Optimizing PAT: Refractometer in Continuous Manufacturing of Lipid-Based Therapeutics

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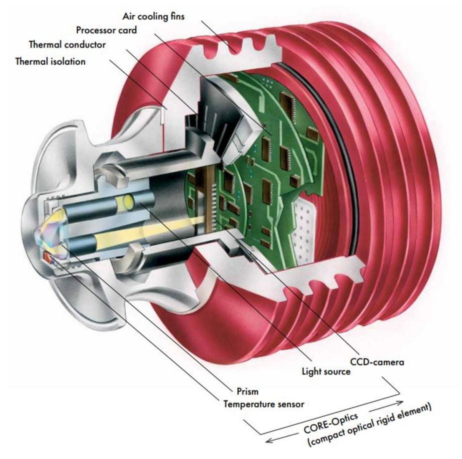
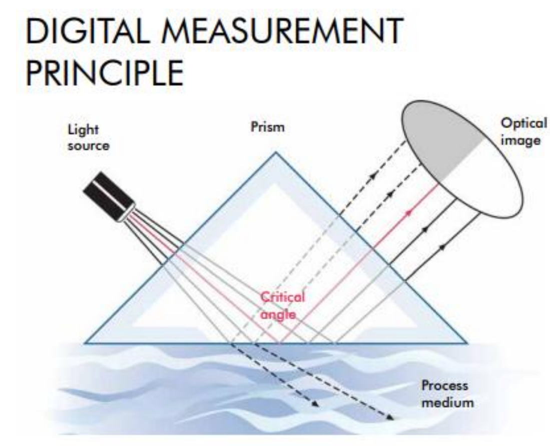
INTRODUCTION

- Continuous manufacturing (CM) makes use of process analytical technology (PAT) to ensure critical quality attributes (CQAs). Process refractometers enable inline concentration monitoring, particularly for cryoprotectants. While refractive index (nD) is well-characterized for single-component systems in the food and beverage industry, multi-component systems for pharmaceutical manufacturing applications remain understudied. This work investigates interactions between formulation components and process parameters to enhance refractometry as a PAT tool, improving the efficiency and reliability of CM for lipid-based therapeutics.

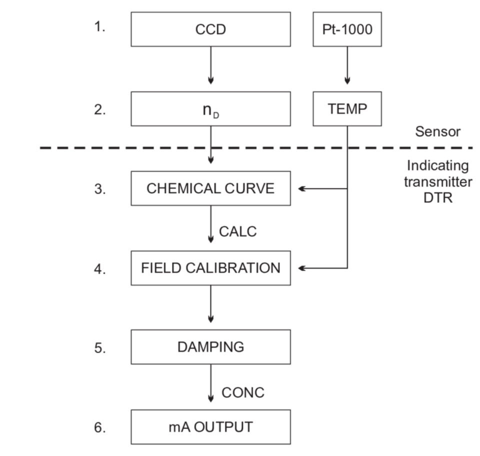
Objectives

1. Install a process refractometer inline for a continuous manufacturing skid
2. Test the ability to determine single component concentrations
3. Explore process parameters that affect refractive index and concentration calculations
4. Look at interactions between formulation components and verify multicomponent concentration nD predictions



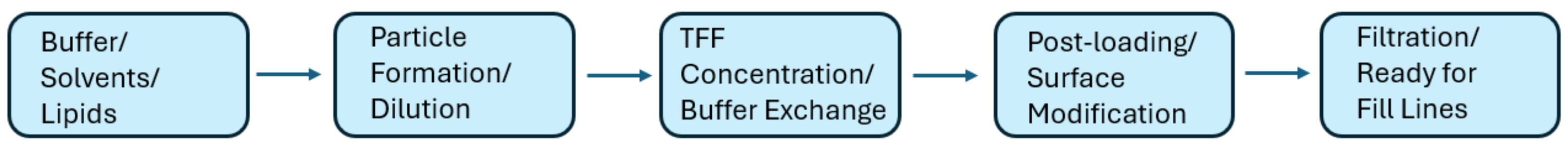


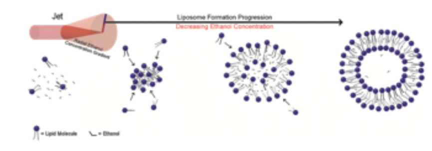
Concentration Calculation



METHODS

- Our laboratory has developed a continuous manufacturing (CM) platform that can produce monodispersed liposomes and LNPs continuously[1,2].
- Measurements were conducted using Vaisala's PR-23 Series Process Refractometer at 589nm (nD), both statically and in-flow with the UConn CM platform[1,2]. Formulations included monodispersed liposomes (50-200 d.nm) with varying charged lipid and PEG-lipid content.





RESULTS

Greater Refractive Index (nD) Influence

Cryoprotectant Concentrations

- Greatest refractive index (nD) impact, accurate concentration determination

Solvent concentrations

- Large nD impact but can be less linear so needs a specific operating range

Temperature

- Higher temperatures decrease nD due to density but many process refractometers can compensate with inbuilt temperature probes although it takes time to adjust and different solutions are impacted by temperature to different degrees

Lipid and Buffer Concentration

- Increased nD with higher concentrations but in practice are a low w/v% of solution

Flow Rate

- Main impact is air bubbles increasing nD during process priming or flow rate change. A slow flow rate may also cake the detector causing drift

pH

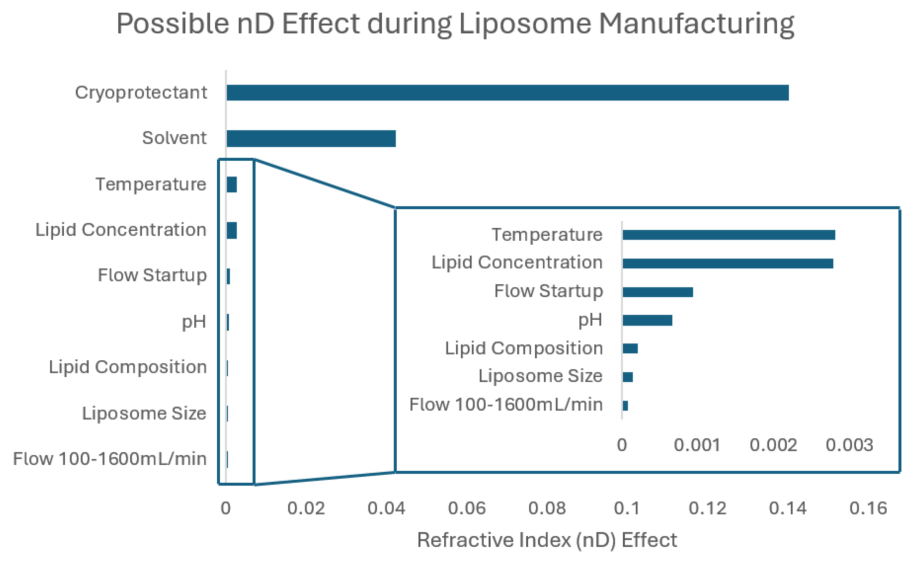
- Very minor effect as higher ion/particle density (e.g., H^+, OH^-, or dissolved salts) slows light propagation, increasing nD

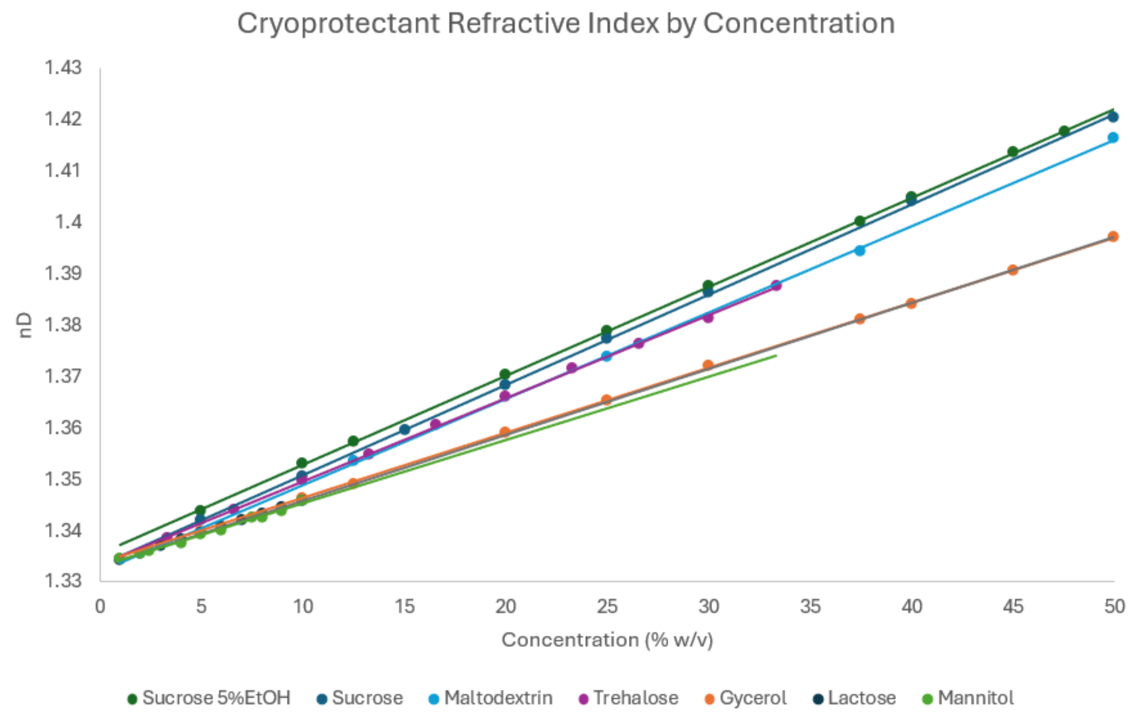
Lipid composition

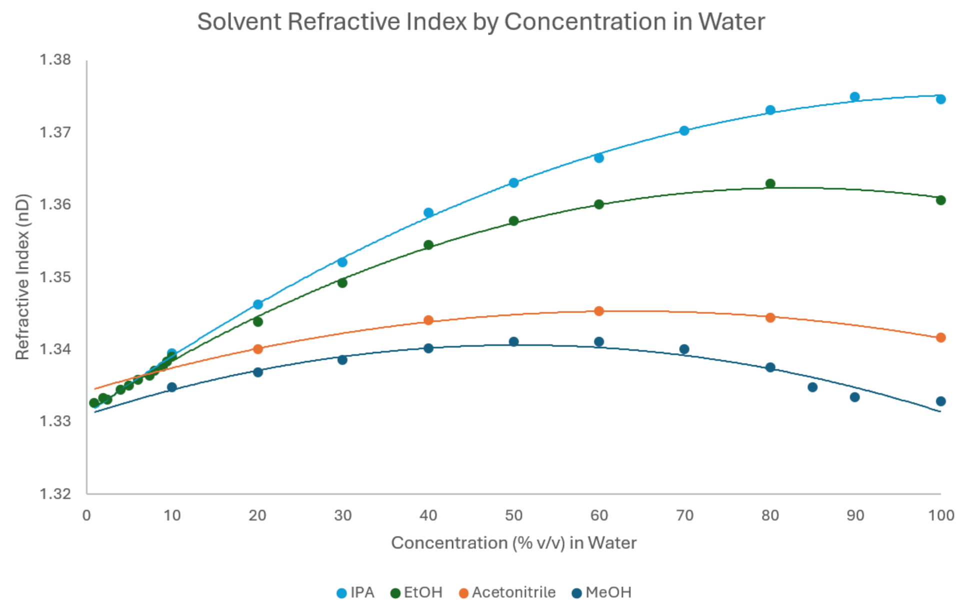
- Lipids only range from 1.45-1.47 nD so little overall effect

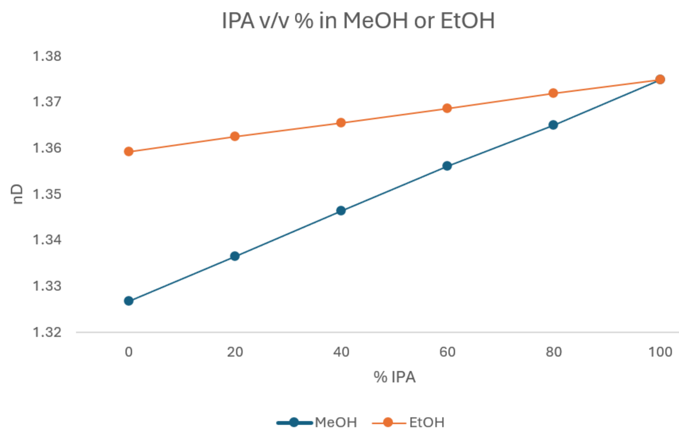
Liposome size

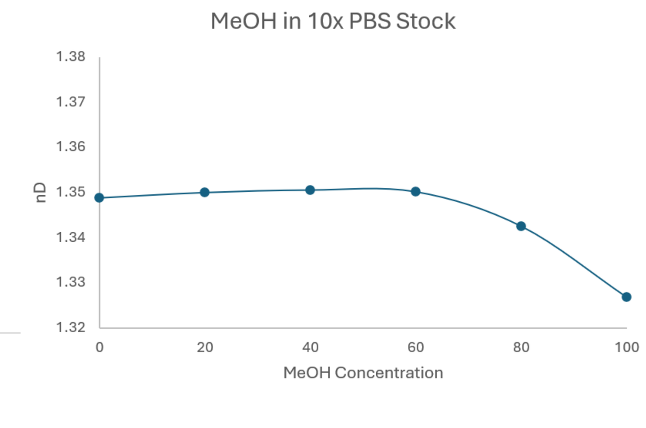
- 50-200 nm showed little difference, aggregations may have more effect

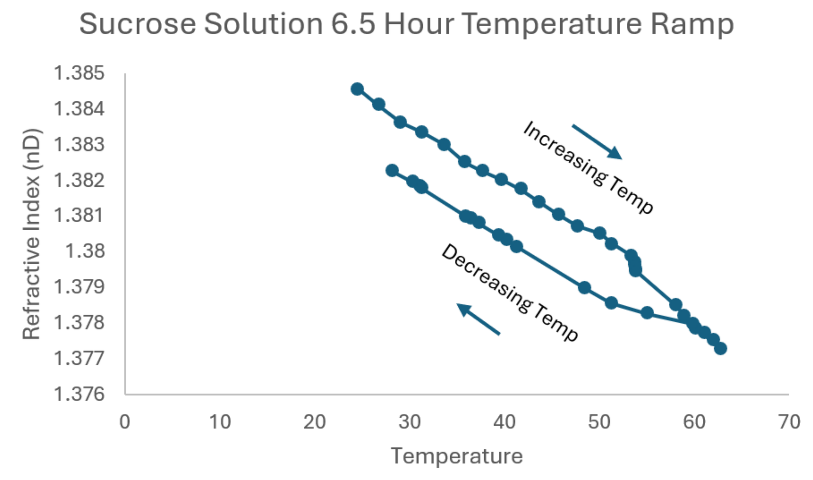


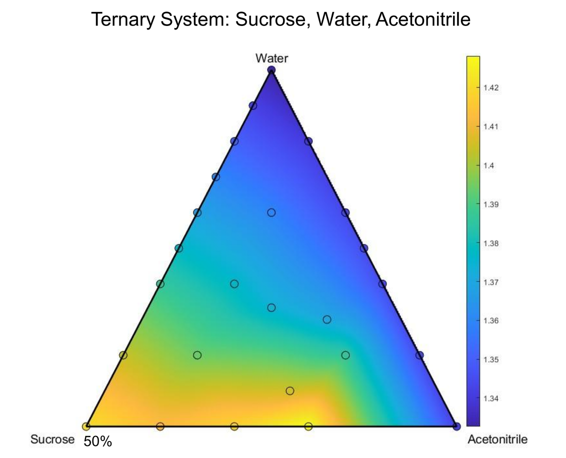


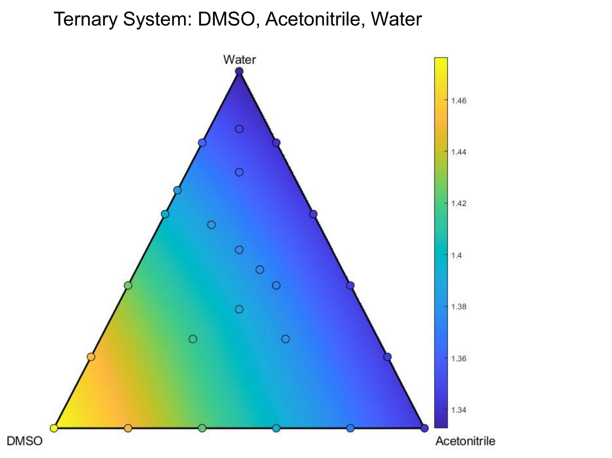


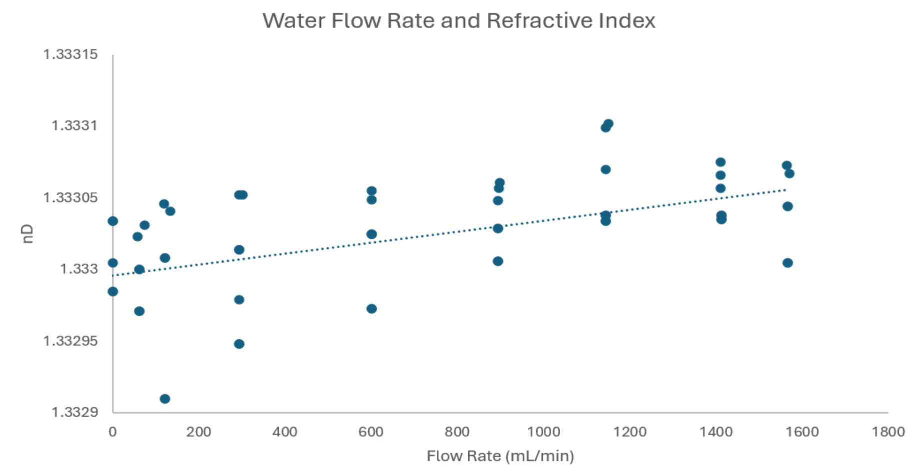












Predicted vs Experimental

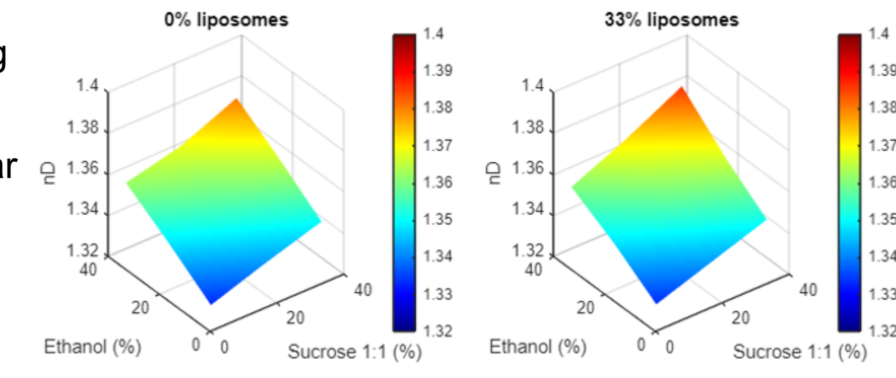
- Combined Lipids, EtOH, and Cryoprotectants and confirmed that single component measurements predicted mixed solution nD.

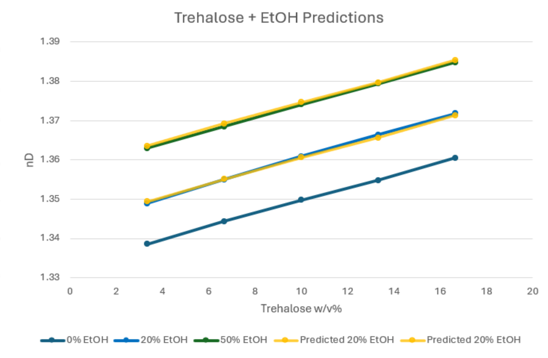
Ternary Systems

- Interactions between three components sometimes influence molecular packing and nD but in general were linear for liposomal systems

Process Recommendations

- Takes time to adapt to sudden process changes so it is useful for process optimization but not as fast at process control as other PAT such as flow sensors





DISCUSSION

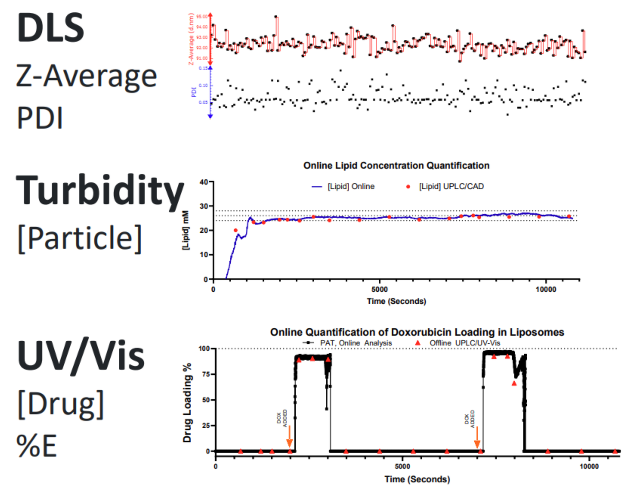
How Molecular Packing Influences Refractive Index

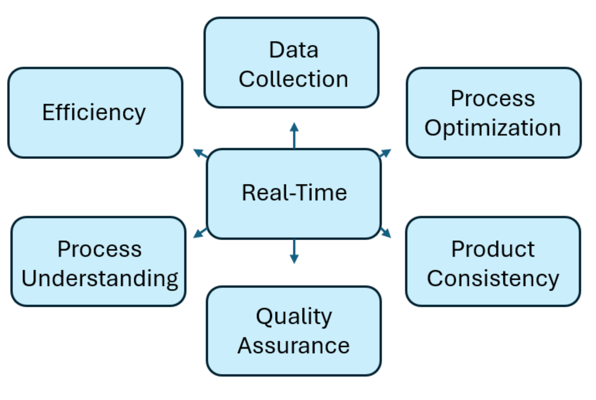
- Refractive index (nD) reflects both the density and polarizability of a material.
- When molecules are packed more closely together, the material becomes more polarizable, resulting in a higher refractive index.
- In mixtures like water–methanol, molecules interact and pack more efficiently than in the pure solvents, increasing both density and polarizability, which leads to a higher refractive index than expected from a simple average.
- In contrast, mixtures such as isopropanol (IPA) or ethanol (EtOH) with methanol do not significantly alter molecular packing. As a result, the refractive index changes linearly with composition.
- Overall: The degree of molecular packing in a solution directly impacts how much the refractive index deviates from linearity, providing insight into molecular interactions within mixtures.

Other Process Analytical Technology (PAT)

While refractive index is a valuable inline tool for monitoring solution concentration, additional PAT methods enable a more comprehensive assessment of liposome sample quality beyond basic parameters like temperature, pressure, flow rate, and pH. More advanced PAT relevant for liposomes includes:

- Spatially Resolved Dynamic Light Scattering (SR-DLS): Particle size and polydispersity index (PDI) for liposome uniformity
- Turbidity Measurements: Particle concentration, especially for TFF concentration
- Raman Spectroscopy: Lipid composition, encapsulated drug content, and other critical quality attributes
- Ultraviolet-Visible (UV-Vis) Spectroscopy: Drug concentration, after drug loading





CONCLUSIONS

1. A process refractometer was successfully implemented into a continuous manufacturing platform and could successfully determine concentrations of solvents and cryoprotectants at different points in the process.
2. Process parameters such as temperature, lipid concentration, and flow rate can noticeably affect refractive index but can be mitigated with good process design.
3. Molecular packing influences refractive index so non-linear concentration curves may occur and require specific operational concentration ranges to remain accurate.
4. Multi-component formulation refractive index can be predicted by adding single component weight averages but for greatest accuracy a field calibration under normal process conditions may be needed.

ACKNOWLEDGEMENTS

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