4:12:18. From these data, at 25% DOPE-PEG(350) concentration we observe the coexistence of two hexagonal phases and a cubic phase. The unit cell parameters of the H_{II} phases are a_1~78 Å and a_2~69 Å, respectively, and the lattice parameter of the *Pn3m* cubic phase is a=133 Å. On increasing the PEG-lipid concentration a transition to a cubic phase becomes apparent. This cubic phase *Pn3m* is observed in the DOPE-PEG(350)/DOPE]-DNA-Mn^{2+} complexes too. Based on these data, a transition from an inverted hexagonal phase to a cubic phase should occur in the three-component mixtures on increasing the PEG-lipid concentration, which is a quite new and striking result in these biological systems. We believe that these findings can open up new exciting perspectives for the understanding of the phase behaviour of these ternary complexes in the presence of PEG-lipid.

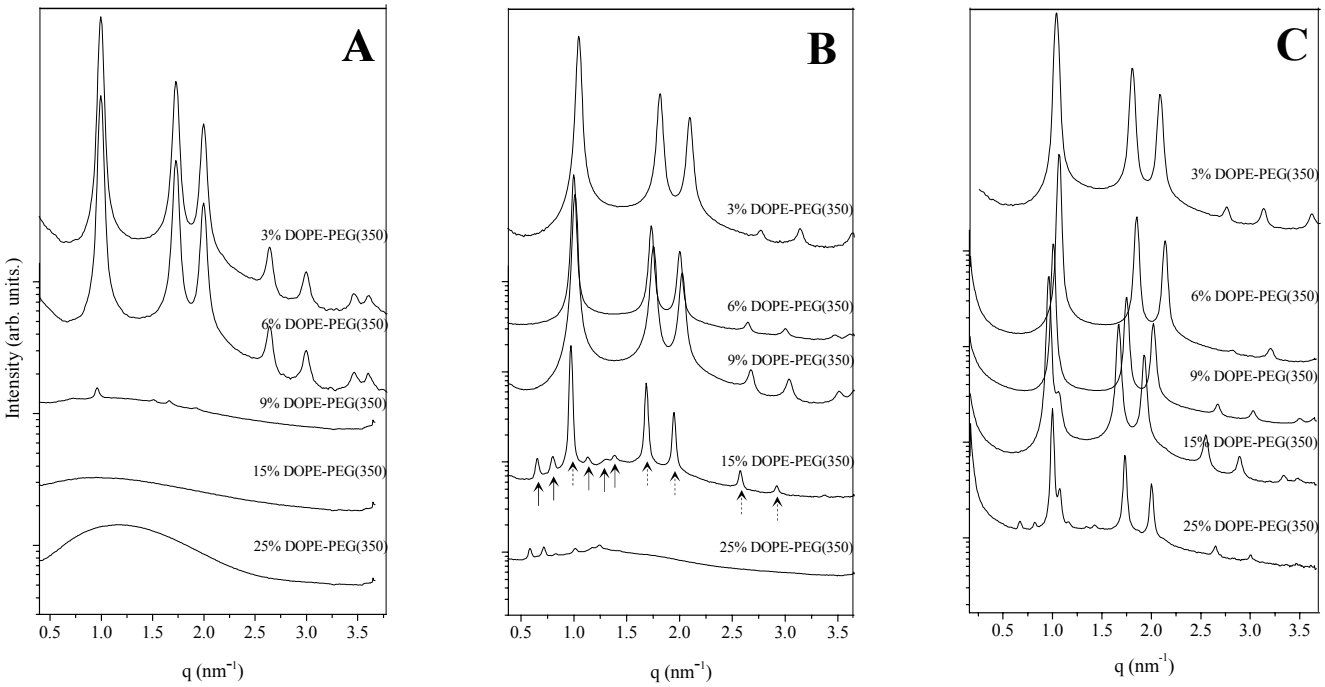

Figure 1: (A) Synchrotron X-ray diffraction patterns of [DOPE-PEG(350)/DOPE] mixture as a function of the concentration of the PEG(350)-lipid. **(B)** Synchrotron X-ray diffraction patterns of [DOPE-PEG(350)/DOPE] mixture as **A** with a fixed concentration of $CaCl_2$ (69 mM). The solid arrows denote the Bragg peaks associated with the Q^{224} (*Pn3m*) cubic phase and the dashed ones refere to the peaks of the H_{II} hexagonal phase. **(C)** Synchrotron X-ray diffraction patterns of [DOPE-PEG(350)/DOPE]-$CaCl_2$ as **B** with DNA (3.8 mg/ml).

References:

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