Scalable Continuous Manufacturing of Multi-drug Liposomes: Tuning Size and PDI *via* Post-loading

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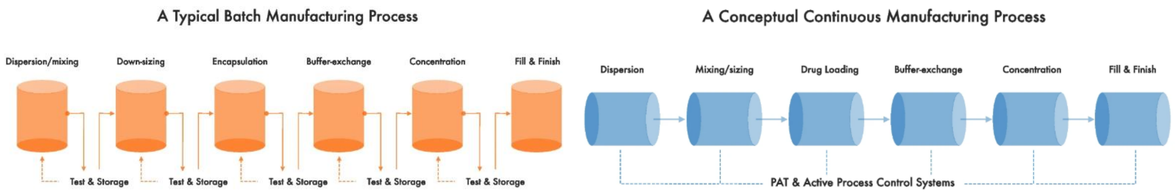
INTRODUCTION

Continuous manufacturing (CM) offers a solution to the inherent scalability and quality consistency challenges that plague traditional batch liposome production, which often result in shear heterogeneity, extensive post processing, inefficiency, and batch waste. Here a fully CM platform was used to produce multi-drug loaded chemotherapeutic liposomes. The objective was to evaluate post loading for particle size and polydispersity (PDI) control while using inline process analytical technology to monitor the effect of process parameters on critical quality attributes (CQAs) in real time. This should enable the manufacturing of highly monodisperse, multi-drug formulations with improved translational potential.



METHODS

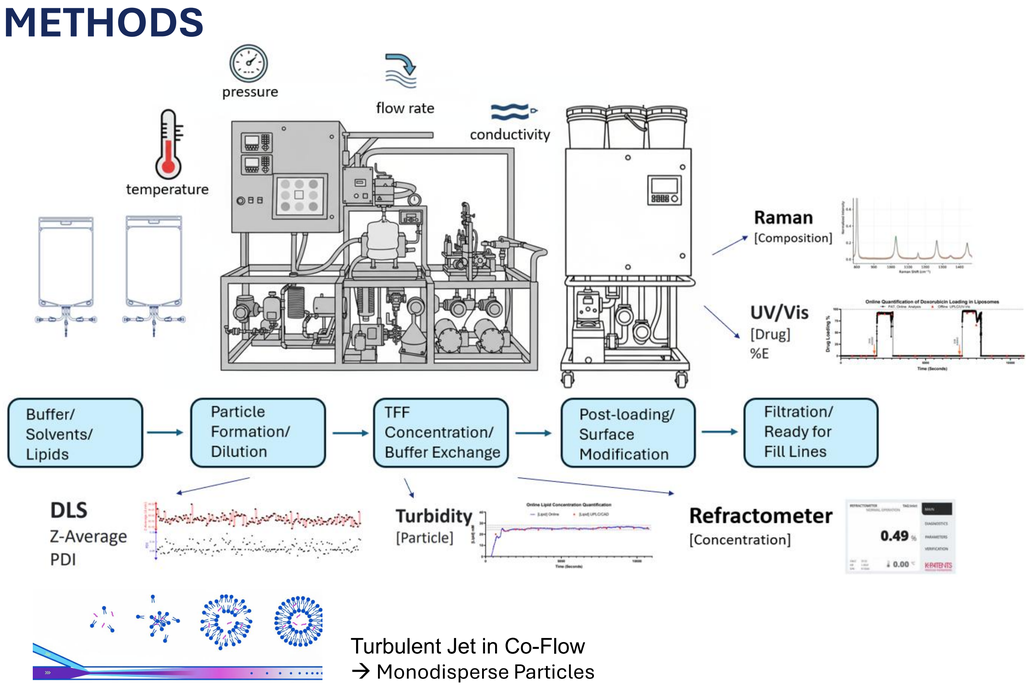


Figure 1: UConn Continuous Manufacturing Platform

The UConn CM platform features a coaxial turbulent jet in co-flow with ethanol injection, which allows for rapid and uniform self-assembly of liposomes.[1]

- Process Control: critical process parameters (*i.e.* temperature and flow rate) were altered to target six sizes (35, 55, 70, 100, 100, 140 nm).
- Unit Operations: particle formation, dilution, buffer exchange (*via* tangential flow filtration, TFF), concentration, API or surface post-loading, and final filtration ready for fill line.
- Scalability: the system demonstrated linear scalability, maintaining CQAs while increasing output from R&D scale (5 mL) to an industrial scale (10 L).

FORMULATION OPTIMIZATION: PARTICLE SIZE AND PDI

Five different formulation conditions were used to explore five particle size (PS) targets. A blank, Paclitaxel (PTX), Docetaxel (DTX), and PTX/DTX formulations were made using each condition.

Formulation ID	Initial Lipid Ratio (HPSC:Chol:PEG2k)	Aq Flow Rate (mL/min)	Aq Temperature (°C)
F1	77:20:03	150	52
F2	77:20:03	130	32
F3	77:20:03	150	43
F4	57:40:00*	150	43
F5	57:40:03	150	43

*PEG2k was post-loaded through heated sonication

Continuous Post-loading Particle Size & PDI

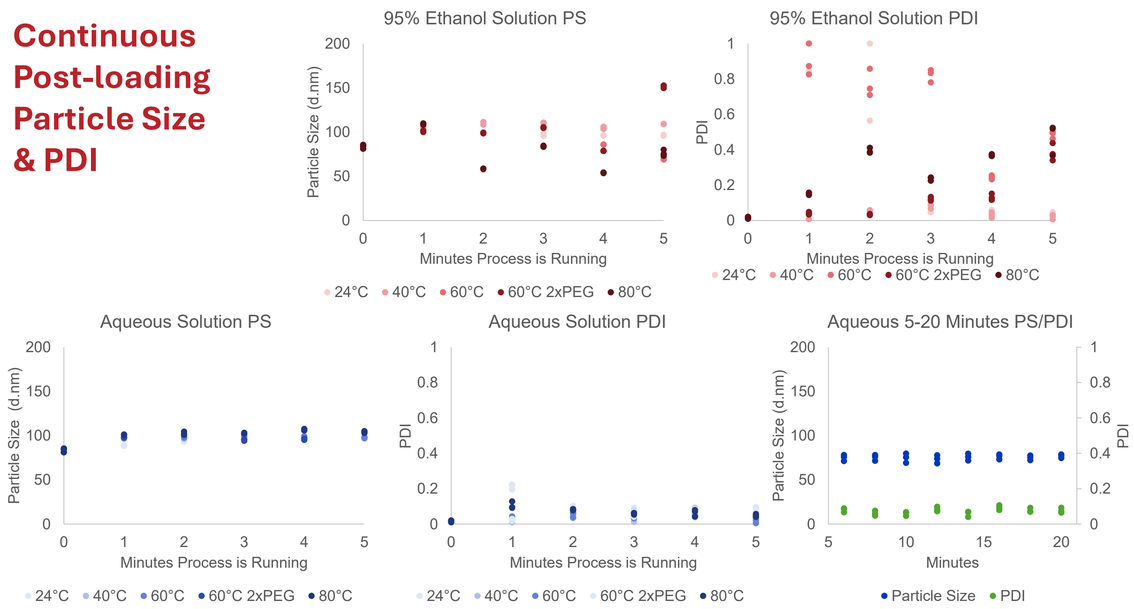


Figure 3: Liposome particle size (nm) and PDI pre- and post-loading of PEG2k in aqueous and ethanolic solutions as a function of process run time at different temperatures

ACKNOWLEDGEMENTS / REFERENCES

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[1] Costa AP, Xu X, Khan MA, Burgess DJ. Liposome formation using a coaxial turbulent jet in co-flow. Pharm Res. 2016;33(2):404-416.

Results

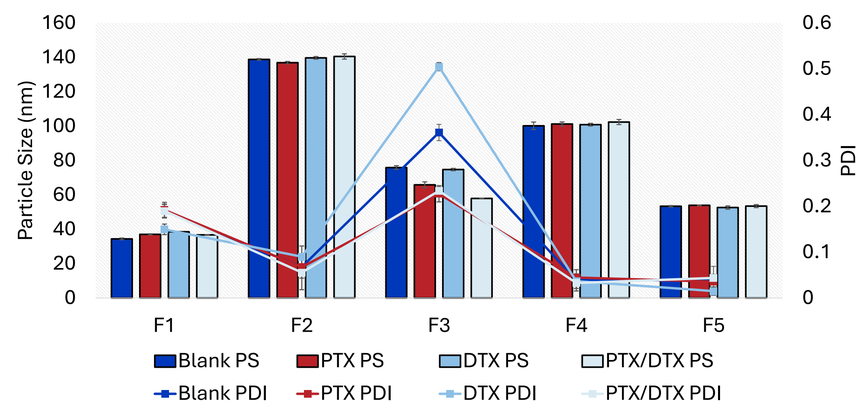

Figure 2: Particle size and PDI through changes in temperature, aqueous flow rate, lipid composition, and drug loading. Formulations were made targeting the PS (35-140 nm) and PDI (<0.1) of model liposomal chemotherapeutics. Increasing cholesterol composition and loading DSPE-PEG2000 into liposomes following particle formation were shown to be most effective in achieving target CQA values.

Effect of Co-Encapsulation

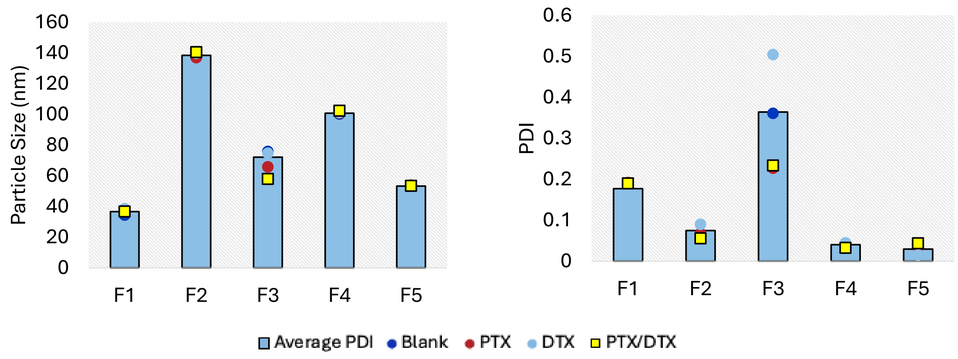

Figure 4: Co-Encapsulation has no effect on particle size or PDI compared to blank and single-drug liposomes.

LOADING METHODS

Pre-TFF

Post-TFF

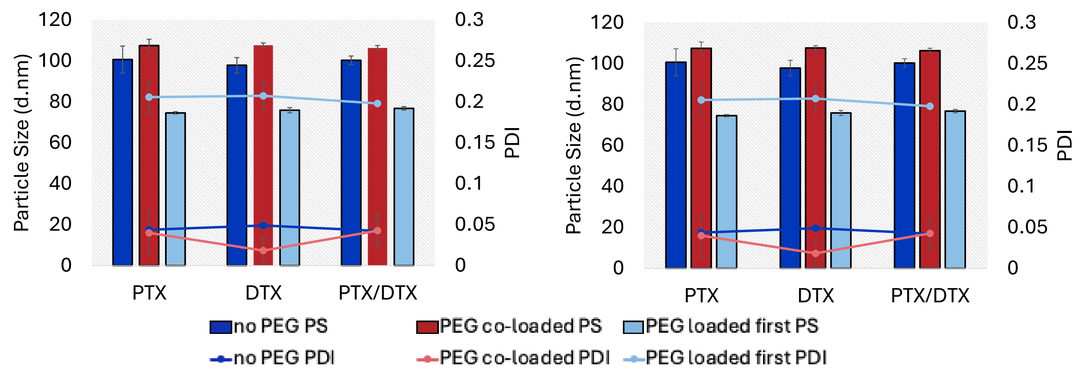

Figure 5: Changes to particle size and PDI were minimized when PEG loading occurred prior to drug-loading. Simultaneous PEG loading and drug loading were shown to mildly increase PS, while PEG loading prior to drug loading decreased PS. PEG-loading prior to drug increased PDI.

Stability

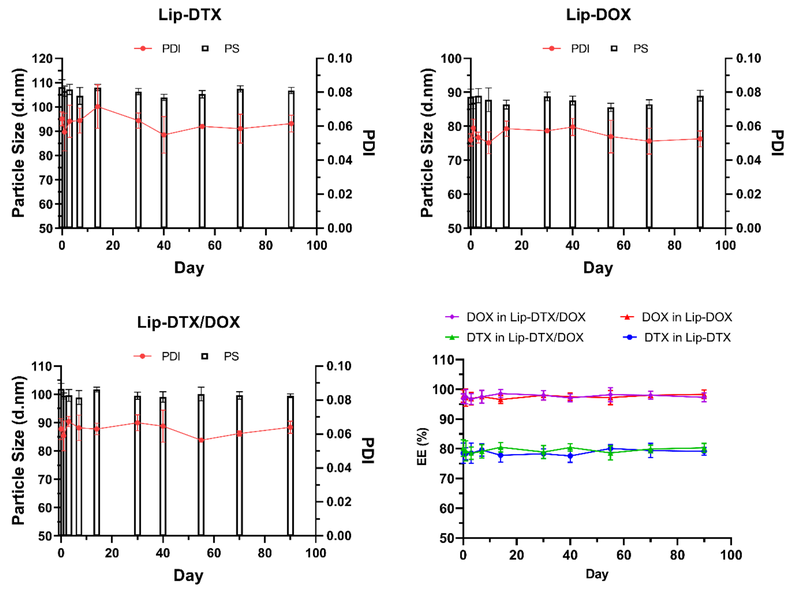

Figure 6: Long term stability study at 4°C using glass vials investigating the change in particle size and PDI (red) for 90 days (top and bottom left). Bottom right graph shows relatively no change in the encapsulation efficiency of both APIs for the duration of the testing period. Error bars were obtained from triplicate samples.

CONCLUSIONS

- The continuous manufacturing platform produced co-loaded Paclitaxel, Docetaxel, and Doxorubicin liposomes at precise sizes by modulating flow rate/temperature for particle formation and post-insertion.
- Co-loading DTX, PTX, and DOX did not have a significant impact on particle size and PDI for pre and post TFF material.
- DSPE-PEG2k post-insertion can have less impact on particle size and PDI than pre-loading during particle formation. Other PEG lipids, degradation products, and APIs can be post-loaded with minimal particle size distribution impact.
- Aqueous post-loading conditions maintain PS/PDI while ethanolic post-loading conditions induce major changes in PS (±50nm) and PDI (>1).
- Co-loaded liposomes are stable during and after processing for 90+ days