IMPLEMENTATION OF A NOVEL MICROFLUIDIC STRUCTURE TO TRANSITION PROTEIN LOADED LIPOSOME PRODUCTION FROM BENCH TO GMP

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Background: Microfluidics is a flexible process that offers scale-independent manufacturing processes for liposomes. Microfluidics offers higher drug loading and better physio-chemical attributes compared to traditional liposomal production processes. These processes are often time-consuming and complex which can be circumvented using microfluidics allowing for easier up-scaling. The aim of this study was to compare different microfluidic architectures and test liposome production from the lab bench to GMP scale.

Methods: 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol were dissolved in methanol or ethanol at 2:1 wt/wt (initial lipid concentration 4 or 16 mg/mL mg/mL). Liposomes were produced with different microfluidic architectures using either a staggered herringbone mixer (SHM) or a toroidal mixer (TrM). The flow rate ratio (FRR) used was 3:1 aqueous:lipid phase at a total flow rate ranging between 12 - 200 mL/min. Ovalbumin dissolved in PBS was added to the aqueous inlet (250 – 4000 µg/mL). After production, liposomes were purified using tangential flow filtration (Krosflow Research Iii system with a 750 KDa mPES column). Liposome size, charge and PDI was measured using a Malvern Zetasizer Nano ZS. Protein encapsulation and protein release kinetics was quantified using reversed-phase high performance chromatography (RP-HPLC, Shimadzu 2010-HT, Milton Keynes, UK) connected with a UV detector at 210 nm using a Jupitar 5 µm C5 300A 4.6 mm i.d x 250 mm length or micro-BCA assay. For GMP production a modified HPLC pump and NxGen cartridge 500 with TrM architecture was used at a TFR of 400 mL/min.

Results: Our results show that liposomes produced by both microfluidic mixers gave high protein loading (30-40%) with small sizes (50-60 nm) and homogenous (<0.2 PDI) liposome formulations with comparable protein release rates. Using the TrM system we are able to produce these liposome formulations at up to 200 mL/min giving the facility for high-throughput scale-independent production with flow rate having no impact on formulation attributes.

Conclusions: From our results we have demonstrated the ease of production at high-throughput using the TrM architecture by maintaining liposome properties across different microfluidic architectures. By increasing the flow rate to 200 mL/min it is now possible to scale-up microfluidic production with the same critical quality attributes across a range of production speeds and volumes using process parameters providing a direct path from bench to GMP.