Experimental report

31/08/2022

Proposal: 9-13-989 **Council:** 10/2020

Title: Lipid nanoparticles under shear stress: mimicking the blood flow

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: lipid nanoparticles
Apolipoprotein E

Instrument	Requested days	Allocated days	From	To
D22	2	2	18/05/2021	20/05/2021

Abstract:

Therapeutic treatments based on the protein production by delivering messenger RNA (mRNA) represent a promising approach. One of the major challenges is to protect mRNA from enzymatic degradation and deliver it into the target cells. Lipid nanoparticles (LNPs) formed by a cationic ionizable lipid (CIL), DSPC, cholesterol and a pegylated lipid were approved for delivery of small interference RNA. In vitro and in vivo studies often draw different conclusions when LNPs are involved and this may be due to the extremely different environments where the LNPs are immersed, not only in terms of molecular composition of the media but also due to responsiveness in the LNP to flow rate and shear. There is little known on the structural effects due to corona formation or due to the shear stress upon intravenous (IV) administration.
Our recent results (currently being prepared for publication) suggest that Apolipoprotein E binding affects the LNP structure. We aim to characterize the LNP structure under shear stress comparable to blood flow both with and without the payload. In addition, we want to investigate if the ApoE binding to LNPs induces a change in the LNP response to shear stress.

Report on Experiment 9-13-989
18th – 20th May 2021

Lipid nanoparticles (LNPs) formed by a cationic ionizable lipid (CIL), DSPC, cholesterol (Chol) and a pegylated (PEG) lipid can be used to deliver mRNA. In our previous experiments, we were able to describe: (#8-13-866) the structure and component distribution of the LNP at pH 7.4 in presence and absence of ApoE[1], and (#9-13-909) the structure of 3 different LNP formulations at both pH 7.4 and 5.5 (Sebastiani et al., *manuscript in preparation*).
In this experiment, we investigated:

(1) the LNP structure under shear stress in presence and absence of payload (polyA) for 3 formulations
(2) the low-density lipoprotein, LDL, structure under shear stress as a reference sample.

We formulated LNPs according to Table 1, for each composition we prepared a sample with 100%mol dDSPC and 100%mol d7cholesterol (both from Avanti Polar Lipids) in order to highlight separately shell and core according to the solvent contrasts. We prepared the 3 formulations with and without payload. For this experiment, we have used polyadenylic acid (polyA) as a model for mRNA. LDL sample was used as a reference sample, since its structure in static conditions is well characterised. Samples were prepared at two solvent contrasts: 27 and 95% D_2O, while LDL was in D_2O. All samples were measured in phosphate buffer saline at 10 mM with 150 mM NaCl pH 7.4. We have measured the samples at 4 shear rate values: 0, 100, 500 and 1000 s^{-1}.

Table 1 LNP compositions

	CIL	DSPC	Chol	PEG-lipid
LNP c1	50	10	38.5	1.5
LNP c2	50	10	39.75	0.25
LNP c3	53.5	4.7	41.2	0.7

In order to fully characterise the samples in static conditions, we have prepared other 2 or 3 solvent contrasts: 46, 70 and 87 % D_2O. These samples were measured in quartz cuvettes with a concentration of 3 mg/ml.

It is noteworthy to say that the required volume for the measurement with the rheometer is quite large (5 mL) and hence the concentration of LNP had to be fairly low (1 mg mL^{-1}), due to the high cost of the compounds. The rheology data acquired show no difference between the water and the LNP solution (Fig. 1B). However, the scattering curve shows a decreased intensity at low q when the LNP c1 (27% D2O) is under shear, which suggests a transient thinning of the shell (Fig. 1A). These results suggest that, even in absence of a macroscopic effect of particle anisotropy induced by shear flow, a microscopic change in the LNP structure is occurring. The data collected at 95% D2O do not show a visible effect of shear stress, however that contrast is not as sensitive to changes in shell as the 27% D_2O. Further data analysis is ongoing.

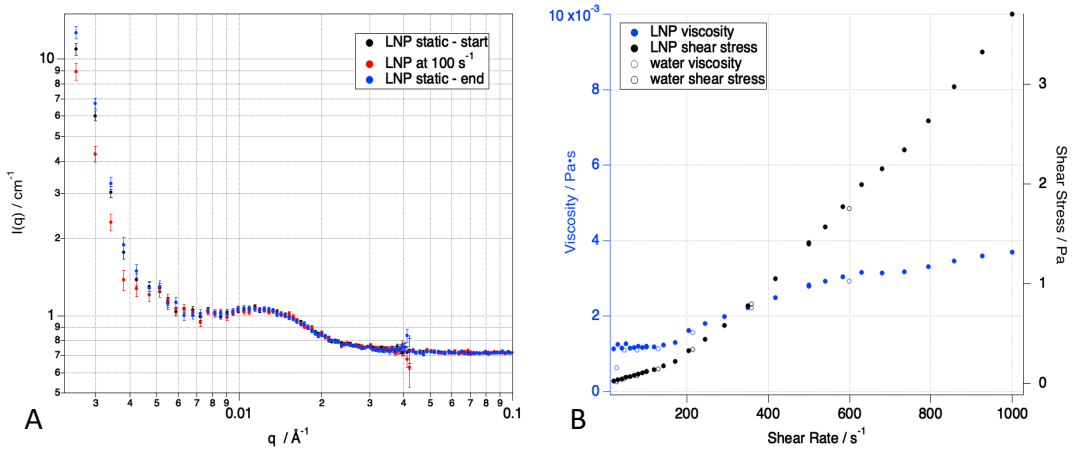

Figure 1 SANS curves acquired with LNP c1 27% D2O solution under shear (Couette geometry), from rest (black) to 100 s^{-1} (red) and back (blue) (A); viscosity (blue) and shear stress (black) measured as a function of shear rate for water (open circles) and LNP solution (filled circles) (B).

1. Sebastiani, F. *et al.* Apolipoprotein E Binding Drives Structural and Compositional Rearrangement of mRNA-Containing Lipid Nanoparticles. *ACS Nano* (2021) doi:10.1021/acsnano.0c10064.