ANNUAL REPORT

2021

ADVANCED LYOPHILIZATION TECHNOLOGY CONSORTIUM

LYOhub.org

2 hr 2 min 4 sec | Tsh= -18 C | Tpr= -2.2 C

2 hr 2 min 5 sec | Tsh= -18 C | Tpr= -2.8 C

2 hr 19 min | Tsh= -23 C | Tpr= -8 C

2 hr 37 min | Tsh= -29 C | Tpr= -23 C

Primary drying starts at 6 hr

(2 hr and 25 min after beginning of primary drying)

8 hr 25 min | Tsh= +20 C | Tpr= -12.8 C

10 hr 27 min | Tsh= +20 C | Tpr= -11.2 C

11 hr 21 min | Tsh= +20 C | Tpr= -9.6 C

16 hr 30 min | Tsh= +20 C | Tpr= 21.1 C

Freeze-drying of pure water (vial on the right) and 5% w/v Mannitol (vial on the left) in 6R vials, 3 ml fill. *Process recipe includes: Cooling for 3.5 hours at 0.3 deg/min from 20C to -45 C; Freezing at -45 C for 2.5 hours; Primary drying at 60 mtorr, Tsh=20 C for 12 hr.*

DIRECTORS' MESSAGE

LYOHUB ANNUAL REPORT 2021

"Every new beginning comes from some other beginning's end."

Seneca the Younger, Roman philosopher and statesman, ca. 4 BC – AD 65

We're one year into the Covid-19 pandemic. As of this writing, three Covid vaccines are being administered in the United States, and authorization of two more is expected soon. Worldwide, nearly 120 million people have been fully vaccinated. This is a scientific and humanitarian triumph. The Covid vaccines have broken barriers, given us the first mRNA drug products, shortened development and regulatory timelines, and achieved global distribution at ultracold temperatures. Many of you have been directly involved in creating these vaccines, and many more belong to the companies and organizations that have supported their development and distribution. We congratulate you on your paradigm shattering accomplishments. With the rest of the world, we are deeply grateful for the skill and dedication that made these accomplishments possible.

This isn't the end of the pandemic, though, or even the beginning of the end. But thanks to your efforts, it might be the end of the beginning.

That means it's time for a new beginning. The first Covid vaccines smashed barriers, but also brought new challenges to light. These include the need to ensure global supply and access, to eliminate the need for a cold chain, and to respond to mutations in the virus with next-generation vaccines. Lyophilization can be an important part of the response to these challenges, with Sputnik V COVID19 vaccine already available in a freeze-dried form and some other vaccine manufacturers developing lyophilized formulations. To ensure global lyophilization capacity and efficiency, however, and the safety and efficacy of lyophilized vaccine products, advances in lyophilization technology will be needed.

LyoHUB is an industry-led consortium dedicated to advancing lyophilization technology. Our members span the lyophilization value chain, and include equipment manufacturers, software developers, analytical instrument companies, contract manufacturers, and members of the biopharmaceutical industry. Together, we work to advance lyophilization technology through training and education, by creating new process modeling tools, through scientific meetings and workshops, by disseminating best practices, and with fundamental research. This annual report highlights some of LyoHUB's accomplishments over the past year.

Thank you for your interest in LyoHUB. We're grateful to the twenty-five member companies who partner with us in this effort, and to Purdue's Colleges of Engineering and Pharmacy for their support. We are grateful for the strong partnership with Davidson School of Chemical Engineering and their Professional Masters program. Thanks, too, to Birck Nanotechnology Center for hosting our Demonstration Facility, a pilot scale lab for lyophilization research and training. Special thanks to the students and postdocs, both past and present, who have helped build LyoHUB and continue to inspire our progress. Finally, we are ever grateful to Jen Gray, our LyoHUB Operations Manager, without whom much would be discussed, but nothing would be accomplished. We look forward to continuing to work with all of you to advance lyophilization technology.

With our gratitude and best wishes,

Alina Alexeenko and Liz Topp

MEMBERSHIP

IMA LIFE
Freeze Drying Solutions

Member Since 2014

Pfizer

Member Since 2014

janssen
PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Member Since 2015

MILLROCK TECHNOLOGY
millrocktech.com

Member Since 2015

INFICON

Member Since 2015

SP SCIENTIFIC

Member Since 2016

Baxter

Member Since 2016

abbvie

Member Since 2016

Allergan

Member Since 2016

Roche

Member Since 2016

THE McCRONE GROUP

Member Since 2016

SIEMENS

Member Since 2017

FRESENIUS KABI
caring for life

Member Since 2017

Bristol-Myers Squibb

Member Since 2017

Abbott

Member Since 2018

OPTIMA
EXCELLENCE IN PHARMA

Member Since 2018

AMGEN®

Member Since 2018

Merck KGaA,
EMD Serano

Member Since 2019

AstraZeneca

Member Since 2019

EVONIK
POWER TO CREATE

Member Since 2019

Daiichi-Sankyo

Member Since 2019

COOK® BIOTECH

Member Since 2019

CORNING

Member Since 2020

SANOFI

Member Since 2020

MERCK
INVENTING FOR LIFE

Member Since 2021

BEST PRACTICES PAPERS

Work was completed on the **Recommended Best Practices for Lyophilization Validation-2021 Part I: Process Design and Modeling and Part II; Process Qualification and Continued Process Verification**

Paper Part I:

This work describes lyophilization process validation and consists of two parts. Part one focuses on the process design and is described in the current paper, while part two is devoted to process qualification and continued process verification. The intent of these papers is to provide readers with recent updates on lyophilization validation in the light of community-based combined opinion on the process and reflect the industrial prospective.

In this paper, the design space approach for process design is described in detail, and examples from practice are provided. The approach shows the relationship between the process inputs. It is based on first principles and gives a thorough scientific understanding of process and product. The lyophilization process modeling and scale-up are also presented showing the impact of facility, equipment, and vial heat transfer coefficient. The case studies demonstrating the effect of batch sizes, fill volume and dose strength to show the importance of modeling as well as the effect of controlled nucleation on product resistance are discussed.

Paper 2:

Paper 2 is devoted to process qualification and continued process verification. The goal of the study is to show the cutting edge of lyophilization validation based on the integrated community-based opinion and the industrial perspective.

This study presents best practices for batch size determination and includes the effect of batch size on drying time, process parameters selection strategies, and batch size overage to compensate for losses during production. It also includes sampling strategies to demonstrate batch uniformity as well as the use of statistical models to ensure adequate sampling.

Based on the LyoHUB member organizations survey, the best practices in determining the number of PPQ runs are developed including the bracketing approach with minimum and maximum loads. Standard practice around CQA and CPP selection is outlined and shows the advantages of using control charts and run charts for process trending and quality control.

The case studies demonstrating the validation strategy for monoclonal body and the impact of the loading process on the lyophilization cycle and product quality as well as the special case of lyophilization for dual-chamber cartridge system are chosen to illustrate the process validation. The standard practices in the validation of the lyophilization process, special lyophilization processes, and their impact on the validation strategy are discussed. Additionally, special cases of the lyophilization in alternate primary packaging systems such as dual-chamber vials, syringes, and cartridges are discussed, and recommended best practices for alternate lyophilization container-closure systems are suggested.

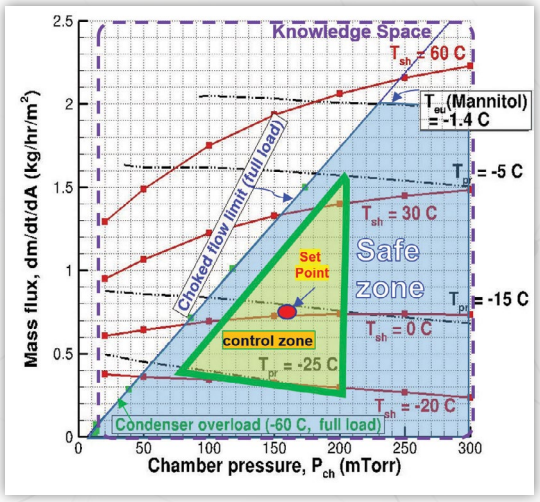


Figure: *Primary Drying Design Space*

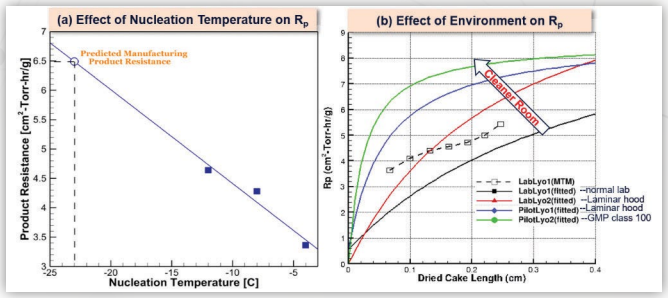


Figure: *Effect of Nucleation Temperature and Environment differences (particle-free/class 100 area) on mass transfer differences (Rp)*

Work began on a new best practice paper, ***Best Practices in the Development of Lyophilized Formulations***. This paper is being led by Dr. Elizabeth Topp (Purdue/NIBRT) and Dr. Greg Sacha (Baxter Healthcare)

LyoHUB's first lyophilization best practices paper, ***"Recommended Best Practices in Instrumentation Process Monitoring in Pharmaceutical Freeze Drying"*** continued to be popular with 13,000 downloads. It is available in open access at http://link.springer.com/article/10.1208/s12249-017-0733-1

Work continues on best practices papers:

- ***Scale Up and Tech Transfer***
  Led by Serguei Tchessalov and Bakul Bhatnagar (Pfizer)
- ***Equipment Performance Qualification***,
  Led by Arnab Ganguly (Amgen)

INDUSTRY VISITS & CONFERENCES

September 2020

Alina Alexeenko presented about Lyophilization Modeling at the 2020 PDA/FDA Joint Regulatory Conference. The conference focused on advancing quality management and the robust manufacturing of innovative drugs, biologics, and combination products. Combined efforts of industry and regulators are necessary to assure uninterrupted supplies of safe and high-quality products while advancing the use of new capabilities.

November 2020

Drew Strongrich from Purdue (PhD candidate of Alina Alexeenko and LyoHUB Superuser) provided a SP Scientific's webinar: ***Next-Generation Process Monitoring in Lyophilization – Applications for Wireless Sensor Technology***

SPECIAL PRESENTATIONS TO LYOHUB

May 2020

Jacqueline Linnes, Assistant Professor of Biomedical Engineering Purdue University, presented: ***Lyophilization use in Near-Patient Molecular Diagnostics***

June 2020

Ivo Backx, Life Sciences industries presented: ***Technology and innovation drive change in pharmaceutical manufacturing***

July 2020

Julie O'Neill, Founder & Principal Consultant, Direxa Consulting LLC, presented: ***Statistics in Formulation Design for Lyophilized Products***

August 2020

Zijie Wu and **Shrikanth Yerragolla**, Purdue University presented: ***Purdue Chemical Engineering Professional Masters' summer 2020 capstone project on Vial Fogging***

September 2020

Feroz Jameel, AbbVie, presented: ***LyoHUB Best Practices Paper on Validation***

October 2020

Petr Kazarin from Purdue (Postdoctoral associate of Alina Alexeenko and LyoHUB Superuser) and **Xiaofan Jiang** presented: ***Virtual Thermocouple: A Non-Invasive Approach to Measure Product Temperature for Controlled Lyophilization***

November 2020

Dr. Brecht Vanbillemont, Lyophilization Scientist, Coriolis Pharma, Munich – Germany presented: ***Application of 3D and 4D MicroCT in Pharmaceutical Freeze-Drying***

January 2021

Christopher Weikart, Chief Scientist, SiO2 presented: ***SiO2 Hybrid Vials for Lyophilized Drug Products*** and **Spencer Holmes**, Applications Engineer, Millrock Technology presented: ***Lyophilization Protocols for using SiO2 Vials***

February 2021

Dr. Brecht Vanbillemont, Lyophilization Scientist, Coriolis Pharma, Munich – Germany presented: ***Lyophilized Orally Dissolving Tablets***

March 2021

Vaibhav Kshirsagar, Technical Product Manager for IMA Life and **Nikunj Mehta**, CEO and Founder of Falkonry, presented: ***Predictive Analytics for Lyophilization***

GRANTS & COLLABORATIONS

Improving Lyophilization of Recombinant Proteins with ssHDX-MS

- Funded by NIIMBL
- $450,000 over 18 months
- **Goal:** Evaluate solid-state hydrogen deuterium exchange with mass spectrometric analysis (ssHDX-MS) as a method to test stability of proteins in solid powders.
- **Investigators:** Lokesh Kumar (Genentech), Ben Walters (Genentech), Andrea Allmendinger (Roche), Deborah Bitterfield (Lindy Biosciences), Michael Doherty (Lindy Biosciences)

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

Software and Hardware Tools for Pharmaceutical Lyophilization and Scale Up

- Funded by NIIMBL
- **Goal:** Develop and test hardware and software tools that will harmonize pharmaceutical lyophilization process development and scale-up.
- **Industry Partners:** Physical Sciences, Inc., Genentech, Merck, University of Massachusetts Lowell, NIPTE-University of Connecticut, Purdue University, Massachusetts Life Sciences Center

Advanced Wireless Sensor (PAT) for Pharmaceutical Lyophilization

- Selected for funding by CESMII (The Smart Manufacturing Institute)
- **Goal:** To apply a real-time, non-invasive, process monitoring system for pharmaceutical lyophilization equipment based on a wireless network of vacuum and temperature sensors.
- **Industry Partners:** Millrock Technology, Purdue University

ASTM LYOPHILIZATION STANDARDS

E55.05 Lyophilization subcommittee of E55 Committee on Manufacture of Pharmaceutical and Biopharmaceutical Products: www.astm.org/COMMITTEE/E55.htm

ASTM INTERNATIONAL

The work item WK63507 on Standard Practice for Product Temperature and Equipment Pressure Instrumentation in Pharmaceutical Freeze Drying via ASTM has been submitted for ballot.

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

LYOHUB IN THE NEWS

LyoHUB Co-Director, Dr. Elizabeth Topp is leading an effort to develop a new drug form which may help treat osteoporosis, calcium-related disorders. Purdue University innovators have developed a stabilized form of human calcitonin, which is a peptide drug already used for people with osteoporosis. Researchers at Purdue created a prodrug form of the peptide hormone to increase its effectiveness as an osteoporosis treatment. To read the full article, visit https://www.purdue.edu/newsroom/releases/2021/Q1/new-drug-form-may-help-treat-osteoporosis,-calcium-related-disorders.html

LyoHUB was featured in the Summer 2020 Chemical Engineering Newsletter.

Read the full article at https://engineering.purdue.edu/ChE/aboutus/publications/newsletters/ChE_Summer-2020-web-new.pdf

LYOHUB: ADVANCING LYOPHILIZATION TECHNOLOGY

University-Industry Center improves efficiency, science, and technology of freeze-drying

By Jennifer Gray

LyoHUB, a university-industry center at Purdue University, is advancing the science of lyophilization and providing the necessary capabilities in the fight against COVID-19 and other illnesses around the globe.

Lyophilization, also called freeze-drying, is a process that gently removes water from materials to produce a dried product. For pharmaceuticals, lyophilization is often used to stabilize sensitive drugs, lengthening the shelf life while preserving the critical efficacy of the medicine. Lyophilization also presents a technology challenge because the process is time consuming, often misunderstood, and inefficient. Despite this challenge, lyophilization is a critical manufacturing process for the pharmaceutical industry, and around 25 percent of new injectable drugs, vaccines, and biological products are formulated in a lyophilized form. With support from the Davidson School of Chemical Engineering, LyoHUB is advancing the science and technology of lyophilization, with the goal of lowering costs and improving the availability of lyophilized products. The need for advancements in this area is magnified as COVID-19 lyophilized treatments, such as Gilead's Remdesivir®, diagnostic reagents and other countermeasures are being developed and evaluated.

"The pharmaceutical industry uses solid forms of drugs to protect them during manufacturing, shipping, and storage. If we tried to remove the water by heating, many drugs would be destroyed," said Elizabeth Topp, a Professor in Purdue's Department of Industrial and Physical Pharmacy and co-Director of LyoHUB. "Lyophilization removes water at low temperature and low pressure, safely drying sensitive drugs."

LyoHUB was funded at Purdue in 2014 with a National Institute of Standards and Technology (NIST) ANTECH grant program. The program's goal was to develop the Lyophilization Technology Roadmap, a project which was published in 2017 with input from over 100 industry leaders, establishing a university-industry consortium that identified trends and developments in lyophilization.

> We are excited that LyoHUB capabilities are being utilized right now to speed up development of novel COVID-19 diagnostics.

LyoHUB's 24 industry members, which include the entire value chain in lyophilization—pharmaceutical companies, equipment manufacturers, software companies and more — work together with university researchers to achieve three goals: identify and disseminate Best Practices for lyophilization including equipment performance, testing, validation and formulation; conduct applied research to advance lyophilization processes and technologies; and develop educational and training programs in lyophilization.

Interested parties can now learn about lyophilization and get involved with LyoHUB through a non-credit online course and the LyoLaunchPad program.

In January 2020, LyoHUB released an innovative online, open-access lyophilization short course which allows participants to learn the basics of freeze drying of pharmaceuticals through eight 20-minute educational modules. The modules were developed and recorded by leading lyophilization experts from around the United States.

Course modules include: Introduction to pharmaceutical lyophilization; Overview of the lyophilization process; Production lyophilizers; Quality attributes of lyophilized products; Glass transition temperature; Freezing; Primary and secondary drying; and Graphical design space. An online laboratory exercise in pharmaceutical lyophilization is also included in the course, allowing students inside the LyoHUB demonstration facility to virtually see how a lyophilization cycle is run. The course was developed through a grant from the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), and is available free of charge.

Elizabeth Topp and Alina Alexeenko, Co-Directors of LyoHUB

Birck Nanotechnology Center at Purdue is home of the LyoHUB Lyophilization Technology Demonstration Facility, a research lab featuring state-of-the-art lyophilization equipment and analytical tools run by several experienced lyophilization "superusers." The facility has hosted almost 900 lyophilization runs since it opened in 2016.

"We are excited that LyoHUB capabilities are being utilized right now to speed up development of novel COVID-19 diagnostics," said Alina Alexeenko, Professor in Purdue's School of Aeronautics and Astronautics and the Davidson School of Chemical Engineering, and co-Director of LyoHUB. "We are ready to offer support for other efforts to improve lyophilization for faster manufacturing of COVID-19 reagents, drugs, or vaccines."

This year, LyoHUB also introduced LyoPRONTO, an Open Source Lyophilization Process Optimization Tool. This user-friendly lyophilization simulation and process optimization tool is freely available under the name LyoPRONTO. It includes freezing, primary drying modeling, and optimization modules, as well as a design space generator (Figure 1). LyoPRONTO can be used to model the lyophilization process and create more efficient cycles. The tool is also capable of determining the vial heat transfer parameters and product resistance characteristics, reducing the number of experiments. LyoPRONTO is available on two platforms, Python Source Code and an online version with graphical user interface.

LyoHUB's "superusers" are students from a variety of disciplines including pharmacy and several engineering fields, who develop freeze drying process & equipment expertise through training and completing projects in the lab. Superusers help Purdue faculty, fellow students and industry members from around the world successfully complete research projects in the LyoHUB demonstration facility.

ChE Professional Master's Program alumna Yirang Park loads a lyophilizer in LyoHUB's lab in Birck Nanotechnology Center. LyoHUB is a university-industry center at Purdue whose goal is the efficiency, science, and technology of freeze-drying in pharmaceuticals and other areas. (Purdue University/Rebecca McElhoe)

LEARN MORE ABOUT LYOHUB

For more information about LyoHUB, visit the website at www.lyohub.org.

LyoPRONTO is available on two platforms: (Python Source Code): lyopronto.org and the online version with graphical user interface (GUI): http://lyopronto.rcac.purdue.edu/.

LyoHUB's new online, open-access lyophilization short course was developed through a grant from the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), and is available free of charge at https://pharmahub.org/courses/lyo101.

Learn more about the LyoHUB Lyophilization Technology Demonstration Facility, including a description of the facility, along with an equipment listing is available at https://pharmahub.org/groups/lyo/demofacility.

LyoHUB offers LyoLaunchPad, a program which allows new users to conduct an approved, non-proprietary, short-term project in the LyoHUB demonstration facility at no charge. Participants are asked to give a short presentation for LyoHUB members at the completion of the project. For more information on LyoLaunchPad, contact Jennifer Gray, LyoHUB Operations Manager, at gray160@purdue.edu.

LYOHUB DEMONSTRATION FACILITY

LyoHUB demo facility is located in Birck Nanotechnology Center, Room 2261.

In February 2016, LyoHUB opened the Lyophilization Technology Demonstration Facility located in the Birck Nanotechnology Center at Purdue Discovery Park. The facility, where collaboration on breakthrough technologies can be advanced with a goal of accelerating adoption and decreasing time to market, is equipped and supported by LyoHUB's industry members. The facility also offers various hands-on training opportunities for academic and industry users. Full equipment listings and capabilities can be found on the LyoHUB website at https://pharmahub.org/groups/lyo/demofacility

Computroc® Vapor Pro®

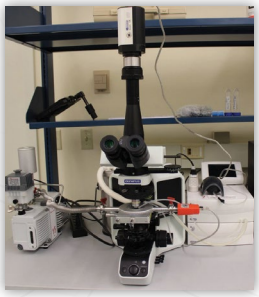
Lighthouse FMS-1400 Headspace Pressure/ Moisture Analyzer

Donation from Baxter

REVO lyophilizer with controlled nucleation and in site mass spectrometer

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

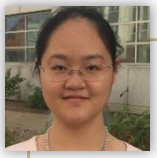
Development Freeze Dryer/Lyophilizer MICROFD



McCrone Freeze Drying Microscope

LYOHUB SUPER USERS

Andrew "Drew" Strongrich
PURDUE AERONAUTICS AND ASTRONAUTICS



JiaMei Hong
Associate Research Engineer
LYOHUB

Michael Sinanis
PURDUE INDUSTRIAL ENGINEERING

Rishabh Tukra
PURDUE INDUSTRIAL AND PHYSICAL PHARMACY

Shrikanth Yerragola
Associate Research Engineer
LYOHUB

USAGE & LYOHUB DEMONSTRATION FACILITY PROJECTS

LyoLaunchPad 2020-2021 Projects:

- Lyophilization of Cellulose Nanocrystals (US Forest Service and Georgia Tech)
- Continuing work on Application of residual gas analysis process gas composition measurement in lyophilization (Bristol Myers Squibb)
- Freeze drying of maize root tissue samples to be used for metabolite extractions. (Dr. Brian Dilkes, Department of Biochemistry, Purdue)
- Freeze drying of biosolids for the analysis of PFAS compounds, preserved to enable extended research. (Dr. Linda Lee, Agronomy, Purdue)
- Transforming hydrogel into aerogel using freeze drying (Wenzhou Wu, Industrial Engineering, Purdue)
- Investigation of soluble metabolites in Arabidopsis and Sorghum leaves and stems (Dr. Clint Chapple, College of Agriculture, Purdue)
- Feasibility study of lyophilizing aqueous block copolymer nano-assemblies for room temperature storage and handling (Dr. You-Yeon Won, Purdue, Davidson School of Chemical Engineering)
- Vial Breakage Study (Corning)

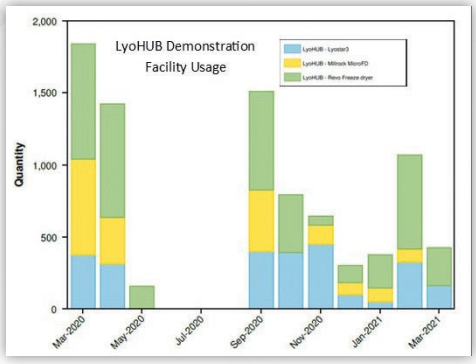
Funded 2020-2021 Projects:

- Wireless pressure and temperature sensor characterization (Purdue AAE/NSF project)
- Development of microwave assisted freeze drying (Purdue AAE/NSF project)
- Lyophilization Scale-Up Data Analysis and Modeling (NIIMBL project)
- ssHDX-MS of proteins and ssHDX-MS under pressure of proteins (Purdue AAE/NSF project)
- ssHDX-MS: Generating a mechanistic understanding of solid state hydrogen deuterium exchange (ssHDX-MS) in lyophilized protein formulations. (Purdue AAE/NSF project)
- ssHDX-MS under pressure: Understanding the effects of pressure on the ssHDX-MS of lyophilized protein formulations. (Purdue AAE/NSF project)
- Wireless Sensor for Vial Headspace Pressure Monitoring During Controlled Ice Nucleation Process (Genentech)
- MicroRAAD modification and lyophilization of CRISPR reagents (CASPR/Linnes)
- RGA-MS Monitoring of Free HCl Vapor during Lyo (Merck)
- Studying pH change and buffer effects in protein formulation (Funded by Merck)
- Studying the effects of electrostatic spray drying and comparing them to lyophilization (Funded by Genentech)

Usage

Total Number of Lyophilization Runs:
(2/26/16-3/26/17): 87
(3/1/17-3/30/18): 178
(4/1/18-3/27/19): 190
(3/1/19-3/1/20): 421
(3/1/20-3/1/21): 184

Total Lyo Run Time:
(2/26/16-3/26/17): 2,971.37 hours
(3/1/17-3/30/18): 9,227.17 hours
(4/1/18-3/27/19): 13,944 hours
(3/1/19-3/1/20): 16,624 hours
(3/1/20-3/1/21): 8,505 hours



PURDUE CHEMICAL ENGINEERING PROFESSIONAL MASTERS CAPSTONE PROJECTS

During the summer of 2020, the following LyoHUB members developed the following projects and mentored several students as part of the Purdue Chemical Engineering Professional Masters Program.

Siemens:

- *StarCCM dissolution prediction analysis for media prep mixing vessels in biopharma industry*
- *Simulating and Validating Fluidized Beds with Kinetic Theory of Granular Flow*

AbbVie:

- *Lyophilization Vial Fogging*

Cook Biotech:

- *Evaluation of a possible product including financial modeling*
- *Environmental Health & Safety/Compliance: ISO 14001 compliance program building*

Pfizer/Allergan:

- *Comparison of lyocalculators*

Figure: *Glass with lyophilized product exhibiting fogging*

Overview of the Lyophilization Vial Fogging Project

Fogging is a phenomenon seen after lyophilization of a pharmaceutical drug. It is due to the residue formed by thin liquid films creeping towards the neck of the vial. Though the quantity is low, the freeze-dried material on the vial inner surface can be regarded as a cosmetic defect which could interfere with optical inspection or Container Closure Integrity (CCI). This leads to higher and unpredictable reject rates which is an economical loss for the pharmaceutical companies.

The two primary factors essential for the occurrence of fogging are the inclusion of a surfactant in the formulation solution and the hydration film on the surface of the glass (increasing contact angles result in a lower degree of fogging). Other factors have little to no clear impact on fogging. Therefore batch - to - batch, vial - to - vial and even variations within one single vial are found.

It's also been found that due to the complex interplay of several factors, having control over fogging in a reliable manner is difficult with the standard procedures. A potential solution would be to use vials with a hydrophobic surface. This single change will reduce fogging to a large extent. It is also known that siliconized glass shows more hydrophobic nature and hence shows poor wetting and larger contact angles with polar liquids when compared with untreated clean glass surfaces. The overall goal of this project is to delve deeper into further understanding the array of contributing factors of fogging and recommend ways to reduce.

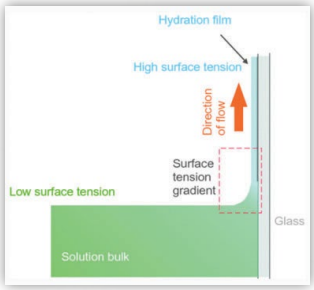


Figure: *Surfactant-driven Marangoni flow in a glass vial*

The team under the mentorship of Ted Tharp from Abbvie Inc. decided to use a higher concentration of sucrose than mannitol in our formulation. It was found that sucrose and mannitol stabilize the protein against aggregation. A 4/1 ratio of mannitol to sucrose was used for cycle development as this is known to be beneficial for complete mannitol crystallization. A molar ratio of protein to sucrose of 360:1 has been shown to be necessary for lyophilized mAb. Along with them 1.55 mg/ml histidine and 0.01% polysorbate 80 was used. It has been shown that the presence of surfactants (like polysorbate) in the formulation reduces the aggregation.

The sample formulations were summarized and highlighted the important factors such as the lyoprotectant (sugars), the surfactant (Polysorbates) and different depyrogenation conditions. The sample concentration was imitated and the generic formulation implemented to reduce fogging in the vial. Fractional factorial design was applied in screening, factors from which caused instability of API in the protein formulation. Fractional factorial design can determine the main effect of each factor and their interactions which helps in designing an experimental setup with fewer runs. the MICROFD was used along with the McCrone Freeze-Drying Microscope in Ompi ez-fill 6R vials to run the experiments.

There are two major findings in the results. Firstly, different degrees of shrinkage of the cake were seen in the vials (Especially for the vials with only Sucrose (amorphous phase) or only Mannitol (crystalline phase). Secondly, the addition of conservative polysorbate - 80 had little fogging while that vial which was devoid of the surfactant did not have any fogging. To summarize, results show that the addition of surfactant is the reason for the fogging, but the overall appearance is not greatly affected by it. The reason for this scenario is still not clear and it might be because of the high viscosity of the formulations caused by addition of Polysorbate 80. The importance of the glass chemistry and the glass structural change by depyrogenation must also be studied thoroughly as a potential factor to vial fogging.

10mL siliconized	*10mL Type 1 plus*	*10mL Toplyo*	*10mL Fiolax (Source 1)*	*20mL Fiolax (Source 2)*	*20mL Fiolax (Source 3)*

Figure: *Fogging behavior of different vial types in lyophilized drug product*

RF-ASSISTED LYOPHILIZATION

Motivation

Efficiency in the pharmaceutical manufacturing of freeze-dried drugs has been of central interest for years, but most recently the need for more effective methods has been investigated. Lyophilization is a time-consuming industrial process with an efficiency less than 5%. Microwave heating of the product during the primary drying has been considered as an alternative method to conventional method of conductive heating. Currently in LyoHub through a modified freeze dryer we have developed a product adaptive microwave heating technique to increase efficiency of the primary drying process.

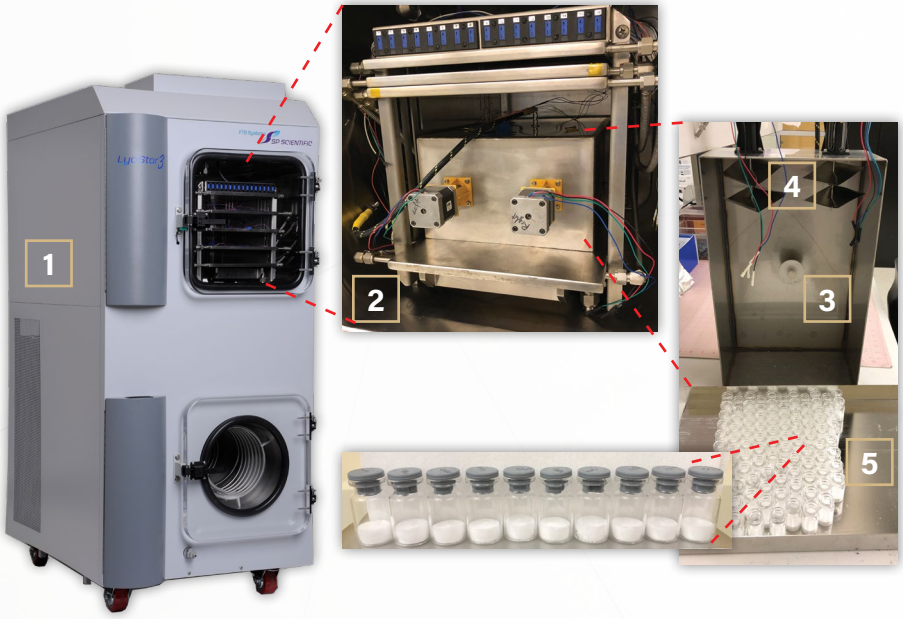


Figure 1: *Modified LyoStar Laboratory Freeze Dryer for RF-assisted lyophilization [1], auxiliary chamber [2], high power antenna [3], stirring elements [4], sucrose 5% in 6R vials.*

RF-assisted lyophilization

This work presents the experimental setup manifesting the RF-assisted lyophilization based on the theory of statistical electromagnetics. As the theory states, a well behaved statistical electromagnetic environment is the key to a statistically uniform RF power distribution. This, in turn, results in statistically uniform heating. The setup contains, a stainless-steel cavity-based environment enclosing all the vials in a metallic chamber designed to accommodate numerous modes at the frequency of interest. The presented lab-scale setup can be readily extended to the industrial scale opening the door to adding the feature of RF heating to the currently utilized industrial freeze dryers.

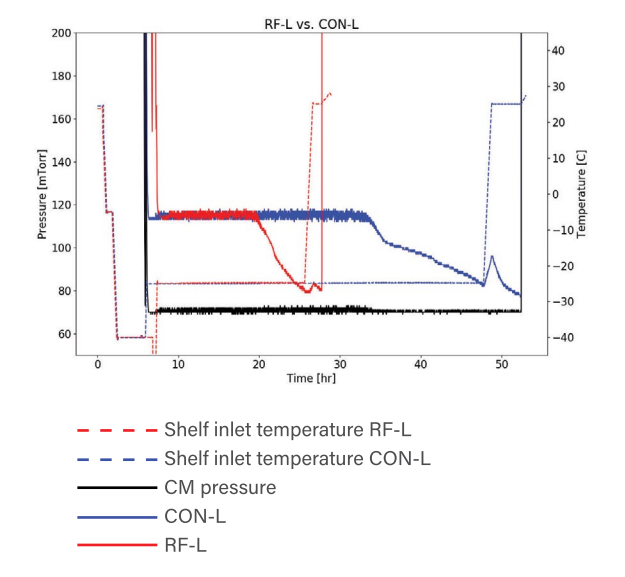


Figure 2: *Sucrose 5%, 3ml in 6R vial. Batch moisture 1.5% (+/- 0.1). Primary drying time for conventional cycle (CON-L) at ~42 hours and RF-assisted cycle (RF-L) at ~18 hours. 57% reduction in primary drying processing time.*

A stainless-steel enclosure is used as the cavity-based environment required to sustain a uniform field for heating the product during primary drying. The pressure is being monitored to define the conclusion of the primary drying cycle and the product is also inspected visually at the end of the secondary cycle. Moisture analysis is performed on samples across the shelf to confirm uniformity and successful end of freeze-drying. A reduction in processing time is established by more than 50% and an average moisture rate of 1.5% with a standard deviation of 0.1%.

WIRELESS SENSOR DEVELOPMENT PROJECTS

Over the last decade, Wireless Sensor Networks (WSNs) have become prolific in a broad range of applications from personal health monitoring to smart manufacturing. Their benefits are now being realized for lyophilization applications in the realm of next-generation process analytical technologies. Two devices currently under active development in the LyoHUB Demonstration Facility include wireless microPirani and wireless multipoint temperature sensors.

Wireless MicroPirani Sensors

A series of Wireless MicroPirani (WMP) sensors have been developed to measure the spatial variations of gas pressure and temperature in the vial pack. The devices are encapsulated in enclosures having an identical footprint to a common pharmaceutical vial. This feature enables direct placement at any location within the vial pack. The WMP devices are calibrated in pure water vapor over a range of pressures and temperatures characteristic of lyophilization. An image of the sensors installed in the vial pack and their corresponding output in terms of pressure are shown in Figure 1. These measurements may be coupled to a heat and mass transfer model to extract relevant process parameters such as the vial heat transfer coefficient and product mass transfer resistance.

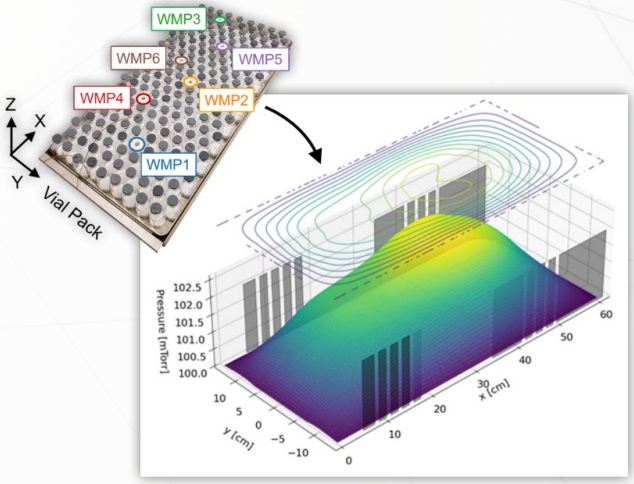


Figure 1: *WMP sensor locations within 20R vial pack (left) and corresponding measured pressure distribution across shelf (right).*

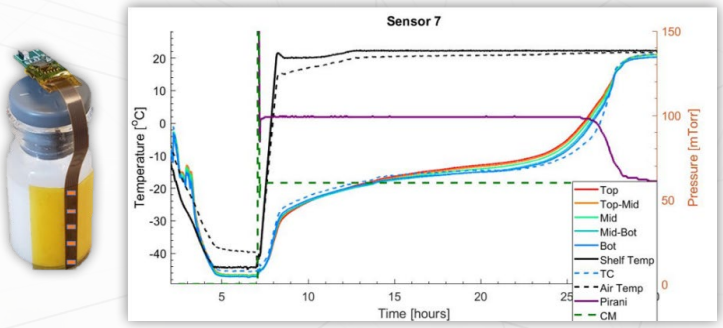


Figure 2: *Wireless multipoint sensor installed on 6R glass tubing vial (left) and the corresponding process data. Results demonstrate good agreement with nearby thermocouple.*

Wireless Vial Temperature Sensors

Wireless multipoint temperature sensors are applied to measure the outside wall temperature of product vials at several discrete locations along the fill height. An image of the multipoint sensor and its corresponding output are shown in Figure 2. The non-invasive nature of the multipoint sensors gives them an advantage over traditional thermocouples due to their inherent compliance with cGMP aseptic manufacturing processes and limitless positioning flexibility. Measurements demonstrate good agreement with a thermocouple installed in a nearby vial.

RESIDUAL GAS ANALYSIS PROJECT

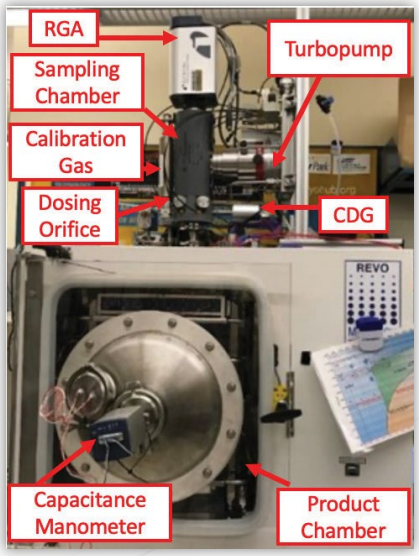


Figure 1. *REVO Freeze-Dryer (Millrock) with RGA unit (INFICON) installed on top.*

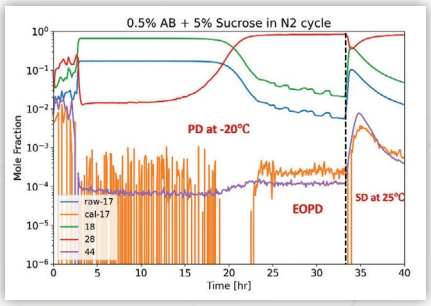


Figure 2. *Processed RGA data, mole fraction over time graph. Legend on bottom right indicates m/z values, best representing the following gas species [17: NH3, 18: H2O, 28: N2, 44: CO2]. The blue and orange lines represent raw 17 signal (huge influence coming from water) and self-calibrated 17 signal (best represent NH3).*

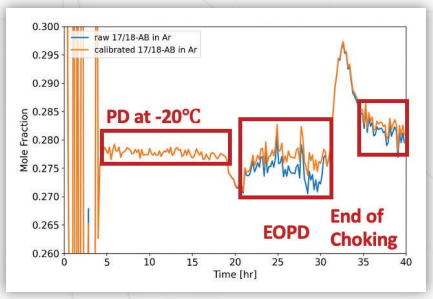


Figure 3. *Calculated raw and calibrated 17/18 ratio over time graph. Solution used in this cycle is 0.5% w/v AB with 5% w/v Sucrose. Ballast gas is 99.99% Argon. The blue and orange represents raw and calibrated 17/18 ratio, cycle conditions same as Figure 2.*

In collaboration with Bristol Myers Squibb, this project aims to explore the application of RGA's (Residual Gas Analyzer) process gas online monitoring capability to examine the off-gassing rates of gaseous species released from chemical decomposition throughout the entire freeze-drying process. RGA is a mass spectrometer commonly used for high vacuum system (pressure lower than 0.1mTorr) leak or contamination detection. By attaching a dosing orifice, a reduction in partial pressure makes RGA possible to perform under higher pressure range of 1mTorr, such as that in lyophilization cycles.

Figure 1 shows the REVO lyophilizer from Millrock with a RGA (INFICON, Transpector CPM) mounted on top, which was used for this project. This RGA unit could monitor the gas composition inside the chamber in-situ in the mass-to-charge (m/z) range from 0-200, with a detection limit of 2-20 ppb. Figure 2 shows an example of processed RGA data extracted from freeze-drying of 0.5% w/v Ammonium Bicarbonate (AB) with 5% w/v Sucrose solution. Based on NIST mass spectrum data, ammonia has its primary peak at 17 m/z value, but due to an overlapping of spectrum data between water and ammonia, water signal influence on the 17-signal must first be subtracted.

During initial primary drying (PD) stage, as shown in Figure 2 between 3-19 hrs, the chamber gas mainly consists of water vapor, due to the high sublimation rate of ice. During which both 17 and 18-signal comes only from water and therefore the average of the relatively constant 17/18 ratio in this steady state phase, as shown in Figure 3, can be used as self-calibration basis to eliminate water influence on 17-signal. In Figure 2, it is observed that starting at hour 19, there's rapid decrease and increase in 18 and 28-signal respectively, indicating the end of primary drying (EOPD) as water sublimation rate slows down and additional ballast gas (N2) is pumped into the chamber to maintain setpoint pressure. At the 33-hour mark, a rapid increase in calibrated signal 17 and 44 was observed, as cycle proceeds into secondary drying (SD) where AB sublimes aggressively. The near 1:1 molar ratio of calibrated signal 17 and 44 (NH3 and CO2) represents the dissociation of AB. It is worth mentioning that the system experienced loss of pressure control/choking as soon as the cycle proceeded to SD at 33-hr.

While it is promising to implement RGA to identify chemical composition change in the process, it should be noted that the ballast gas used could have a huge interference on certain species. It has been shown that using pure Argon (Ar) gas could affect the 18-signal, due to the relatively high abundance of doubly-charged 36Ar (m/z = 18). Figure 3 shows the comparison between raw and calibrated 17/18 ratio in a system using pure Ar as ballast gas using the same solution (0.5% AB + 5% Sucrose) and cycle conditions as in Figure 2. In this case, 18-signal is first calibrated by remove Argon influence, and then 17-signal is calibrated based on 17/18 ratio in PD stage. The ballast gas influence is most obvious when the system requires additional pumping of Ar to maintain pressure setpoint, such as EOPD and end of choking in the case of AB solution. As such, deconvolution technique needs to be modified based on the characteristics of a specific system.

EFFECTS OF PRESSURE

ON SSHDX-MS OF LYOPHILIZED PROTEIN FORMULATIONS

Lyophilized biopharmaceuticals need to undergo accelerated stability studies to ensure their long term stability, safety and efficacy. Since these stability studies take a few months to complete, there has been an increasing interest in developing faster methods that can be used to screen formulations in the early stages of product development. ssHDX-MS can be used as a quick screening method to assess product stability that can be correlated to long term stability studies. Previously, the effects of temperature and RH on ssHDX-MS kinetics were studied and currently efforts are underway to understand how pressure affects the kinetics.

Pressure chamber correlation to conventional desiccator

Figure 1 compares the cross section of the custom pressure chamber with a conventional desiccator. The chamber was constructed of metal to withhold no more than 5 atm gauge pressure. Industry grade dry air was used to build pressure. RH equilibration between the chamber and the desiccator were compared (Figure 2). The RH value in the chamber at the end of a 9 hour observation period was within 1.5% of the desiccator RH. This is also within the error limits of the hygrometers.

ssHDX-MS kinetics of myoglobin formulations under pressure

A formulation of 1:1 w/w myoglobin:sucrose was subjected to ssHDX-MS experiments for 10 days (240 hours) under 1 atm in the desiccator, 1 atm in the chamber, 2 atm in the chamber and 4 atm in the chamber. The data from 1 atm chamber coincided almost perfectly with the 1 atm desiccator. For experiments done at higher pressures, a decrease in the rate of deuterium uptake was observed for the first 24 hours. The rate of deuterium uptake was found to be fastest for samples incubated at 1 atm followed by 2 atm and 4 atm respectively. This difference, however, diminished at 48 hours between 2 atm and 4 atm samples and disappeared almost completely for 120 and 240 hour time points for all conditions. Further experiments testing the effect of pressure at peptide level deuterium uptake are currently underway.

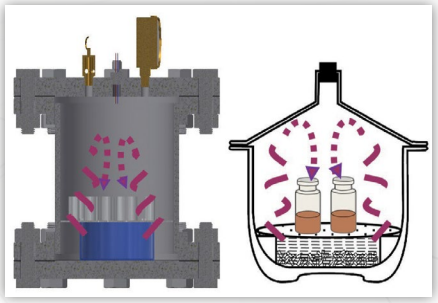


Figure 1: *Cross section of chamber(left) and desiccator (right)*

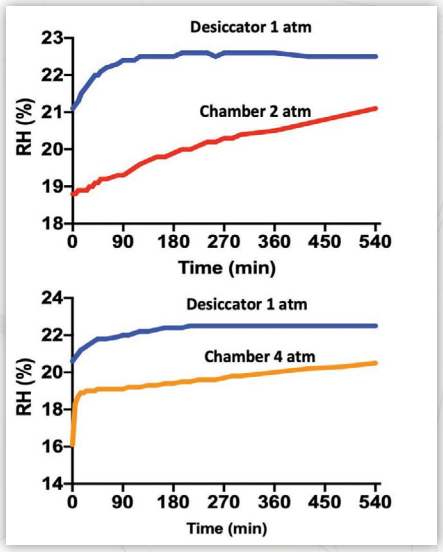


Figure 2: *RH equilibration comparison between 2 atm chamber (top) and 4 atm chamber (bottom) to 1 atm desiccator between 1 atm desiccator*

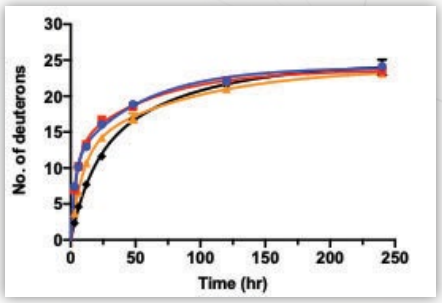


Figure 3: *Pressure kinetic curves for 1 atm desiccator (blue); 1 atm chamber (red); 2 atm chamber (yellow); 4 atm chamber (black)*

EXPERIMENTAL DESIGN AND MODELING OF SECONDARY DRYING

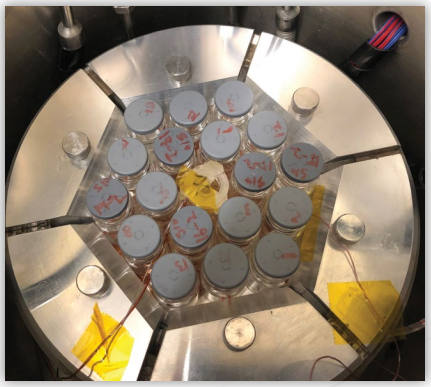


Figure 1 *Micro-freeze dryer (MicroFD) and experimental setup*

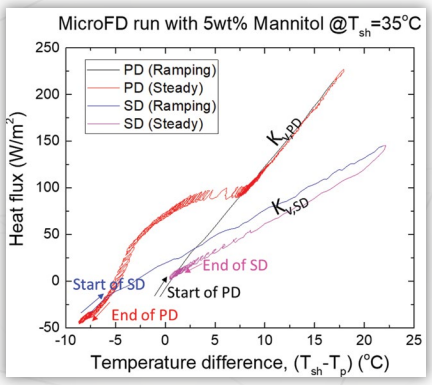


Figure 2 *The heat flux plot for a 5wt% mannitol solution during the lyophilization process.*

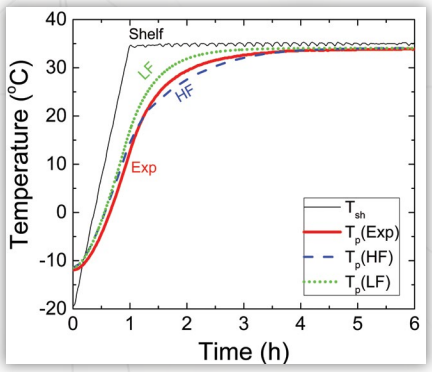


Figure 3 *Average temperature for a mannitol cake during secondary drying (2 mL fill volume, 5% initial weight, 6R vial in a micro freeze dryer). Red = experiment, blue = high fidelity model, green = low fidelity model, and black = shelf temperature profile.*

Conventional freeze-drying consists of two sequential steps: (1) primary drying where ice sublimates from the product to leave behind a porous cake (10-30 hr) and (2) secondary drying where the remaining bound water is removed from the porous cake (2-10 hr). For primary drying, LyoHUB previously developed a modeling toolbox LyoPRONTO that includes optimization routines to determine the transient, shelf temperature and chamber pressure profiles that minimize primary drying time given user-defined constraints. However, there currently lack robust models for secondary drying with comparable accuracy and flexibility as primary drying models. To alleviate this issue, LyoHUB has performed modeling and experiments of secondary drying. The laboratory scale freeze dryer (MicroFD, Millrock Technology, Kingston, NY) that is used in this project is shown in Fig 1.

Experiments involved lyophilizing various sugars (e.g., sucrose and mannitol) under different operating conditions to determine the effects they have on energy transfer during secondary drying stage. The first step conducted at LyoHUB was to determine the heat transfer coefficient of the vial by using a sensor to measure the slope of heat flux versus temperature difference between the shelf and the cake (Fig 2). We find that the heat transfer coefficient during secondary drying for a 6R vial at 100 mTorr chamber pressure is a factor of ~2 smaller than the reported values during the initial stages of primary drying. This unreported observation is likely due to the fact that the overall heat transfer coefficient of the system is affected by materials such as glass vial, ice, and lyophilized cake.

We further developed two models for secondary drying -- a high-fidelity model that captures the detailed, 3D temperature profile of the vial and the moisture content of the cake, and a simpler "lumped capacitance" model that assumes uniform temperature in the cake. The high fidelity model assumes that the energy in the cake can be transferred by conduction, absorbed into internal energy, or used to desorb bound water, with the kinetic parameters of desorption determined from literature. Both models reasonably capture the experimental temperature profiles (Fig 3). We note that both models show that the heat flux during secondary drying is predominantly routed through the vial wall rather than the cake. This means that drying is predominantly determined by the thermal properties of the vial rather than the cake, and that the drying is highly inefficient. Ongoing projects at LyoHUB are looking at alternative heating technologies (like microwaves) to specifically target heating the cake's bound water and accelerate drying.

WEBSITE RESOURCES & TRAINING

LYO101 COURSE

Open Access Online Introduction to Lyophilization course

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

Current enrollment: 274

LYOPRONTO

An Open-Source Lyophilization Process Optimization Tool

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

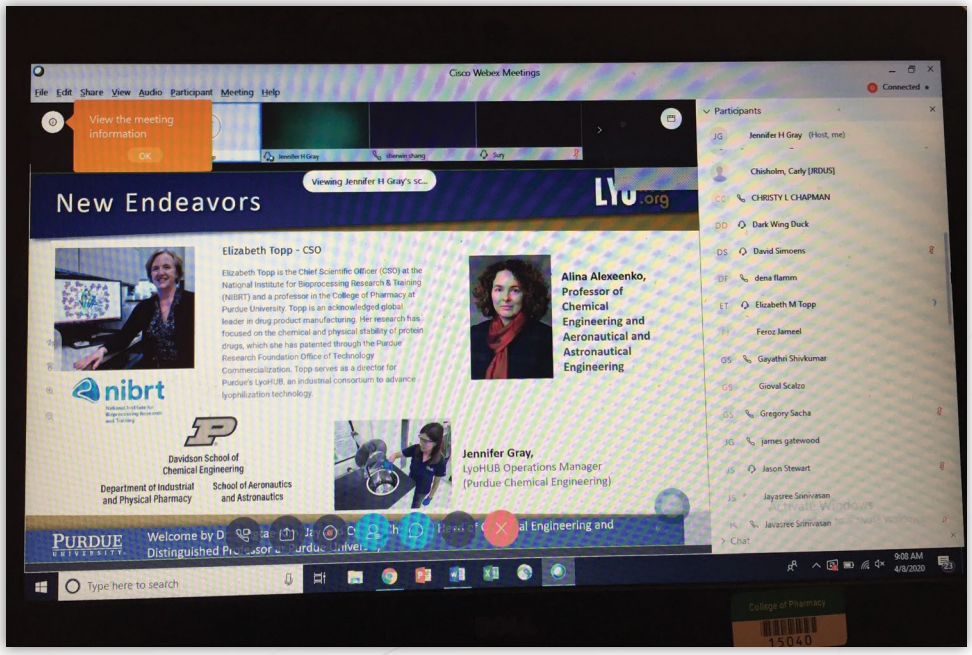
Online version with GUI
http://lyopronto.rcac.purdue.edu

Website Tools
pharmahub.org/groups/lyo/tools

- YouTube videos
- Lyo University Lyophilization Short Course
  https://pharmahub.org/courses/lyo101
- LyoPRONTO Lyophilization Process Optimization Tool
  www.lyopronto.org
- 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 Best Practice Paper, Recommended Best Practices for Process Monitoring in Pharmaceutical Freeze Drying
  http://link.springer.com/article/10.1208/s12249-017-0733-1
- 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

New Users Trained on Lyophilization Equipment from March 2020-2021

- **JiaMei Hong** | LyoHUB Research Engineer
- **Shrikanth Yerragola** | LyoHUB Research Engineer
- **Patrick Williams** | Purdue Chemical Engineering Student
- **Jack Van Wingen** | Purdue Chemical Engineering Student
- **Zachary Mora** | Purdue Chemical Engineering Student
- **Kinnari Arte** | Purdue IPPH/Topp lab
- **Kyu Yoon** | Purdue Chemical Engineering/ Narsimhan lab
- **Sungwan Park** | Purdue Chemical Engineering Student
- **Brett Walker** | Purdue/ECE/Peroulis lab
- **Siyue Shen** | Purdue Electrical and Computer Engineering Student
- **Bobby Santoso** | Purdue Industrial Engineering Student
- **Zijie Wu** | Purdue Engineering Graduate Student
- **Cole Tower** | Purdue IPPH/Munson lab
- **Jeffrey Simpson** | Purdue Research Scientist/Biochemistry
- **Yuan Chen** | Purdue IPPH/Topp lab



The 2020 LyoHUB Annual Meeting – *held virtually due to COVID-19 pandemic*

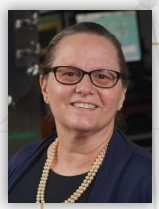
CONTACTS

Alina Alexeenko
Co-Director | LYOHUB
Professor of Aeronautics & Astronautics and Professor of Chemical Engineering
PURDUE

Elizabeth Topp
Co-Director | LYOHUB
Professor
THE COLLEGE OF PHARMACY, PURDUE
Chief Scientific Officer
THE NATIONAL INSTITUTE FOR BIOPROCESSING RESEARCH & TRAINING, DUBLIN

Jennifer Gray
Operations Manager
LYOHUB

LYOHUB SCIENTIFIC ADVISORY BOARD MEMBERS



Robin Bogner
Professor of Pharmaceutics
UNIVERSITY OF CONNECTICUT

Steven Nail
Principle Scientist
BAXTER BIOPHARMA
Adjunct Professor
PURDUE UNIVERSITY

Raj Suryanarayanan
Professor of Pharmaceutics
UNIVERSITY OF MINNESOTA

LYOhub.org

LyoHUB.org
linkedin.com/company/lyohub/
@lyohub
LyoHUB