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ESRF	**Experiment title:** SAXS Analysis of Supramolecular Organization of siRNA Delivery Systems in Suspension and in Tablets	**Experiment number**: LS-2647
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Report:

Small Angle X-Ray Scattering (SAXS) was used here to identify the supramolecular structure of our nucleic acid delivery systems either in suspension in aqueous buffer or in solid state, in lyophilisate and tablets. For us it is crucial to identify the supramolecular of these particles since it directly impacts on their efficiency. SAXS using synchrotron beam is the only analysis technology that provides access to structure from various dosage forms, suspension or powder as well.

As previously shown by others, this kind of delivery systems, namely lipoplexes (cationic lipid-based nucleic acid complexes), build up with plasmid DNA or siRNA, can display lamellar, hexagonal, or even cubic structures [1-3].

Fig. 1A summarizes the SAXS results obtained for cationic liposome, basic building block of our delivery system and siRNA lipoplexes in suspension in saline buffer. Liposomes present peaks compatible with a combination of a lamellar phase and a cubic phase. When siRNA is added to liposome, to form siRNA lipoplexes, a cubic phase exhibiting higher and more define peak is observed. An additional phase is observed with undetermined packing symmetry. This results show that the formation of lipoplexes upon addition of nucleic acid and electrostatic interaction between negative charged groups of nucleic acid and positive charged groups of cationic lipids modify the supramolecular structure of lipids while retaining the cubic phase. We also examined the effect of varying the composition of lipoplexes, such as the type of nucleic acid (plasmid DNA versus siRNA) or the addition of anionic polymer adjuvant (sodium alginate or

sodium polyglutamate) on the supramolecular structure. We previously showed that the addition of anionic polymer enhanced the gene silencing efficiency of siRNA lipoplexes [4]. As the formulation may be, we observed either a unique cubic phase or a mixture of lamellar and cubic phases. The deciphering of the precise effect of formulation on the supramolecular structure will require more investigation.

This study aimed also to explore the behaviour of our delivery systems upon lyophilisation and compression steps applying for tableting these particles. We wanted to know whether freeze-drying and subsequence compression have an impact on the internal structure of our particles. **Fig. 1B** shows the SAXS results obtained for siRNA lipoplexes in solid form, either in lyophilisate powder (in green) or in tablet powder (in red). Lipoplexes in lyophilisate and tablets present peaks compatible with a lamellar phase, showing that upon freeze-drying the cubic phase observed when lipoplexes are prepared in suspension disappeared and gave way to a lamellar phase. We observed for the first time the structure of lipoplexes in solid form and demonstrated that they keep their typical structure upon dehydration and compression.

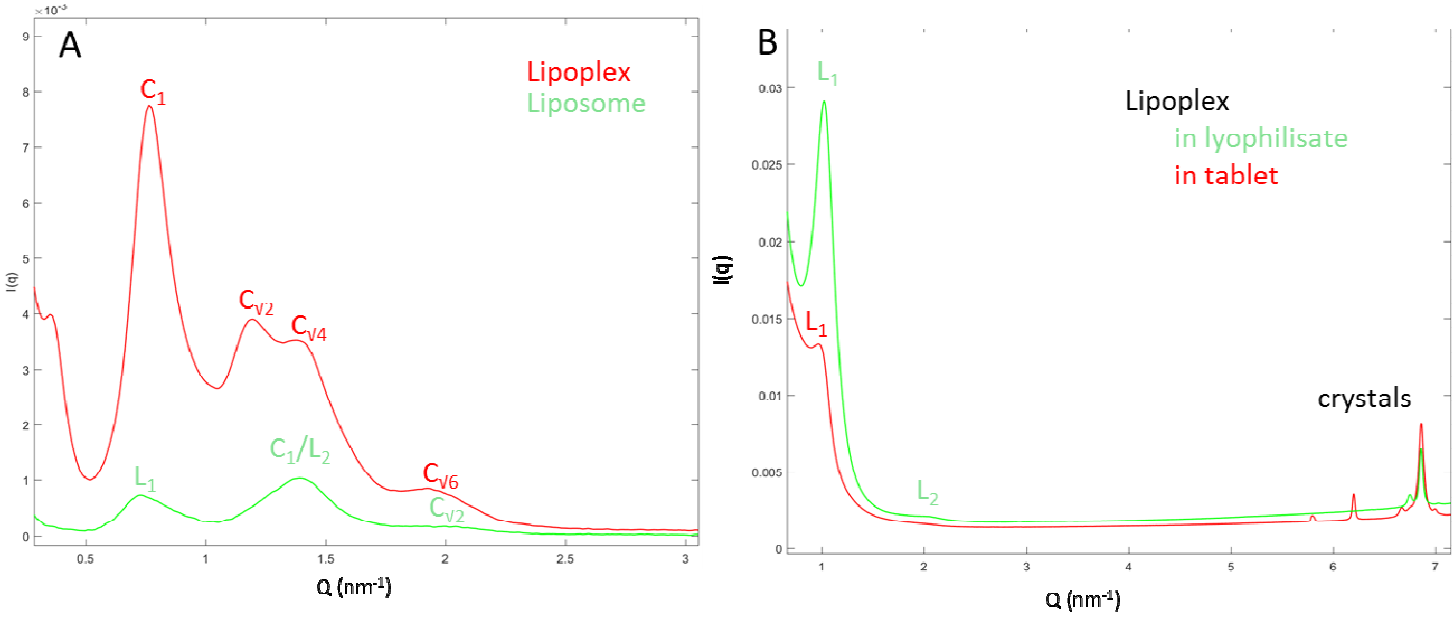

Fig. 1: SAXS profiles of cationic liposome and siRNA lipoplexes in suspension (A) and in solid form (B).

In summary, this SAXS experiment on siRNA lipoplexes provided data on internal structure of these particles and demonstrated the feasibility of studying this strucutre once the particles have been freeze-dried and/or compressed in tablets.

References

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