Effect of Process Parameters and Buffer Exchange on the Critical Quality Attributes of siRNA Loaded Lipid Nanoparticles

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PURPOSE

To explore the effect of process parameters and buffer exchange on the critical quality attributes (CQAs) of siRNA loaded lipid nanoparticles (siRNA-LNPs). Onpattro®, partisiran sodium intravenous solution, was used as the model drug in this study.

1. Investigate the process parameters and conditions including total flow rate (TFR), flow rate ratio (FRR), and temperature on CQAs of siRNA-LNPs, including size, size distribution, surface charge, morphology, lipid composition, encapsulation efficiency (EE%), cell viability, and efficiency of target mRNA knockdown.
2. Investigate the effects of buffer exchange on CQAs of siRNA-LNPs including size, size distribution, zeta potential, EE%, and lipid composition.

METHODS

A two-level full factorial design was employed to assess the impact of three variables: TFR (1, 3 mL/min), FRR (1, 5), and temperature (8, 45°C) on the CQAs of siRNA-LNP formulations. Eight siRNA-LNPs maintaining the same molar ratio and composition as Onpattro® were prepared accordingly, utilizing the NanoGenerator® Flex-M, PreciGenome microfluidic system. The resulting siRNA-LNPs were dialyzed by 10 kDa dialysis cassette over phosphate buffered saline (PBS) to remove ethanol residues and to exchange buffers. The CQAs of formulations were evaluated using different techniques, which are summarized in Table 1.

CQA	Method
Morphology	Transmission Electron Microscopy (TEM)
Size, Polydispersity Index (PDI)	Dynamic Light Scattering (DLS)
Surface Charge	Electrophoretic Light Scattering
Encapsulation Efficiency (EE%)	Ribogreen Assay
Transthyretin (TTR) mRNA Knockdown	Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)
Lipid Content	Liquid Chromatography-Charged Aerosol Detector (LC-CAD)

Table 1. siRNA-LNP CQAs and the analytical methods to investigate them.

RESULTS

1. Effect of temperature on morphology of siRNA-LNPs:

Particles made at 8°C show spherical structure, while particles made at 45°C show irregular morphology, as depicted in Figure 1.

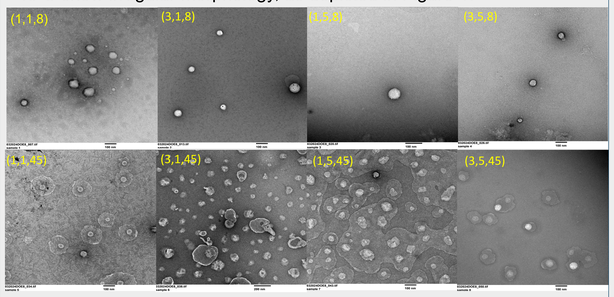

Figure 1. TEM images of siRNA-LNP particles made at different process parameters as shown in parenthesis in order of TFR, aqueous to organic phase FRR, and temperature.

2. Process parameter screening for "size" response:

As shown in Figure 2, FRR and temperature have the most significant effect on size of the particles. It is also shown that particle size reduces at higher temperature, higher FRR, and after dialysis. The interaction profile of parameters, highlighted by the red circles shows that size reduction after dialysis is more significant for particles made at lower FRR.

Source	Logworth	PValue
FRR(1,5)	6.005	0.00000
temperature(8,45)	3.881	0.00013
dialysis	2.444	0.00360
FRR*dialysis	2.053	0.00885
FRR*temperature	1.142	0.07211
TFR(1,3)	1.054	0.08836

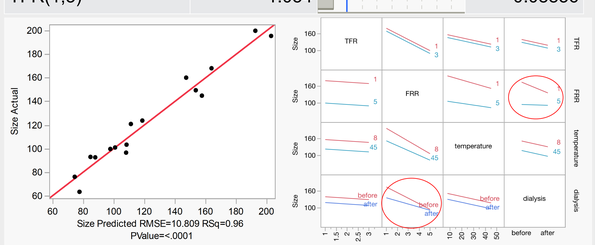

Figure 2. Process parameters ranking, actual vs. predicted plot, and interaction profile of parameters for "size" response. Blue line in the table shows the threshold of statistical significance of Logworth (-Log of P-value) defined as 2, which means P-value < 0.01.

3. Process parameter screening for "PDI" and "EE%" response:

FRR is shown in Figure 3 to have the most significant effect on PDI and EE%. The prediction profile of FRR shows an inverse correlation with PDI and a positive correlation with EE%.

Source	Logworth	PValue
FRR(1,5)	3.401	0.00040
FRR*temperature	1.311	0.04883
TFR(1,3)	1.270	0.05371
TFR*dialysis	1.146	0.07140
dialysis	1.105	0.07845
temperature(8,45)	0.711	0.19473

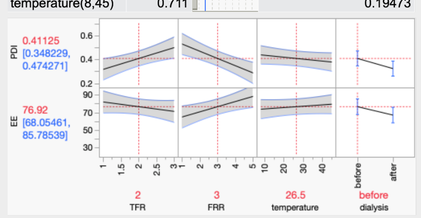

Figure 3. Process parameters ranking, and prediction profiler for "PDI" and "EE%" response. Blue line in the table shows the threshold of statistical significance of Logworth (-Log of P-value) defined as 2, which means P-value < 0.01.

4. *In vitro* TTR mRNA knockdown of siRNA-LNPs:

Data shown in Figure 4, shows very low TTR mRNA expression levels for all samples, highlighting very high TTR mRNA knockdown efficiencies. The other observation is that particles made at lower temperature have a lower TTR expression level, and thus higher gene silencing efficiency. The reason to this could be owing to the probable higher cellular uptake of these particles due to more spherical morphology, compared to those made at 45°C.

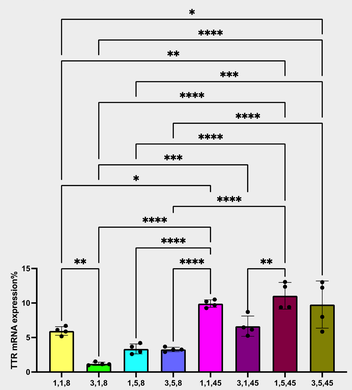

Figure 4. TTR mRNA expression levels of Hep G2 cells after 48 hours following treatment with siRNA-LNPs encapsulated with 60 nM siRNA, made at different process parameters as shown in sample labels in order of TFR, aqueous to organic phase FRR, and temperature. The negative control which was 60 nM non-targeting siRNA delivered by lipofectamine showed ~100% of TTR expression (data not shown here).

5. Lipid concentrations and molar ratios of siRNA-LNPs:
Data in Figure 5, shows that the lipid concentrations and molar compositions relatively remain constant during buffer exchange for all samples except for those made at FRR1, highlighted by brackets. This effect could be due to the less efficient ethanol dilution at lower FRR, which could lead to the loss of unincorporated lipids in LNP during dialysis.

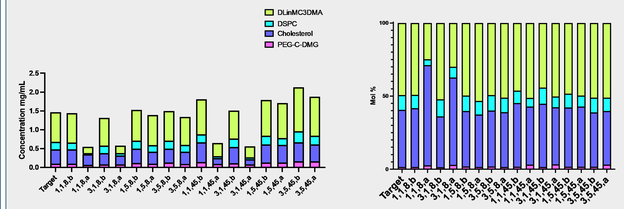

Figure 5. Compositional lipid concentration and molar ratios in siRNA-LNPs made at different process parameters as shown in sample labels in order of TFR, aqueous to organic phase FRR, temperature, and before dialysis (b) and after dialysis (a).

CONCLUSION

- In this study, FRR was found to be the most critical process parameter during microfluidic preparation of siRNA-LNPs to control CQAs of formulations.
- Some CQAs of particles may change during buffer exchange including size and lipid content/composition. The mentioned changes are more significant in particles made at lower FRR, highlighting the importance of optimization of FRR during microfluidic preparation of siRNA-LNPs. Such difference could be attributed to less efficient ethanol dilution and higher ethanol content pre dialysis for particles made at lower FRR.
- Temperature was shown to affect the morphology and gene silencing efficiency of siRNA-LNPs, as particles made at lower temperature, are shown to have a more spherical morphology, potentially enabling higher cellular uptake, and hence the higher observed TTR mRNA knockdown efficiency.

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