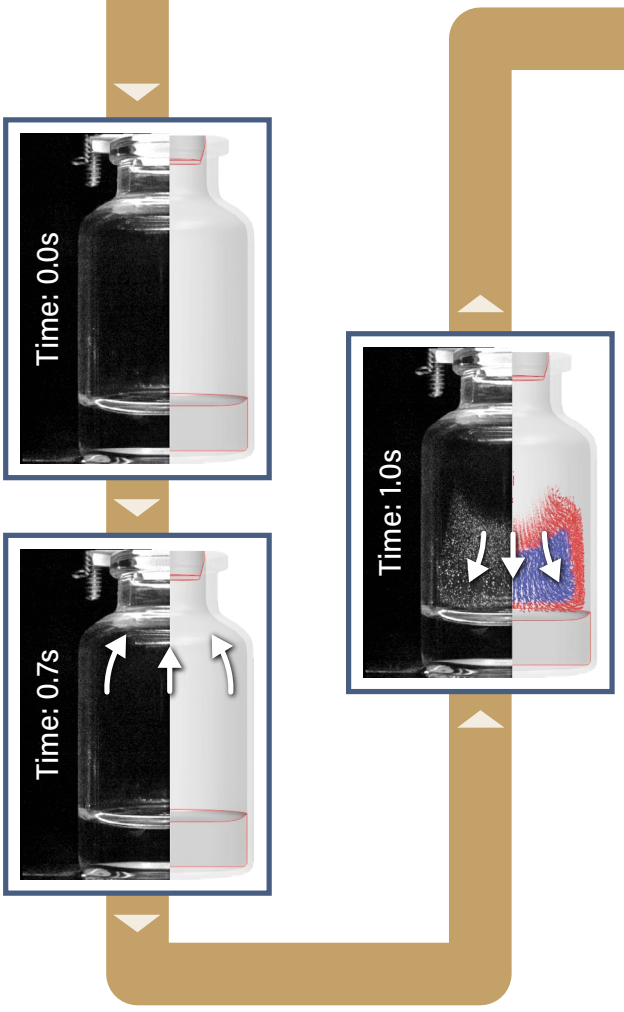
ANNUAL REPORT

ADVANCED LYOPHILIZATION TECHNOLOGY CONSORTIUM

2022

BLOWING THE LID OFF

of Rapid Depressurization Controlled Ice Nucleation in Pharmaceutical Freeze-Drying



LYOhub.org

Pharmaceutical Freeze-Drying

Freezing is a critical phase of the pharmaceutical freezedrying/ lyophilization process due to its influence on primary drying duration, batch homogeneity, and reconstitution time.

Controlled ice nucleation is a process capable of overcoming many of these difficulties by causing all vials to undergo nucleation simultaneously.

The rapid depressurization method is one of the most widely applied technologies but is not yet well understood.

These images show, for the first time, a combination of experimental and computational methods to reveal the highly coupled and complex flow inside the vial headspace during the discharge, providing the evidence necessary to unravel the method's mysteries.

KEY

Ice

Liquid

Flow visualization and simulation data produced by Drew Strongrich in the LyoHUB Demonstration Facility as part of research supported by Genentech Inc. Additional details can be found in Strongrich, A., Lim, F., Kumar, L., Alexeenko, A., "Rapid Depressurization Based Controlled Ice Nucleation in Pharmaceutical Freeze-drying: The Roles of the Ballast Gas and the Vial." Journal of Pharmaceutical Sciences 110.11 (2021).

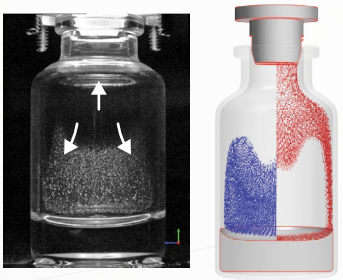
https://doi.org/10.1016/j.xphs.2021.07.011

Valve Open: Discharge valve opens and the flow visualization system is activated.

TIME: 0.00s

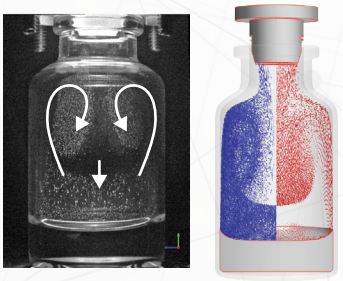
Expansion: Depressurization drives the gas from the vial headspace to the chamber. The gas cools down as it expands at a rate of around 60°C per second, causing water vapor to condense into droplets.

TIME: 0.17s



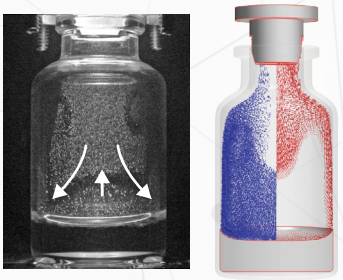
Condensation: The gas in the center of the headspace continues to cool, reaching a terminal value of about -42°C (which is about 36°C cooler than the liquid product). Convective cooling from the gas causes the water droplets in the headspace to freeze into ice particles (having an average size of a few microns) as they are driven down towards the meniscus.

TIME: 1.00s



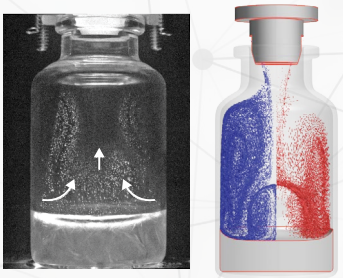
Rollup: A vortical flow sets up in the headspace with ice particles moving downward at the center and upward along the sides of vial. The average gas temperature in the headspace is around -40°C (34°C cooler than the bulk liquid) and remains relatively constant as the latent heat release from condensation and freezing balances the expansion cooling.

TIME: 1.46s



Nucleation: Impinging flows split into upward and downward moving streams in the neck region. The gas in the center of the vial headspace has warmed up to -28.5°C (22°C cooler than the liquid product). Warm gas rises from the liquid surface and diverts ice particles radially outward where they collide with the meniscus and induce nucleation.

TIME: 1.76s



Freezing: Gas near the warmer ice surface rises up towards the stopper by natural convection. The ice temperature is near the equilibrium freezing temperature of water and the gas in the center of the headspace is approximately -13°C. The chamber pressure stabilizes while the headspace gas exchanges heat with the surroundings such as vial walls.

TIME: 2.23s

DIRECTORS' MESSAGE

LYOHUB ANNUAL REPORT 2022

Before the first company joined LyoHUB in 2014, we imagined a consortium focused on lyophilization. That was a goal without a plan—Saint-Exupéry would call it a wish. In 2015, we received a grant from NIST to build a lyophilization consortium and conduct technology roadmapping. That was the beginning of a plan.

The pages of this annual report show just how far that plan has taken us. Today, LyoHUB has twenty-seven member companies that span the lyophilization value chain (p. 4). Consortium members conduct research in our pilot facility ("Demonstration Center", pp. 12-22). They write best practices papers to summarize the state of the art (pp. 5-6) and convert those papers into recognized consensus standards (p. 7). They participate in lyophilization training programs as students and instructors (pp. 11, 23). They create simulation tools to improve lyophilizer performance (pp. 14, 15, 23). Above all, they're a vibrant and well-connected community, working together to advance lyophilization technology.

All these activities can be traced back to the goals and priorities identified during technology roadmapping. So now for true confessions: We (Alina and Liz) didn't like technology roadmapping at first. We didn't know what it was. And we didn't want to do it.

"A goal without a plan is just a wish."

— Antoine de Saint-Exupéry

But we've come around.

That's why we're very excited to launch a new technology roadmapping process, with the goal of expanding LyoHUB's remit to better serve our members. We imagine that the new roadmap will expand the scope of LyoHUB technologies to include freezing and alternative aseptic drying methods. We also imagine that we'll expand the scope of products we support, to include cell and gene therapies, DNA and RNA-based products, and vaccines. But those are wishes. Exactly what the new technology roadmap will be, and the direction that LyoHUB will take over the next 5-10 years, will be decided by our members in the 2022-23 roadmapping process.

A big "thank you" to the twenty-seven member companies who are the core of LyoHUB, and to Purdue's Colleges of Engineering and Pharmacy for their support. We're grateful to Birck Nanotechnology Center for continuing to host our Demonstration Facility. Special thanks to all the students and postdocs who have helped build LyoHUB over the years and who inspire our progress. We are ever grateful to our wonderful LyoHUB Operations Manager Jen Gray and LyoHUB Research Scientist Drew Strongrich, who have a knack for turning straw into gold and plans into reality.

Finally, thanks for your interest in LyoHUB and in this annual report. We hope you'll join us as we continue to advance lyophilization technology and create a new technology roadmap. Bring a wish!

All the best,

Alina Alexeenko and Liz Topp

MEMBERSHIP

IMA LIFE Aseptic Processing & Freeze Drying Solutions	Pfizer	janssen PHARMACEUTICAL COMPANIES OF Johnson & Johnson	MILLROCK TECHNOLOGY millrocktech.com
Member Since 2014	Member Since 2014	Member Since 2015	Member Since 2015
INFICON	Baxter	ESP	Allergan
Member Since 2015	Member Since 2016	Member Since 2016	Member Since 2016
abbvie	Roche	THE McCRONE GROUP	SIEMENS
Member Since 2016	Member Since 2016	Member Since 2016	Member Since 2017
FRESENIUS KABI	Bristol Myers Squibb™	OPTIMA EXCELLENCE IN PHARMA	AMGEN
Member Since 2017	Member Since 2017	Member Since 2018	Member Since 2018
Merck KGaA EMD SERONO	AstraZeneca	EVONIK POWER TO CREATE	Daiichi-Sankyo
Member Since 2019	Member Since 2019	Member Since 2019	Member Since 2019
COOK® BIOTECH	CORNING	SANOFI	MERCK
Member Since 2019	Member Since 2020	Member Since 2020	Member Since 2021
FUJIFILM	Elanco	Jazz Pharmaceuticals	
Member Since 2021	Member Since 2021	Member Since 2021	

BEST PRACTICES PAPERS

Recommended Best Practices for Lyophilization Validation 2021

Part I: Process Design and Modeling

Part II: Process Qualification and Continued Process Verification

Both these papers were published in 2021 and have already been accessed over 3,900 times (Part I) and over 2,300 times (Part II).

These papers are a collective work by authors who are experts in their fields from several organizations engaged in pharmaceutical lyophilization process R&D and pharmaceutical manufacturing. This work describes lyophilization process validation and consists of two parts.

Part I focuses on the process design and is described in the current paper, while **Part II** is devoted to process qualification and continued process verification. The intent of these articles is to provide readers with recent updates on lyophilization validation in light of recent technological development, improved process understanding and includes the current practical aspects from the industrial perspective.

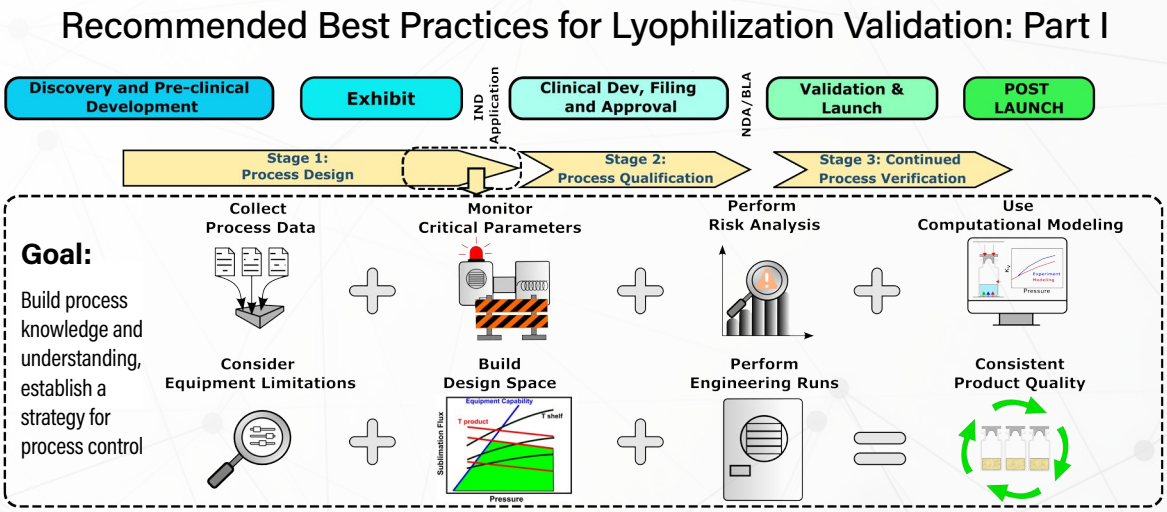


Figure: *"The Best Practices for Lyophilization Validation Part I" is available in open access at https://link.springer.com/article/10.1208/s12249-021-02086-8.*

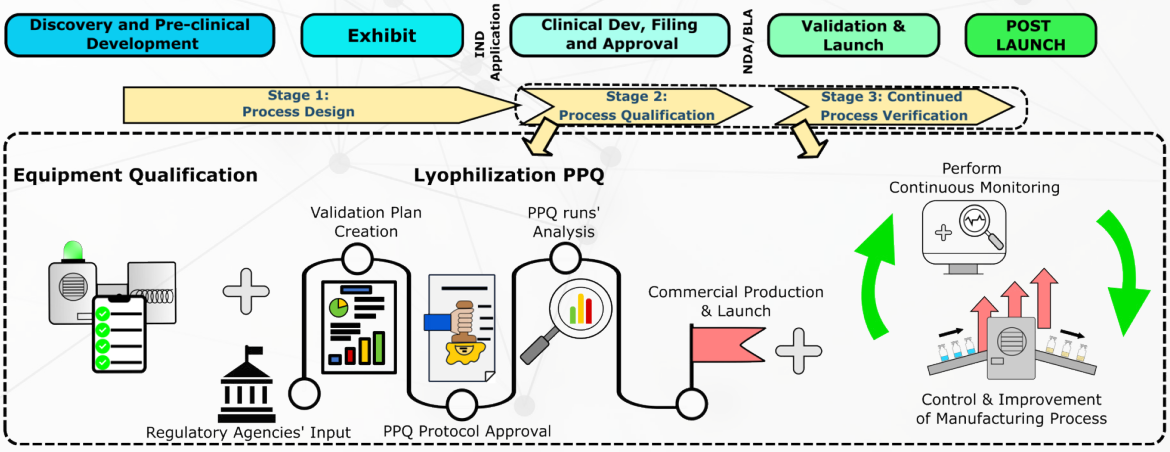


Figure: *"The Best Practices for Lyophilization Validation Part II" is available in open access at https://link.springer.com/article/10.1208/s12249-021-02107-6.*

BEST PRACTICES PAPERS (Cont'd)

LyoHUB's first lyophilization best practices paper, ***Recommended Best Practices in Instrumentation Process Monitoring in Pharmaceutical Freeze Drying***, continues to be popular with over 16,000 downloads. It is available in open access at http://link.springer.com/article/10.1208/s12249-017-0733-1.

Work continues on the Best Practices Paper on Lyophilization Process Scale Up and Tech Transfer

Under the direction of Dr. Serguei Tchessalov, Research Fellow at Pfizer, over 35 LyoHUB members representing over 18 member companies, have been working in collaboration with BioPhorum, a global biopharmaceutical industry collaborative organization, to write a best practice paper on scale up and tech transfer for the lyophilization process. This collaboration was established to help deliver a better solution for the biopharmaceutical industry and accelerate adoption of good practices. More specifically, BioPhorum and LyoHUB have identified a shared interest in developing best practices for technology transfer and scale up for lyophilization processes. This work is particularly important as lyophilization is an important part of the fill finish process and at present there are no harmonized processes for scale up or transfers from one manufacturer to another.

Freeze-drying process scale up and transfer remains an empirical effort, often driven by historical experience and involves non-uniform approaches across the industry. Scientists engaged in freeze-drying within the LyoHUB consortium and BioPhorum, are sharing experiences, defining best practices and proposing the development of rational scale up/tech transfer strategies. The initial effort focuses on identifying the challenges in enabling the transfer from the laboratory scale to the pilot or commercial dryers. The impact of differences in the small vs. larger scale dryers (heat and mass transfer differences, dryer design, and dryer capability) as well as assessing the impact of the GMP clean environment on product quality. Scale up based on the use of the same process set points and duration between laboratory and commercial scale dryers could lead to compromised product quality (collapse, higher water content) and/or vial breakage. Emerging modeling tools reveal the advantage of a prior generation of critical input parameters (container heat transfer coefficient, minimum controllable pressure, and maximum sublimation rate) to ensure successful application of modeling-based approaches.

This team is also summarizing both inefficient and best practices and providing some practical advice. With the significant progress achieved during last year, the team is targeting submission of the first part of the paper during the summer of 2022.

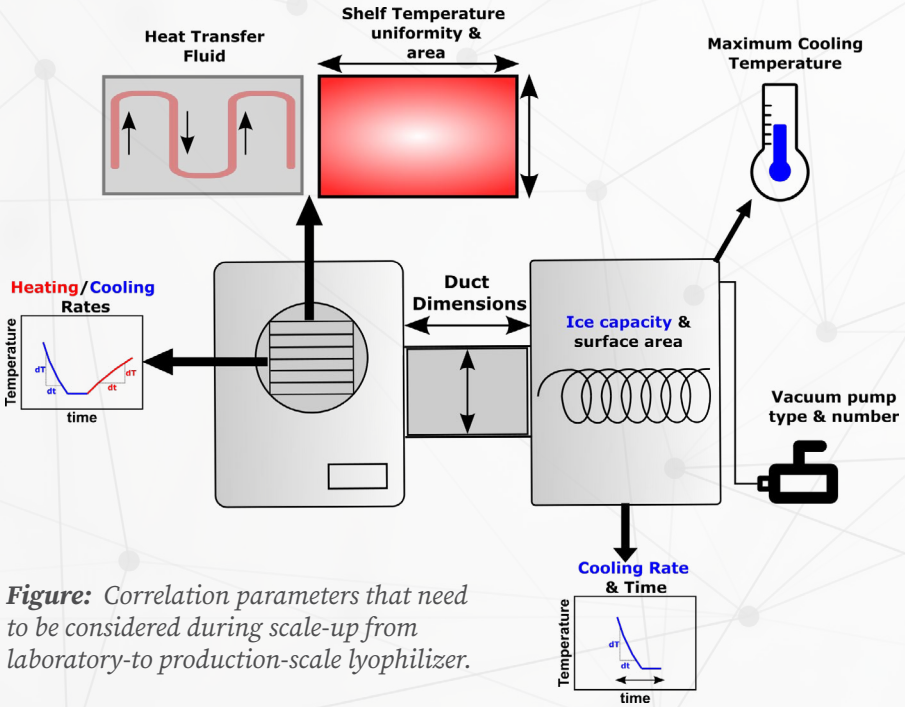


Figure: *Correlation parameters that need to be considered during scale-up from laboratory-to production-scale lyophilizer.*

Work also continues on:

Best Practices in the Development of Lyophilized Formulations led by Dr. Elizabeth Topp (Purdue/NIBRT) and Dr. Greg Sacha (Baxter Healthcare).

Equipment Performance Qualification led by Arnab Ganguly (Amgen).

FIRST CONSENSUS STANDARD FOR FREEZE DRIED PHARMACEUTICALS

In 2021, the American Society for Testing and Materials (ASTM) issued a new standard, ASTM E3250-21, Standard Practice for Product Temperature and Equipment Pressure Instrumentation in Pharmaceutical Freeze Drying. Much of the standard incorporated findings are laid out in a paper co-authored by LyoHUB Co-Director Alina Alexeenko, a collaborative effort among members of LyoHUB, which was aided in its consortium development with support from the National Institute of Standards and Technology (NIST).

The paper, Recommended Best Practices for Pharmaceutical Freeze-Drying Process Instrumentation, while recognizing the need to protect intellectual property, emphasizes that there are areas where industry can benefit from information sharing and standards—in this case, for pharmaceutical freeze-drying. The paper sets out recommendations for measuring and monitoring product temperature and pressure within the freeze dryer, along with equipment capability testing. Temperature and pressure are vital variables that affect things like heat transfer, process efficiency, and product quality.

The ASTM standard, taking the paper's lead, focuses on best practices for freeze dryer instrumentation, with emphasis on monitoring the status of product for temperature and pressure. It examines and clarifies sources of uncertainty around measurement probes, and the differences between temperature-measuring instruments like thermocouples and resistance temperature detectors. On the pressure side, it considers and issues recommendations for pressure transducers—specifically, thermal conductivity type gauges and capacitance manometers for the dryer's product chamber and condenser.

LyoHUB led the multi-year effort to deliberate the details of this first recognized consensus standard for pharmaceutical lyophilization.

The participants' collegial, cooperative approach was vital to forging agreement on the best practices that would constitute key elements of the standard. "The consensus-building process took time, to carefully review and consider all aspects of the process across the extended value chain of stakeholders, through a series of workshops and collaborative events with ASTM at Purdue," said Arnab Ganguly, principal engineer for pivotal drug product technologies at Amgen, chair of ASTM subcommittee E55.05.

LyoHUB also worked to establish an ongoing ASTM subcommittee on lyophilization. This subcommittee's mission is to develop, disseminate, and educate standard practices and guidance relevant to lyophilization of parenterals and other pharmaceutics and biologics for the manufacture of pharmaceutical and biopharmaceutical products. ASTM E55.05 is currently working on establishing best-practice guidance on equipment performance validation and commercial scale-up, and are working on converting these best practices into consensus standards as well.

ASTM INTERNATIONAL

ASTM LYOPHILIZATION STANDARDS

E55.05 Lyophilization subcommittee of E55 Committee on Manufacture of Pharmaceutical and Biopharmaceutical Products:

www.astm.org/COMMITTEE/E55.html

Dr. Arnab Ganguly
Chair | E55.05
IMA LIFE

Dr. Serguei Tchessalov
Vice-Chair | E55.05
PFIZER

Jennifer Gray
Recording Secretary 2022-2024
E55 Executive Committee
PURDUE

INDUSTRY VISITS

- **July 1, 2021:** Cook Biotech visit to LyoHUB
- **July 26, 2021:** Elanco visit to LyoHUB
- **February 8, 2022:** Millrock visit to LyoHUB
- **March 10, 2022:** Metrohm visit to LyoHUB

SPECIAL PRESENTATIONS TO LYOHUB

April 2021

- **Kumar Janoria** | FDA Branch Chief OPQ/CDER
- **Steve Rhieu** | FDA Branch Chief OPQ/CDER

A Regulatory Perspective on Manufacturing Processes Pertaining to Lyophilized Injectable Products

May 2021

- **Drew Strongrich** | Research Scientist, LyoHUB
- **Jack Van Wingen** | Purdue Chemical Eng. student
- **Patrick Williams** | Purdue Chemical Eng. student, VIP program

Corning-LyoHUB "Lyo Breakage Reduction" LyoLaunchPad project report

June 2021

- **GV (Rex) Reklaitis** | Burton and Kathryn Gedge Distinguished Prof. of Chemical Eng., Purdue

Innovations in Pharmaceutical Manufacturing on the Horizon: Technical Challenges, Regulatory Issues, and Recommendations

July 2021

- **Denis Dowling** | Director, I-Form Advanced Manufacturing Research Centre, University College, Dublin

Application of Industry 4.0 for Enhancing Manufacturing Processing Efficiencies

August 2021

- **Kyu Yoon** | Postdoctoral Research Associate at Purdue in the group of Professor Vivek Narsimhan

Modeling and Experiment during Secondary Drying Process for Accurate Temperature Prediction of the Freeze-Dried Cake

September 2021

- **Tatsuhiro Kodama** | Senior Researcher in Formulation Technology Research Laboratories, DAIICHI SANKYO CO., LTD. (Japan)

Lyophilization Study with μ-CT and Pirani Vacuum Gauge

October 2021

- **Sarah Ehlers** | Senior Scientist I, AbbVie Deutschland GmbH & Co. KG

Nests and Chamber Wall Temperature Control as Tools in Pharmaceutical Freeze Drying—Evaluation of Heat Transfer, Edge-Vial-Effect and Drying Kinetics

November 2021—Project Proposals

1) **Drew Strongrich** | Research Scientist, LyoHUB
   Quantitative Analysis of Vial Breakage During Lyophilization Using Wireless Strain Sensors
2) **Kyu Yoon** | Postdoc Research Associate at Purdue
   Excel-based Lyo Calculator for Secondary Drying
3) **Zane Baird** | Research Associate, Baxter Pharmaceutical Solutions
   Co-Solvent Lyophilization Residual Gas Analysis

December 2021

- **Tom Anchordoquy** | Prof., Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado

Challenges in the Development of Lyophilized Lipid-based Formulations for Nucleic Acid Therapeutics

January 2022

- **Jens Philipp** | Head of Process-Technology at Optima pharma Mornshausen
- **Shreyas Bhatt**, Process Design Engineer at Optima pharma Mornshausen

Using Simulation to Optimize Ice Condensers

February 2022

- **Gayathri Shivkumar** | Senior Scientist, Engineering Combination Products, AbbVie

Characterization of Nitrogen Flow in Lab-Scale Lyophilizers and its impact on Primary Drying

March 2022

- **Petr Kazarin** | Aeronautics & Astronautics Post-Doc at Purdue, LyoHUB
- **Jack Van Wingen** | Purdue Chemical Eng. student, LyoHUB

Machine Learning for Prediction and Optimization of Lyophilizer Equipment Performance

- **Chris Weikart** | Executive VP, Chief Scientist at SiO2
- **Drew Strongrich** | Research Scientist, LyoHUB

A Moisture Stability Study of Lyophilized Formulations Stored SiO2 Hybrid Vials

GRANTS & COLLABORATIONS

PFI-RP: Sensors, Computational Modeling, and Bioanalytical Technologies for Closed-Loop Lyophilization

- Funded by NSF, Partnership for Innovation program
- $750,000 over 3 years
- **Goal:** (i) Noninvasive product temperature monitoring using wireless probes that are compatible with aseptic processing requirements; (ii) Accelerated biomolecule stability analytics by solid-state hydrogen-deuterium exchange mass spectrometry; and (iii) Real-time lyophilization rate measurement and closed-loop process control based on distributed wireless probes and computational modeling of the heat and mass transfer in the product, container, and the lyophilizer equipment.
- **Investigators:** Alina Alexeenko (PI, Purdue/AAE), Timothy Peoples (Co-PI, Purdue Foundry), Elizabeth Topp (Co-PI, Purdue/IPPH), Dimitrios Peroulis (Co-PI, Purdue/ECE)
- **Industry Partner:** Millrock Technology

Advanced Characterization and Manufacturing Methods for mRNA Vaccine Development

- Funded by NIIMBL (National Institute for Innovation in Manufacturing Biopharmaceuticals) as part of the American Rescue Plan
- $500,000 over 1 year
- **Goal:** This project involves constructing/developing mRNA LNP formulations through available historical scientific literature. Furthermore, these mRNA LNP's will be used to produce frozen, lyophilized, and spray dried formulations. These formulations will be further characterized for drug product stability. New analytical approaches solid-state nuclear magnetic resonance (SSNMR spectroscopy), solid-state hydrogen-deuterium exchange mass spectrometry (ssHDX-MS), and Fourier transform infrared spectroscopy (FTIR-ATR) will be used to characterize the frozen, lyophilized, and spray dried mRNA vaccine formulations. Analytical data related to mRNA degradation on storage in the solid state (~3-6 months) will be evaluated, with the goal of identifying stability-indicating methods that can be used to accelerate formulation and process development. Emergence of new variants of SARS-COV-2 may require new mRNA vaccines. The knowledge gained from characterizing different formulations as part of this project will help prepare and respond to the future coronavirus waves by enabling the rapid development and manufacture of mRNA vaccines that do not require ultracold shipping and storage.
- **Investigators:** Eric Munson (PI, Purdue IPPH), Alina Alexeenko (Co-PI, Purdue ChemE/AAE), Elizabeth Topp (Co-PI, Purdue IPPH), Tony Zhou (Co-PI, Purdue IPPH)

Lyophilization and Aseptic Drying Technology Roadmap for Biotechnology and Pharmaceutical Manufacturing

- Funded by the U.S. Department of Commerce's National Institute of Standards and Technology (NIST)
- $296,000 over 18 months
- **Goal:** The funding will expand the existing Advanced Lyophilization Technology Hub, or LyoHUB, consortium's technology roadmap, first published in 2017, to include novel freeze-drying technologies and applications for emerging classes of stable drug products (therapeutics) that will allow the pharmaceutical industry to deploy effective medicines and vaccines rapidly.
- **Investigators:** Alina Alexeenko (Co-PI, Purdue ChemE/AAE), Robin Bogner (University of Connecticut), Eric Munson (Purdue/IPPH), Steve Shade (Purdue/EEE), Raj Suryanarayanan (University of Minnesota), Serguei Tchessalov (Pfizer), Elizabeth Topp (Co-PI, Purdue IPPH), Tony Zhou (Co-PI, Purdue IPPH)

LYOHUB DEMONSTRATION FACILITY

In 2022, two Basler ACE 2 Pro high-resolution cameras were acquired to capture high speed and time-lapse videos during lyophilization. Cameras are mounted to the doors of the REVO and Lyostar 3 lyophilizers to provide a direct view of the product during lyophilization process. The devices support both software or hardware triggering, offering a high degree of acquisition flexibility.

Basler ACE 2 Pro high-resolution camera

Camera setup to record time-lapses in REVO to discover any anomalies like observe collapse dynamics, nucleation performance, and shrinkage behavior. The arm reaching from REVO to the camera is rigid enough to hold the camera in place, but able to be adjusted easily to find the ideal location for recording.

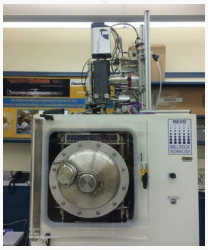
Snapshot from a time-lapse as the cycle runs through freezing and primary and secondary drying. Nineteen vials are held within a hexagonal ring that is taped down to the shelf. The center vial in the pack has a temperature probe in the cake.

Development Freeze Dryer/Lyophilizer MICROFD

Computrac® Vapor Pro®

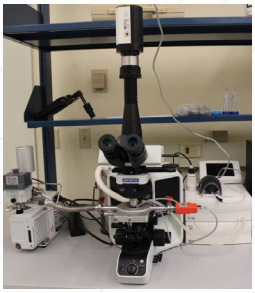
Lighthouse FMS-1400 Headspace Pressure/Moisture Analyzer

Donation from Baxter



REVO lyophilizer with controlled nucleation and in situ mass spectrometer

LYOSTAR™ 3 Freeze Dryer with controlled nucleation and mass flow meter



McCrone Freeze Drying Microscope

TRAINING

June 2021:

Python for Lyophilization Tutorial

- **Drew Strongrich** | Research Scientist, LyoHUB
  https://youtu.be/4TKmgoz6VsM
  LyoPRONTO

July 2021:

LyoPRONTO Tutorial for LyoHUB

- **Petr Kazarin** | Purdue Aeronautics & Astronautics Post-Doc, LyoHUB
  https://youtu.be/MIvSRNMIDuw

2021 PURDUE PROFESSIONAL MASTERS CHEMICAL ENGINEERING PROGRAM

Summer Capstone Projects with LyoHUB Members

Allergan Pfizer

Crystallization during freeze-drying: Scientific design of formulations and manufacturing processes with the aid of solid-liquid state

abbvie

Evaluating and Analyzing Pharmaceutical Products Mixing with Increased Viscosity by Tank-Impeller Manufacturing Process for Products Homogeneity

FRESENIUS KABI

Optimization of Controlled Nucleation & Lyophilization Cycles for Multiple Antibiotics

Bristol Myers Squibb™

Development and Optimization of a Lyophilization Cycle for Co-formulated Biologics

LyoLaunchPad PROJECTS

- Lyophilization Event Detection with Machine Learning (Falkonry)
- Usage Report for Headspace Gas Analysis Detection System (Gasporox)
- Nanox Release Freeze Dry Microscopy (Nanox Release)
- Characterization of nitrogen pressure control and nitrogen distribution within a laboratory scale lyophilizer under typical operating conditions (AbbVIe)
- Continuation of Capstone project: Optimization of Controlled Nucleation & Lyophilization Cycles for Multiple Antibiotics (Fresenius Kabi)
- Optimized Lyophilization of Gelatin Solutions (Cook Biotech)
- Lyophilization Breakage Reduction Collaboration (Corning)
- Freeze drying of a cellulose solution made of methyl cellulose and water to make it fit for SEM microscopy (Tian Li lab, Mechanical Engineering)
- Stimulated Growth of Biofilms in Microgravity to Fuel a Microbial Fuel Cell (Quest Institute)

INDUSTRY-SPONSORED PROJECTS

- Lyophilization Stability Study in SiO2 Hybrid Vials Residual (SiO2)
- Protein Surrogate Models (AbbVie)
- Lyophilization of Proprietary Formulation (Clinigen)
- IVF Formulation Review and Testing (Cook Medical Technologies)
- Quantifying free HCl in the vapor phase during lyophilization using a mass spectrometer (Merck)
- Experimental Measurement and Computational Modeling of the Rapid Depressurization Controlled Ice Nucleation Method in Pharmaceutical Freeze-Drying (Genentech)

CHARACTERIZATION OF NITROGEN FLOW IN LAB-SCALE LYOPHILIZERS AND ITS IMPACT ON PRIMARY DRYING

Investigators: *Drew Strongrich (Purdue, LyoHUB), Petr Kazarin (Purdue AAE)*

A series of experiments were carried out in partnership with AbbVie to investigate the role of the inert ballast gas on process and condenser chamber pressures during lyophilization. The study began by comparing chamber pressure throughout the cycle both with and without nitrogen ballast. In the latter case, the nitrogen ballast control valve was disconnected from the system, removing the lyophilizer's ability to regulate pressure. A second capacitance manometer was installed on the condenser chamber for both experiments to monitor the pressure drop across the duct. A comparison of the experiments is shown in **Figure 1**.

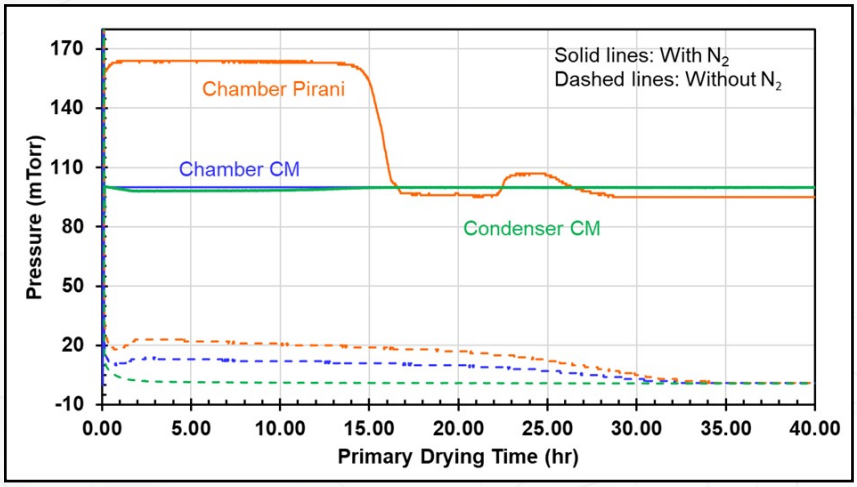


Figure 1: *Comparison of process and condenser chamber pressures with and without inert nitrogen ballast.*

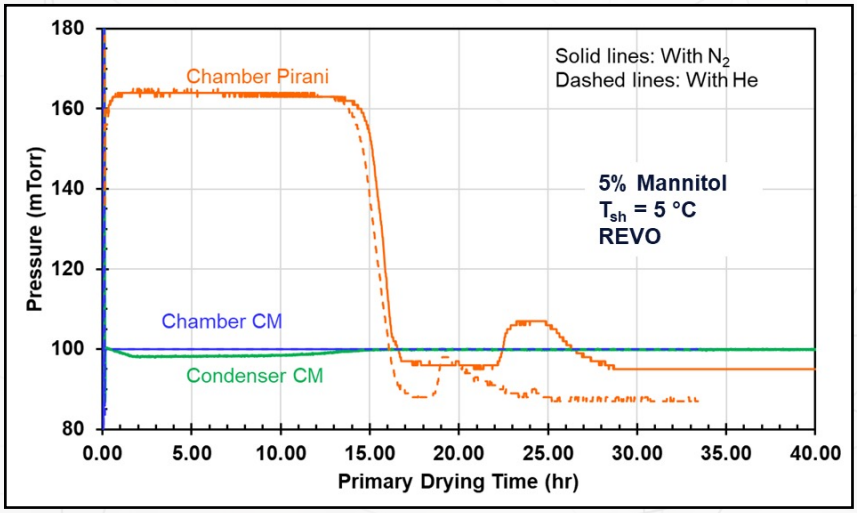


Figure 2: *Comparison of drying performance using nitrogen and helium ballast.*

The absence of nitrogen led to large (~10x) deviations from the pressure setpoint and increased the primary drying time by a factor of two, confirming the accepted view that the inert ballast is critical for both maintaining the chamber pressure and regulating heat flow into the vial. The reduction in base pressure also led to an increase in the pressure difference between the process and condenser chambers. This behavior is attributed to the reduction in Reynolds number, a nondimensional parameter representing the ratio of inertial and viscous forces acting on a fluid element, at lower base pressures and flow rates. The experimental study also explored the pumping dynamics of nitrogen and water vapor. The reduction in pressure drop across the duct following primary drying suggests the ballast flow rate needed to sustain the target chamber pressure is lower than water vapor. In this case, the flow of nitrogen is primarily dictated by the vacuum pump and foreline characteristics.

Finally, the influence of inert ballast on drying performance was investigated. A comparison between nitrogen and helium is shown in **Figure 2**. Helium was chosen as a contrast species due to its high thermal conductivity and low molecular mass (large mean free path) relative to nitrogen. The data suggest that the primary drying and Pirani convergence rates are relatively insensitive to the gas composition. Instead, the role of the ballast appears to be the regulation of back pressure, and ultimately, the partial pressure of water vapor in the vicinity of the vial pack.

VIAL HEAT TRANSFER MEASUREMENTS DURING PRIMARY AND SECONDARY DRYING

Investigators: *Kyu Yoon (Purdue/ChemE), Vivek Narsimhan (Purdue/ChemE)*

Vial heat transfer coefficients have been measured during primary drying by the gravimetric method, which estimates the energy transfer between the shelf and vial by calculating the mass change of the frozen solution during sublimation. Historically, most freeze drying models have used this value for secondary drying as well, due to the fact that it is difficult to perform gravimetric tests during secondary drying and it was believed that there was no significant change in heat transfer between the shelf and product (i.e., radiation and conduction through glass and gas). However, a recent study found that the vial heat transfer coefficient shows different values for primary drying and secondary drying. To clarify this gap in the vial heat transfer coefficient, LyoHUB has performed experiments to measure the heat transfer coefficients during primary drying and secondary drying.

Figure 1 shows the schematic of the laboratory scale freeze dryer (MicroFD, Millrock Technology, Kingston, NY) that is used in this project. Experiments involved lyophilizing various sugars (e.g., sucrose and mannitol) under different operating conditions in primary and secondary drying stages. The vial heat transfer coefficient was determined by heat flux analysis method. We first define the overall heat transfer coefficient of the sensor K_{tot} as the ratio of the measured heat flux and the temperature difference (i.e., $K_{tot} = q/(T_{sh} - T_V)$). This heat transfer coefficient K_{tot} contains contributions from the vial and the air since the sensor is partially occupied by a hexagonal array of vials. After substituting the contribution of heat flux between sensor and air from K_{tot}, the vial heat transfer coefficient K_V is estimated.

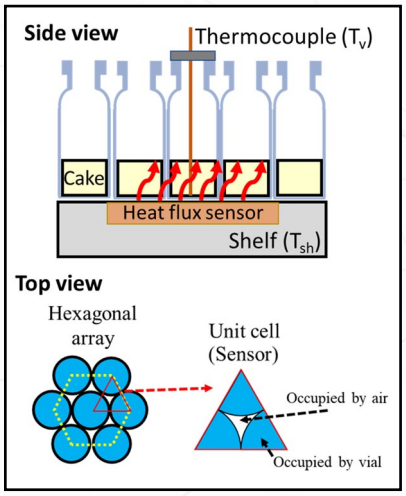


Figure 1: *Schematics of perimental setup (side view and top view).*

Figure 2 shows that both glass and SiO2 vials exhibit unique heat transfer coefficients for primary and secondary drying for a given chamber pressure. K_V measured in primary drying is comparable with the reported value, which is measured by gravimetric method, while K_V for secondary drying is about 40-60% of heat transfer coefficient in primary drying stage. This discovery might be explained by the presence of water vapor during primary drying, which modifies the conductivity of gas between the vial bottom and shelf, as well as the flow rate in the chamber due to sublimation. Following experimental measurements (**Figure 3**) also showed distinct heat transfer characteristics in primary and secondary drying processes for 6R vials at varying shelf temperatures. We also note that if one performs a heat transfer coefficient measurement on empty vials in a dry chamber, one obtains the same value as in secondary drying.

Further experimental tests are underway for an approximation of heat transfer parameters K_C, K_P, and K_D in both primary and secondary drying.

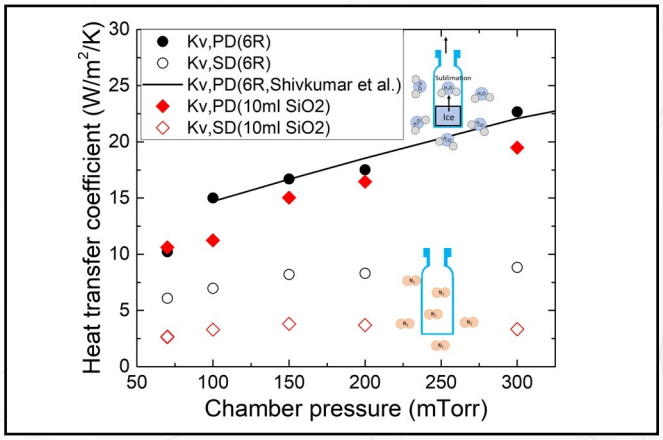


Figure 2: *Vial heat transfer of 6R and 10ml SiO2 vial during primary and secondary drying stages at the different chamber pressure (70 mTorr < P_{ch} < 300 mTorr).*

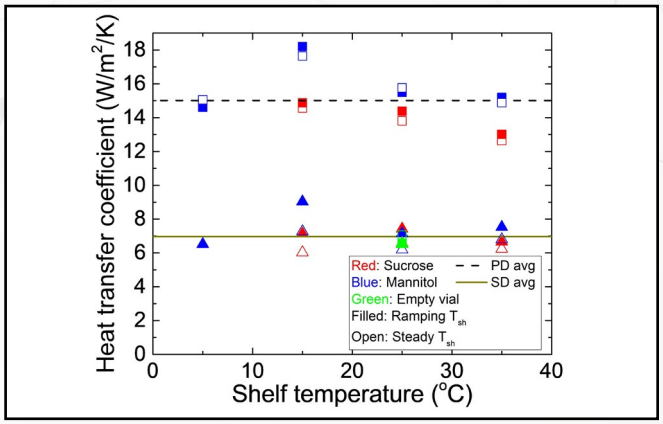


Figure 3: *Vial heat transfer coefficient of 6R vial measured during primary and secondary drying stages for sucrose and mannitol at the different final shelf temperatures (5oC ≤ T_{sh} ≤ 35oC).*

MACHINE LEARNING FOR PREDICTION AND OPTIMIZATION OF LYOPHILIZER EQUIPMENT PERFORMANCE

Investigators: *Petr Kazarin (Purdue AAE), Jack Van Wingen (Purdue ChemE)*

The machine learning (ML) algorithms can be applied to the lyophilization process and be used to predict and quantify different phenomena in the lyophilizer during all stages of freeze-drying: freezing, primary drying, and secondary drying. The information can be further applied for cycle optimization and reduction of operational costs. Notably, the machine learning algorithms' potential "on-the-fly" usage for equipment failure detection is demonstrated. An example of big data analysis is shown based on the lyophilization cycle data recorded during the several-year period.

The Falkonry machine learning system is used to perform the data analysis, and its effectiveness is demonstrated on the lab-scale freeze-dryers using the output data recorded during four years of operation. The ML algorithms allowed to deeply analyze the data and extract various metrics used to estimate the performance of the equipment. Specifically, the system successfully detected all the phases of lyophilization: freezing, primary drying, and secondary drying. Eventually, the duration of each phase was calculated, and the efficiency of equipment usage was inferred. In addition, the ML system was trained to determine the excipient being dried and to detect the equipment failure due to the issue with shelf temperature control. It was shown how the obtained information could be potentially used to increase the efficiency of the lyophilization cycles.

Effective ML-based models for predicting and optimizing lyophilization cycles can correctly identify specific lyophilization events and differentiate between solutions used in freeze-drying cycles. The developed models will be adapted for identifying and alerting the lyophilizer users of issues in real-time ("on-the-fly"), which paves the way to more time-and cost-effective freeze-drying cycles.

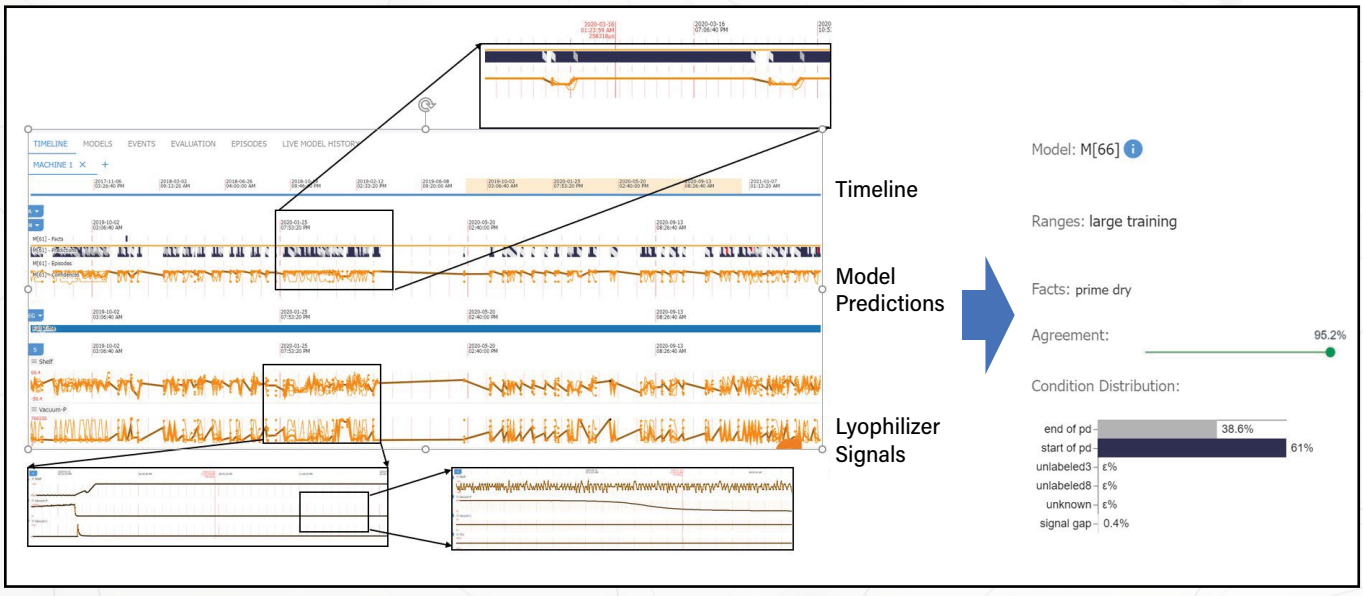


Figure 1: *Falkonry ML interface: events detection during the operation of lyophilizer.*

CLOSED-LOOP MODEL-PREDICTIVE CONTROL OF PHARMACEUTICAL LYOPHILIZATION

Investigators: *Drew Strongrich (Purdue, LyoHUB), Petr Kazarin (Purdue AAE)*

Since its inception, the lyophilization process has been carried out using an open-loop control strategy. That is, process setpoints (e.g. chamber pressure and shelf temperature) are predefined by the user and executed in sequence by the lyophilizer. Although simple and robust, this technique commonly leads to unnecessarily long cycle times. Improvements can be made through iterative optimization. However, this process is time consuming and requires a certain level of expertise. To overcome this limitation, a closed-loop control strategy has been developed in partnership with Millrock Technology Inc. as part of the NSF PFI program.

The nonlinear model predictive control (MPC) strategy [1] built in the GEKKO environment [2] was chosen to control the product temperature and minimize the primary drying time. The illustration of the MPC control algorithm is demonstrated in **Figure 1**. In our case, the algorithm is based on the predictions of the model (LyoPRONTO primary drying calculator [3]), which predicts the product temperature over the specific prediction horizon at every time step of the process. Based on these predictions, the algorithm changes the manipulated variable (shelf temperature) to make the product temperature follow the predicted trajectory as closely as possible.

A mathematical model mimicking Millrock REVO lyophilizer was used for demonstration purposes instead of the actual equipment. The primary drying control simulation of 5ml 5% mannitol solution filled in 6R SCHOTT vials is shown. The algorithm adjusts the shelf temperature profile to keep product temperature close to the critical value during the whole primary drying stage (**Figure 2**).

The model-predictive control algorithm has been partially implemented as a control strategy in the Millrock REVO lyophilizer. The Python OpenOPC module is used to bypass the control software and interface directly with the Programmable Logic Controller (PLC). The control system uses a multithreaded approach, allowing all read and write operations to be queued and executed asynchronously. Product temperature data is read directly from the lyophilizer and passed to the model-predictive controller. Here, the most optimal chamber pressure and shelf temperature are computed (while obeying the constraints imposed by equipment capabilities) and returned to the PLC as setpoints. The controller computes and writes setpoint data at 60-second intervals with the goal of maintaining product temperature at its critical temperature over the duration of primary drying. However, additional robustness is still needed to accommodate expected (e.g. loss of thermocouple contact with ice) and unexpected events. In addition to shelf temperature-based control additional control strategies will be considered such as chamber pressure control and mixed control.

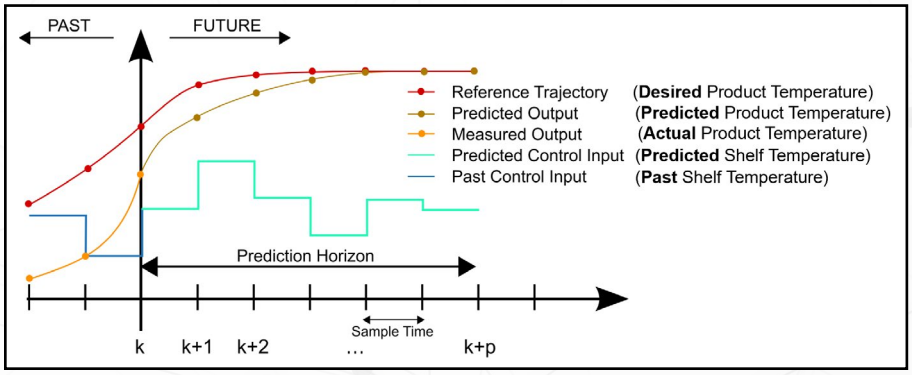


Figure 1: *MPC algorithm illustration [Ref: https://electronics360.globalspec.com/article/17088/advantages-and-applications-of-model-predictive-control].*

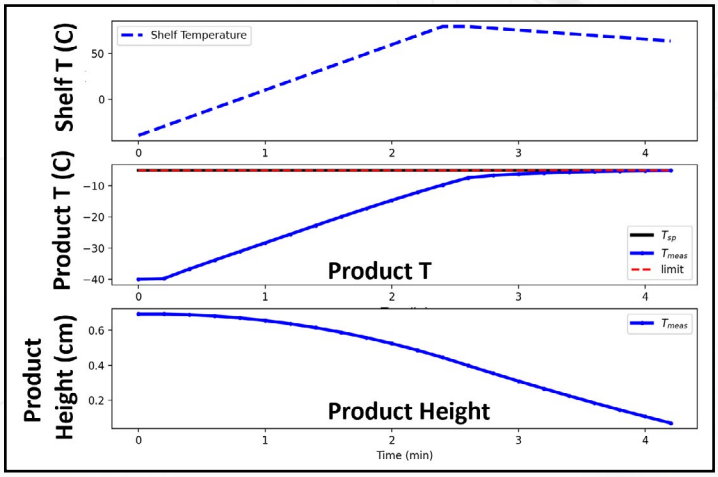


Figure 2: *Demonstration of the MPC controller work using 6R Schott Vials with 5 ml fill 5% mannitol solution.*

References:

[1] Camacho, E. F., & Alba, C. B. (2013). Model predictive control. Springer science & business media.

[2] Beal, L. D., Hill, D. C., Martin, R. A., & Hedengren, J. D. (2018). Gekko optimization suite. Processes, 6(8), 106.

[3] Shivkumar, G., Kazarin, P. S., Strongrich, A. D., & Alexeenko, A. A. (2019). LyoPRONTO: an open-source Lyophilization process optimization tool. AAPS PharmSciTech, 20(8), 1-17.

RF/MICROWAVE-ASSISTED LYOPHILIZATION

Purdue Investigators: *Alina Alexeenko (AAE/ChemE), Vivek Narsinham (ChemE), Dimitrios Peroulis (ECE), Elizabeth Topp (IPPH), Qi (Tony) Zhou (IPPH), Anthony Cofer (AAE), Ahmad Darwish (ECE), Petr Kazarin (AAE), Tarun Mutukuri (IPPH), Michael Sinanis (ECE), Drew Strongrich (AAE), Isaac Wheeler (ChemE), Kyu Yoon (ChemE).*

Owing to the increased demand for lyophilized injectable products over the past few decades, especially with the current COVID-19 pandemic, freeze-drying, or lyophilization process, has been recently given great attention. Freeze-drying is extensively employed in the pharmaceutical industries as it allows the processing of thermolabile products in sterile conditions, notwithstanding it is one of the longest industrial processes with a significant energy waste with an efficiency of less than 5%. To that end, RF/microwave-based lyophilization is currently being actively pursued as it shows a significant acceleration of such processes. Currently, a product adaptive RF heating technique to reduce the duration of the primary drying process has been developed in LyoHUB.

The statistical microwave setup integrated with the lab-scale lyophilizer is depicted in **Figure 1**. The experimental setup comprises five main components, namely, an auxiliary or reverberation chamber (RC), a network analyzer, a power amplifier, an antenna inside the RC, and two stepper motors connected to stirrers, which are placed inside the RC. The RC is a metallic box containing a transmitting antenna, which acts as a source of the RF power inside the chamber. The antenna is connected, through coaxial cables, to a power amplifier, which is used to amplify a high-frequency (18GHz in this application) electromagnetic signal obtained from the signal generator. When the antenna radiates electromagnetic waves inside the RC, waves experience multiple reflections, creating

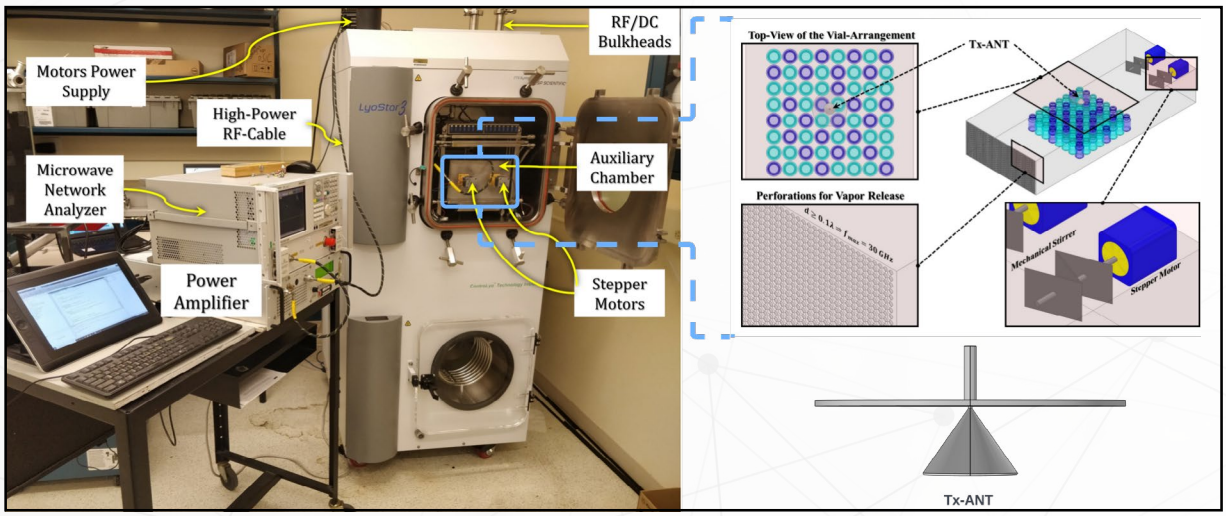


Figure 1: *Modified Millrock LyoStar 3 Laboratory Freeze Dryer for RF-assisted lyophilization.*

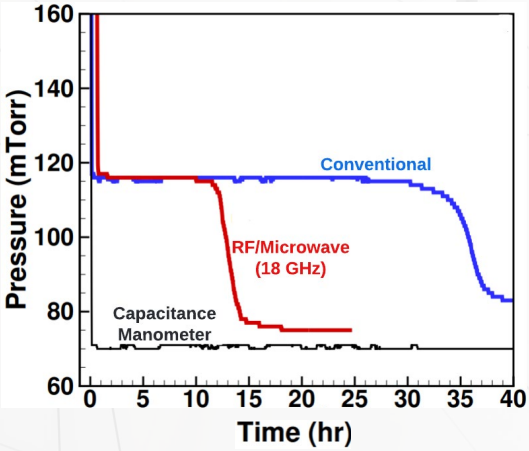


Figure 2: *Capacitance Manometer (CM) and Pirani gauge pressure measurements versus primary drying time for conventional and microwave-assisted lyophilization cycles of sucrose (5% v/v, 6R SCHOTT Vial, 3 ml fill volume, 64 vials).*

many resonances inside the chamber. The number of resonances, and hence, field uniformity increases with frequency. If the stirrers inside the chamber are static, the field distribution would retain a fixed distribution, creating hot and cold spots inside the RC and yielding field nonuniformity. We continuously rotate the two stirrers inside the RC to change the field distribution at different time instants to overcome this problem.

The pressure is continuously monitored inside the lyophilizer to define the conclusion of the primary drying cycle and the product is also inspected visually at the end of the secondary cycle. The Capacitance Manometer (CM) and Pirani gauge pressure measurements versus primary drying time for different lyophilization cycles are shown in **Figure 2**. The primary drying durations are 38 hours and 16 hours, respectively.

Reference: "Statistical electromagnetics for industrial pharmaceutical lyophilization." PNAS Nexus (2022). *Ahmed Abdelraheem, Rishabh Tukra, Petr Kazarin, Michael D. Sinanis, Elizabeth M. Topp, Alina Alexeenko, Dimitrios Peroulis.*

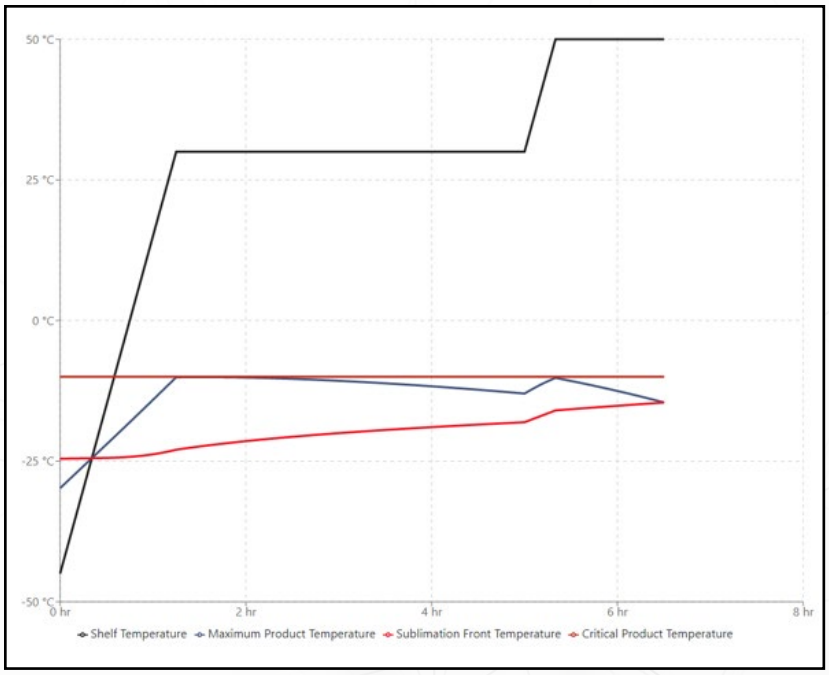
https://academic.oup.com/pnasnexus/advance-article/doi/10.1093/pnasnexus/pgac052/6586350?login=true

OPTIMIZING FREEZE DRYING USING CONTROLLED ICE NUCLEATION

Investigator: *This LyoLaunchPad was conducted by Robert Kirisits, a student in the Purdue Chemical Engineering Professional Masters in Pharmaceutical Manufacturing graduate program and a tech transfer scientist intern with Fresenius Kabi.*

Objective/Description

As part of a LyoLauchPad project, LyoHUB facilities were used to optimize an antibiotic formulation produced by Fresenius Kabi. Several strategies were used to accomplish this task including Controlled Nucleation and primary drying modeling. Controlled Ice Nucleation improves uniformity from vial-to-vial as well as creates larger ice crystals for lower mass transfer resistance. Another strategy involved modeling with LyoPRONTO to characterize the formulations heat/mass transfer parameters.



Using LyoPronto to approximate primary drying temperature profile with set shelf temperature 1 of 30°C and set shelf temperature 2 of 50°C. Duration of each set shelf temperature is changed so that bottom temperature is kept as close to -10°C.

With knowledge of these parameters, the product temperature can be predicted in accordance with the shelf temperature and chamber pressure combination inputted. Also, LyoPRONTO's optimizer assists in selecting the optimal shelf temperature and chamber pressure for primary drying.

Methods

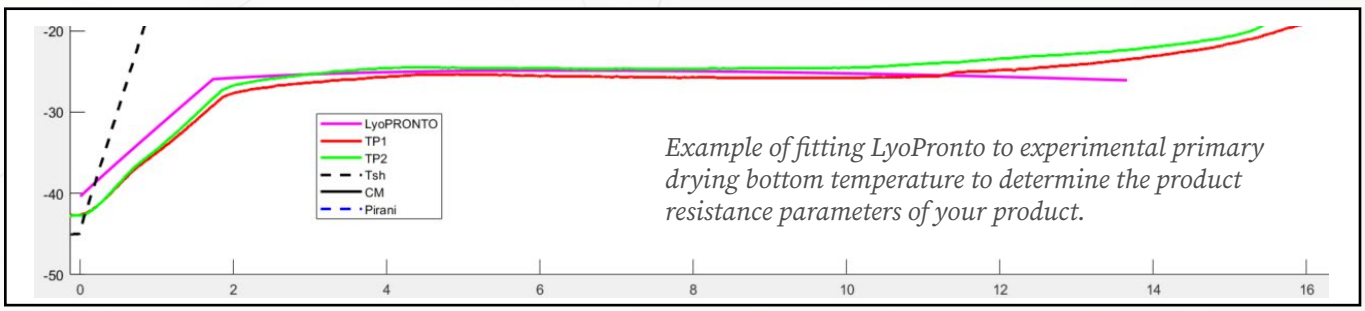
The formulation collapse temperature was determined using freeze drying microscopy. This established a boundary limit that product temperature can reach during primary drying. Using the MicroFD for quick small-scale freeze drying, the vial heat transfer coefficient for the vial used by the antibiotic was found using Mannitol which has a known product resistance. After vial heat transfer coefficient is determined for the vial, LyoPRONTO's product resistance fitting tool can be used to fit the model's temperature profile to an experimental temperature profile by varying product resistance parameters.

Results

As a result of controlled ice nucleation, the primary drying time variation among vials decreased by 5% compared to without controlled ice nucleation. Also, the primary drying time was reduced by over 50% of Fresenius Kabi's current primary drying cycle time. As compared to current moisture content of the antibiotic formulation, the moisture content of optimized vials resulted in a marked decrease by as much as a 75%.

Future Work/Current Work

Work continues to establish failure conditions for the antibiotic formulation during freeze drying as well as scale up of the freeze-drying cycle using LyoHUB's pilot scale freeze driers.



Example of fitting LyoPronto to experimental primary drying bottom temperature to determine the product resistance parameters of your product.

LONG TERM MOISTURE CONTENT ANALYSIS FOR LYOPHILIZED PRODUCTS IN SIO2 HYBRID POLYMER COATED VIALS

Investigators: *Drew Strongrich (Purdue, LyoHUB), Zack Mora (Purdue ChemE), Hallie Harrison (Purdue ChemE)*

Currently, borosilicate glass vials are the most common packaging option for lyophilized drugs. Polymer vials are not a viable option to store lyophilized drugs because oxygen and water vapor can easily permeate through the polymer and reduce the stability of the lyophilized product. SiO2 Materials Science has manufactured a hybrid polymer coated vial that combines benefits of polymer and glass vials. Ideally, these hybrid vials should have a greater breakage resistance, lower adsorption, tighter dimensional tolerances, and increased chemical resistance while still maintaining a low moisture content in a lyophilized cake during long term product storage. The SiO2 project was developed to analyze how effective these new vials are at keeping water vapor and oxygen out of the lyophilized product. The three vials tested in this experiment were Type I borosilicate glass serum vials, 10cc cyclic olefin polymer (COP) vials coated using plasma enhanced chemical vapor deposition (PECVD), and 10cc FB-ALD vials coated by atomic layer deposition (ALD). After lyophilization, vials were placed in a high humidity (75% RH) desiccator at both room temperature and at 40°C using an oven. Two different fill volumes, 3mL (**Figure 1**) and 5mL (**Figure 2**), were also used for each vial type. Data was collected over varying storage lengths to monitor how each vial behaved in a high moisture environment. Data points were collected on day 0, 7, 14, 30, and 60 days after lyophilization.

For each data collection day, three vials of each different type and fill volume were randomly chosen for each temperature. 18 room temperature vials and 18 vials at 40°C were tested for each storage period. For each vial, the stopper was pierced with a needle and the cake was broken up into a powder to help increase the flow rate of water vapor leaving the cake. The vial was then loaded into the Computrac® Vapor Pro® XL machine for it to be analyzed. The weight percent and mass in µg of water was recorded for each vial.

After the 60th day data set was collected, the trends of the residual moisture were plotted based on the fill volume. It should be noted that between the 30 and 60 day recordings, the 3mL fill PECVD vials in the oven all collapsed. Based on the trends in the data, all samples in oven exhibit consistently higher moisture than their room temperature counterparts. FB-ALD samples consistently indicate the lowest moisture content, regardless of fill or storage temp. PECVD appeared to have the highest moisture content when in the oven, while borosilicate had slightly higher moisture content at the room temperature setting.

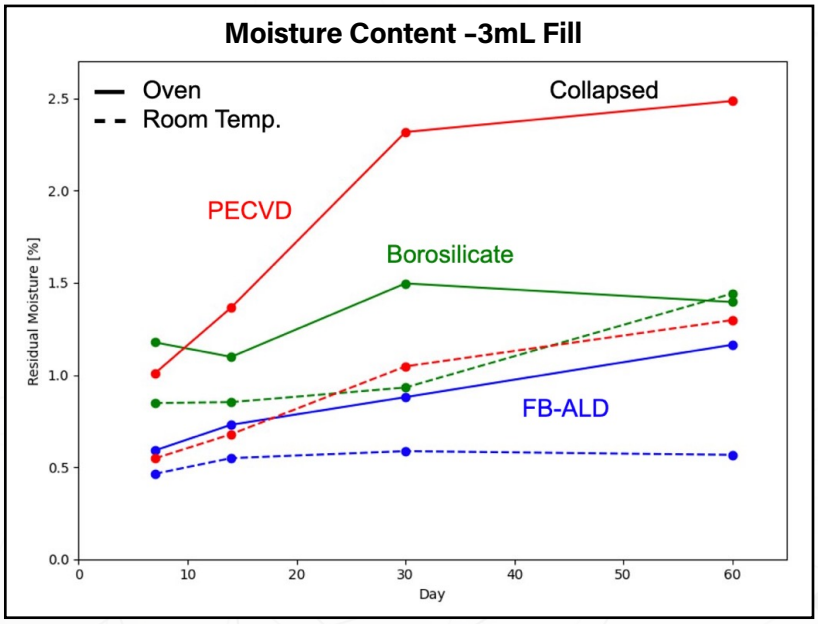


Figure 1: *Plot of residual moisture vs. storage period for 3mL fill PECVD, Borosilicate, and FB-ALD vials.*

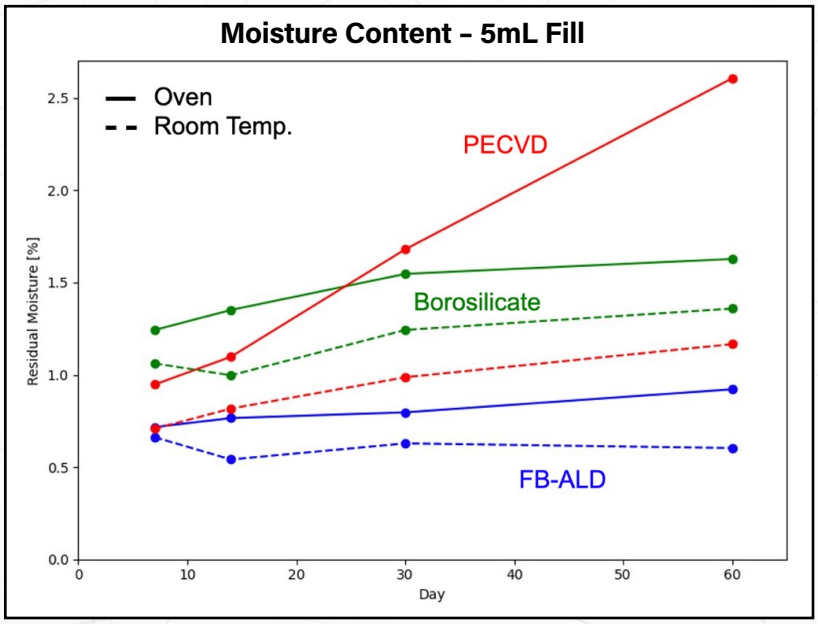


Figure 2: *Plot of residual moisture vs. storage period for 5mL fill PECVD, Borosilicate, and FB-ALD vials.*

VIAL MECHANICAL STATE CHARACTERIZATION DURING LYOPHILIZATION USING WIRELESS STRAIN GAUGES

Investigators: *Matt Hall (Corning), Christy Chapman (Corning), Lirong Pei (Corning), Drew Strongrich (Purdue, LyoHUB), Patrick Williams (Purdue ChemE), Jack Van Wingen (Purdue ChemE)*

The addition of certain crystallizable bulking agents to lyophilized formulations can improve robustness and reduce drying time by significantly increasing the critical temperature. However, care must be taken when using these materials to ensure that complete crystallization occurs since a phase change during storage can lead to instabilities. Additionally, this crystallization process can place a significant amount of stress on the vial. In many cases, this stress can exceed the critical strength and lead to breakage. Vial breakage during lyophilization can be catastrophic to the process due to the potential for batch contamination and loss of process pressure control. Additionally, the cleanup costs following a breakage event can be significant, requiring thorough manual cleaning by a skilled technician. Therefore, the ability to monitor the crystallization characteristics of new formulations is critical to effect process development.

A series of custom wireless strain sensors were developed in partnership with Corning to investigate the influence of fill volume, shelf temperature ramp rate, and excipient (mannitol) concentration on vial stress throughout the lyophilization process. Strain-sensitive elements were bonded directly to the outer surface of 20mL vials, roughly 1 cm from the heel. Gages were arranged in a "full bridge" configuration to minimize the influence of parasitic thermal stresses. Battery-powered electronics modules, having an identical footprint to the 20mL vials, sampled the strain response of the gages and broadcast the data to an external host in real time with a measurement period of 30 seconds. An image of the assembled sensor is shown in **Figure 1**. The sensors were placed in a full rack of 148 vials. Target vials were isolated from the electronics modules by an inert sample to reduce parasitic heating. A comparison of the strain response between 15% w/v mannitol formulations and empty borosilicate and Corning Valor® vials is shown in **Figure 2**. Thermocouples were placed inside the target vials to synchronize strain and temperature data. During nucleation, a clear step response in strain signal results from the simultaneous influence of ice crystal growth and increase in product temperature. Following solidification, the mannitol formulations produced a sharp decrease in strain magnitude as the mannitol crystallized. A second crystallization event occurred during warming as the cycle entered primary drying. Here, the ultimate stress in the borosilicate vial was exceeded which ultimately led to failure. The strain sensors developed during this study demonstrated several applications as a process analytical technology. Specifically, the devices can be directly applied to process development to both verify excipient crystallization and ensure vial ultimate stress is not exceeded.

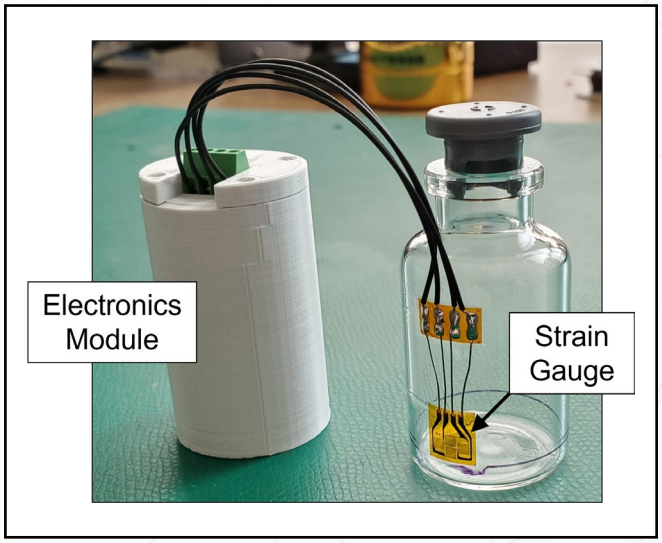


Figure 1: *Image of assembled strain sensor. Strain-sensitive elements were bonded directly to the glass vial and sampled by the electronics module.*

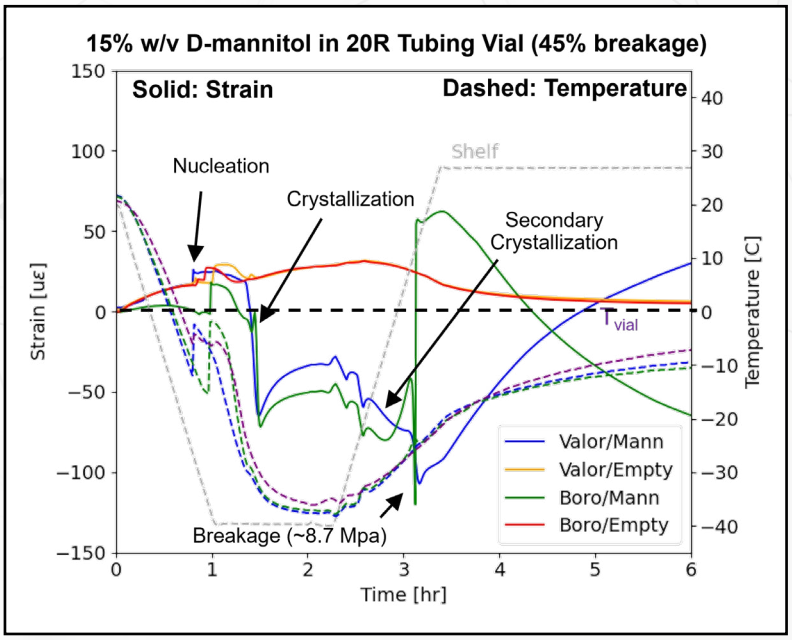


Figure 2: *Strain data from 15%w/v mannitol formulation. Nucleation, crystallization, and breakage events are clearly visible.*

THE LYOPHILIZATION OF WATERMELON

Purdue Students working on project: Aidan Jennewein (ChemE), Christine Mayo (CHemE), Michael Fidler (ABE), Vedha Srigiri (Purdue ECE)

Purdue Coordinating Professors: Alina Alexeenko (ChemE/AAE). Nathan Schultheiss (Director of Unconventional Energy/ChemE)

Can freeze-drying, or lyophilization, be a profitable solution for reducing watermelon wasted in fields? This was the question presented to a group of Purdue undergraduate students who are part of the LyoHUB VIP team. The Vertically Integrated Projects (VIP) Program at Purdue University provides an opportunity for undergraduate students to earn academic credit while engaging in authentic and extended research and design projects related to active research areas of Purdue faculty members and national, international, and industry-sponsored design challenges. Students can participate on interdisciplinary and vertically-integrated teams (first-year through seniors) with faculty and graduate student mentors for multiple semesters to address these real-world research and design challenges.

According to the USDA, over 100,000 acres of watermelons were grown in the US, producing approximately 38 million pounds in 2021. Over half of watermelons grown in the state of Indiana rot in their fields every year due, in large part, to the high water content and fragility of the fruit. Watermelon contains numerous health benefiting nutrients such as lycopene and citrulline, but has multiple storage drawbacks such as a short shelf life and a high freezing point which makes cold storage difficult. Surprisingly, with an ever-growing demand for freeze-dried products, the amount of freeze-dried watermelon on the market is quite limited. Under the guidance of LyoHUB, the team is focused on determining the optimal lyophilization conditions for the individual watermelon parts, e.g., rind, flesh and juice, for the purposes of determining if desirable products can be produced for consumption while still containing the healthiest components of the fruit.

First step was performing freeze-drying microscopy of watermelon juice to determine its freeze-drying behavior. In **Figure 1**, the gray gas is the sublimation front, the illuminated bubbles represent the collapse of the juice, and the purple tones beyond are the ice crystals of the frozen juice.

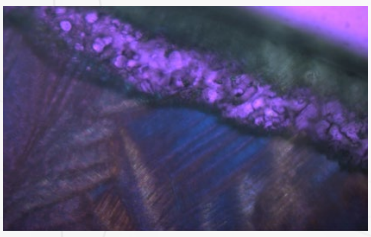


Figure 1: *Freeze drying microscopy of freshly squeezed watermelon juice.*

After observing the behavior of the watermelon juice, it was lyophilized in vials within the MicroFD so that a full pilot cycle could be analyzed with the assistance of run time data and attached thermocouples.

The next run performed with watermelon was done using watermelon flesh of different dimensions, all approximately one half-inch thick. The flesh was placed directly inside of the LyoStar 3 on top of aluminum foil for easier post-lab cleanup (**Figure 2**).

Figure 2: *Watermelon flesh, cut into near even squares. Batch Moisture 1.5% (+/- .2).*

RF-Assisted Lyophilization

Due to the higher water content as compared to other fruits, lyophilizing watermelon pulp takes considerably longer amounts of time. Within a commercial setting this is not optimal, so the team is exploring the drying process using RF-Assisted lyophilization, a technique currently being investigated within LyoHUB. Using microwaves, the heat from the waves are evenly distributed throughout the watermelon being dried in the freeze-dryer. Using RF-Assisted lyophilization, one experiment showed a reduction in drying time from over two days (utilizing traditional lyophilization) to only 20 hours to reach the same level of moisture content (**Figure 3**). With a feasible process for lyophilizing watermelon flesh in place, the team is now focusing on a full economic assessment to produce this product at scale. Additionally, continued research is ongoing to improve the product creation through shorter dry times.

In addition to the traditional freeze drying experiments, the VIP team is studying edibility, marketability, chemical composition, and the economics for each of the products created.

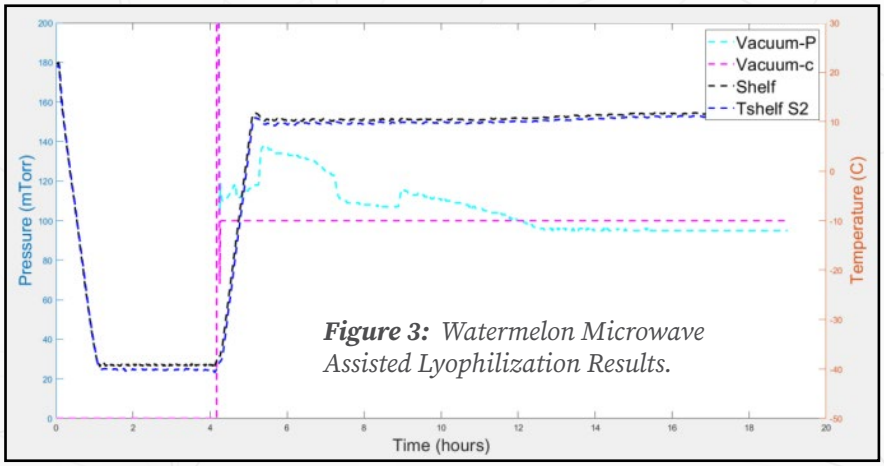


Figure 3: *Watermelon Microwave Assisted Lyophilization Results.*

STIMULATED GROWTH OF BIOFILMS IN MICROGRAVITY TO FUEL A MICROBIAL FUEL CELL

Students from Valley High School in California approached LyoHUB as part of a project through the Quest Institute, an educational non-profit organization that develops and markets STEM educational programs and support materials for K-12 schools globally. The mission of the Quest Institute is to introduce, intrigue, inspire and ultimately engage students to understand what it means to pursue STEM-based careers.
https://thequestinstitute.com

Description

This experiment compares the performance of a biofilm-based microbial fuel cell on Earth with a biofilm-based microbial fuel cell in microgravity on the International Space Station (ISS), using Shewanella Oneidensis, an electrochemically active bacteria. Biofilms oftentimes have different properties than the bacteria they are made up of in terms of their ability to handle stressful environments and other characteristics. An electroactive biofilm could be more efficient than a bacterial colony in the making of a microbial fuel cell. They are measuring the voltage difference across both MFCs (microbial fuel cells) amplified by an operational amplifier to determine how the MFCs compare to each other over time using an analog-to-digital converter which then feeds the voltage data to a microcontroller.

Procedure

A biofilm of S. Oneidensis was grown on graphite felt, sent to LyoHUB, and lyophilized. A chamber was then prepared with lyophilized S. Oneidensis graphite felt, Nafion proton exchange membrane, potassium ferricyanide solution, and copper wires. The chamber was sealed with epoxy, and bags filled with sodium lactate and tryptic soy broth. The tubing and pumps were connected to the chamber and liquid bags. Non-inverting amplification circuits were constructed using operational amplifiers.

Lyophilization

LyoHUB lyophilized two trials of graphite anodes with Shewanella Oneidensis (**Figure 1**). Within each trial, the anodes were lyophilized with a specific excipient or no excipient to test the viability of the lyophilized bacteria. Each independent variable of the excipient was run in triplicate to determine with sufficient certainty whether Shewanella Oneidensis was able to survive after lyophilization. For excipients, 0.3 g of whey powder, 0.75 g of trehalose and 0.75 g of milk powder were used with no excipients.

Figure 1: *Lyophilized product.*

Experimental Goals

The goal of the experiment is to measure the difference between the magnitude and duration of the voltage output of a microbial fuel cell on earth compared to one in microgravity. In a world with an increasing demand for new energy sources, microbial fuel cells show huge potential as a renewable energy source.

Challenges/Solutions

A primary challenge with this experiment was the inability of their initial test chamber to carry a voltage across it. In order to analyze why the test chamber failed to operate as expected, they utilized a dremel to open the test chamber and identify any issues. The primary issues entailed an insufficient quantity of C6N6FeK3 (aq) and an insufficient amount of bacteria on the graphite felt. In order to remedy these concerns, they adopted a new strategy to better fill the chamber with C6N6FeK3 (aq) and ensured the bacteria was sufficiently adhered to the graphite felt. Upon implementation, the new chamber carried an expected voltage and operated within expected parameters (**Figure 2**).

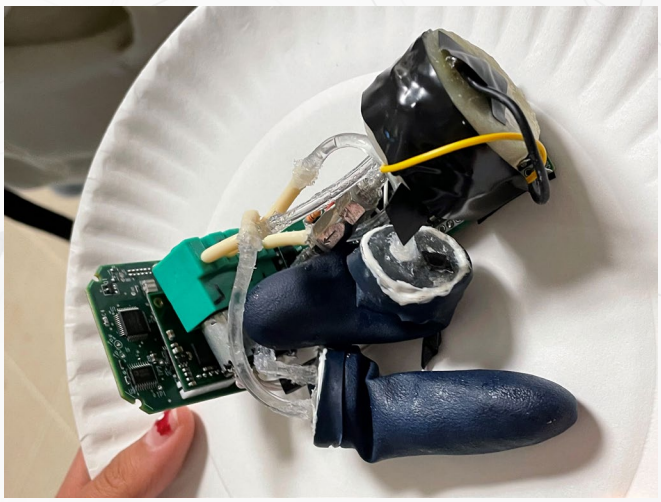


Figure 2: *Experimental set up.*

Next Steps

Their experiment's launch date is scheduled for early May, 2022. They expect to receive results by late June.

FREEZE DRY MICROSCOPY LYOLAUNCHPAD PROJECTS

Nanox Release Freeze Dry Microscopy Project

Nanox is a nano-biotechnology company engaged in the research and development of advanced discovery, testing, and commercialization of nano-pharmaceutical drugs. The company has developed and specializes in conventional liposomes, pegylated liposomes, and microspheres depot with PLGA (polylactide – polyglycolide copolymers) formulations.

Slight shrinkage/ sagging in Nanox product during lyophilization was seen. This is common for amorphous formulations.

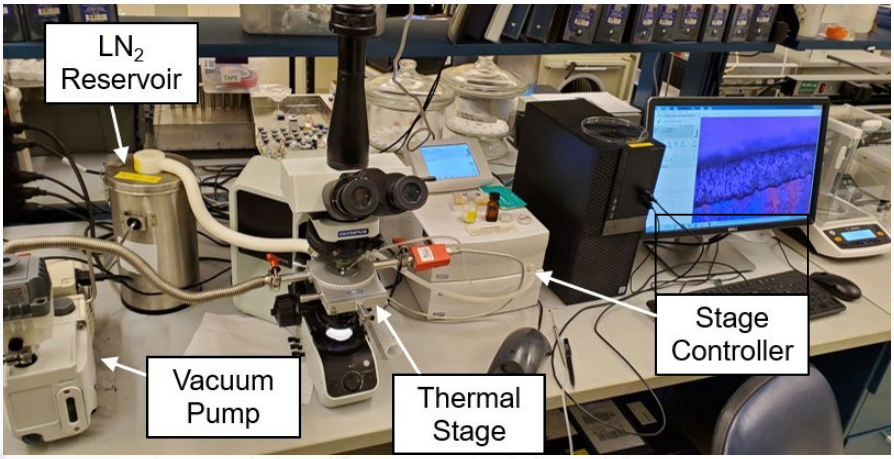
Nanox asked LyoHUB to address the transition temperatures of a liposomal aqueous injectable formulation. The lyophilization cycle they were using was effective and robust, but they felt that there was room for improvement in terms of duration which was nearly 100 hours/cycle.

LyoHUB arranged vials in a hexagonal pattern with a thermocouple at the center and lyophilized.

LyoHUB provided Nanox with heat and mass transfer parameters to be used by Nanox to maximize their cycle.

Optimized Lyophilization of Gelatin Solutions

LyoHUB member, Cook Biotech, is the arm of Cook that manufactures medical devices to provide advanced tissue-repair products for improving patient outcomes worldwide. LyoHUB was tasked with assisting Cook to optimize lyophilization cycle for one of their gelatin solutions. The project involved determining the critical temperatures of the gelatin solutions by performing freeze dry microscopy on ≤10 µL of a liquid low molecular weight gelatin, native high molecular weight gelatin, and modified high molecular weight gelatin solutions (gelatins dissolved in salt buffers). Dry samples were provided in vials, along with the appropriate reconstitution buffers) and instructions for sample preparation. FDM results of the gelatin solutions were then compared at Cook to determine if the low molecular weight or native high molecular weight solution could be used for a follow-up study.



Typical Freeze Dry Microscopy Experimental Set Up.

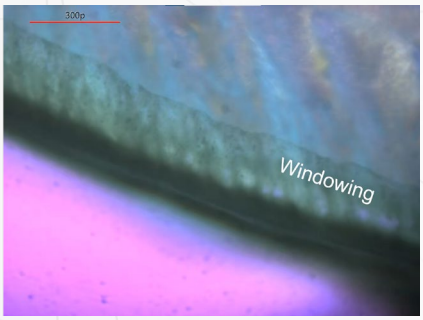


Figure 1: *Freeze-drying microscopy of liposomal formulation with a critical temperature of -27°C.*

Figure 2: *Freeze-drying microscopy of gelatin matrix. No critical temperature could be identified. However, the dried material exhibited a "wood grain" appearance at temperatures above -25°C.*

FDM analyses were conducted to evaluate the critical temperatures of various compounds. Liposomal material from Nanox at the collapse temperature of -27°C is shown in **Figure 1**. Here, collapse is indicated by the classical windowing behavior as the material transitions from a glassy to rubbery state. Gelatin matrix samples from Cook Biotech are shown in **Figure 2** and demonstrated no evidence of catastrophic failure. However, a macroscopically-visible "wood grain" effect was observed at -25°C. This patterning has no influence on the efficacy of the final product.

WEBSITE RESOURCES & TRAINING

LYO101 COURSE

Open Access Online Introduction to Lyophilization Course

Free
https://pharmahub.org/courses/lyo101

Current enrollment: Over 500

LYOPRONTO

An Open-Source Lyophilization Process Optimization Tool

Freely available (Python Source Code)
http://lyopronto.org

Website Tools
(https://pharmahub.org/groups/lyo/tools)

- YouTube videos: https://www.youtube.com/channel/UCFhNxcSLJf1Fx86Zh_gODWw
- LyoHUB Training, July 2018: Freeze drying: https://pharmahub.org/resources/773
- LyoHUB Training, July 2018: CFD: https://pharmahub.org/resources/778
- LyoCalculator: https://pharmahub.org/resources/lyocalculator
- Lyo Chamber Pressure Variation Calculator: https://pharmahub.org/resources/pressurevar
- LyoHUB Lyophilization Technology Roadmap: https://pharmahub.org/groups/lyo/lyohub_roadmapping
- Presentations, such as "Developing Transferable Freeze Drying Protocols using Accuflux® and a MicroFD®": https://pharmahub.org/groups/lyo/tools

Published Best Practices Papers

- "Recommended Best Practices for Process Monitoring in Pharmaceutical Freeze Drying": https://link.springer.com/article/10.1208/s12249-017-0733-1
- "The Best Practices for Lyophilization Validation Part I": https://link.springer.com/article/10.1208/s12249-021-02086-8
- "The Best Practices for Lyophilization Validation Part II": https://link.springer.com/article/10.1208/s12249-021-02107-6

New Users Trained on Lyophilization Equipment from March 2021–2022

- **Nirajan Adhikari** | Purdue Aeronautics and Astronautics Post-Doc
- **Ashwani Agarwal** | Purdue Polytechnic Masters Student
- **Ritvik Agrawal** | Purdue Research Engineer
- **Jeongyeon Cho** | Purdue ChemE Student
- **Anthony Cofer** | Purdue Spacecraft Laboratory Engineer
- **Ahmad Darwish** | Purdue Electrical and Computer Engineering Post-Doc
- **Michael Fidler** | Purdue Agriculture and Biological Engineering Student
- **Hallie Harrison** | Purdue ChemE Student
- **Aidan Jennewein** | Purdue First Year Engineering
- **Robert Kirisits** | Purdue ChemE Masters Student
- **Christine Mayo** | Purdue First Year Engineering Student
- **Nicholas Ritchie** | Purdue ChemE Student
- **Josiah Rockey** | Purdue ChemE Masters Student
- **Kaustabh Sarkar** | Purdue PhD student
- **Nicholas Sierzputowski** | Purdue ChemE Masters Student
- **Vehda Srigiri** | Purdue Electrical and Computer Engineering Student
- **Peter Stark** | Purdue ChemE Masters Student
- **Isaac Wheeler** | Purdue PhD student
- **Lijun Zhu** | Purdue Electrical and Computer Engineering Student

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