Levitronix® Life Science
LNP Manufacturing using Ultrasonic Concentration Measurement

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Glossary

AOI	Analyte of Interest
DF	Diafiltration
DF_C	DF Step with model including Temperature compensation
DF_Tc	DF Step keeping Temperature constant
EtOH	Ethanol
LNP	Lipid Nano Particle
PAT	Process Analytical Tool
Std. Dev	Standard Deviation
SU	Single Use
T	Temperature
TFF	Tangential Flow Filtration
UF	Ultrafiltration
UF_C	UF Step with model including Temperature compensation
UF_Tc	UF Step keeping Temperature constant

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Motivation

- Goal is process performance improvement of LNP formulation applications as well in LNP TFF UF/DF steps to bring the LNP to target concentration and into final buffer. In addition, a general sensor calibration approach is presented allowing to track concentration of process components in real-time.

- The extended focus is on where Levitronix components can be used in these processes to bring a process improvement.

Theory and Background

LNP Manufacturing Process

Step 1: LNP Formulation

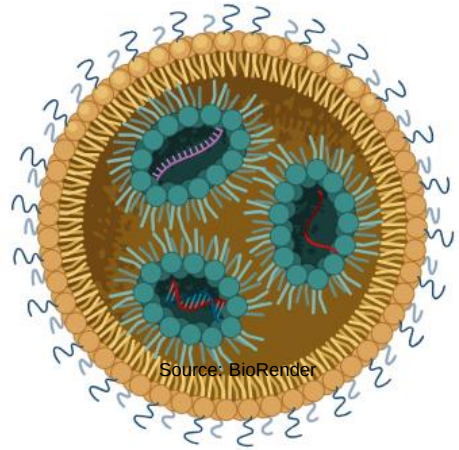
Organic Phase
(Lipids in EtOH)

Aqueous Phase
(Buffer, mRNA)

Step 2: LNP TFF (UF/DF)

Removing EtOH and
reach target concentration

LNPs



Source: BioRender

Step 1: Organic and Aqueaous Phase are blended together, LNPs are generated

Step 2: LNPs are concentrated and buffer is exchanged (especially EtOH is removed) in a TFF process
UF: Ultrafiltration = Concentration of LNPs
DF: Diafiltration = Buffer Exchange

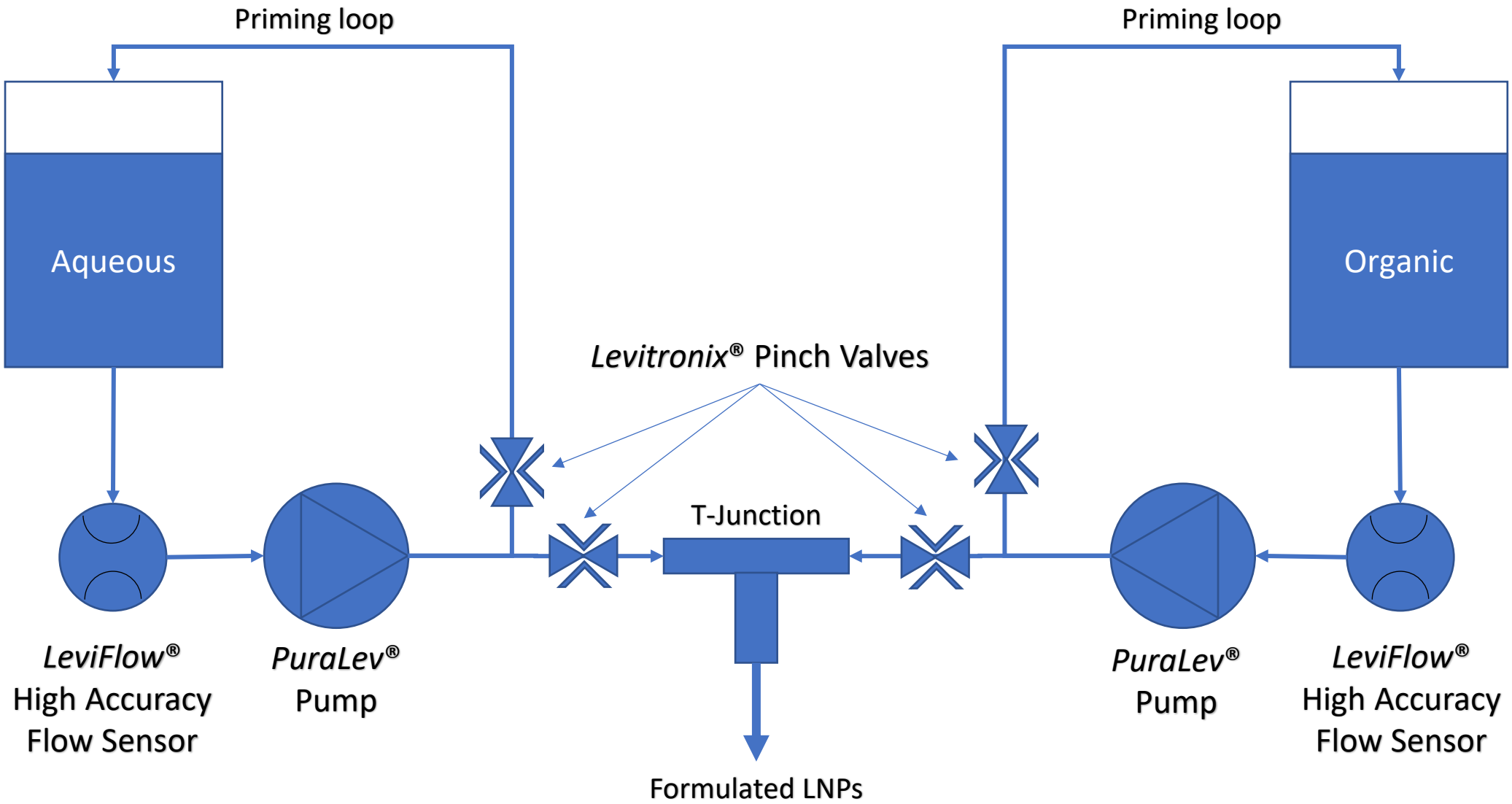
LNP Formulation using Levitronix Products

Step 1: LNP Formulation

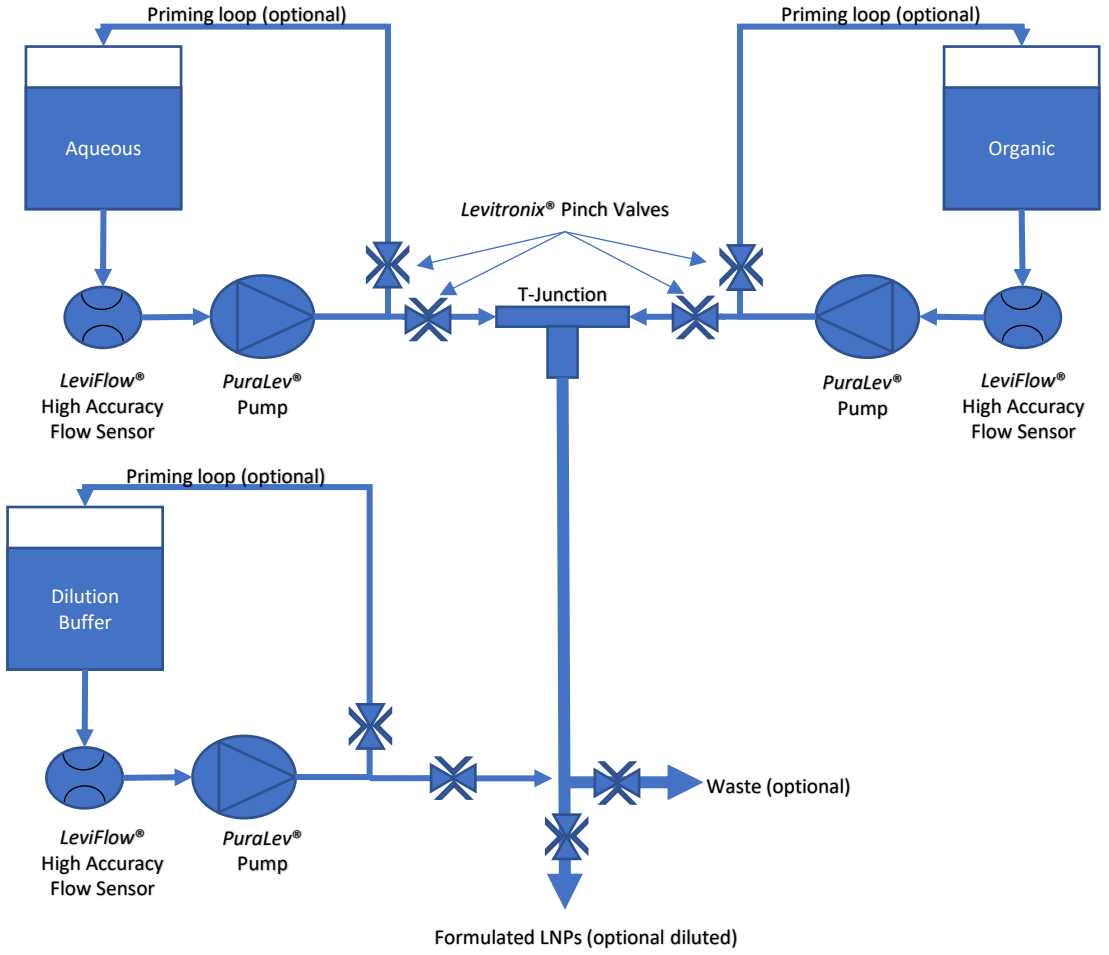
- LNP formulation processes have specific needs for exact, and smooth flow delivery, in specified ratios.
  - Levitronix Pumps, flow sensors, and pinch valves are naturally suited for these applications, due to their ability to provide smooth, accurate flow rates when used in conjunction with on another.

- The formulation process generally consists of two streams of fluid (one RNA in an Aqueous Solvent, and the other lipids in an Organic Solvent), coming together at an inline mixing element at a specified ratio of flow rates. The process results in encapsulation of the RNA product, in the LNP delivery vessel.
  - I.e. 300mL/min Aqueous mixes with 100 ml/min Organic to create 400 ml/min output of encapsulated product.
  - The success of formulation process is directly related to how well the mixing ratio was held throughout. This makes it imperative to provide the right flows, and to provide them as instantaneously and simultaneously as possible.

Generic LNP Formulation Setup



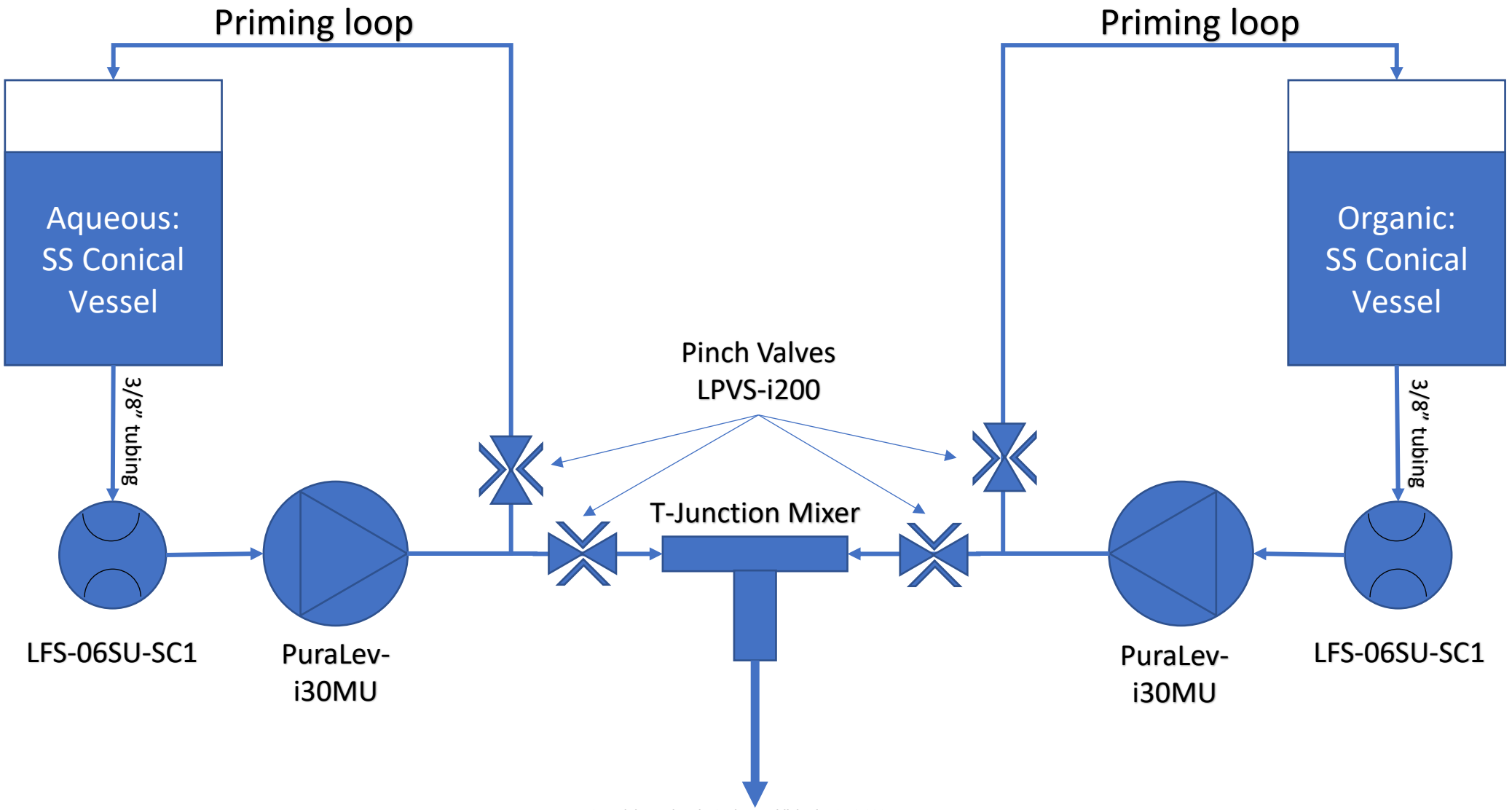
Extended LNP Formulation Setup



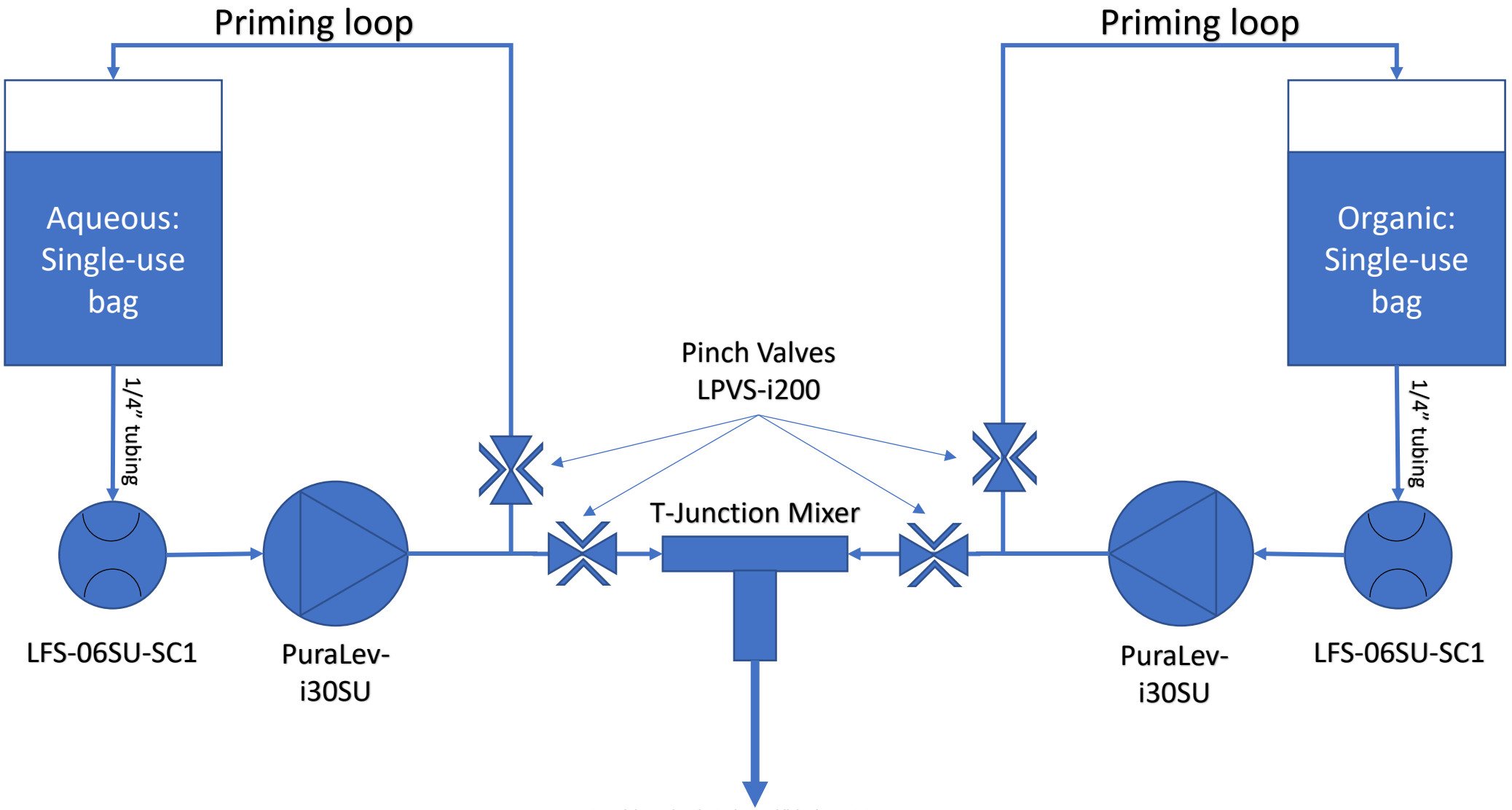
Extended Setup:

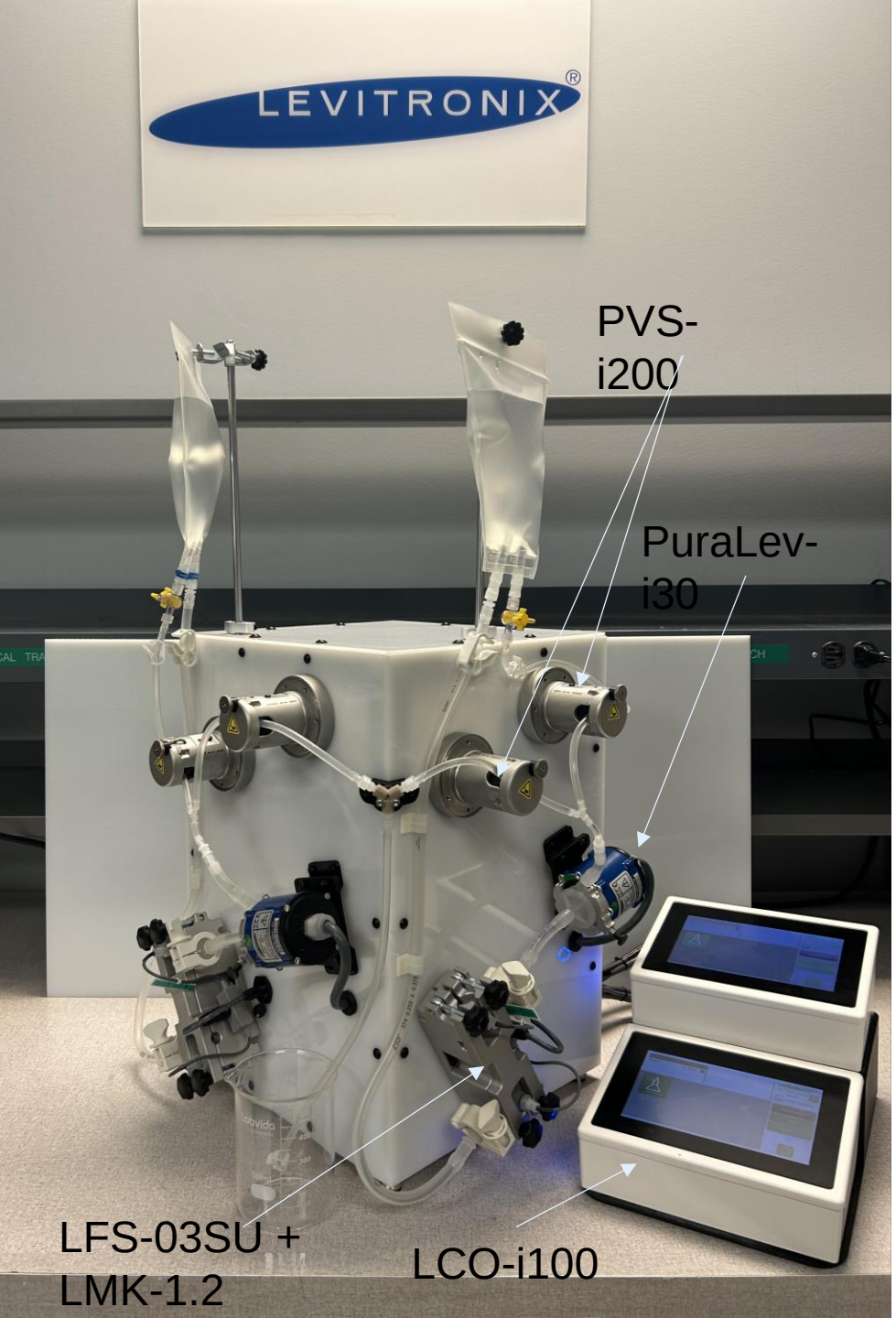
- Additional dilution buffer line which can be primed.
- Optional waste line such that undesirable process fluid can be discarded
- Flow sensors can be added in all lines to track concentrations of process components (not shown in setup)

Small Scale Multi-use Setup



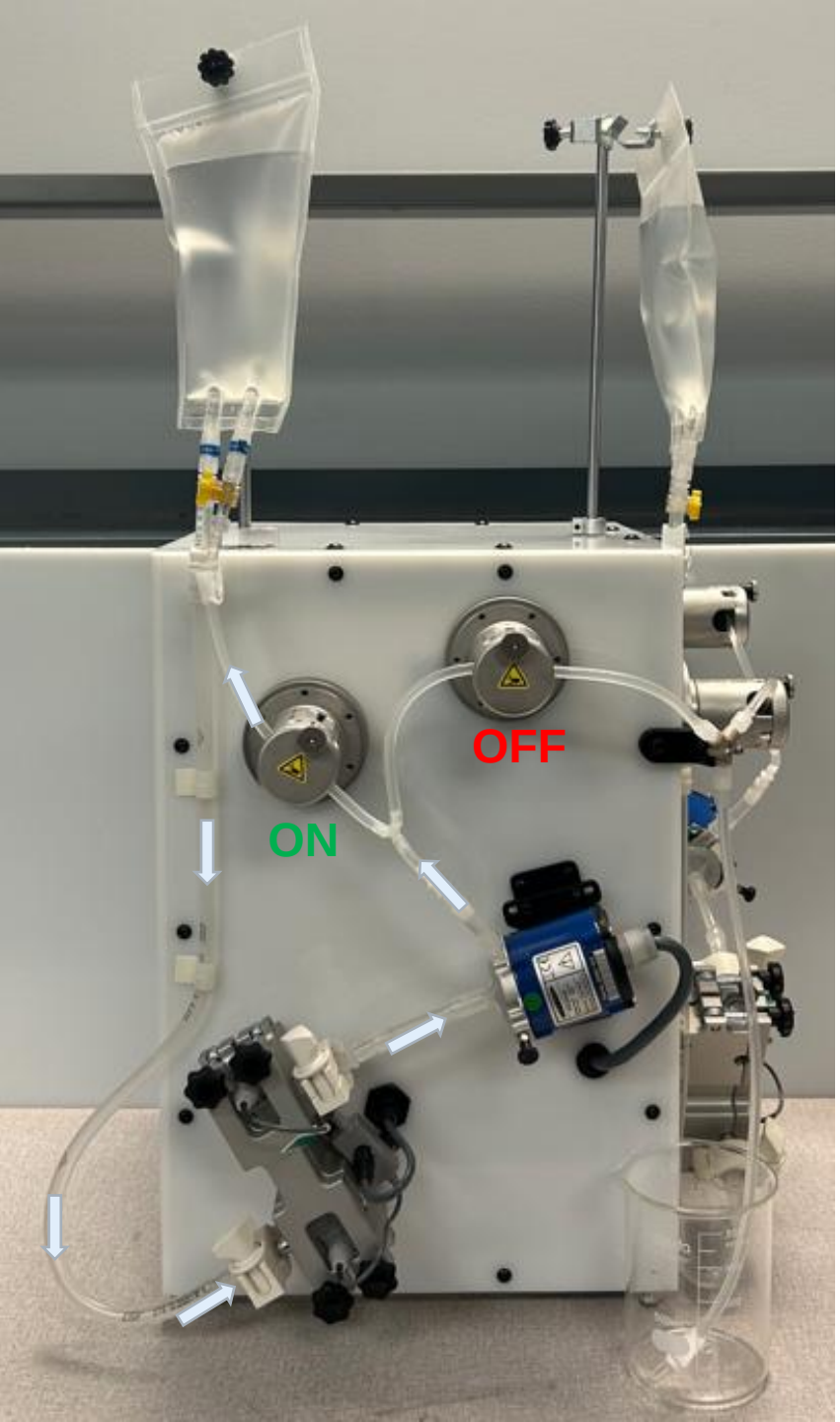
Small Scale Single-use





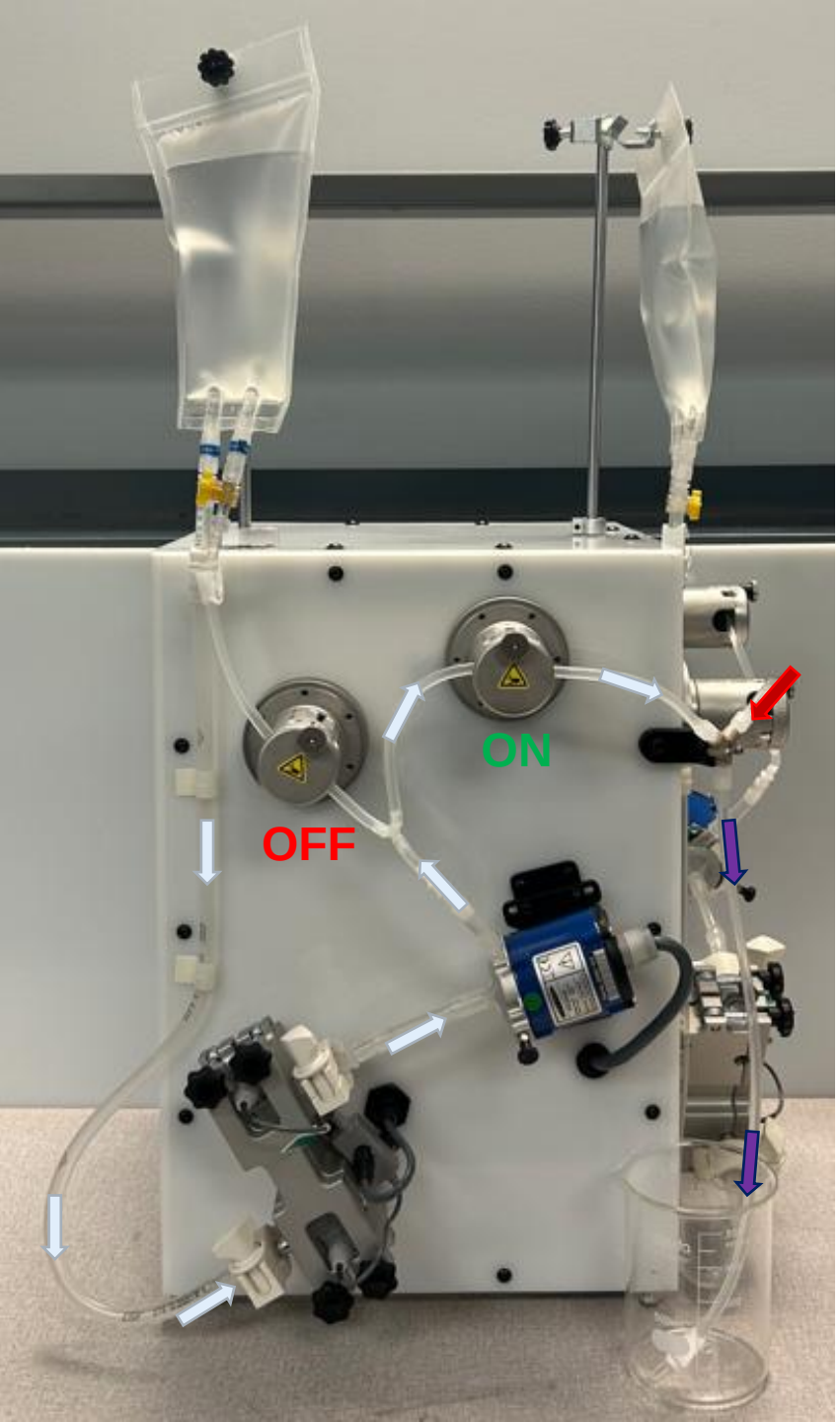
At-A-Glance

- Levitronix Parts Included:
  - 2x LCO-i100 Consoles
  - 2x IPD-30 Drives (Compatible with i100 as well)
  - 2x LFS-06SU-Z-SC1 Sensors (Compatible with 03SU as well)
  - 2x LMK-1.2
  - 4x PVS-i200 Pinch Valves
- 12” x 12” x 18” Body
  - Serves as mount for hydraulic components, and enclosure for electrical components.
- Recipe function utilized on both consoles to set flow rates, and control valves switching.
- SU Bags can be replaced with bottles and other containers



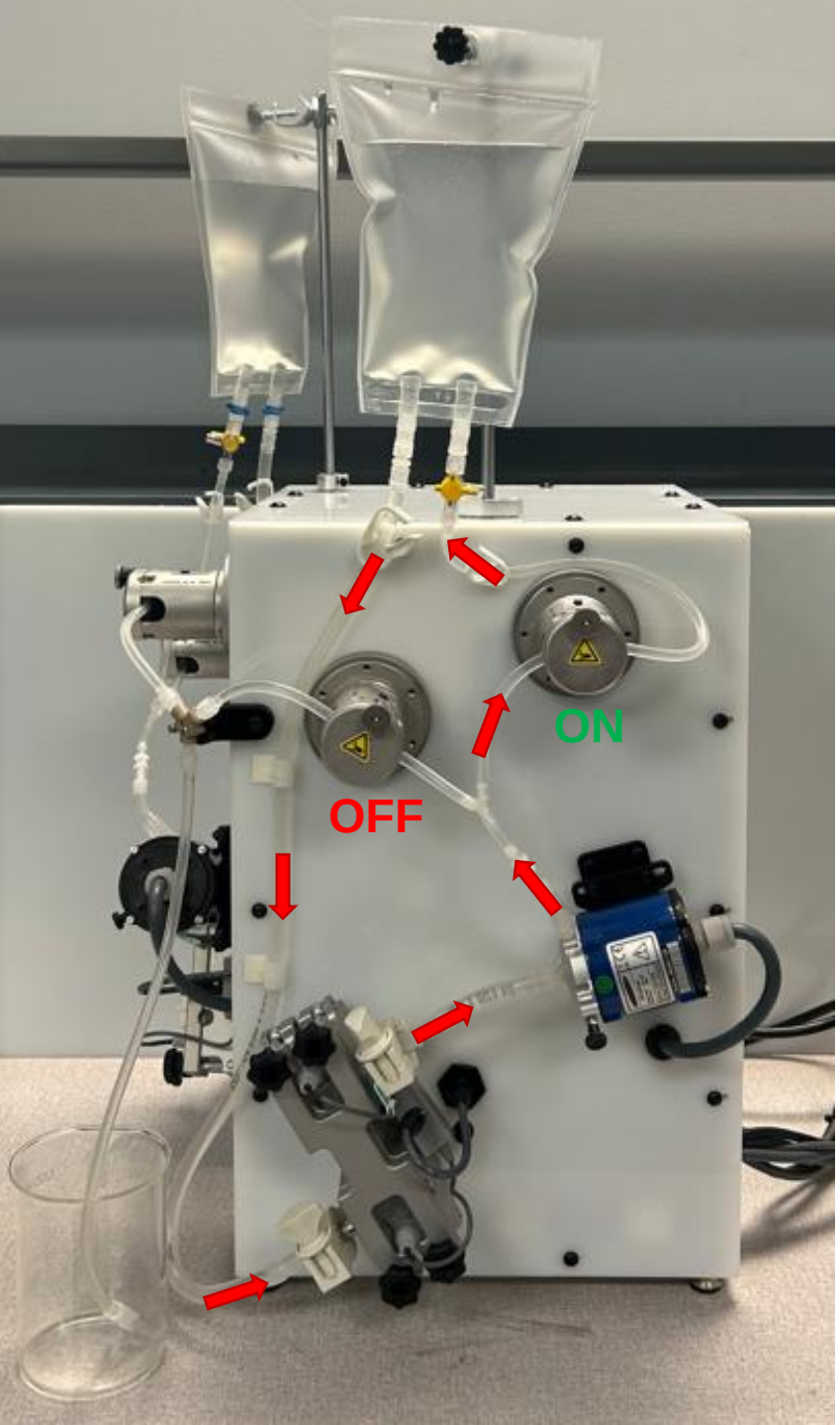
Aqueous Flow Path – Recirc Path

- PVS Valves allow for easy switching between mixing and Recirculation
- Recirc loop feeds flow back into the bag/reservoir
- This allows pump to be at speed, with a flow, prior to switching into mix mode.
  - This ensures a “running start” to the mixing.



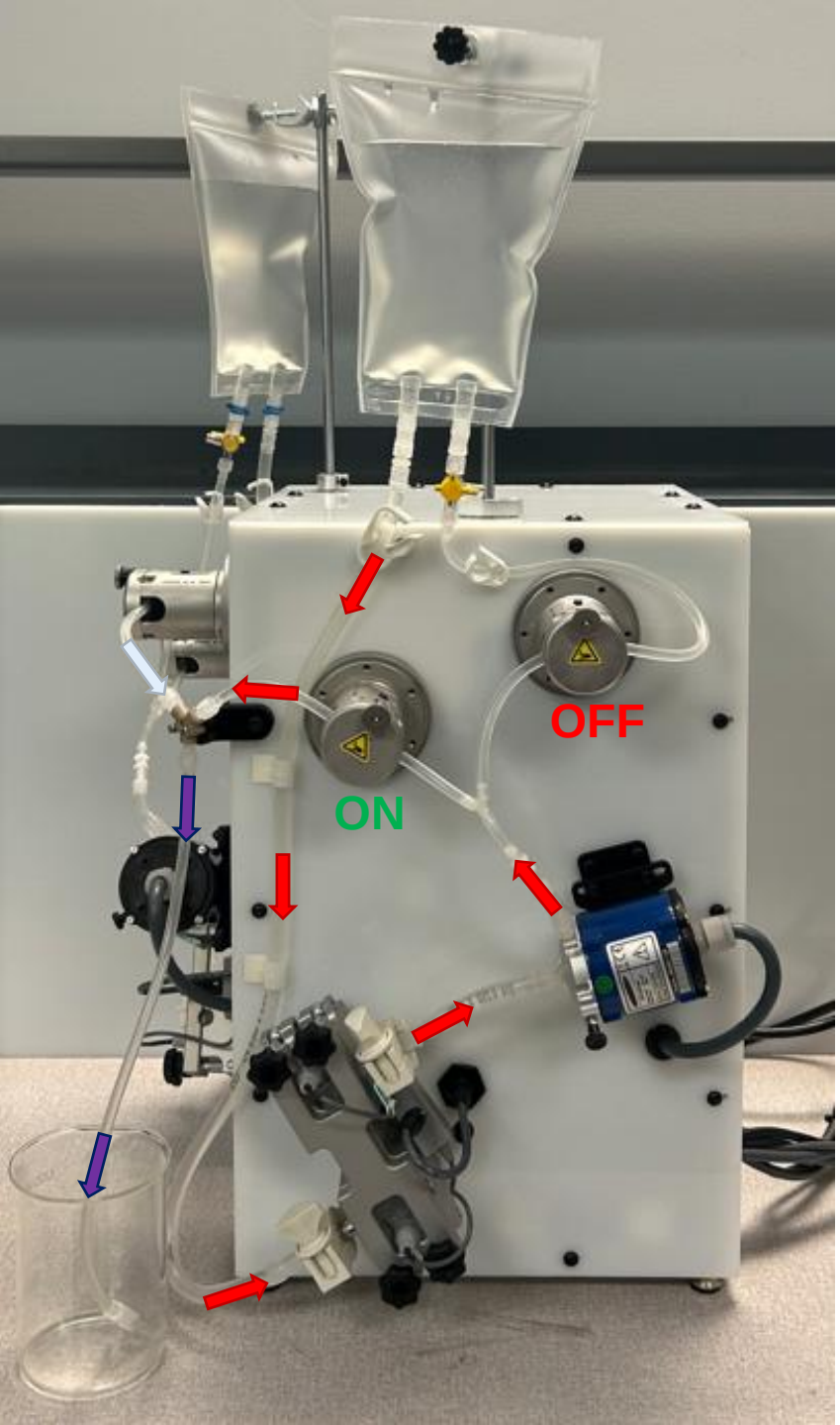
Aqueous Flow Path – Mixing Path

- PVS valves allow for easy switching to mixing path
- Mixing path directs flow to the mixer (T, Y, etc.)
  - Flow meets the organic side flow, and this initiates the actual LNP formulation step.



Organic Flow Path – Recirc Path

- PVS valves allow for easy switching between mixing and Recirculation
- Recirc loop feeds flow back into the bag/reservoir
- This allows pump to be at speed, with a flow, prior to switching into mix mode.
  - This ensures a “running start” to the mixing.



Organic Flow Path – Mixing Path

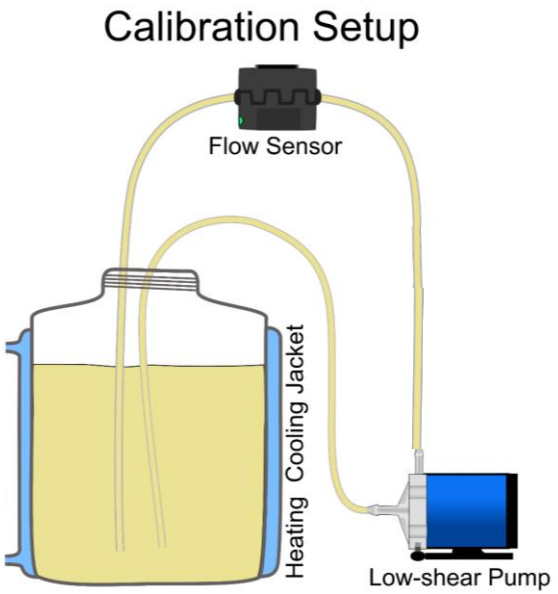
- PVS Valves allow for easy switching to mixing path
- Mixing path directs flow to the mixer (T, Y, etc.)
  - Flow meets the aqueous side flow, and this initiates the actual LNP formulation step.

Levitronix Sensors as PAT: General Calibration Approach

Calibration Workflow

Calibration of Quantification Model:

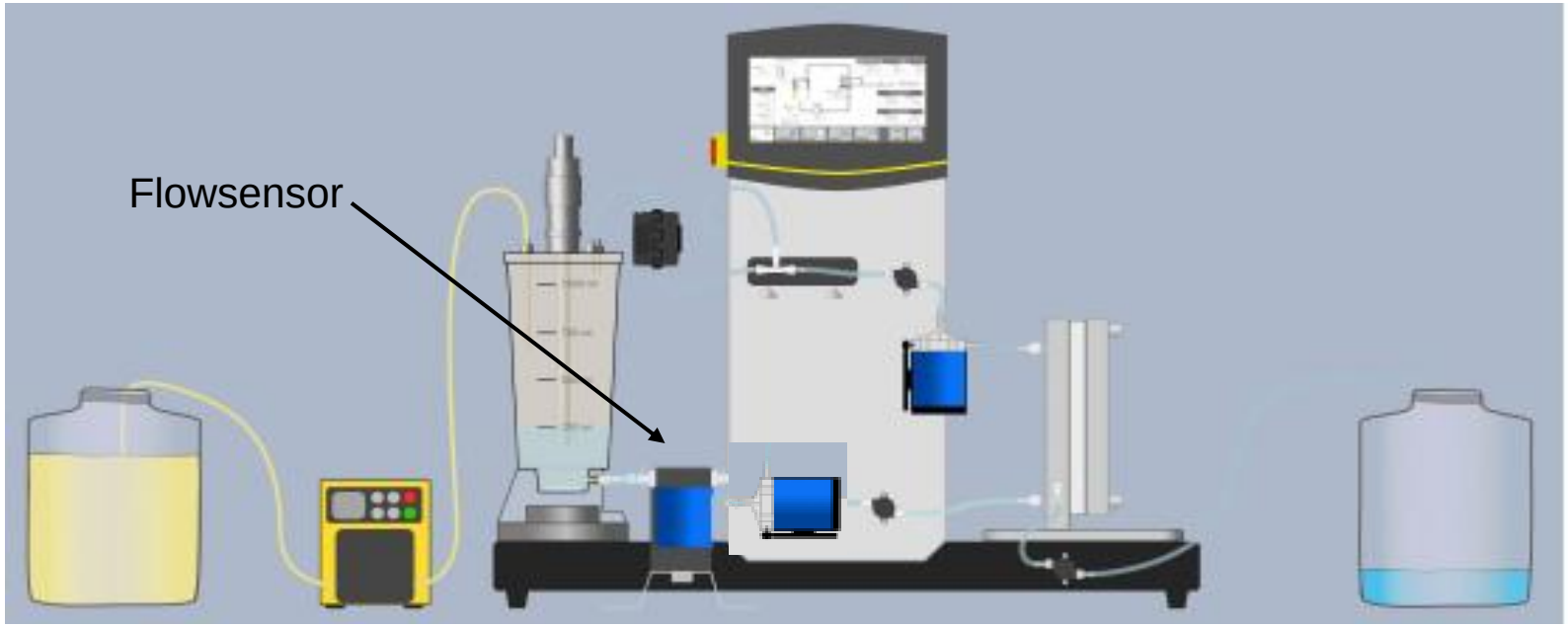
- (a) Analyte of interest (AOI) in buffer was recirculated in a calibration setup including a heating and cooling device, starting at the highest concentration (also possible to start at any other concentration).
- (b) Temperature was ramped from low temperature to high temperature (depending on the range of interest), while measuring the resulting sound velocity for each temp-conc combination.
- (c) AOI was diluted / concentrated to the next concentration and steps (a) – (c) were repeated until all the concentrations of interest were covered.
- A reliable correlation between AOI concentration and sound velocity could be established for each buffer by linear regression, resulting in a polynomial quantification formula (the order of the polynomial function can be defined by user, e.g. linear, quadratic, cubic, but also higher order, and all combinations thereof).
- The experimental setup can be modified (e.g. more sensors can be integrated into the loop, the position of the sensors in the loop can be varied, …)



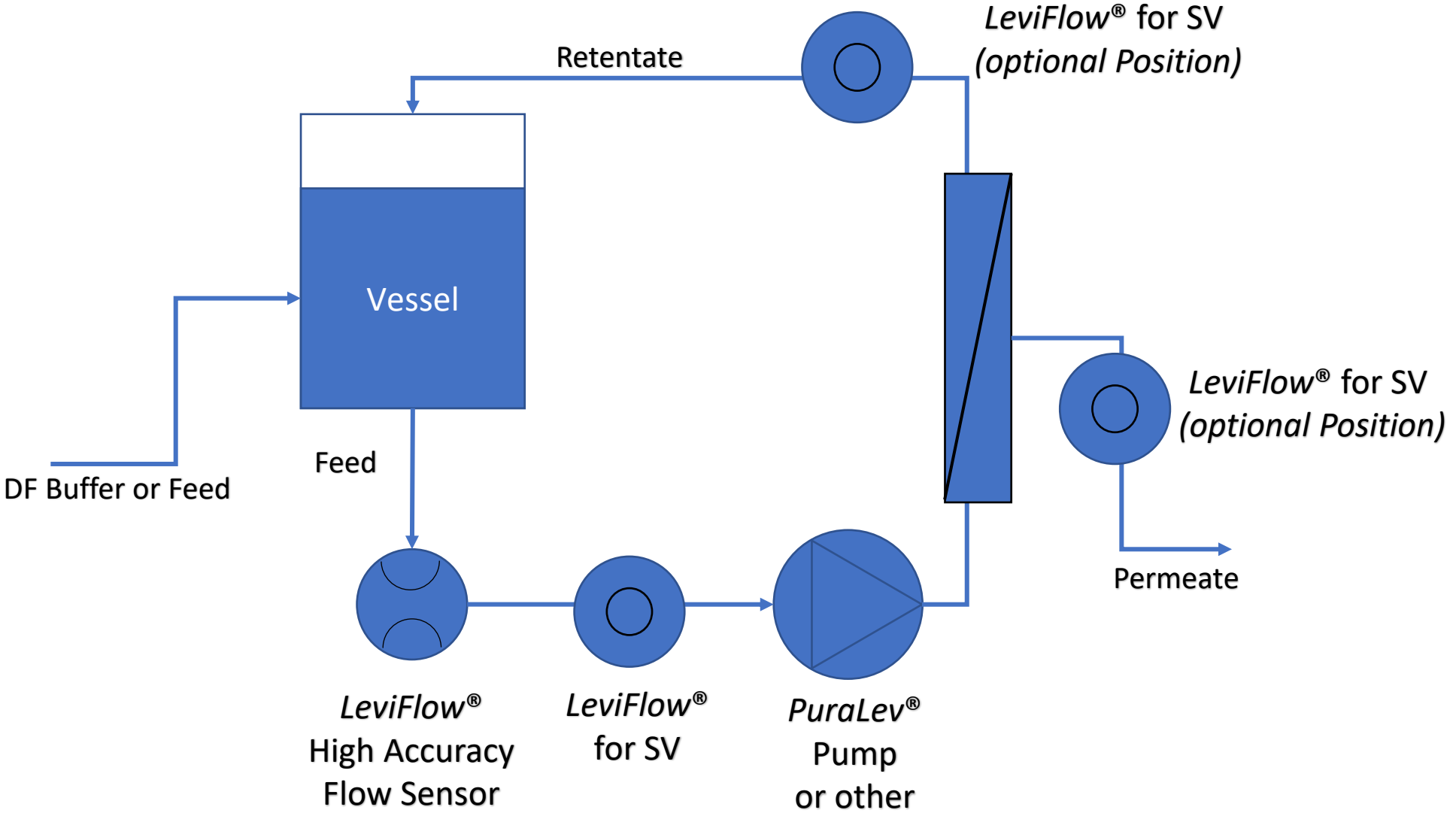
Sound Velocity as PAT for LNP Manufacturing

Flowsensor integration into TFF UF/DF

- The single use sensor can easily be integrated into the TFF flow path.
- Preferentially, the sensor is integrated into the flow path prior to the filter as this allows correct concentration determination in the vessel. If positioned behind the filter, then the concentration will be increased due to the concentration process in the filter.
- The sensor might be even used on the permeate side to track protein leakage



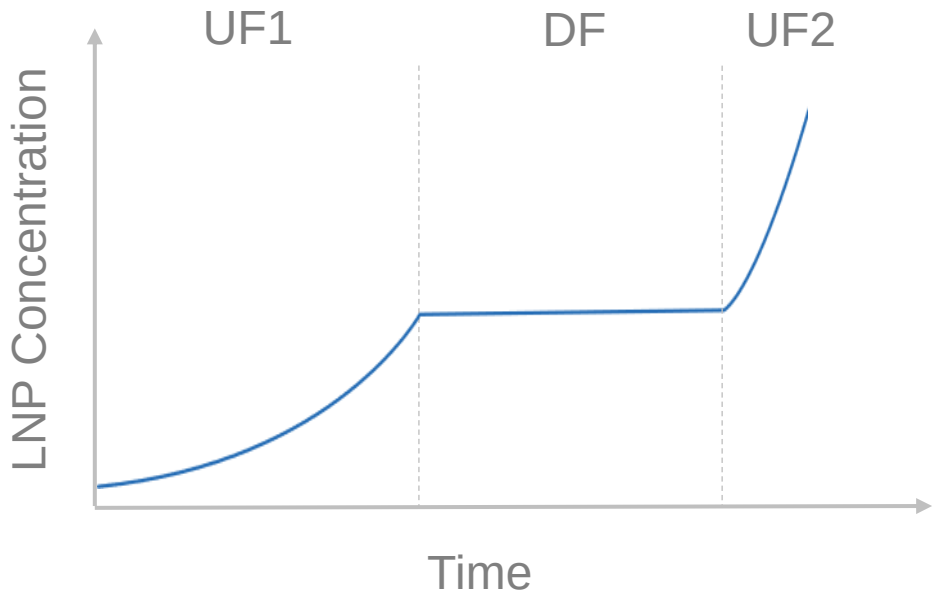
Generic LNP TFF Setup



Step 2: LNP TFF (UF/DF)

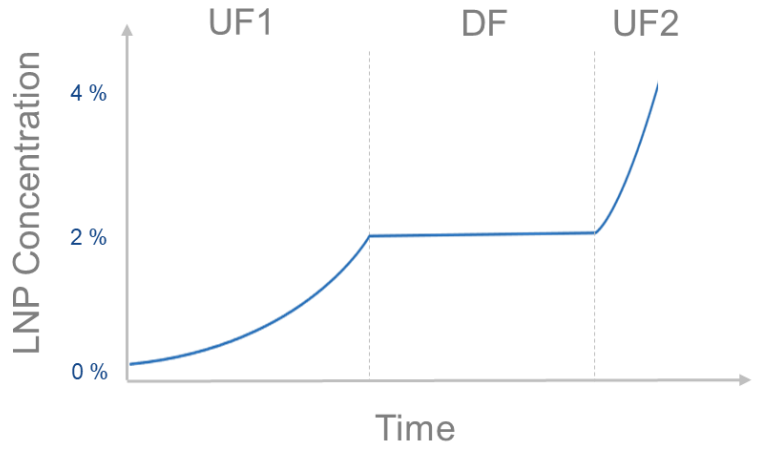
- Ultra-filtration (UF): There can be either 1 or 2 UF steps in the process (potentially even more options)
  - UF1: Initial concentration of LNPs to reduce volume prior to DF
  - UF2: UF after DF to reach target concentration of LNPs for final formulation

- Diafiltration (DF): Removal of residual formulation buffer containing EtOH (e.g. 25%) into the final storage buffer (0% EtOH). The LNP concentration does not change during this step.

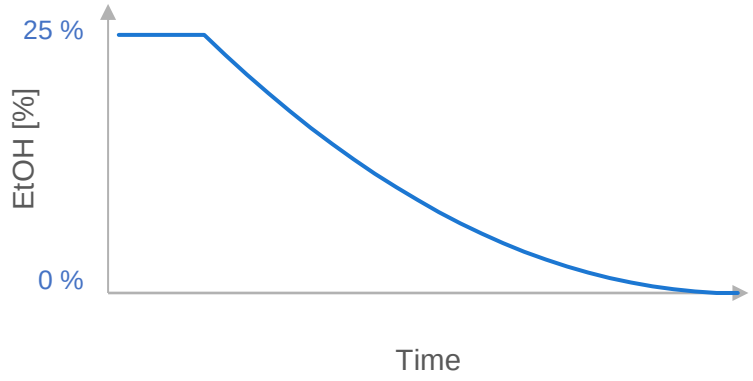


Inline Concentration Measurement

- UF: During UF, an accurate real-time measurement of LNP concentration would be desirable in order to avoid time consuming operator intervention for offline analysis. Further, real-time LNP concentration would allow for process automation.



- DF: During UF, the buffer is exchanged to the target storage buffer. Tracking the progess of buffer exchange in real-time would allow to optimize diafiltration volumes and automation of the process.



Model System Lecithin

Lecithin is a suitable model for LNPs because of:

- It’s similar lipid composition: Lecithin is a naturally occurring phospholipid, similar in composition and structure to the lipids used to form LNPs.
- Lecithin’s self-assembly behavior: It’s amphiphilic nature leads to formation of lipid bilayer in aqueous solution, representing the structure of LNPs
- Both, LNPs and lecithin affect the sound velocity.

Lecithin used in study contained: Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol, Phosphatidic acid

Phospholipids in LNPs: Phosphatidylcholine, Phosphatidylethanolamine, Cholesterol, Ionizable lipids

Potential Control Strategies

UF:

- UF_Tc: *T = constant, [EtOH] = constant, [Lecithin] = decreasing: Simplified approach tracking sound velocity*

- UF_C: Prediction Model Calibration for Lecithin: *T= ramping from 20°C to 30°C, [EtOH] = constant, [Lecithin] = Changing between defined concentrations*

DF:

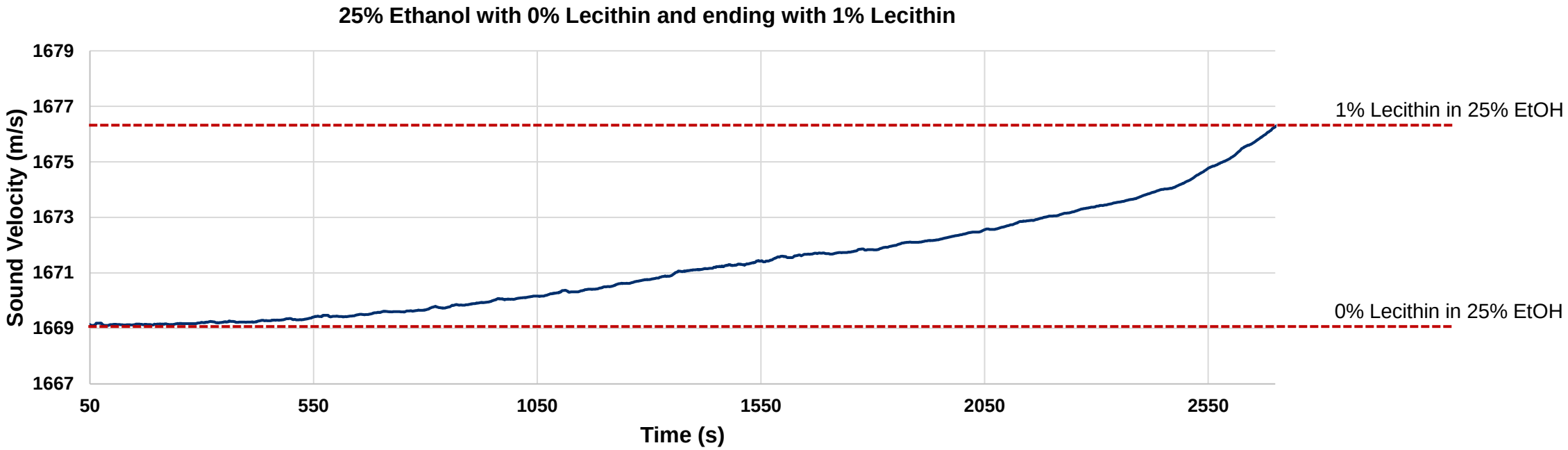
- DF_Tc: *T= constant, [EtOH] = decreasing, [Lecithin] = constant: Simplified approach tracking sound velocity*

- DF_C: Prediction Model Calibration for EtOH: *T= ramping from 20°C to 30°C, [EtOH] = Changing between defined concentrations, [Lecithin] = constant*

→ Only UF_Tc and DF_Tc control strategies are shown in the following, but UF_C and DF_C can similarily be performed using the flowsensor.

UF_Tc
Sound Velocity

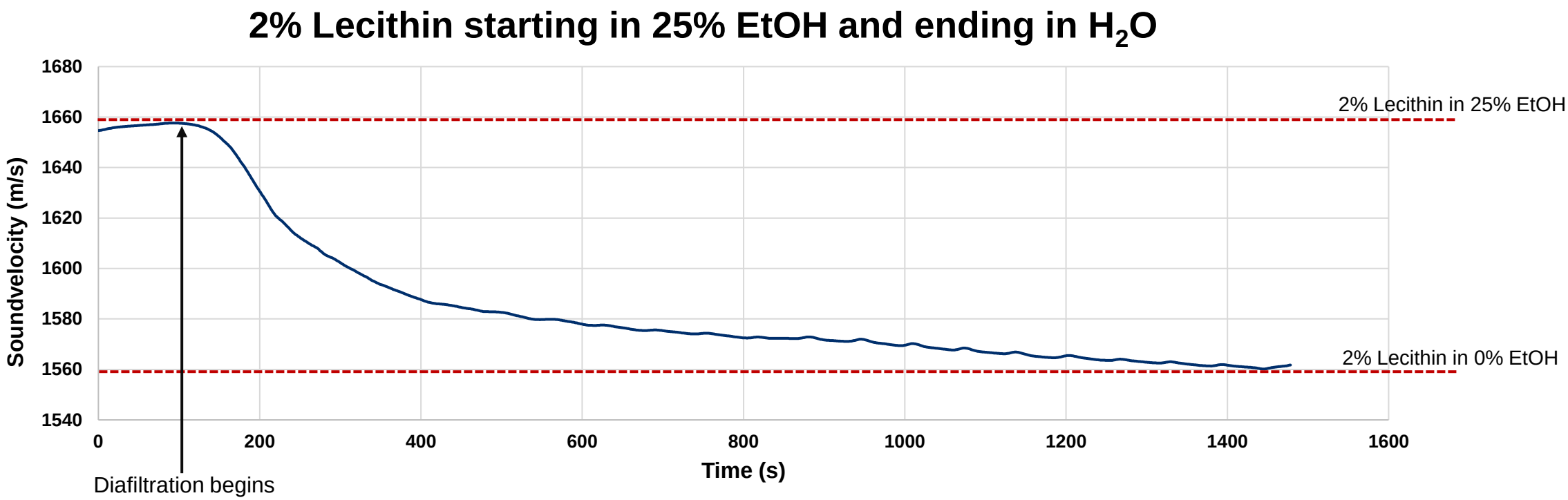
UF: Increasing Lecithin Concentration



- Sound Velocity increases with increasing Lecithin concentration
- Knowing sound velocity of a target Lecithin (LNP) concentration allows control strategies

DF_Tc
Sound Velocity

DF: Decreasing EtOH Concentration



- Sound Velocity decreases with decreasing EtOH concentration
- Knowing sound velocity of a target EtOH concentration allows control strategies

Sound Velocity as PAT in LNP Formulation

- Sound Velocity as PAT for LNP Manufacturing can not only be used in the TFF step (step 2), but also during formulation (step 1) at all process flow positions.

Acknowledgements

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