Application note Short summary

Benchmarking TAMARA against mainstream toroidal microfluidic mixer

The efficient formulation of RNA-loaded lipid nanoparticles (RNA-LNPs) plays a pivotal role in advancing mRNA-based vaccines and therapeutics. Microfluidics-based systems have become the leading technology for RNA-LNP formulation during discovery phase, thanks to their ability to minimize the consumption of expensive reagents by operating at sub milliliter scale, while maintaining precise control over key nanoparticle characteristics.

This study compares two leading microfluidic platforms for RNA-LNP formulation at < 1 mL volumes: TAMARA by Inside Therapeutics and Ignite by Precision Nanosystems/Cytiva. Their performance is evaluated using standard mRNA-LNP protocols, focusing on critical factors such as particle size, encapsulation efficiency, yield, transcription efficiency, cell viability, and protein expression. The goal is to determine which platform optimizes RNA-LNP formulation for preclinical applications.

This work was carried out together by Inside Therapeutics supported by the University of Orleans, France.

The formulation parameters for the Ignite system were previously determined and optimized through a Design of Experiments (DOE) approach, with all experiments conducted at the smallest feasible volume of 700 µL.

Influence of Formulation Parameters on Size & PDI

Polydispersity Index (PDI): Nanoparticle size distribution (0 homogeneous to 1 highly heterogeneous)

TFR and FRR impact on TAMARA

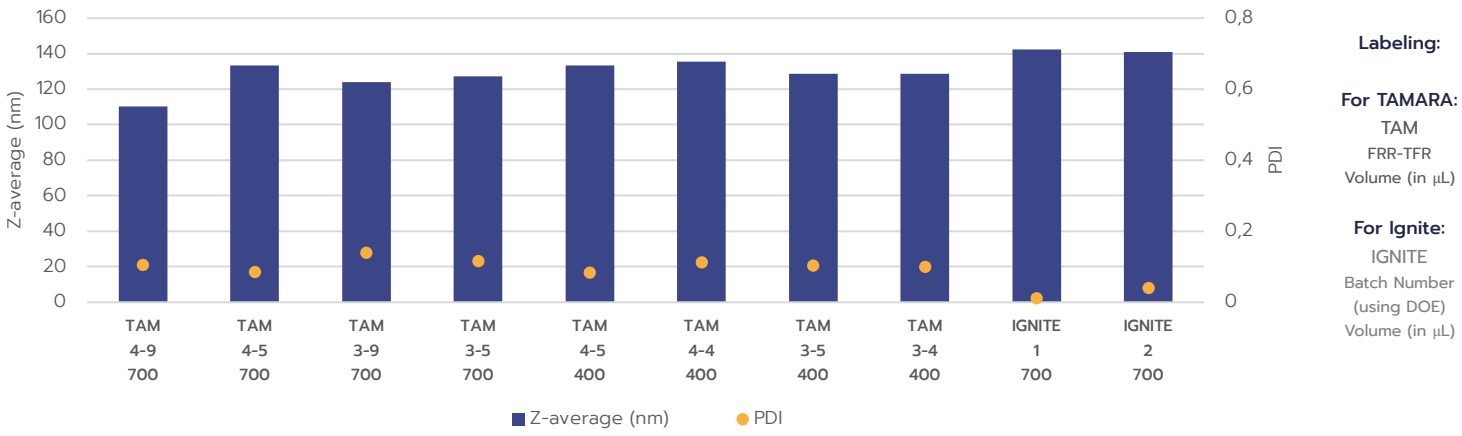
→ Increasing the **Total Flow Rate** (TFR) reduces nanoparticle size; Flow Rate Ratio (FRR) has less impact.

Total Flow Rate (TFR): Overall flow rate.
Flow Rate Ratio (FRR): The ratio between the aqueous and solvent phases.

Systems comparisons

→ Both systems exhibit excellent quality with size in ideal range and **PDI < 0.2 meeting industry standards**.

→ Optimization of TFR & FRR parameters through DOE – such as done with Ignite - is critical to optimize formulation quality.



Labeling:

For TAMARA:
TAM
FRR-TFR
Volume (in µL)

For Ignite:
IGNITE
Batch Number (using DOE)
Volume (in µL)

Comparison of Encapsulation Efficiency (EE%) and Encapsulation Yield (EY%)

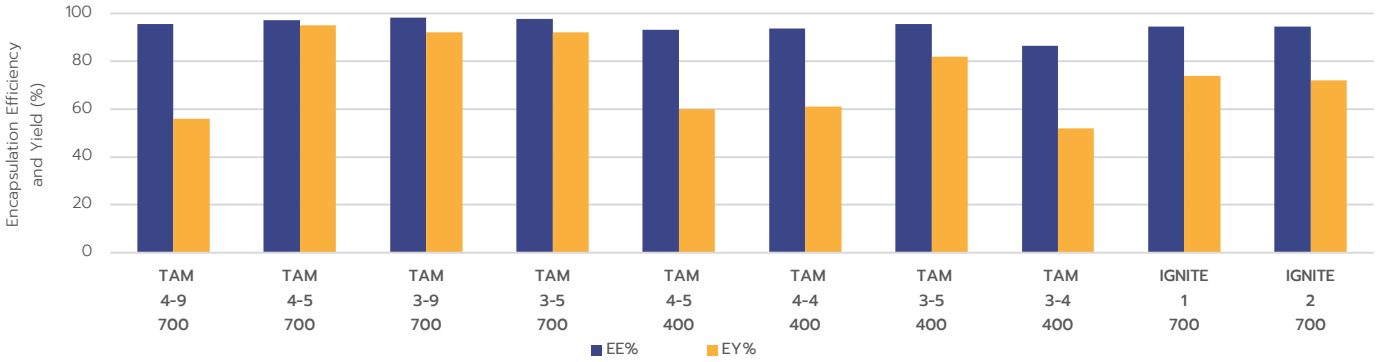
Encapsulation Efficiency (EE%): Trapped RNA/RNA at the end of the experiment
Encapsulation Yield (EY%): Trapped RNA/Input RNA.Similar to recovery

TFR and FRR impact on TAMARA

- **EE%:** TAMARA consistently achieved over 95% encapsulation efficiency, both at 700 and 400 µL.
- **EY%:** Encapsulation yields also high, reaching 95% for 700 µL, except for one outlier (TAM 4-9 700). Lower volumes lead to lower EY%.

Systems comparisons

- **EE%:** Both systems achieve comparable EE% levels.
- **EY%:** TAMARA demonstrates much higher level of yield than Ignite at similar volumes, maximizing RNA recovery.



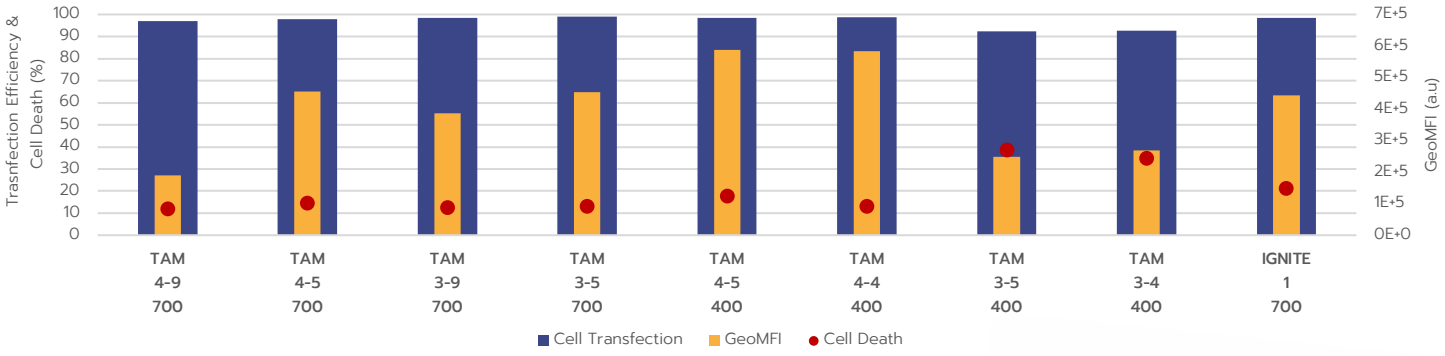
Comparison of Transcription Efficiency, Cell Viability, and Protein Expression

TFR and FRR impact on TAMARA

- **Transfection efficiency** remained consistently high (~100%) across samples.
- **Cell viability** stays high with low cytotoxicity at 700 µL. Can decrease at lower volumes.
- **Protein expression** generally remains highs across samples, though vary from formulation parameter to formulation parameter and closely align with encapsulation yield.

Systems comparisons

- **Transfection efficiency** remained consistently high (>90%) for both systems.
- **Cytotoxicity** was acceptable with both systems, though TAMARA showed lower cytotoxicity at equivalent volumes.
- **Protein expression** was strongly correlated with encapsulation yield.
- TAMARA achieved up to 30% higher **protein expression** compared to Ignite, even at lower volumes. This can be attributed to TAMARA's higher encapsulation yield and reduced cytotoxicity.



Conclusion

- **Size & PDI:** Both systems met regulatory standards (PDI < 0.2) and effectively allow for nanoparticle size control.
- **EE% & EY%:** Encapsulation efficiency is similar, but TAMARA achieves much yields over 90% at 700 µL, compared to the Ignite's 75%.
- **In cell studies:** Both systems had similar transcription efficiencies (~100%) and low cytotoxicity, but TAMARA produced up to 30% higher protein expression.
- **RNA usage:** TAMARA's performance at 400 µL and 700 µL matched Ignite's 700 µL, meaning near half the reagent use.
- **Cost-effectiveness:** TAMARA used only two reusable chips, demonstrating superior cost-efficiency.
- **Scalability:** Formulation with TAMARA can be brought from 400 µL to 30 mL with identical performances.

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Read the full Application Note