An Alternative Method to RNA Lipid Nanoparticle Production

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Abstract

Introduction

RNA-LNP are a promising nucleic acid delivery modality for a wide variety of therapeutic applications. However, current methods of LNP production are limited by the need for complicated mixing and buffer exchange techniques (1). Here we present a method for producing RNA-LNP by loading pre-formed vesicles (PFV) with RNA, without the use of solvents or heating to encapsulate the RNA payload. The resulting RNA-LNP are essentially physically identical to those made by conventional LNP production methods and demonstrate equivalent biological response.

Methods

To prepare PFV, component lipids (ALC-315™/ Distearoylphosphatidylcholine/ Cholesterol / ALC-159™) were dissolved in ethanol and combined with an acidic aqueous buffer in a controlled manner and dialyzed in the same buffer to remove ethanol (2). The resultant PFV were assessed for particle size and polydispersity (PDI). PFV were mixed with RNA and incubated at ambient temperature from 10 minutes to 24 hours. After incubation, the RNA-LNP mixture was neutralized with phosphate-buffered saline and characterized for particle size, PDI and loading efficiency. Formulations were tested for efficacy by vaccinating mice intramuscularly (IM) using a 2-dose prime/boost regimen. Blood was harvested 28 days post-dose and serum was collected to determine neutralizing antibody titer.

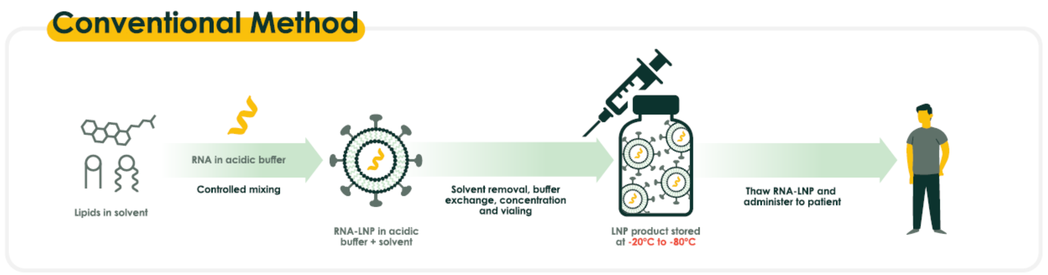
Results

LNP were loaded under different conditions to generate formulations with target particle size, PDI and RNA loading efficiencies. Most notably, it was established that use of a buffer pH within 1 pH unit of the apparent pKa of the ionizable lipid achieved optimal particle size, PDI and loading efficiency. Particle size/PDI of PFV stored at the optimized pH remained consistent during storage in liquid format at 2–8°C and at room temperature for at least 4 weeks (study ongoing) for ALC-315™ PFV and up to 1 year for Lipid A PFV. Loading efficiency for these stored PFV remained high (>90%) throughout. *In vivo* efficacy was tested in an immunogenicity study assessing the functional antibody titer of mice vaccinated IM with a 0.2 µg dose of LNP loaded with mRNA encoding A/Puerto Rico/8/1934 hemagglutinin (HA) influenza antigen. Mean hemagglutination inhibition (HAI) values were 504 (SD=350) and 400 (SD=212) for the conventionally mixed LNP and the PFV, respectively. This result was determined to be statistically non-significant via two-way ANOVA (P = 0.9933), thus demonstrating comparable immunogenicity between the two LNP.

Conclusion

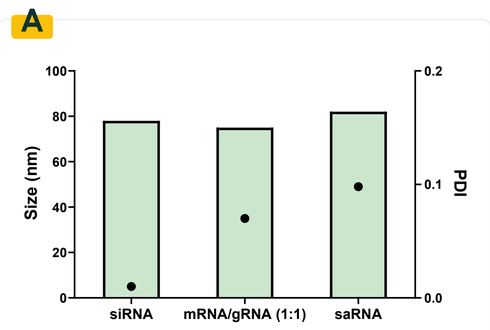
This method allows for the formulation of RNA-LNP without the need for disruptive agents or heat to encapsulate the RNA payload. LNP prepared using this method can be stored for at least 4 weeks in liquid format with no significant change in particle size or loading efficiency. *In vivo* evaluation shows comparable immunogenicity relative to conventionally prepared RNA-LNP.

Conventional LNP and PFV Mixing Methods



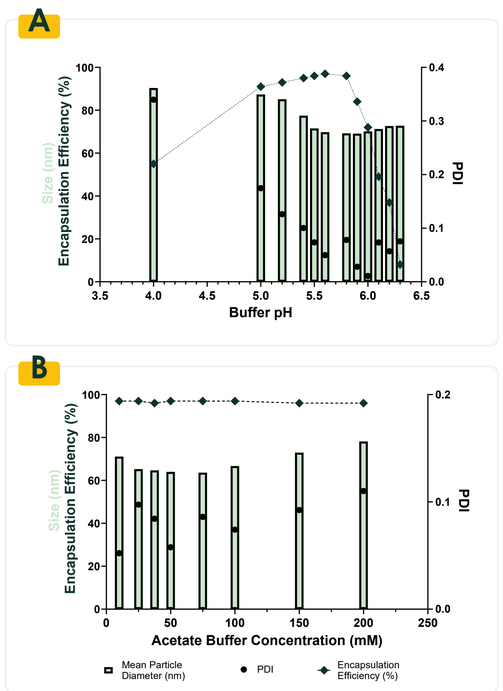
An illustrative comparison of conventional and PFV loading method. Conventional RNA-LNP are prepared via controlled mixing of an organic (lipid) phase and an aqueous (nucleic acid) phase, followed by additional processing (buffer exchange, concentration) to produce a single batch of RNA-LNP. The PFV method enables RNA-LNP production by simply mixing the PFV with the RNA payload. This approach allows for storage of the LNP product in liquid form, eliminating the need for an expensive and storage-prohibitive ultra-cold chain. Additionally, this approach enables encapsulation of different RNA payloads just prior to administration using the same batch of PFV. This flexibility allows healthcare providers to prepare RNA-LNP as needed for a variety of therapeutic applications. This could include multivalent infectious disease vaccines where antigens are adapted to the region/season, use in a personalized cancer vaccine approach where patient-derived tumor antigen is available to generate corresponding mRNA, or use as a gene-editing therapy to treat rare genetic diseases by which guide RNAs are designed to target genes of interest.

PFV formulations are agnostic to nucleic acid species



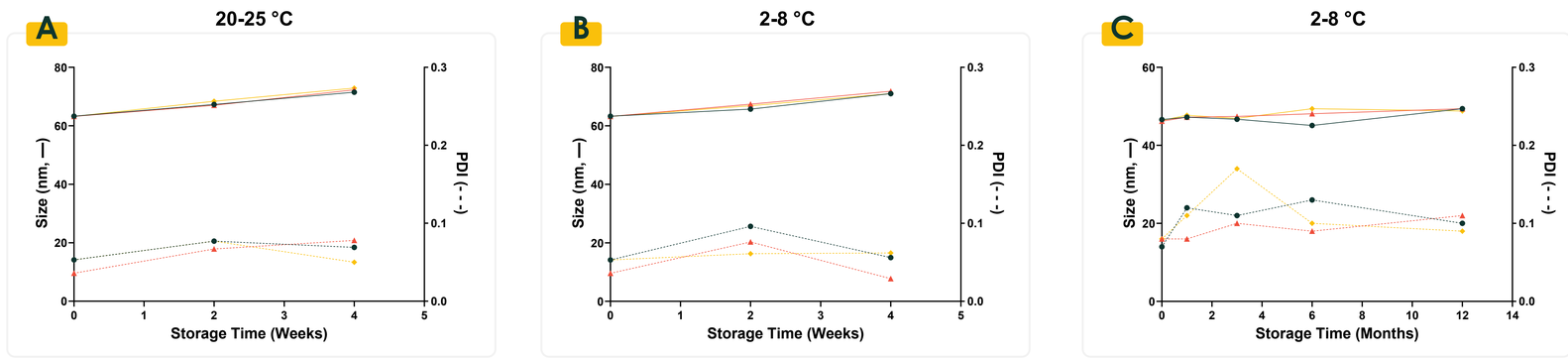
RNA-LNPs with distinct payloads prepared using the PFV method. ALC-315™ PFV were loaded at an RNA concentration of 0.075 mg/mL with siRNA, saRNA or an mRNA/guide mix at a weight-to-weight ratio of (1:1). Particle size (bars), PDI (●) (**A**) and encapsulation efficiency (**bars**) (**B**) was measured.

PFV buffer pH influences RNA-LNP physical characteristics

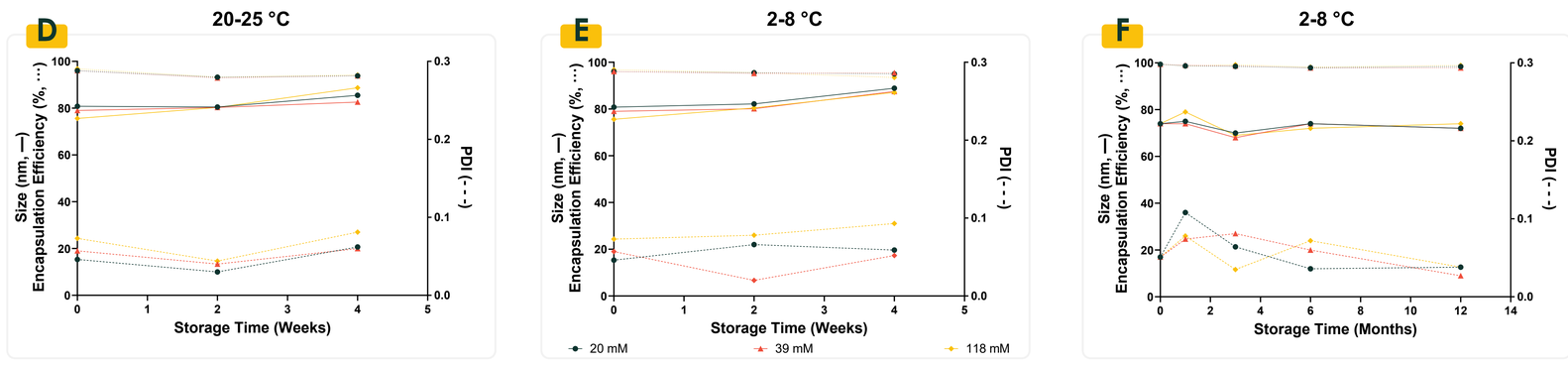


Optimizing acetate buffer pH and concentration for PFV formulation with ALC-315™ PFV. ALC-315™ RNA-LNP were formulated with mRNA encoding firefly luciferase (fLuc) using the PFV method in 25 mM acetate buffer at different pHs. Particle size (bars), PDI (●) and encapsulation efficiency (♦) were measured (**A**). Buffer concentration was also optimized for ALC-315™ LNP; RNA-LNP were formulated with fLuc mRNA at varying buffer concentrations at a pH of 5.5. Particle size (bars), PDI (●) and encapsulation efficiency (♦) were measured (**B**).

PFV are stable in liquid format at time points tested

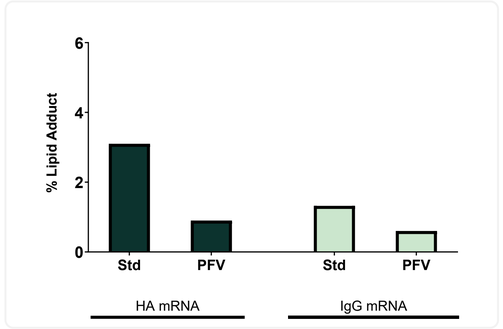


Resultant RNA-Loading of PFV (Post-Storage)



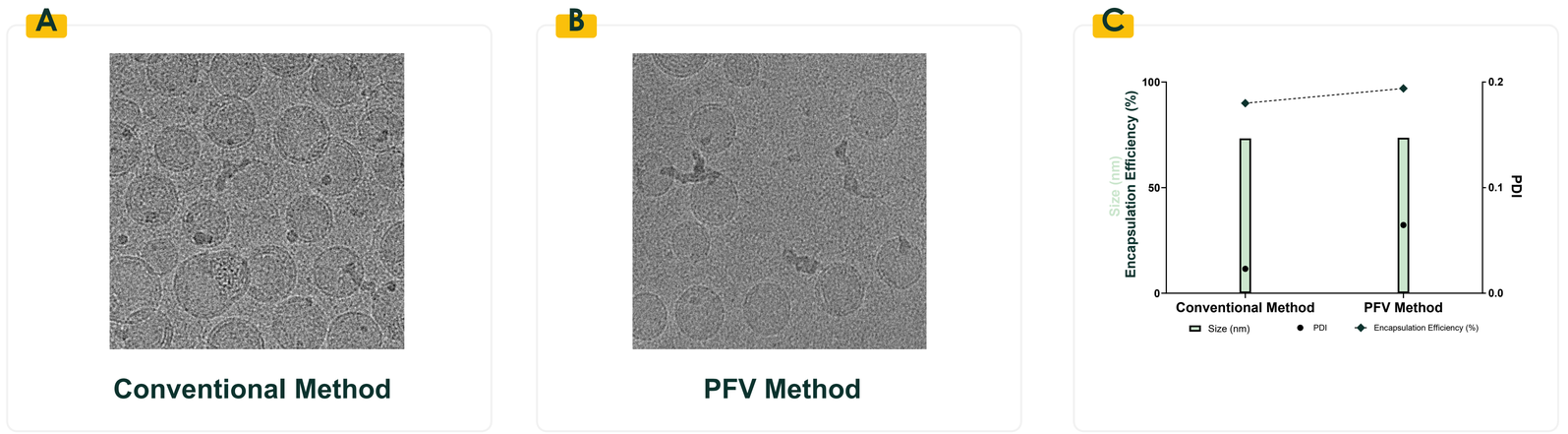
PFV stability after storage at room temperature and 2-8 °C. ALC-315™ PFV were stored in acidic buffer at total lipid concentrations of 20 mM (●) , 39 mM (▲) and 118 mM (♦) at either 20-25 °C (**A, D**) or 2-8 °C (**B, E**), particle size (solid lines) and PDI (dashed lines) were measured at the indicated time points. Lipid A PFV were stored in acidic buffer at total lipid concentrations of 20 mM, 39 mM and 118 mM at 2-8 °C (**C, F**), particle size (solid lines) and PDI (dashed lines) were measured at the time points indicated. PFV were loaded with fluc mRNA at a mixing concentration of 0.075 mg/mL RNA at each time point. Particle size (solid lines), PDI (dashed lines) and encapsulation efficiency (dotted lines) were measured for each of the storage conditions (**D, E, F**).

PFV method shows decrease in RNA-lipid adduct formation when compared to conventional method



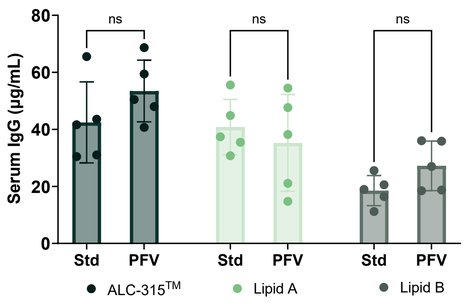
RNA-lipid adduct analysis. The percentage of RNA-lipid adducts was measured in ALC-315™ RNA-LNP encapsulating either an mRNA encoding a human IgG antibody or an mRNA encoding an HA antigen prepared with PFV or conventional methods.

PFV and conventionally mixed LNP show similar particle morphology and physical characteristics



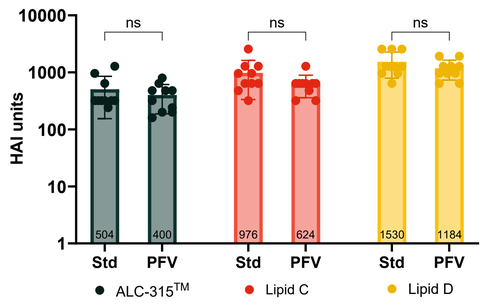
RNA-LNP particle characterization: RNA-LNP encapsulating mRNA encoding a human IgG were prepared using either the conventional (**A**) or PFV method (**B**), Cryo-EM images were obtained to assess particle morphology (**A,B**). Particle size, (bars) , PDI (●) and encapsulation efficiency (♦) were also measured (**C**).

Intravenously delivered PFV containing mRNA encoding for human anti-HA IgG show serum IgG expression comparable to standard LNP



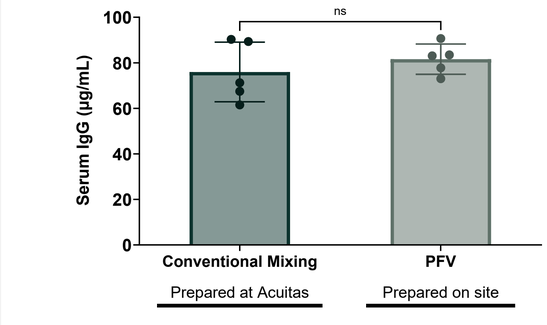
PFV intravenous efficacy study. Mice (n=5) were administered a 0.3 mg/kg dose of RNA-LNP encapsulating mRNA encoding a human anti-HA IgG. 3 different ionizable lipids were tested (ALC-315™, Lipid A and Lipid B), and each were prepared using either conventional mixing (Std) or the PFV method. Two-way ANOVA with Šídák's multiple comparisons test; ns, not significant. Blood was collected (24 h post-administration) and serum IgG titers were measured.

PFV method shows vaccine efficacy comparable to standard LNP in mice vaccinated with mRNA encoding an HA antigen



PFV vaccine efficacy study. Mice (n=10) were administered a 0.2 µg dose of LNP loaded with mRNA encoding HA antigen using a prime/boost dosing regimen administered 14 days apart. Three different ionizable lipids were tested (ALC-315™, Lipid C and Lipid D), each were prepared using either conventional mixing (Std) or the PFV method. Blood was collected at day 28 and functional anti-viral antibody titers were measured using an HAI assay. Two-way ANOVA with Šídák's multiple comparisons test; ns, not significant.

Bedside care demonstration of RNA-LNP prepared ahead of administration shows activity comparable to conventionally mixed LNP



PFV point-of-care study. Mice (n=5) were administered a 0.5 mg/kg dose of RNA-LNP encapsulating mRNA encoding an anti-HA IgG. Lipid E PFV was mixed with mRNA immediately prior to dosing, while conventionally mixed RNA-LNP was prepared and stored at -80 °C until use. Blood was collected (24 h post-administration) and serum IgG levels were measured. Unpaired t-test; ns, not significant.

Summary

1. Buffer optimization experiments indicate that there is an optimal pH range at which the PFV method generates monodisperse RNA-LNP formulations with high encapsulation efficiency (>90%), whereas buffer concentration does not meaningfully impact RNA-LNP size or encapsulation efficiency.
2. Both Lipid A and ALC-315™ PFV show stable size and PDI for 1 year and 4 weeks, respectively. Both also remain capable of successfully encapsulating RNA at >90% during that time.
3. PFV are capable of encapsulating different nucleic acid payloads. Specifically, we demonstrate that siRNA, saRNA and gRNA/mRNA can be loaded with >90% encapsulation efficiency, while also generating monodisperse RNA-LNP at sizes <100 nm.
4. RNA-LNP produced using the PFV method generate a lower percentage of RNA-lipid adducts compared to conventionally mixed RNA-LNP.
5. ALC-315™ RNA-LNP made using the PFV method show comparable size and morphology to conventionally mixed LNP. Encapsulation efficiency was improved for the PFV method.
6. PFV and conventionally mixed LNP showed comparable activity across two separate efficacy studies and across different LNP formulations, demonstrating the method's utility in both vaccine and intravenous therapeutic contexts.
7. The PFV method can be used to prepare RNA-LNP at the point of care just before dosing and yields activity comparable to a conventionally prepared RNA-LNP (frozen at -80 °C).

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References

1. Webb C et al. Current Status and Future Perspectives on mRNA Drug Manufacturing. Mol Pharmaceutics. 2022:1047-1058
2. Pardi N et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. J Control Rel. 2015:345-351