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| **Stability study of PolyA lipid nanoparticles stored under different conditions based on the evaluation of their critical quality attributes** |
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| **Background:** Lipid nanoparticles (LNPs) are emerging new modalities for mRNA therapeutics and have been in the spotlight for the past decade. Since they are a relatively new delivery system compared to conventional medicines, new analytical techniques for the robust characterization of their critical quality attributes (CQAs) such as size distribution, polydispersity, zeta potential, encapsulation efficiency and morphology are needed. It has been reported that a number of stimuli can affect the stability of the LNPs such as leaking of cargo nucleic acid from the nanoparticle and LNP aggregation, resulting in low translation efficiency. Hence, understanding the duration of stability is key during formulation development. The current work aims to evaluate the stability of PolyA LNPs when stored under different temperatures and when using cryoprotectant by determining their physicochemical properties.  |
| **Methods:** PolyA LNPs were manufactured with standard lipid composition using DOTAP: DSPC: Cholesterol: DMG-PEG2000 = 50:10:38.5:1.5 mol%. The formulation was purified via the dialysis method. We tested storage temperature and addition of cryoprotectant as the study variables which could affect the stability of the LNP formulation by storing the formulation in the fridge (4 oC) and at room temperature (25 oC), as well as dialyzing the formulation with 20 % w/v sucrose to determine the stability following freeze-thaw, when stored at -80 oC. CQAs such as particle size, polydispersity index (PDI) and zeta potential were measured using Dynamic Light Scattering (DLS), size distribution using Nanoparticle Tracking Analysis (NTA) and encapsulation efficiency and mass balance using the Ribogreen Assay. |
| **Results**: Relating to the 10 days temperature study, the particle size and PDI values of PolyA LNPs were maintained between 60-80 nm and below 0.3 respectively. ZP for both temperatures was 5-8 mV. EE at day 10 of the stability study was 99 ± 0.2 % for both temperatures and MB 80 ± 8.4 % at 25 oC and 87 ± 6.0 % at 4 oC. Relating to the cryoprotectant study, the addition of sucrose for one freeze-thaw cycle at has led to an increase in particle size (154 ± 58.3 nm) compared to day 0 of manufacture (56.63 ± 3.2 nm). However, storing the formulation at -80 oC without sucrose for one freeze-thaw cycle has resulted in almost 12 fold increase in particle size. The ZP, EE and MB did not change with and without the addition of sucrose. |
| **Conclusions:** This study demonstrated that storing PolyA-loaded LNP formulation for 10 days at ambient temperature and in the refrigerator did not result in changes in its CQAs namely size, ZP, EE and MB of the nanoparticle. However, storing the nanoparticle at -80 oC without a cryoprotectant caused an increase in particle size possibly attributed to particle aggregation.  |