inside Therapeutics

Template
LNP formulation Protocol – SM102 5mg/mL

2024

General protocol for the formulation of lipid nanoparticles using TAMARA

LNP formulation protocol

Before starting a formulation :

- Make sure you have the right amount of all the chemicals needed
- Likewise, be sure to have enough buffer to conduct your formulations
- Be mindful of the analysis you want to perform after formulation and their requirements
- It is best to use filtered solvents (ultrapure water and ethanol)

Materials and equipment

Equipment/machines	Reagents/chemicals	Materials/consumables
TAMARA formulation platform Magnetic stirrer (**if dialysis**) DLS or NTA for colloidal analysis Plate reader for payload analysis (Ribogreen)	mRNA stock solution Lipids (stock solutions or solids) Citrate buffer (e.g. pH 4.0, 10 mM) Phosphate buffer saline (1X) Ultra-pure ethanol	Eppendorfs Falcons (15 mL and 50 mL) Dialysis membrane (**if dialysis**)

Procedure

1- Preliminary preparation

Take out the RNA stock solution out of the freezer (-80°C) and let it thaw (if possible, let it thaw on ice).

Take out the lipids out of the freezer and let them warm up to room temperature (at least 20 minutes) without opening the lid. If working with stock solutions (and not solids) make sure that the solutions are homogenous (no precipitation or crystallization) by gently heating them, vortexing them and/or sonicating them.

2- Lipid mix solution (5mg/mL)

You will either work with lipids as powders or lipid stock solutions.

DSPC/Cholesterol/SM-102/DMG-PEG2000 (molar ratios 10:38.5:50:1.5).

Either weigh the right amount of solid lipids in a vial or pipette the right amount of lipid solutions in a vial, and add the right amount of ethanol to complete the lipid mix solution.

For one triplicate of 1mL Volume Total at FFR of 3, prepare the following lipid mix solution :

Lipids	MW (g/mol)	molar ratio	mass (mg)	Stock solution oncentration (mg/mL)	Volume to pipette (µL)
DSPC	790	10,00%	0,64	25,00	25,461
Cholesterol	386,6	38,50%	1,20	25,00	47,969
SM-102	710,18	50,00%	2,86	25,00	114,440
DMG-PEG2000	2509,2	1,50%	0,30	25,00	12,130
total	620,569	100,00%	5	**Total**	200,000
				Ethanol to add (µL) :	800,000

Make sure that this lipid mix is homogeneous, vortex/gently heat it if needed.

3- RNA solution

Be careful to **remain as RNAse free as possible** throughout this process, and from this point on.

Calculate the amount of RNA solution needed for your formulation according to your N/P ratio (we usually use 6).

Pipette the right amount of RNA stock solution and add the right amount of buffer (citrate buffer) to complete the RNA solution.

4- Formulation of LNPs

1. Turn on TAMARA
2. Make sure you are using a **clean and dry chip** and a **clean and dry reservoir**
3. If it is your first run, or if you need it, perform a pressure test before the experiment (page 23 of TAMARA user Guide)

4. Before selecting your formulation parameters, make sure that you are using the right design for the chip (here **Herringbone**), the right organic solvent (**ethanol**) and set in the right temperature of your lab. You can change those parameters in the top left-hand corner menu.
5. Select the parameters of your experiment by clicking on "new run" : **for this example, FRR 3, TFR either 1mL/min or 5mL/min, Total Volume 1mL**.
6. Place the collection tube on the holder
7. Pipette the right volume of each solution in the corresponding tank (in this case 750µL of aqueous buffer containing your RNA and 250µL of lipid mix in ethanol, for one run). **As highlighted on p29 of the user guide, pipette gently in the centre at the bottom of the reservoir to avoid bubble formation**. Pipette in the aqueous solution first.
8. Place the silicone gasket and the chip on the designated slot.
9. Make sure you align the chip correctly **to use the right design**.
10. Close the lid
11. Run the desired experiment and collect the sample (you can check your parameters in the upper part of the screen)
12. Place a 50 mL falcon tube as a waste collector in the holder and run the whole cleaning procedure (consisting of a purge and a cleanse with cleaning solutions)
13. Run the purge
14. Run at least one washing sequence with ultrapure water in the aqueous tank and pure ethanol in the organic tank.
15. Make sure that the system is dry and that the chip is dry and in good condition.

5- Purification

We are using **dialysis** as a downstream process.

Rehydrate the dialysis membrane according to instructions.

Carefully add the whole sample in the membrane, and make sure it is properly sealed.

Put the dialysis bag in a beaker containing enough PBS (1X) and let it stir overnight at room temperature or 4°C, depending on your formulation.

You can change the bath several times if necessary (we usually change the bath twice, leaving one overnight at RT).

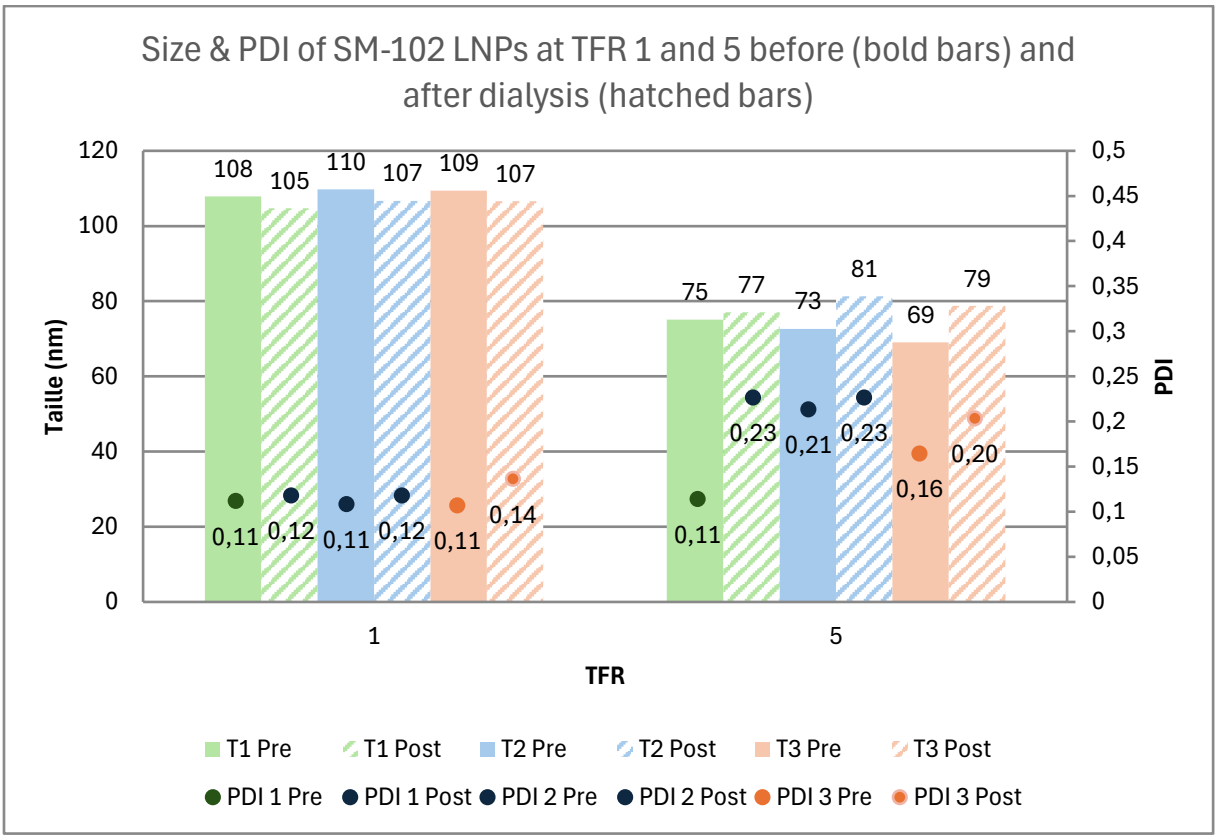
After dialysis, retrieve your sample and store it in a new tube.

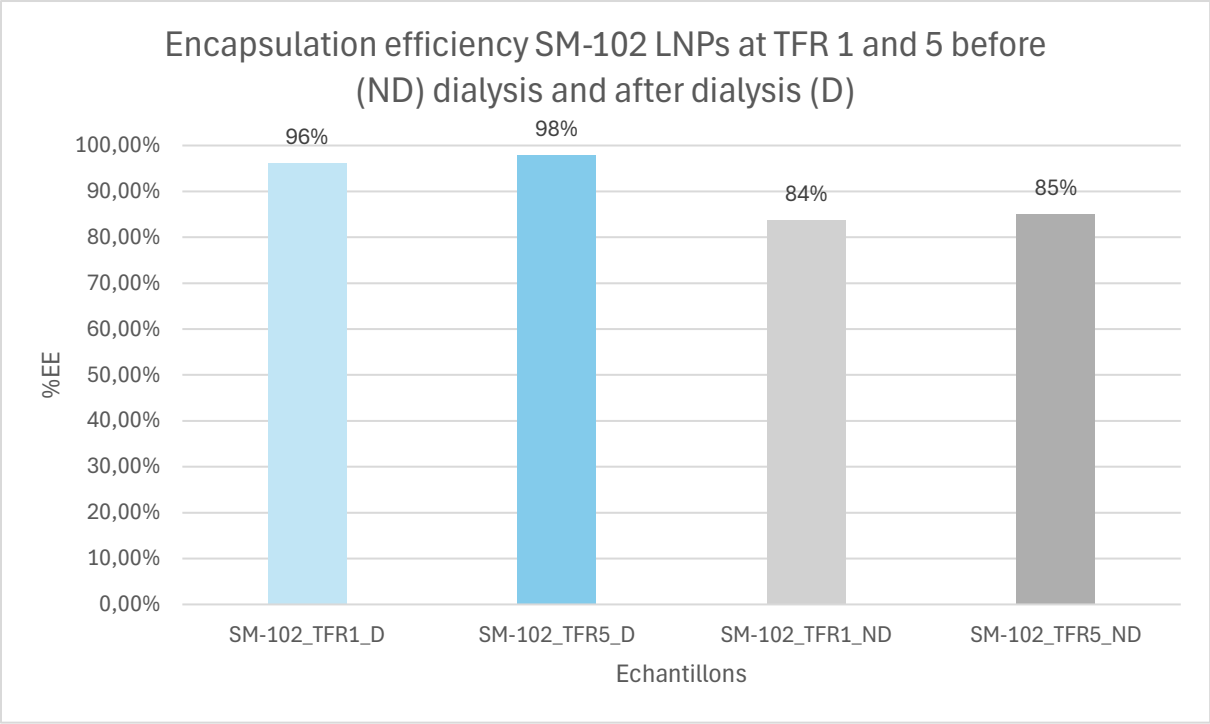
Analysis

You can perform several analysis after formulation, depending on your needs. The easiest way to characterize your nanoparticles, is to use Dynamic Light Scattering technique. It will provide you with information about the size distribution and give you an estimate of sample's homogeneity through the Polydispersity Index (PDI). You can also reach those basic information using a Nanoparticle Tracking Analyzer (NTA).

To have information about the content of your lipid nanoparticles, it's the gold standard to perform a Ribogreen assay. This assay uses a fluorescent probe that will interact with the RNA strands. With the proper protocol and an appropriate plate reader, you will be able to determine the amount of free RNA outside your nanoparticles, and the amount of RNA encapsulated inside your nanoparticles. It is needed to calculate two important metrics for nanoparticles : the encapsulation efficiency and the encapsulation yield.

Here are the data obtained for the formulation of LNPs using SM-102 as the ionizable lipid :





We wholeheartedly thank Nabila Laroui and Chantal Pichon from ART-ARNm (Inserm US-55) whose initial template served as groundwork for this protocol.