LYOPHILIZATION CYCLE DESIGN AND OPTIMIZATION OF AQUEOUS CO-SOLVENT FORMULATIONS USING RESIDUAL GAS ANALYSIS

Lipid Nano Particle (LNP) encapsulated mRNA vaccines are generally unstable at room temperature and require storage under cryogenic temperature prior to administration. Although freezing is simple, proven, and effective, the cold chain dependency of these substances is unsustainable in the long term. Preparation of LNP-based vaccines typically requires high concentrations of ethanol to prevent agglomeration prior to encapsulation. This makes lyophilization highly difficult due to the severe freezing point depression and large differences in vapor pressure between water and organic solvents. Additional dialysis steps are therefore required to adjust solvent concentrations to levels that are feasible for freeze-drying. As part of NIIMBL American Rescue Plan grant 11, **Advanced Characterization and Manufacturing Methods for mRNA Vaccine Development**, we applied in-situ residual gas analysis using an Inficon Transpector CPM3 on a Millrock REVO lyophilizer to quantify the relative concentrations of the co-solvents in vapor phase during lyophilization of LNP encapsulated yeast RNA vaccine placebos. Time lapse imaging was also used to identify abnormal behaviors throughout the cycle. The spectral data is shown in figure 1 and an image of the product during primary drying in **Figure 2**. The RGA is clearly able to identify the principal peaks of water and ethanol in the formulation as well as the nitrogen ballast gas. This capability allows lyophilization cycles to be developed that selectively target solvents of different vapor pressures throughout the process. In this case, ethanol was removed using a series of setpoints that lie between ice sublimation and ethanol evaporation thresholds. Once extracted, a more aggressive and standard set of pressures and temperatures was used to remove bulk ice, the time lapse images indicated cake lifting near the beginning of primary drying. However, both lifting height and lifted duration were significantly reduced when compared to a reference single-step lyophilization process. The study is ongoing but has already demonstrated the utility of using in-situ residual gas analysis and unconventional imaging techniques for lyophilization cycle development.

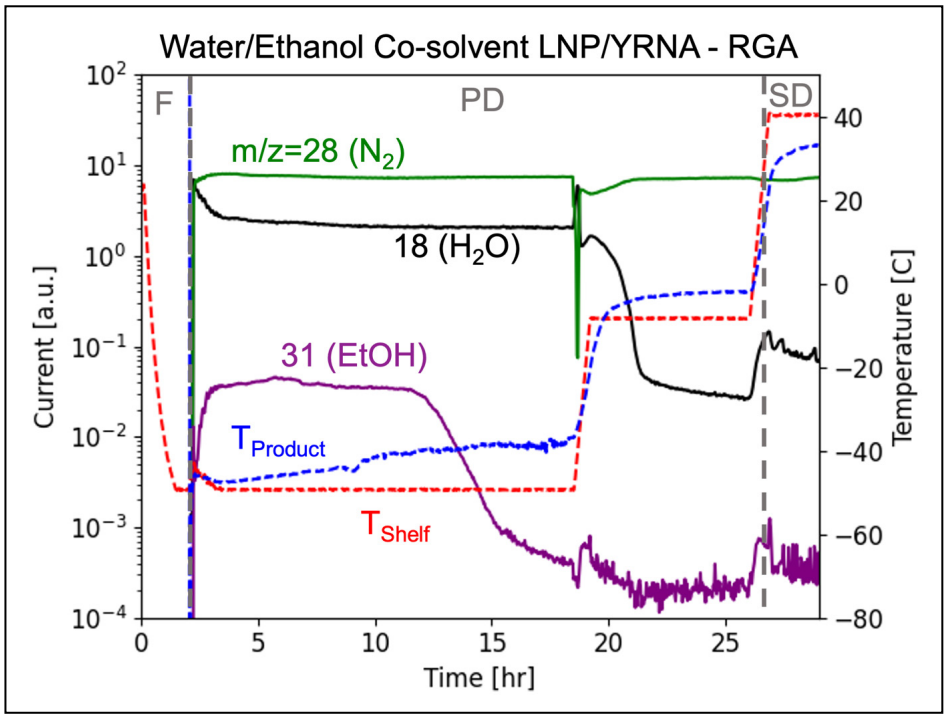

Figure 1: *RGA process data for an LNP encapsulated yeast RNA formulation containing 1% ethanol.*

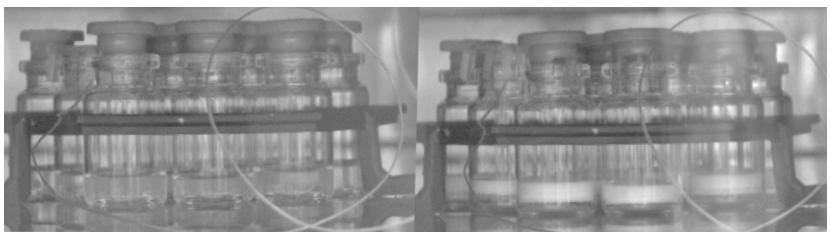

Figure 2: *Time lapse images from the lyophilization of LNP-encapsulated yeast RNA formulation containing 1% ethanol prior to freezing (left) and during primary drying (right).*