LYO 10
hub.org
Celebrating 10 years
EST. 2014

Annual Report 2024

Advanced Lyophilization Technology Consortium

10 Years at a Glance

> *Centers like LyoHUB are a great example of how we draw upon Purdue's deep research strengths and state-of-the-art facilities, and leverage the expertise of industry and government to improve our world.*
>
> — **Karen Plaut,** Purdue's Executive Vice President of Research

$5M
Government/ Non-profit Funding

$5.5M
Industry Funding

11
Patents

825
LEARNERS
Lyo101 Online Short Course
Since 2020

Best Practice Papers

54,000+ Downloads

6 Papers

1
ASTM Standards

2
New LyoHUB Mobile Apps

35
LyoLaunchPad Projects

100+
Publications Referring to Lyohub

LyoHUB Demonstration Facility

480
Hands-on Lyo Trainees

25
Professional Masters Capstone Projects

22
Undergrad Researchers

$1M+
In-kind Equipment

1,786 Lyo Runs

87,000+ Hours of Lyo Run Time

Founders' Message — 2024

"First Who, Then What"

— Jim Collins, "Good to Great"

We are grateful to be able to share with you this 10th anniversary annual report, highlighting the trajectory to date and this year's activities at LyoHUB, an industry-university consortium dedicated to advancing pharmaceutical lyophilization technology. The collage on the cover reflects the essence of these 10 years of our combined collaborative efforts: first The Who, followed by a circle of The What.

The Who are the people that made LyoHUB the vibrant community that it is, gathering experts and practitioners from 30+ companies and many academic disciplines, whose experience, ideas, hard work and critical feedback led to LyoHUB's existence and accomplishments over the past 10 years. In the early days of LyoHUB, Mike Pikal and Steve Nail passionately and with good humor persuaded many of us to focus efforts on identifying and meeting industry needs. Fostering and developing new talent through education and training has been a particularly rewarding activity. We delight in serendipitously meeting some of the hundreds of students who were introduced to lyophilization through LyoHUB's Lyo101 online short course. We rejoice in the professional and personal strides of those students who grew up in the LyoHUB family. One of them is Nick Huls who had his first undergraduate research experience as a ChE sophomore in 2016, in what was then a sparsely populated LyoHUB facility. Nick has returned this

year in a research mentoring role after completing his PhD at the College of Pharmacy.

The What in LyoHUB is the expanding circle of opportunity areas for advancing lyophilization and related technologies. Areas such as new product modalities and new lyophilization technologies have been identified during this year's technology roadmap project supported by NIST. LyoHUB has had a significant influence on defining "what's best" in the current lyophilization practice through the development of best practices and technical standards in collaboration with ASTM. The technology roadmap has brought new momentum for LyoHUB to define and enable "what's next" in lyophilization. As we embark on the journey ahead, we expect that the rocket science of freeze-drying will enable fast, continuous and more energy efficient ways of making safe and effective lyophilized medicines—although that now appears to be a distant moonshot. We look forward to making it happen together in the next decade of LyoHUB!

With our gratitude and best wishes,
Alina Alexeenko and Liz Topp

Membership

IMA LIFE Aseptic Processing & 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	Simtra BioPharma Solutions Member Since 2016	ATS Life Sciences Systems Member Since 2016	Allergan Member Since 2016
abbvie Member Since 2016	Roche Member Since 2016	SIEMENS Member Since 2017	FRESENIUS KABI Member Since 2017
Bristol Myers Squibb™ Member Since 2017	OPTIMA EXCELLENCE IN PHARMA Member Since 2018	AMGEN Member Since 2018	Merck KGaA SERONO 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 Member Since 2021	FUJIFILM Member Since 2021
Elanco Member Since 2021	Jazz Pharmaceuticals Member Since 2021	Metrohm Member Since 2022	PSI Physical Sciences Inc. Member Since 2022
REGENERON Member Since 2022	West Member Since 2022	tempris® Member Since 2023	FLUID AIR Member Since 2023
nibrt Member Since 2024	SG Stevanato Group Member Since 2024		

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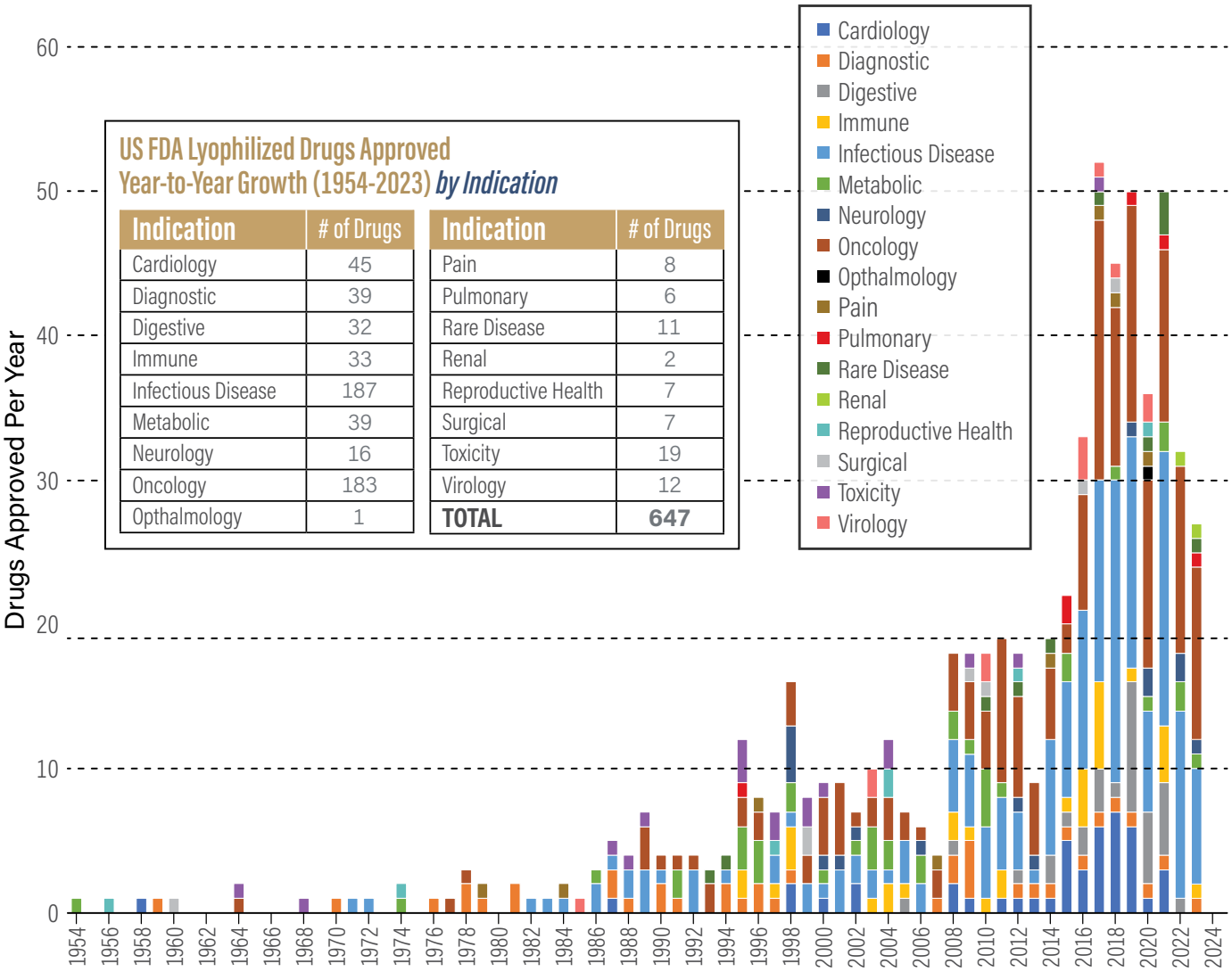
Lyo in Review 2023 *Historical and New Lyophilized Drug Products*

Yearly FDA-Approved Lyophilized Drugs *by Indication*

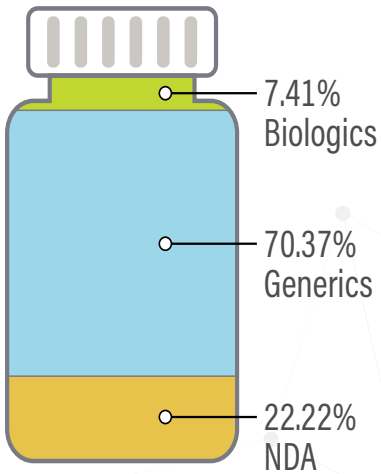


US FDA Lyophilized Drugs Approved Year-to-Year Growth (1954-2023) *by Indication*

Indication	# of Drugs	Indication	# of Drugs
Cardiology	45	Pain	8
Diagnostic	39	Pulmonary	6
Digestive	32	Rare Disease	11
Immune	33	Renal	2
Infectious Disease	187	Reproductive Health	7
Metabolic	39	Surgical	7
Neurology	16	Toxicity	19
Oncology	183	Virology	12
Opthalmology	1	**TOTAL**	**647**

Summary of 2023 FDA Drug Approvals

Twenty-seven lyophilized drugs were approved by the US FDA in 2023 submitted by a total of 22 pharmaceutical companies. This excludes drugs which are discontinued, distributed in a solution dosage form, or administered by inhalation, oral, spinal, and intrathecal routes. 92% of the approvals were small molecule drugs and the remaining 8% were enzymes. Among these approvals, oncology emerged as the dominant category by indication, accounting for ~44% of the total approved drugs. Following closely, infectious diseases represented the second largest category, comprising of ~30% of the approvals. The remaining categories—including diagnostic, rare disease, immune, pulmonary, metabolic, renal, and neurology—each constituted approximately 3.7% of the approved drugs.

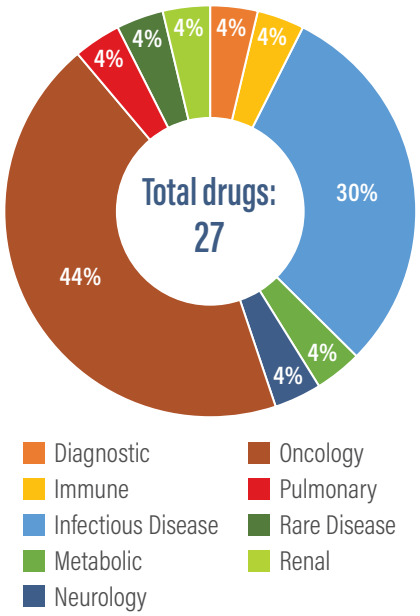


FDA-Approved Lyophilized Drugs in 2023 *by Company*

Company List (FDA)	# of Drugs
Alembic Pharmaceutical	1
Amicus Therapeutics	1
APOTEX	1
BioLineRx	1
BPI Labs	1
CHIESI Farmaceutici S.p.A.	1
Cidara Therapeutics	1
Delcath Systems	1
Entasis Therapeutics	1
Eugia Pharma	5
Gland Pharma Limited	1
Hainan Poly Pharm	1

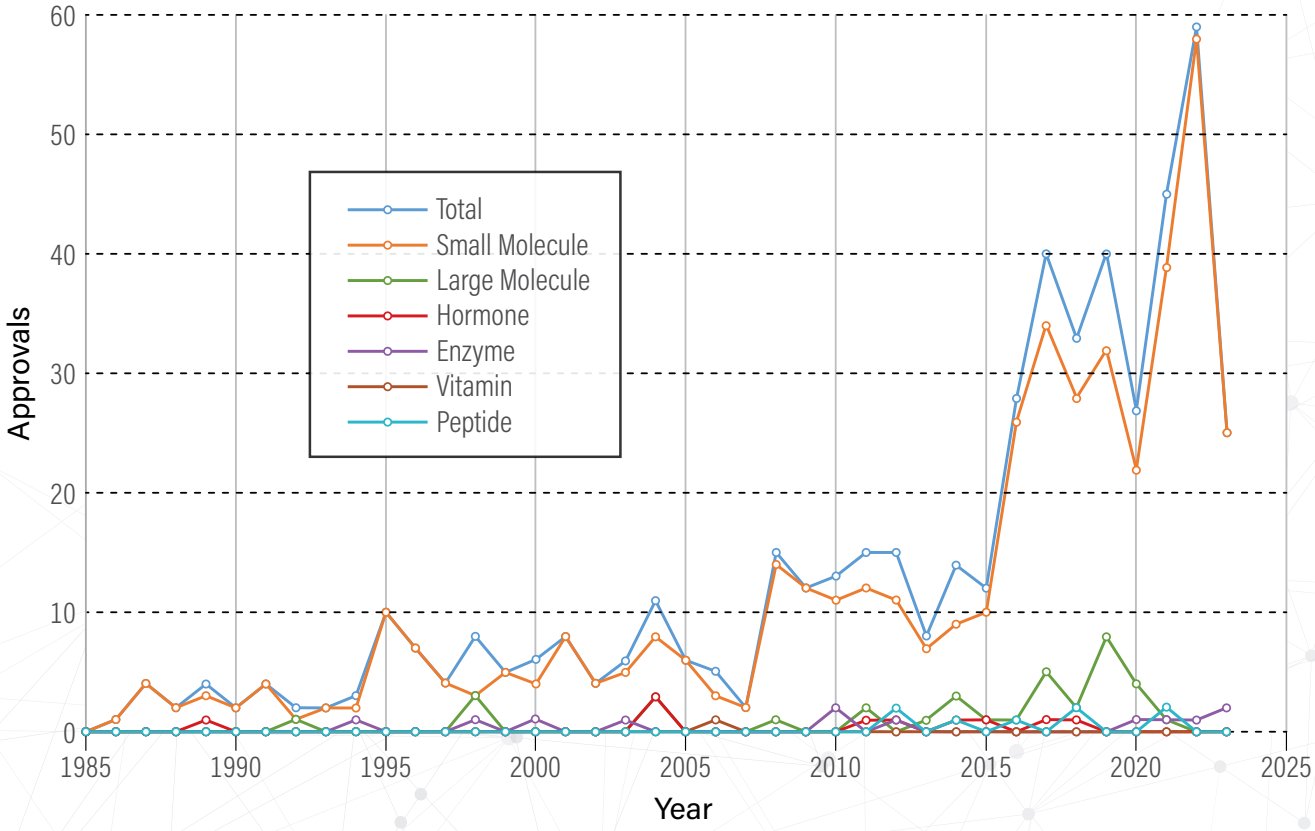
Company List (FDA)	# of Drugs
Hikma Pharmaceuticals	1
Jiangsu Hansoh Pharmaceutical Group	1
Jubilant Pharma	1
Jubilant Draximage	1
Kindos Pharmaceuticals	2
MSN Laboratories	1
Mylan	1
Shandong Pharmaceutical	1
Shandong Luye	1
UBI Pharma	1
TOTAL (22 companies)	**27**

FDA-Approved Lyophilized Drugs in 2023 *by Indication*



NDA/BLA Approvals *by Year and Molecule Type*

The prominence of lyophilized small molecule drugs has surged notably, with significant peaks observed in 2017 and 2023. In 2023, 92% of drug approvals for lyophilized formulations belonged to this category, emphasizing their enduring dominance and efficacy, while enzymes accounted for the remaining 8%. This trend suggests that small molecule drugs are expected to keep growing, highlighting their ongoing importance and lasting impact on pharmaceutical advancements and lyophilization.



Technology Roadmap

Driving future lyophilization efforts through collaborative engagement dominated the forefront of LyoHUB's efforts this past year, as 2023 brought with it an exciting and engaging series of workshops continuing the work begun in 2022 on the development of the NIST Lyophilization, Freeze-Thaw and Aseptic Drying Technology Roadmap for Pharma/Biotech Manufacturing.

February 2023 → Topical Roadmap in Washington DC at the National Academy of Science

PARTICIPANTS

36 Industry | 5 Government | 11 University

Subject matter experts worked in small groups to develop high-level outlines for 4 of the 5 outcome topics identified through trends and driver workshops held in 2022:

1. Lyophilization of New Product Modalities (e.g. mRNA LNP, Cell and Gene therapies)
2. Automation and Digitalization for Lyophilization
3. Freeze/Thaw Technologies
4. Emerging Drying Technologies

The objective was to develop "first-cut" roadmaps for review and discussion, and to agree on a set of priorities and a path forward. Two templates were used to guide this process: a project template and a requirements template.

These roadmaps were then used to guide next steps (e.g., proposal development, public-private partnerships) to achieve the defined impacts.

April 2023 → Topical Roadmap in Chicago: *Education/Workforce Training*

PARTICIPANTS

40 Industry | **13** University

Topical roadmaps focused on **EDUCATION** and **WORKFORCE TRAINING** were created for the following areas:

1. Lyophilization of New Product Modalities (e.g. mRNA LNP, Cell and Gene therapies)
2. Automation and Digitalization for Lyophilization
3. Freeze/Thaw Technologies
4. Emerging Drying Technologies

May 2023 → Synthesis Workshop

Essential information from the strategic landscape and the topic roadmaps were presented on a "key facts roadmap" for stakeholder engagement (both internally and with external stakeholders). Alina Alexeenko (Purdue), Ehab Moussa (AbbVie), Serguei Tchessalov (Pfizer), Steve Nail (Consultant), Steve Shade (Purdue).

July 2023 → Pfizer, Andover MA

Following synthesis workshop, the core team developed a companion set of key performance indicators (KPIs) in a single day workshop.

The following questions were explored during this workshop:

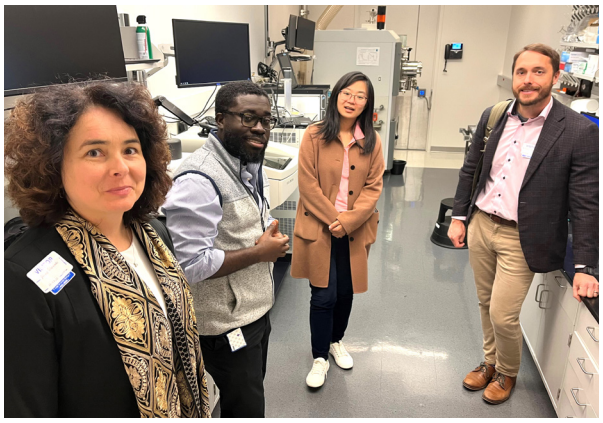
- Does this measurement support the vision and strategy?
- What does success look like?
- Will it be known if performance is trending toward/away from goals?
- What information is needed by stakeholders and sponsors and how will it be used?
- Is the measurement data available to the team?

Technology Roadmap *(Cont'd)*

From the wealth of input and information gathered during all workshops, this summary graphic was created and widely disseminated in order to ensure the Roadmap covers all applicable areas.

October 2023 → Presentations

Presentations were made at NIST and the FDA in order to further gather input and feedback.



2022-2023 → Virtual Participation

Several virtual workshops and individual calls took place throughout 2022 and 2023 which added the "voices" of nearly 2 dozen additional experts.

The following organizations were represented during the virtual workshops:

- AbbVie
- FDA
- Merck
- NIIMBL
- Pfizer
- Politecnico di Torino
- Purdue University
- Rhea Vita
- Sanofi
- Seran Bio
- University of Connecticut
- University of Minnesota

Lyophilization, Freeze-Thaw, and Aseptic Drying **Technology Roadmap** for Pharma/Biotech Manufacturing

Trends and Drivers

- Demographics *(aging/workforce/ chronic disease)*
- New Product Modalities
- Pricing Demographics
- AI/Digital/4.0
- Geopolitics *(onshoring/ anti-globalization)*
- Environment and Climate

Opportunity Areas	NEAR TERM 2024 - 2027	MID TERM 2028 - 2033	LONG TERM 2034 +
Lyophilization of New Product Modalities *(e.g., mRNA LNP, cell and gene therapies)*	AI use in drug formulation and process discovery	CQAs for new DP modalities and related new analytical tools Combination drugs Primary packaging technologies	
Automation and Digitalization for Lyophilization	Closed loop Virtual reality/ augmented reality Cloud/data management Cyber security	Digital twin	AI for manufacturing and operations
Freeze/Thaw Technologies	High fidelity modeling/digital twin	Systems: containers, novel freezing and thawing equipment PAT for freeze-thaw systems Investigating drugs coming off patent/generics Alternatives to freeze thaw (e.g., bulk dried material)	
Alternative Drying Systems Technologies	Aseptic spray drying Lyo bead and bulk products Reduced carbon footprint/green CQA monitoring/PAT Vacuum/foam drying Accelerated heat transfer: IR/microwave/spin	Continuous vial freeze-drying	

Enablers

- Transferable Credentials
- Shared Facilities
- Science and Standards
- Incentives for Tech Adoption
- Public-Private Partnerships

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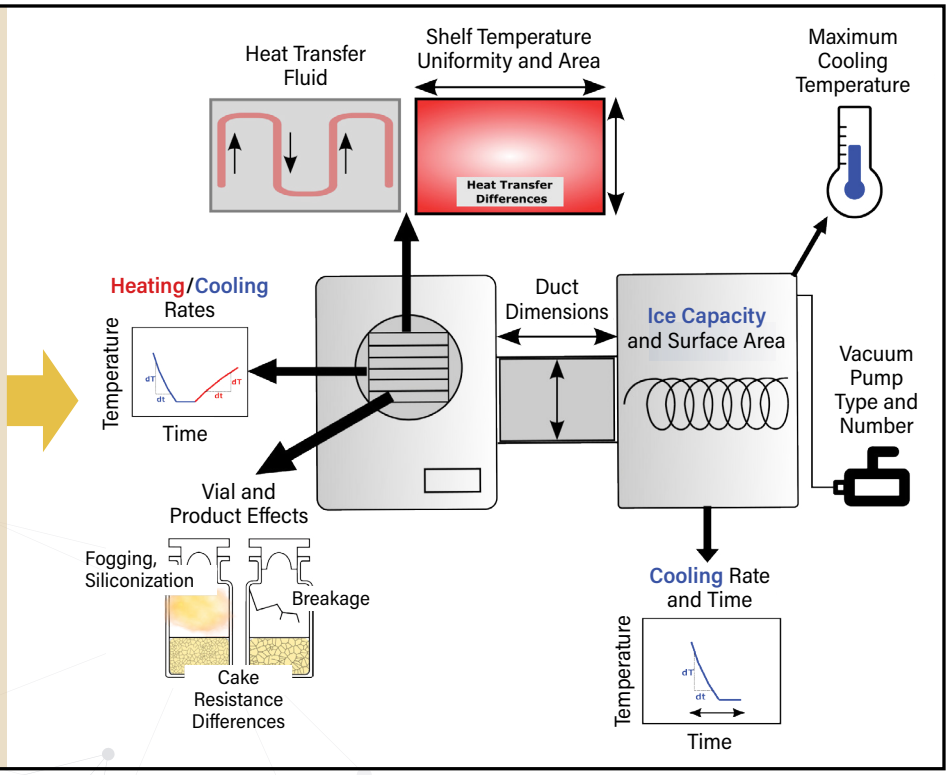
Best Practices

All LyoHUB best practice papers are available in open access. They can be downloaded by going directly to the links below or from the LyoHUB website.

LyoHUB Published Best Practice Papers (in order of publishing):

- *Process Monitoring Instrumentation in Pharmaceutical Freeze Drying (2017)*
  Number of Accesses to Date: **19,000+** (https://link.springer.com/article/10.1208/s12249-017-0733-1)
- *Recommended Best Practices for Lyophilization Validation 2021* — Part I: Process Design and Modeling
  Number of Accesses to Date: **10,000+** (https://link.springer.com/article/10.1208/s12249-021-02086-8)
- *Recommended Best Practices for Lyophilization Validation 2021* — Part II: Process Qualification and Continued Process Verification
  Number of Accesses to Date: **10,000+** (https://link.springer.com/content/pdf/10.1208/s12249-021-02107-6.pdf)
- *Best Practices and Guidelines (2022) for Scale-Up and Tech Transfer in Freeze Drying* — Part 1: Challenges during Scale Up and Tech Transfer *(This paper was developed in collaboration with BioPhorum [BPOG])*
  Number of Accesses to Date: **5,000+** (https://link.springer.com/article/10.1208/s12249-022-02463-x)
- *Recommended Best Practices for Equipment Performance Qualification* — Published in January, 2023
  Number of Accesses to Date: **5,000+** (https://link.springer.com/article/10.1208/s12249-023-02506-x)
- *Best Practices and Guidelines (2022) for Scale-up and Tech Transfer in Freeze Drying* — Part II: Past Practices, Current Best Practices, and Recommendations *(This paper was developed in collaboration with BioPhorum [BPOG])*
  Number of Accesses to Date: **5,000+** (https://doi.org/10.1208/s12249-023-02553-4)

- Freeze-drying is a complicated and non-uniform process.
- Knowledge of factors affecting the scale-up and transfer during freezing, primary and secondary drying states is essential.
- **Part 1** of this work presents the summary of such factors.
- Best practices and recommendation with practical test cases are demonstrated in Part 2.



Work also continues on →

- *Best Practices in the Development of Lyophilized Formulations, led by Dr. Elizabeth Topp (Purdue/ NIBRT) and Dr. Greg Sacha (Simtra BioPharma Solutions)*
  Paper 1: Excipients for Lyophilized Pharmaceuticals, **Paper 2:** Quality Assessment for Lyophilized Pharmaceuticals, **Paper 3:** Formulation of Lyophilized Pharmaceuticals

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
AMGEN

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

Jennifer Gray
Recording Secretary 2022-2024
E55 Executive Committee
PURDUE

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*. This **First Consensus Standard for Freeze Dried Pharmaceuticals** incorporates many of its findings from the LyoHUB *Recommended Best Practices for Pharmaceutical Freeze-Drying Process Instrumentation* Best Practice Paper. LyoHUB led the multi-year effort to deliberate the details of this first recognized consensus standard for pharmaceutical lyophilization and is in the process of working on several other ASTM standards.

Celebrating ASTM's 125th year at the Spring 2023 ASTM Committee week in Denver, CO

Special Presentations to LyoHUB

April 2023

- **Annual Meeting and Roadmapping at the Big Ten Conference Center in Chicago**

May 2023

- **Nate Hartman** | Dauch Family Professor of Advanced Manufacturing and Head of Computer Graphics Technology, Purdue University

  Data Management (Digital Thread) and AR/VR use in Manufacturing

June 2023

- **Frank DeMarco** | Freeze Drying Product Manager at IMA Life

  Eco-friendly, Air-based Refrigeration System for Freeze Drying Technology

July 2023

Presentations at Freeze Dry Conference, Breckenridge, Colorado

- **Drew Strongrich** | Research Scientist, LyoHUB

  Cloudy with a Chance of Freezing Rain: Vial Headspace Meteorology During Depressurization Controlled Ice Nucleation

- **Alina Alexeenko** | Co-Director, LyoHUB

  A Quantitative Study of Volatile Byproducts Generated During Primary and Secondary Drying using RGA

- **Alina Alexeenko** | Co-Director, LyoHUB

  LyoHUB Technology Roadmapping Project Summary

August 2023

- **Ashutosh Sharma** | Drug Product Technical Scientist, Biologics Technical Development at Horizon Therapeutics

  Innovative Drying Technologies for Biopharmaceuticals

  Work on this project was completed in collaboration with Sanofi and South East Technological University (SETU) Waterford, Ireland

October 2023

- **Serguei Tchessalov** | Pfizer
- **Alina Alexeenko** | Purdue University

  Progress on the Roadmap, Lyophilization, Freeze-Thaw and Aseptic Drying Technology Roadmap for Pharma/Biotech Manufacturing

November 2023

- **Jaume Vallet Xicoy** | Managing Director, COMSER
- **Pere Tapiolas** | Innovation Leader and Business Development, COMSER
- **Alfons Ubach** | Head of Business Development, COMSER

  Vapour flow monitoring in primary drying. The new PAT tool to complete and monitor lyophilisation process under QbD approach

December 2023

- **Jean-René Authelin** | Senior Scientific Advisor Global CMC, Sanofi

  Modeling Lyophilization Behavior using Delta P and Delta T Information: a free of charge PAT

January 2024

- **Gintaras V. (Rex) Reklaitis** | Burton and Kathryn Gedge Distinguished Professor of Chemical Engineering, Purdue

  New FDA draft guidance for the Advanced Manufacturing Designation Program

February 2024

- **Kurt Ristroph** | Assistant Professor, Agricultural and Biological Engineering, Purdue

  An Integrated Platform for Continuous RNA Nanoparticle Formulation and Drying

LyoHUB Working Groups:

Three new LyoHUB Working groups were started in 2023. These group are still in early stages, developing their project scopes:

- **Evaluation of Emerging Technologies:** Developing mechanisms by which to evaluate emerging technologies for application and adoption readiness.
- **Extractables and Leachables Study:** Container closure integrity is critical to long-term stability of freeze-dried materials. Contributing factors are largely unknown and/or uncharacterized.
- **Digital Twin for Lyophilization:** Life cycle process data for a given lyophilizer system can be used to generate valuable insights to: 1) identify performance issues, 2) develop possible improvements including in process simulation and design, and 3) inform digital twin.

LyoHUB Demonstration Facility *Through the Years*

The LyoHUB demonstration facility opened in 2016 on the second floor of Purdue's Birck Nanotechnology Center. The entire lab housed one piece of equipment, a REVO lyophilizer, donated by Millrock Technologies.

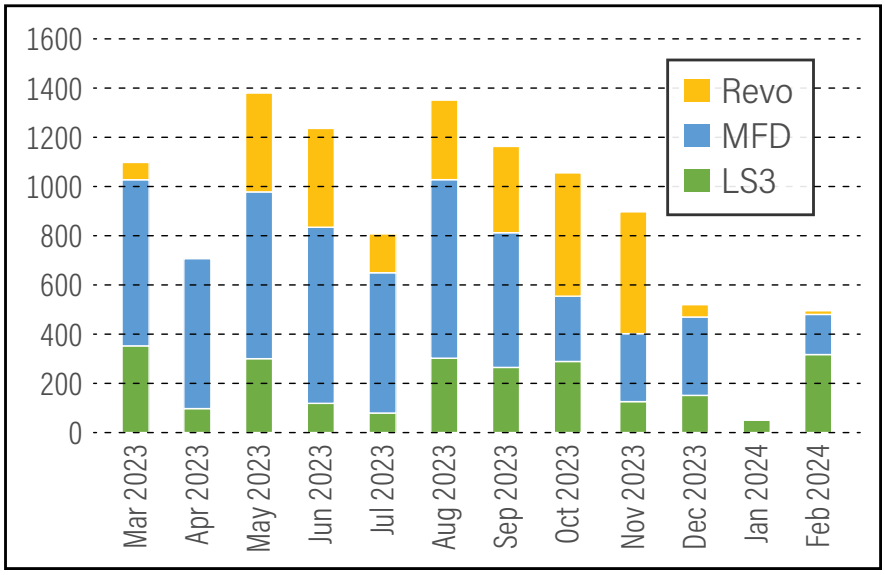
By 2019, the LyoHUB demonstration facility was home to three lyophilizers, adding a LyoStar 3, donated by ATS and a MicroFD (Millrock Technologies), as well as a freeze dry microscope donated by McCrone.

2020-2021 were marked by extra masks, floor spacers, and full face shields in response to the COVID-19 outbreak.

The LyoHUB demonstration facility of 2024 is home not only to lyophilizers and freeze drying microscopy, but a whole host of analytical tools including: residual gas analysis, mass flow metering, product moisture measurement, differential scanning calorimetry (DSC), vial headspace moisture analysis, as well as specialty equipment such as high-resolution cameras, 3D printers, a biosafety cabinet, and an infrared camera.

LyoHUB Demonstration Facility *SuperUsers and Lyo Use Data*

Summary of Lyophilizer Use (March 2023 – February 2024)



This chart summarizes the total time the machines were active on a monthly basis from 2023-2024.

Years	Total Number of Lyophilization Runs	Total Run Time
2016-2017	87	2971
2017-2018	178	9227
2018-2019	190	13944
2019-2020	421	16624
2020-2021	184	8505
2021-2022	217	10614
2022-2023	266	13542
2023-2024	243	11780

Superusers

Dr. Tony Cofer
Spacecraft Engineer

Dr. Ahmad Darwish
Electrical and Computer Engineering Postdoctoral Associate

Ian Flynn
LyoHUB Engineering Trainee
(now at Pfizer)

Dr. Petr Kazarin
Aeronautics and Astronautics Postdoctoral Associate
(now at IMA Life)

Evgeniia Vorozhbit
Aeronautics and Astronautics Graduate Research Assistant

Isaac Wheeler
Chemical Engineering Ph.D. Student

Dr. Kyu Yoon
Chemical Engineering Research Scientist

LyoHUB Software Developer

Nishchal Jagadeesha
Computer Science Graduate Student

LyoHUB Social Media and Website Coordinator

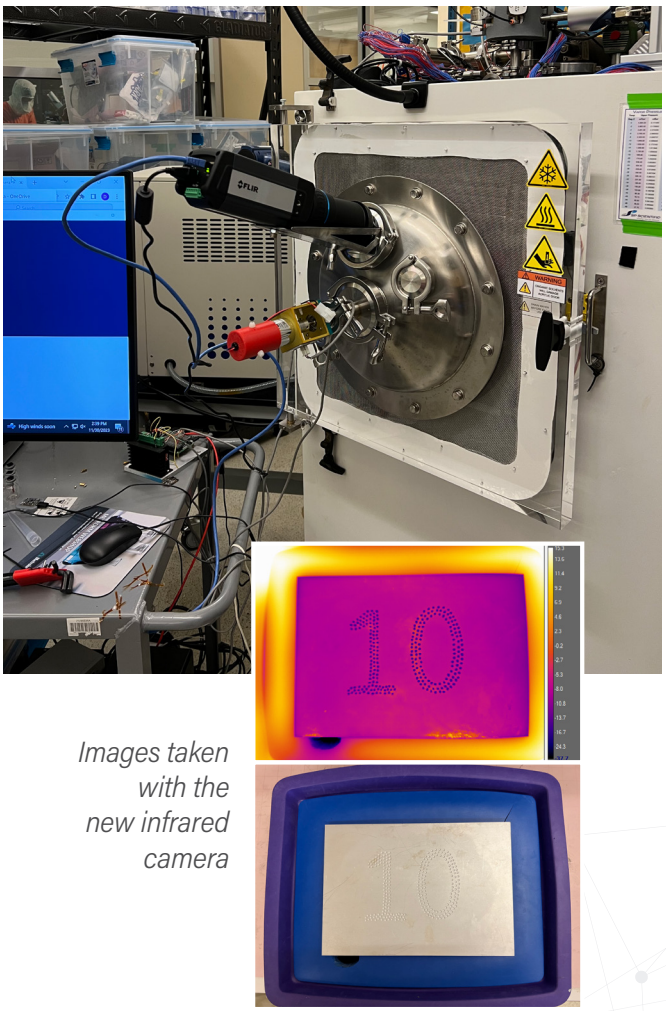
Shama Shankar
Marketing Graduate Student

LyoHUB Demonstration Facility *New Equipment 2023-2024*

Several new pieces of equipment were acquired by the LyoHUB Demonstration Facility in 2023

1. *Infrared Cameras*

The FLIR A655sc long wave infrared camera (LWIR) provides highly accurate and spatially resolved thermography measurements throughout the product chamber during the freeze-drying process. The device has a calibrated range of -40C to +150C with a sensitivity of +/- 0.03C over a spectral range of 7-14µm. A custom germanium viewport offers transmission in the infrared spectrum while simultaneously providing the necessary electromagnetic isolation when used with the microwave heating system.



Images taken with the new infrared camera

2. *Heating and Stirring Plate*

A new stir plate was purchased following decommissioning of an older unit. The device is equipped with an immersible RTD to provide accurate temperature regulation of sensitive materials during dissolution or staging.

3. *SLA 3D Printer*

The Form 3 printer from Formlabs compliments existing additive manufacturing tools in LyoHUB by offering the capability of producing small and highly intricate components (e.g., thermocouple fixtures). The system is compatible with a wide variety of resin materials, offering a high degree of design flexibility for a diverse range of projects with unique mechanical needs.

4. *Biosafety Cabinet*

A biosafety cabinet was acquired as part of NIIMBL PC4.1-307 to assess the compatibility of an attenuated live virus vaccine with LyoHUB's microwave freeze-drying system. The LyoHUB Demonstration Facility can now accommodate most BSL1 level materials for non-sterile testing.

5. *Blast Freezer (coming in Summer, 2024)*

The new blast freezing system extends the capabilities of the LyoHUB Demonstration Facility beyond traditional ramped shelf freezing. The benchtop unit will be used for new collaborative projects aimed at characterizing the effects of the high ice crystal growth rates that are known to have a significant impact on drying performance, morphology, and product efficacy.

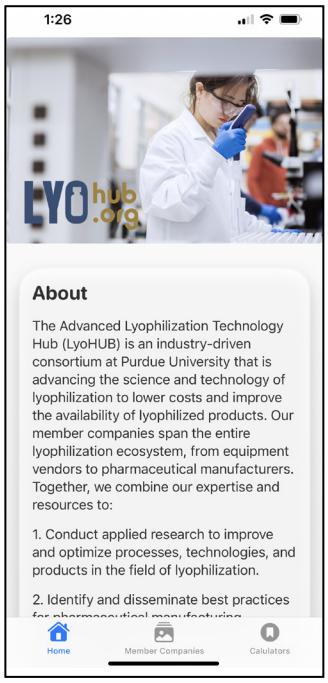
LyoHUB App Now Available for Apple & Android

2023 marked the release of the new LyoHUB app!

The app is available from the App Store (Apple) and Google Play (Android). The software is completely free to download to all iOS and Android devices and gives you instant access to information about the LyoHUB consortium, links to our open-access publications and standards, as well as a mobile version of our LyoPRONTO lyophilization process simulation tool, all in the palm of your hand.

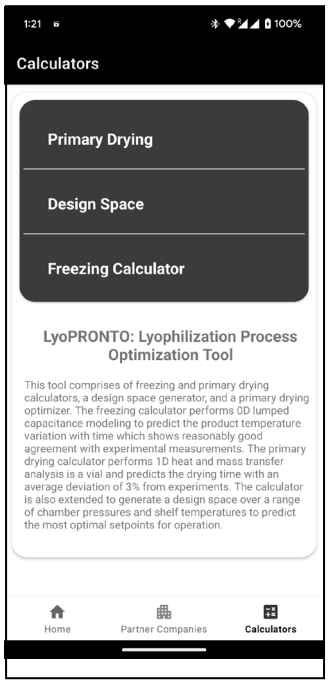
The mobile LyoPRONTO tool is a lightweight and streamlined version of our very own freeze-drying modeling suite. Modules include a primary drying calculator, freezing calculator, and design space analyzer. Input custom modeling parameters, visualize your results with interactive graphs, save inputs and outputs for future use, and share data directly from the app with peers or colleagues via email, WhatsApp, AirDrop, or text message. Download it to start mastering your freeze-drying workflow, "pronto"!

LyoPRONTO Home Screen (APPLE)



Get the **Apple** LyoHUB app here →

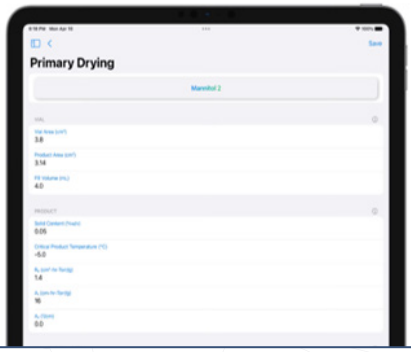
LyoPRONTO Calculator (ANDROID)



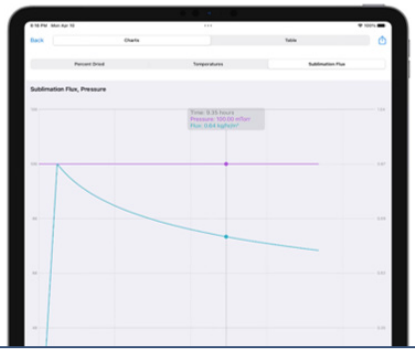
Get the **Android** LyoHUB app here →

APPLE Version View

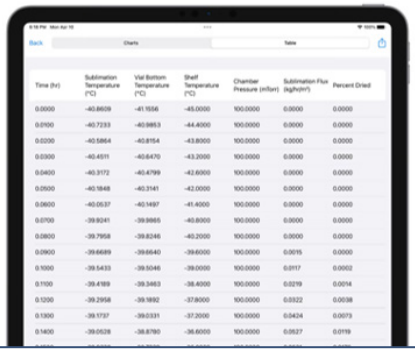
Three Calculators: Primary Design, Design Space, and Freezing



Visualize Your Results: Multi-Chart Visualizations

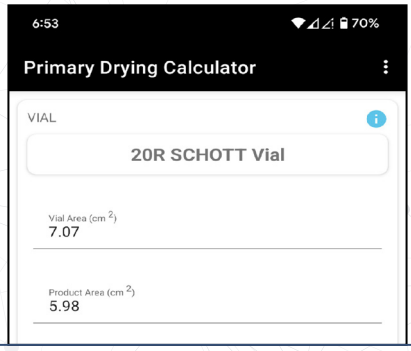


Summarize Your Results: Comprehensive Tabular Outputs

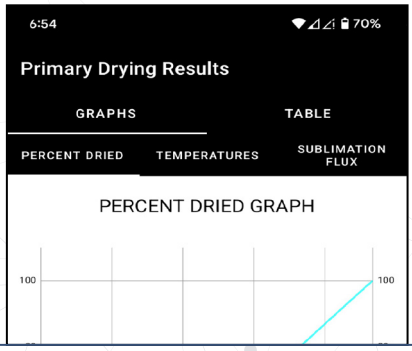


ANDROID Version View

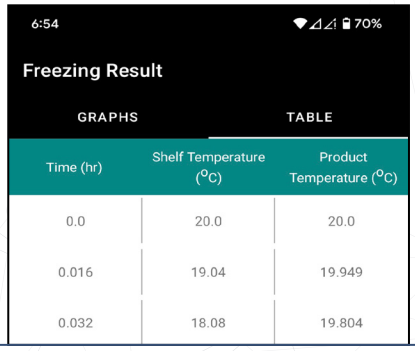
Three Calculators: Primary Design, Design Space, and Freezing



Visualize Your Results: Multi-Chart Visualizations



Summarize Your Results: Comprehensive Tabular Outputs



Education & Outreach

Online Lyophilization Short Course

Online course continues to provide an excellent introduction to lyophilization, supported by a grant from NIIMBL.

Available on LyoHUB Website:

- Open Source
- Free of Charge

Featuring eight online lyophilization 101 learning modules, together with assessment tools and instructions for a virtual laboratory exercise.

https://pharmahub.org/courses/lyo101

Education starts early in LyoHUB!

Teaching students about lyophilization for the Nano Expo at Birck Nanotechnology Center.

Coming in 2024: *LyoHUB Short Course on Pharmaceutical Freeze Drying*

Steve Nail and Drew Strongrich have developed a new course: **"Freeze Drying: Principles and Practice"** to be offered through Purdue Online beginning in Summer 2024. The project began following completion of the highly successful LyoHUB summer school hosted in 2021. The introductory short course is composed of a series of lectures, quizzes, and hands-on practical exercises designed to familiarize students and professionals with the world of pharmaceutical freeze-drying. Following completion of the course, students will be able to:

1. Identify and describe the three fundamental phases of the freeze-drying process.
2. Outline key unit operations, processes, equipment, and process monitoring methods associated with the lyophilization of parenteral products.
3. Describe product critical quality attributes, how they are measured, and the roles they play in short and long-term stability.
4. Provide a broad overview of an approach to developing and optimizing a freeze-drying process, including the use of mathematical models for common formulations and containers.
5. Discuss common deficiencies in the development and manufacturing of freeze-dried pharmaceuticals and provide information on current best-practices in industry.

For course details and enrollment information, visit http://www.eventreg.purdue.edu/online/lyophilization

COMING SOON

Welcome and Introduction Video

In this video, you will meet Dr. Strongrich and Dr. Nail as well as learn more about the course.

2023 Purdue Professional Master's Chemical Engineering Program

Summer Capstone Projects with LyoHUB Members

Capstone Project: Evaluation of Strain Sensor to Assess Phase Transitions and Mechanical Stresses During Freeze-Drying of Pharmaceutical Solutions

Investigators: *Abhishek Naik (Purdue ChemE graduate student), Evgenyi Shalaev (AbbVie), Serguei Tchessalov (Pfizer), Bakul Bhatnagar (Pfizer)*

Objective: Investigate the application of strain sensors in understanding and quantifying the effects of phase transitions and mechanical stresses in freeze-drying systems.

Conclusion: The sensor voltage generates the strain gauge readings which are vial temperature and chip temperature dependent. Compensating the vial temperature and chip temperature from the sensor readings is essential to determine accurate strain count values. The chip temperature is highly sensitive to the change in gain values of the sensor; and is affected more severely at lower gain values of 223. The method for controlled ice nucleation also plays a major role in determining the strain profile either with the jet spray technique or the rapid depressurization technique. The size of the ice particles in the vial generates equivalent hoop strain on the glass vials which is determined by the technique used for the controlled ice nucleation. If there is some doubt regarding heat transfer from within the vials, then ethanol-filled vials can act as a baseline instead of empty vials which should show near zero strain. The sensor, the gauge, the thermocouple and the RTD are the mechanical components associated with these freeze thaw experiments. If any one of these components undergoes breakdown it causes inaccurate data generation.

Capstone Project: Study of Biological Formulations and Constructing a Design Space for a Sterile Injectable Placebo

Investigators: *Sanket Borad (Purdue ChemE graduate student), Jun Xu (Merck)*

Objective: This project examined the different excipients and carriers utilized in formulations, including their compatibility, stability, and safety characteristics. Furthermore, properties, such as pH, conductivity, surface tension, osmolality, and viscosity, were reviewed to see if the placebo closely mimicked the active medicine in appearance and handling.

Conclusion: The investigation, which included experimental analysis and a review of the literature on various formulations and additives, provided valuable insights into the characteristics of biological fluids. The efficacy of various components were classified, to provide researchers with information to use when creating formulations with regulated features for specific uses. The comparison of the "Final Formulation" with the "Citric acid Buffer" revealed no significant variations in osmolality, surface tension, conductivity, or viscosity. The osmolality of the "Final Formulation" was the same. Both solutions had similar conductivities but slightly higher viscosities at different temperatures, indicating potential differences in flow behavior compared to the "Citric acid Buffer." Experiment results aided knowledge of the interactions between various components and their qualities in the context of formulation design. Combining literature analysis and experimental data has broadened the information base available to researchers working on new biological formulations.

Capstone Project: Experimental Study of Freezing Induced Concentration Gradients in a Container

Investigators: *Jigar Navadiya (Purdue ChemE graduate student), Jie Wang (AbbVie)*

Objective: To study the freezing effect on storing the protein solution, and explore the study of protein instability due to progressive freeze concentration.

Conclusion: To keep protein intact, freezing should be conducted in a controlled manner with a stable freezing rate, constant ramp rate, and unidirectional freezing. These help to lower the concentration aggregation during freezing which then reduces the risk of unfolding and structural damages. The controlled freezing environment also brings the solution to its supercool stage and minimizes the resistance for the solute to flow. Due to this, uniform concentration can be obtained throughout the volume. As the temperature goes lower, the homogeneity increases because at very low temperatures, the time required to freeze the solution is less and the freezing rate is high which helps to form small crystals compared to large ones in the case of low freezing rate. As a result, the chances of degradation of material also reduce, however, maintaining the very low temperature below -40°C is expensive, so the optimum temperature and freezing conditions are needed to keep material properties unchanged.

Website Resources & Training

LYO101 COURSE

Open Access Online Introduction to Lyophilization Course

Free

https://pharmahub.org/courses/lyo101

Current enrollment: Over 800

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
- LyoHUB Excipient Database: https://pharmahub.org/resources/lyodrugdatabase

LYOHUB APP

Get the Apple LyoHUB app here

Get the Android LyoHUB app here

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
- "Best Practices and Guidelines (2022) for Scale-Up and Tech Transfer in Freeze Drying — Part 1: Challenges during Scale Up and Transfer" *(This paper was developed in collaboration with BioPhorum):* https://link.springer.com/article/10.1208/s12249-022-02463-x
- "Recommended Best Practices for Equipment Performance Qualification": https://link.springer.com/article/10.1208/s12249-023-02506-x
- "Best Practices and Guidelines (2022) for Scale-up and Tech Transfer in Freeze Drying — Part II: Past Practices, Current Best Practices, and Recommendations": https://doi.org/10.1208/s12249-023-02553-4

New Users Trained on Lyophilization Equipment from March 2023–2024

Jeongyeon Cho | Purdue ChemE

Shruti Irap | Purdue ChemE

Chanakya Patil | Purdue IMPH

Vaibhav Pathak | Purdue IMPH

Chun Yuan (Phil) Kung | Purdue ChemE

Sai Bhusurapalli | Purdue ChemE

Marilyn Padua | Purdue ChemE

Wheeler Isaac Stonewall | Purdue ChemE

Vorozhbit Evgeniia | Purdue Aero/Astro

Erik Lopez | Purdue Aero/Astro

Jesus Meza Galvan | Purdue Aero/Astro

Victor Saca | Purdue Pharmaceutical Sciences

Grants & Collaborations

Advanced Characterization and Manufacturing Methods for mRNA Vaccine Development

NIIMBL

- 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 involved constructing/developing mRNA LNP formulations through available historical scientific literature sources then using these mRNA LNPs to produce frozen, lyophilized, and spray dried formulations which were characterized for drug product stability.
- **Investigators:** *Eric Munson (PI, Purdue IMPH), Alina Alexeenko (Co-PI, Purdue ChemE/AAE), Elizabeth Topp (Co-PI, Purdue IMPH/ChemE), Tony Zhou (Co-PI, Purdue IMPH)*

Lyophilization and Aseptic Drying Technology Roadmap for Biotechnology and Pharmaceutical Manufacturing

NIST

- Funded by the U.S. Department of Commerce's National Institute of Standards and Technology (NIST)
- $296,000 over 18 months
- **Goal:** This project expands the existing LyoHUB 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/IMPH), Steve Shade (Purdue/EEE), Raj Suryanarayanan (University of Minnesota), Serguei Tchessalov (Pfizer), Elizabeth Topp (Co-PI, Purdue IMPH/ChemE), Tony Zhou (Co-PI, Purdue IPPH)*

Tunable RF/Microwave Drying of Biologics

NIIMBL MERCK IMA LIFE

- Funded by NIIMBL (National Institute for Innovation in Manufacturing Biopharmaceuticals)
- $1.9M over 18 months
- **Goal:** The RF/Microwave drying offers significantly increased throughput for manufacturing of vaccines and biopharmaceuticals with more than 3x speed up for tunable power input in batch mode. The technology is compatible with the existing lyophilization equipment and amenable to integration as a heating method for novel bulk drying methods such as bulk dynamic spray freeze-drying and other spray drying methods as well as Lyosphere drying technology by Merck. It provides contactless, efficient, and highly controllable heat transfer. The cost of components for the solid-state RF/microwave power sources ($50-75k) is about the same as a weekly cost of operation of production scale lyophilizer and is a fraction of the cost of a conventional laboratory-scale lyophilizer leading to a ROI within a year.

Work Continues on these LyoHUB Sponsored Projects:

- Strain Gauges
- Excel-based Calculator for Secondary Drying

Industry Sponsored Projects for 2023-2024:

- Rapid Depressurization Controlled Ice Nucleation Phase 2 (Genentech)
- Solid-State Stability of mRNA Vaccines (Pfizer)

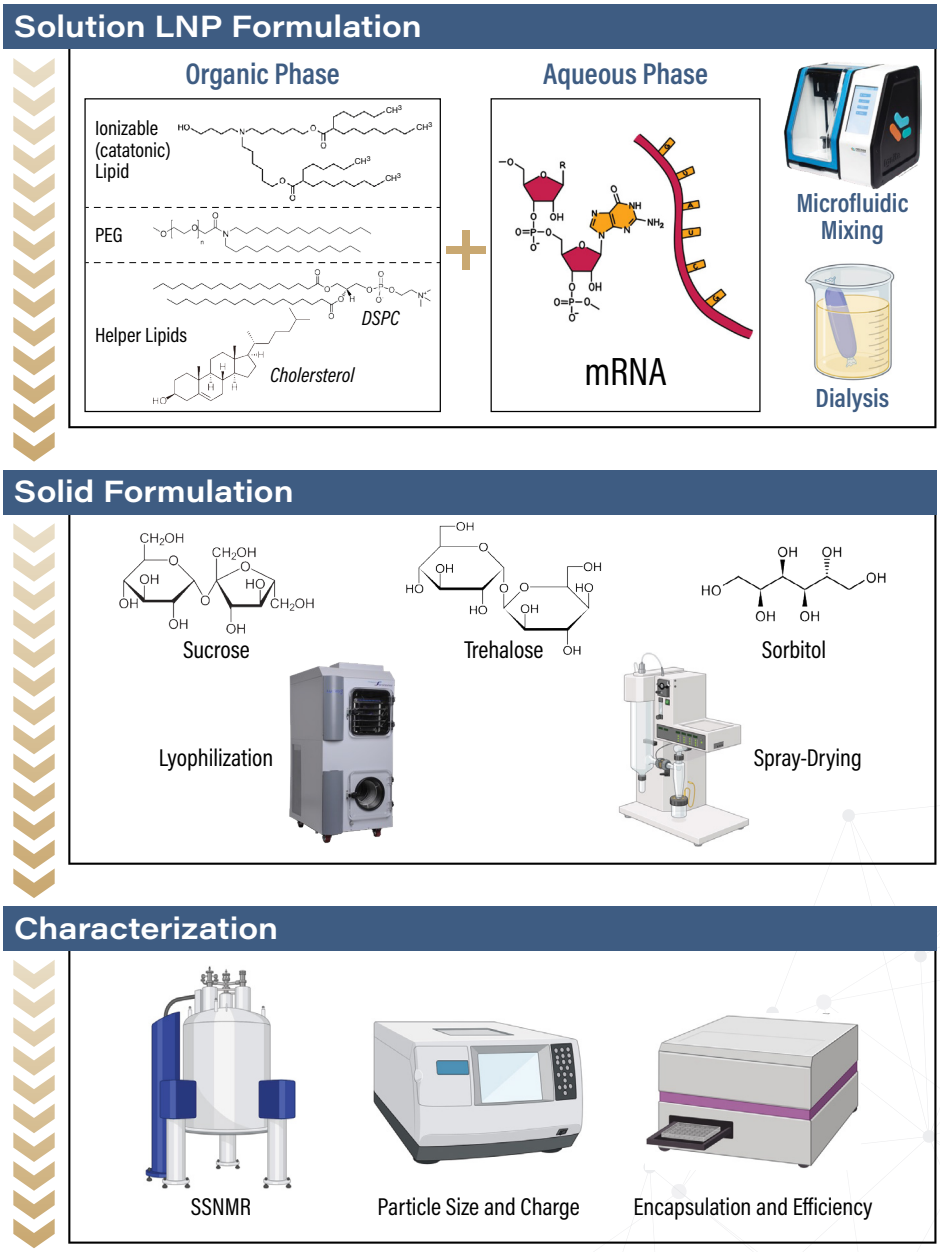
Advanced Characterization and Manufacturing Methods for mRNA Vaccine Development

Funded by:
NIIMBL (National Institute for Innovation in Manufacturing Biopharmaceuticals) as part of the American Rescue Plan

Completed in 2023

Investigators: *Eric Munson (PI, Purdue IMPH), Alina Alexeenko (Co-PI, Purdue ChemE/AAE), Elizabeth Topp (Co-PI, Purdue IMPH), Tony Zhou (Co-PI, Purdue IMPH)*

Current mRNA/LNP vaccines are solutions which must be stored at -80 °C to remain stable. Refrigeration accounts for a large portion of the costs associated with vaccine shipment and storage. In this project, formulation and manufacturing methods for solid room-temperature stable vaccines were explored.



This project explored advanced analytical and manufacturing methods which can be implemented rapidly and used to quickly develop next-generation vaccines with improved manufacturing and stability performance. Various formulation excipients were explored to understand their impact on stability. New analytical approaches, such as solid-state nuclear magnetic resonance (SSNMR) spectroscopy were used to understand the structural and molecular nature of mRNA LNP complexes. Lyophilization, spray drying and spray freeze drying were evaluated as manufacturing methods for mRNA vaccines. This was significant as neither lyophilization nor spray drying have been thoroughly developed and characterized for manufacturing mRNA formulations.

It was shown that freeze dried formulations using 5% (w/v) or 10% (w/v) sucrose stored at room temperature maintained better integrity when compared to solution formulations stored at the same temperature. Spray drying and spray-freeze drying techniques were evaluated but did not produce viable mRNA/ LNP vaccines. Advanced characterization methods provided insightful information about the structure of mRNA LNP vaccines in the solid state.

Random Field Radio Frequency Lyophilization

Investigators: *Alina Alexeenko (PI, Purdue), Ahmad Darwish (Purdue), Drew Strongrich (Purdue), Petr Kazarin (IMA Life), Chanakya Patil (Purdue), Cole Tower (Purdue), Isaac Wheeler (Purdue), Eric Munson (Co-PI, Purdue), Qi Zhou (Co-PI, Purdue), Vivek Narsimhan (Co-PI, Purdue), Kyu Yoon (Purdue), Steven L. Nail (Consultant), Anthony Cofer (Purdue), Justin Stanbro (IMA Life), Harshil Renawala (Merck), Daniel Roth (Merck), Francis DeMarco (IMA Life), Justin Griffiths (IMA Life), and Dimitrios Peroulis (Co-PI, Purdue)*

There has recently been a surge in the demand for lyophilized injectable products. The rapidly expanding portfolio of new biologics, particularly in the aftermath of the COVID-19 pandemic, highlighted the challenges associated with freeze-drying since such a process is very time-consuming, taking anywhere from days to weeks. Toward that end, this random field radio frequency lyophilization system addresses these shortcomings by applying highly controllable volumetric heating capable of accelerating freeze-drying processes and improving batch homogeneity while retaining products' physical and chemical properties. **Figure 1** shows the current experimental setup and a block diagram of the proposed lyophilization system, enabling both open-loop and closed-loop (highlighted in green) lyophilization processes.

Figure 2 summarizes the primary drying time of different formulations to verify the effectiveness of the proposed system. The primary drying time is proportional to the applied RF power. Such behavior is attributed to the increased electric field intensity (E) inside the chamber, allowing for an increased absorbed power density by the products.

Additionally, batch homogeneity, measured through residual moisture content, is improved using the proposed microwave system. To get batch homogeneity comparable to that obtained with the proposed system, secondary drying, which increases the total drying time, is needed in conventional freeze drying.

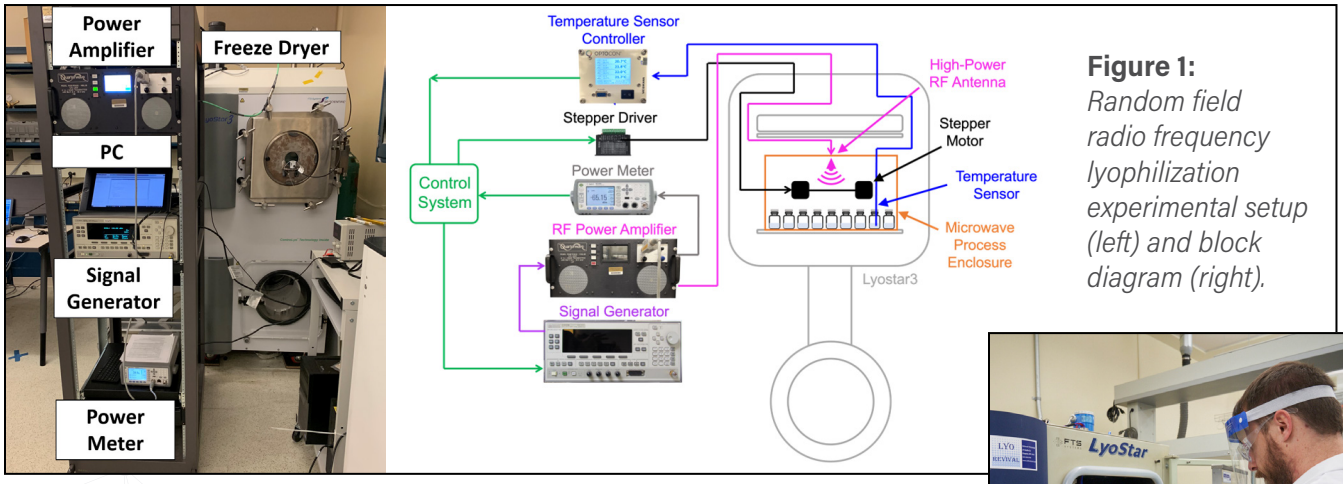


Figure 1: *Random field radio frequency lyophilization experimental setup (left) and block diagram (right).*

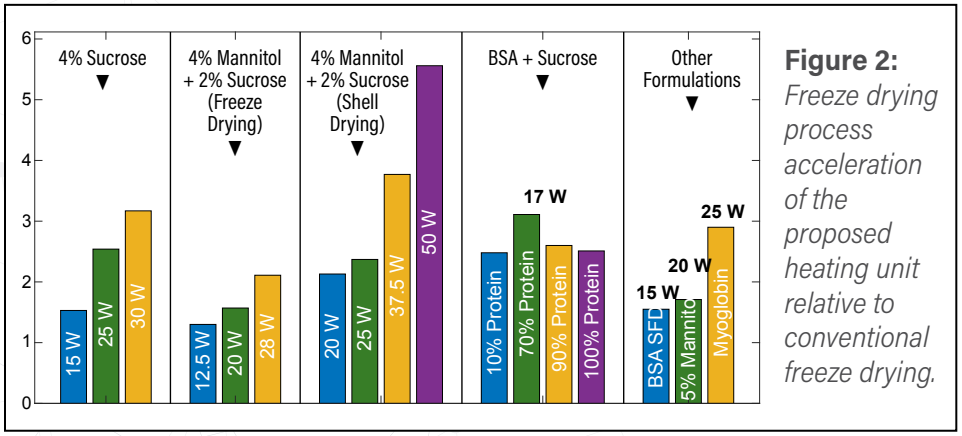
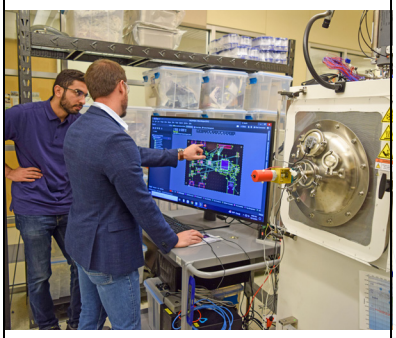


Figure 2: *Freeze drying process acceleration of the proposed heating unit relative to conventional freeze drying.*

FD Process	Avg Moisture Content (%)	Std of Moisture Content
Conventional (without SD)	7.41	0.55
Conventional (with SD)	0.19	0.13
RF (without SD)	0.84	0.15

Table 1: *Summary of residual moisture content for 5% BSA cycles.*



Top photo: *2021 RF set up*
Bottom photo: *2023 RF set up*

Simulating Sublimation Front Shapes in Microwave-Assisted Freeze Drying

Investigators: *Isaac Wheeler (Purdue ChemE Ph.D. student), Alina Alexeenko (PI, Purdue), Dimitrios Peroulis (Purdue Electrical and Computer Engineering), Ahmad Darwish (Purdue Electrical and Computer Engineering), Vivek Narsimhan (Purdue ChemE), Drew Strongrich (Purdue LyoHUB), and Petr Kazarin (Purdue AAE, now at IMA Life)*

Microwave-assisted freeze drying is an exciting new method of accelerating the primary drying stage of lyophilization. One advantage of the technology is the ability to bypass the container and heat the frozen product directly, since electromagnetic heating is applied throughout the chamber volume (rather than from a surface, e.g. the top of a shelf). However, as everyday experience with microwave ovens would suggest, glass vials and similar containers may also be heated by the microwave field. This is schematically illustrated in **Figure 1**.

As with conventional lyophilization, models for the heat transfer in MW-assisted lyophilization aid in ensuring product temperatures stay below appropriate limits while accelerating the process. 1D pseudosteady vial-scale models (as typically applied in e.g. LyoPRONTO) are attractive for their simplicity, but assume that there are no temperature gradients in the radial direction (i.e., from vial wall to product center). Experimental measurements indicate that for some microwave-assisted drying conditions, vial walls may be tens of degrees hotter than frozen product, which violates the assumptions necessary for a 1D model. To assess the impact of this effect, a 2D axisymmetric pseudosteady simulation has been implemented in the Julia programming language. This simulation uses the level set method to represent the sublimation front, which allows arbitrary front behavior (including separation from the wall or the vial bottom). Simulations already indicate that for some sets of parameters, the assumption of a planar sublimation front in microwave-assisted lyophilization is reasonable, but not for all process conditions, as in the case shown in **Figure 2** where the front curves noticeably. Future work will establish the delineation in this behavior.

With this simulation method, a variety of other physical effects can also be explored. One is the variation in temperature readings as a function of thermocouple placement in the vial; another is the shape of the sublimation front due to a spatially-varying mass transfer resistance Rp from different pore sizes in the cake (the latter as can be imaged with microCT scanning techniques). The simulation code itself will be published as open source, allowing others to explore these physics in more detail.

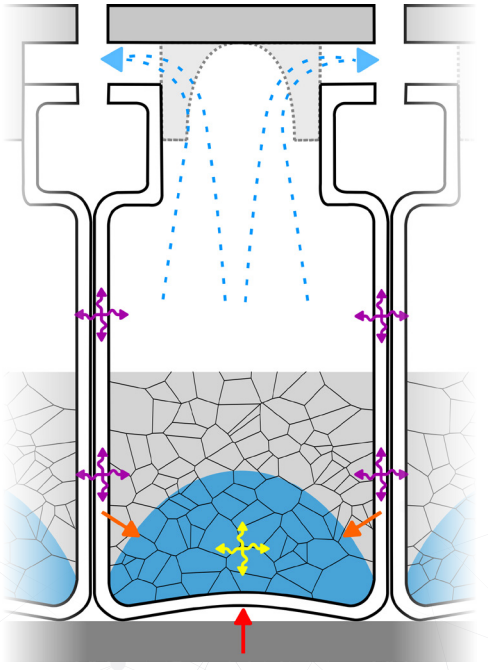


Figure 1: *Schematic of the heat transfer behavior in microwave-assisted lyophilization*

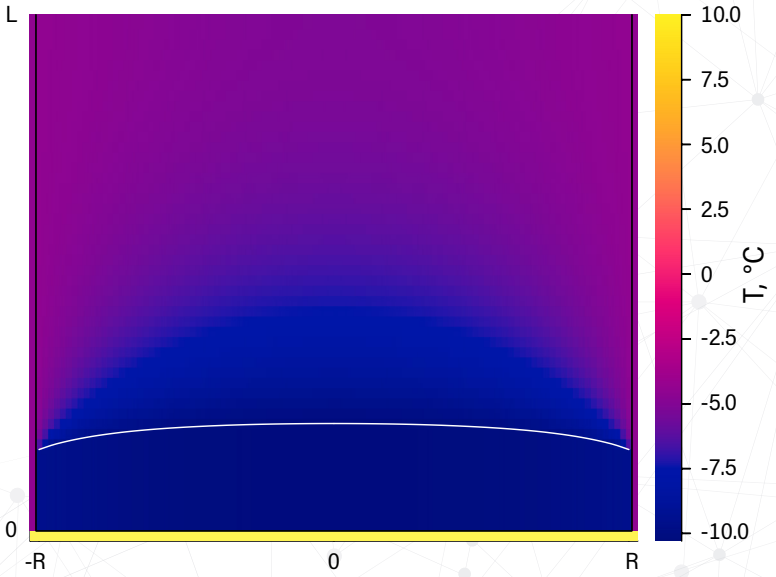


Figure 2: *Simulated temperature throughout the product near the end of primary drying. White line is the sublimation front and the color represents temperature (including shelf and vial wall temperatures), with the ice being the coldest region.*

Empirical Correlation for Predicting Equilibrium Moisture Content in Sugar/Protein Mixtures During Freeze-Drying

Investigators: *Kyu Yoon (Purdue ChemE Research Scientist) and Vivek Narsimhan (Purdue ChemE)*

Predicting the equilibrium moisture content of a dried cake during freeze-drying is essential for understanding the desorption kinetics of bound water and ensuring the final product's quality and stability. The weighted-average model [1] is frequently used for mixture formulations; however, this correlation often falls short in complex systems, such as sugar/protein mixtures, due to interactions between sugar and water monolayer sites on dried proteins [2,3].

The Sucrose/BSA mixture is used as a model formulation, focusing on the protein fraction range between 0 to 0.7. Experimental data, shown in **Figure 1**, shows the same trend as in literature [2,3]: the moisture content at the end of the freeze-drying cycle is not a static figure, but is subject to dynamic changes influenced by the protein composition of the mixture and the temperature conditions during the freeze-drying process. This observation indicates that moisture content may need to be expressed differently than previously acknowledged by linear correlations.

Using scaled moisture content data converted from Figure 1, an empirical correlation is introduced to more accurately predict the equilibrium moisture content for different protein fractions and temperatures. This method has been validated by extensive freeze-drying experiments conducted on a variety of protein fractions at different secondary drying temperatures, using a laboratory-scale lyophilizer. **Figure 2** presents comparisons between measured equilibrium moisture content and model-predicted values. The input for the model is moisture content data of pure-components (BSA or Sucrose). The results underscore the limitations of using linear models and the necessity for an empirical approach in predicting the equilibrium moisture content of the freeze-dried mixture formulations. The equilibrium moisture content measured at two different temperatures supports the notion that it is not merely a function of temperature but also of the specific interactions within the protein/sugar matrix. This study lays the groundwork for a more refined understanding of moisture dynamics in freeze-drying and opens up avenues for further investigation into the molecular interactions at play in the lyophilization of complex mixtures.

References:

[1] Lang, K.W. & Steinberg, M.P. (1980) Calculation of moisture content of a formulated food system to any given water activity, J. Food Sci. 45(5), 1228-1230.

[2] Wang, B., Tchessalov, S., Cicerone M.T., Warne N.W. & Pikal, M.J. (2009) Impact of sucrose level on storage stability of proteins in freeze-dried solids: II. Correlation of aggregation rate with protein structure and molecular mobility, J. Pharm. Sci., 98(9), 3145-3166.

[3] Costantino, H.R., Curley, J.G., Wu, S. & Hsu C.C. (1998) Water sorption behavior of lyophilized protein–sugar systems and implications for solid-state interactions, Int. J. Pharm. 166(2), 211–221.

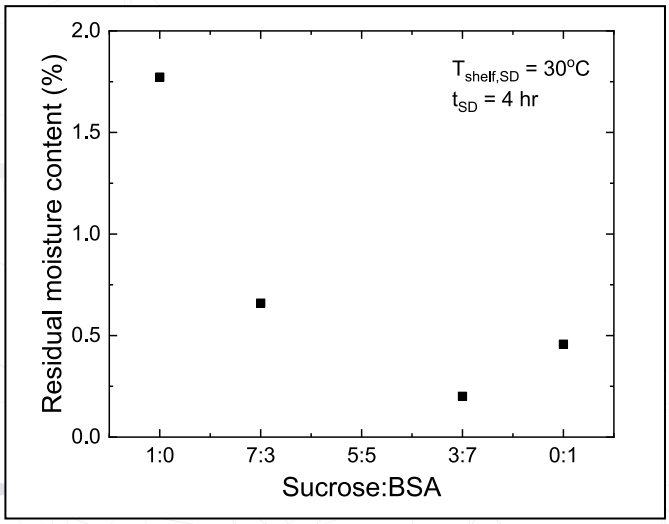


Figure 1: *Residual moisture content (t_{SD} = 4hr) of sucrose/BSA mixtures for different mass ratios of sucrose:BSA.*

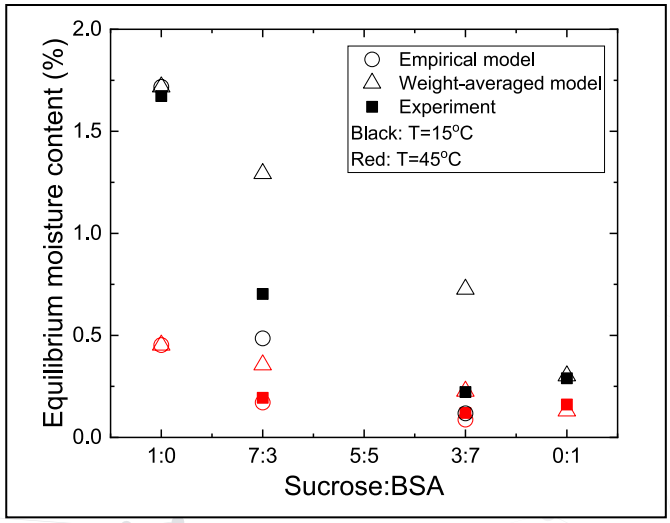


Figure 2: *Measured equilibrium moisture content data versus predicted values using an empirical model (developed in LyoHUB) and a weight-averaged model (linear correlation). Data shows different ratios of sucrose:BSA.*

Mechanical Characteristics of Vial Strain During Freezing and Thawing Operations Using Amorphous Excipients

Investigators: *Ian Flynn (Purdue LyoHUB), Drew Strongrich (Purdue LyoHUB), Serguei Tchessalov (Pfizer), Bakul Bhatnagar (Pfizer), and Evgenyi Shalaev (Abbvie)*

Wireless strain sensors developed in LyoHUB are being evaluated for their unique ability to detect mechanical stresses and strains in primary packaging during freezing, thawing, and freeze drying. Prior research in the LyoHUB Demonstration Facility has identified key differences in strain response for amorphous excipients such as sucrose and trehalose at different concentrations. For example, the temperature and strain profiles of sucrose at concentrations from 5-80% are shown in **Figure 1**. Recent studies are focused on leveraging computational modeling to understand the implication of these differences in strain behavior.

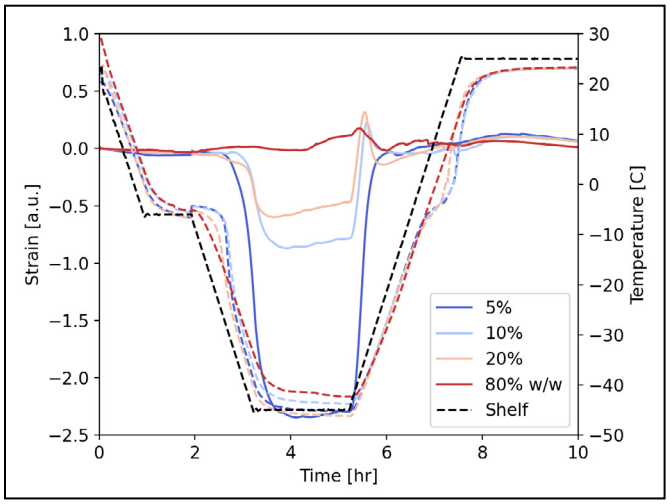


Figure 1: *Measured temperature and strain during freezing and thawing of sucrose at different concentrations.*

A finite element model of the vial was developed to better understand the relationship between principal strain components. The model was verified using an experimental apparatus that allowed the vials to be pressurized (uniformly) with a gas. Model results for a pressure of 50 psig are shown in **Figure 2**. These results were used to understand the vial response to a load as well as identify the appropriate locations for mounting the strain gages.

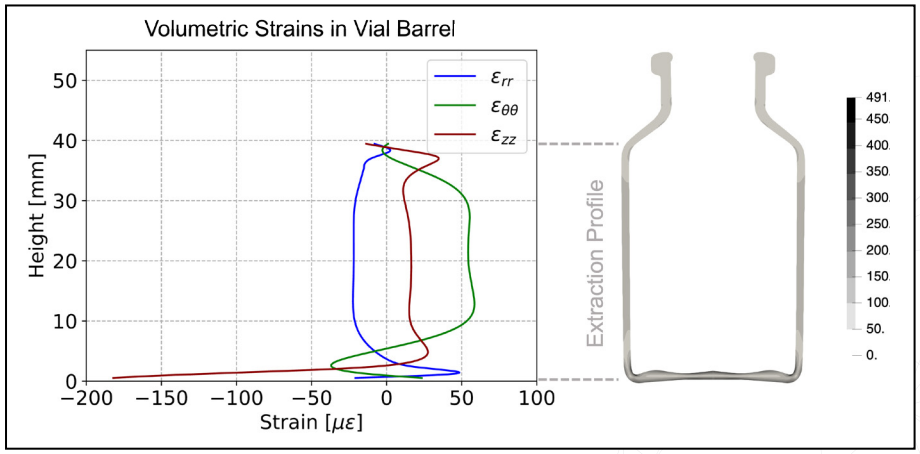


Figure 2: *Computed radial, axial, and hoop strains for a vial under uniform internal pressure.*

One of the key goals of the study is to use the strain sensors and computational modeling to identify the constitutive parameters of the various formulations. To accomplish this, a new experimental setup using a metal pipe with strain and temperature sensors attached to the outer wall has been developed. The apparatus is shown in **Figure 3**.

This approach was selected to reduce the influence of high strain gradients that, due to the configuration of the strain gages, can be difficult to couple to the computation model. In principle, the information collected using the new setup can be used to identify the stress and strain distribution throughout the frozen matrix itself. Future studies will investigate the implications of these mechanical effects on various critical quality attributes.

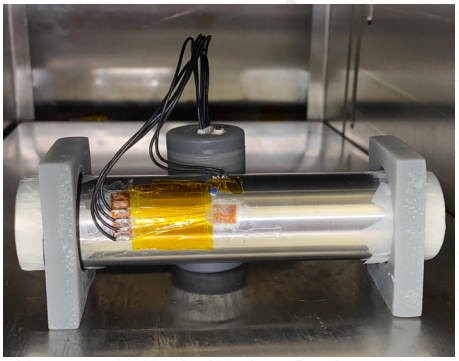


Figure 3: *Image of model metal pipe apparatus used for the identification of a material's constitutive parameters.*

Experimental Study of Freezing-Induced Concentration Gradients in a Container

Investigators: *Marilyn Padua (Purdue PMP student), Sai Bhusurapalli (Purdue PMP student), Jie Wang (AbbVie), Sherwin Shang (AbbVie), Kyu Yoon (Purdue ChemE Research Scientist), and Vivek Narsimhan (Purdue ChemE)*

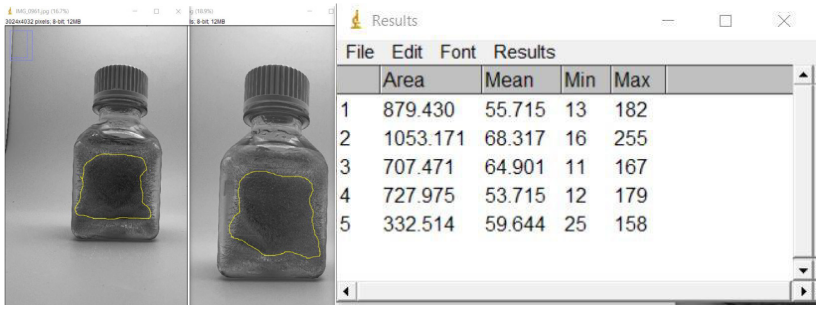


Figure 1: *Screenshot of ImageJ Software with ROI's selected (left); measured mean intensity values (right).*

Freezing is currently a common unit operation in the production and storage of biopharmaceuticals. Although biopharmaceuticals are preferentially stored in the frozen state, degradation due to aggregation, protein unfolding, air bubbles formed on the ice crystallization front, and local pressure and mechanical stresses due to volume expansion during water-to-ice transformation, complications may affect the stability of frozen biopharmaceuticals. The stability of frozen biologicals may also depend on the sample size due to its impact on the freezing kinetics, cryoconcentration effects, and mechanical stresses associated with freezing.

It is well known that the freezing process can generate concentration gradients throughout the storage container. In this study, small molecule solutions were used to estimate the concentration of the cryoconcentrated regions using Image Analysis.

Image analysis was conducted using ImageJ Software, an image processing program developed by the National Institutes of Health, to establish the relationship between absorbance and pixel intensity. This software enabled the calibration of pixel images into millimeters.

Pictures of bottles with frozen solutions were taken, after which, specific regions of interest (ROI) were selected within the images to measure their mean intensities. The mean intensity values were adjusted by subtracting the background intensity, similar to a "blank" reference, before measuring absorbance in spectroscopy. Each ROI's adjusted mean intensity values was compared to the absorbance values of each section. This methodology facilitated the correlation between pixel intensity, corrected for background, and the corresponding absorbance values across different regions.

One sample of each concentration (10, 20, 40, 60, 80, 100ppm) of methylene blue/water was frozen in a -35°C freezer, then cut into three regions, top, middle, and bottom. Further cuts were made to create three sections, left, center and right (**Figure 2**). These nine sliced sections of each concentration were thawed separately in 20 mL vials. Their absorbance was measured using Thermo Scientific NanoDrop One at a wavelength of 662 nm.

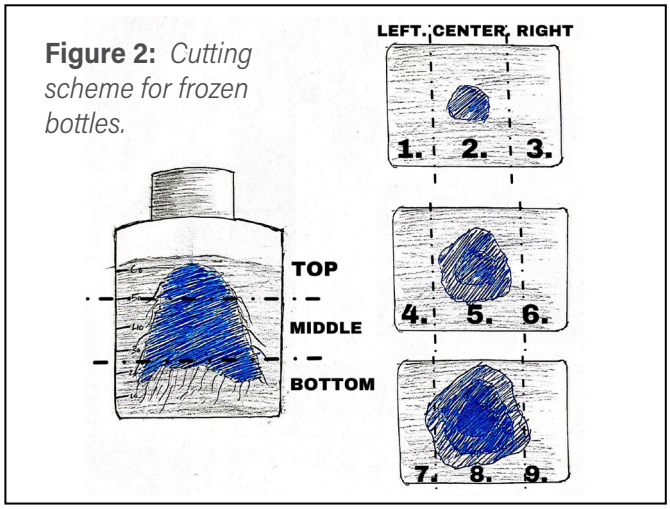


Figure 2: *Cutting scheme for frozen bottles.*

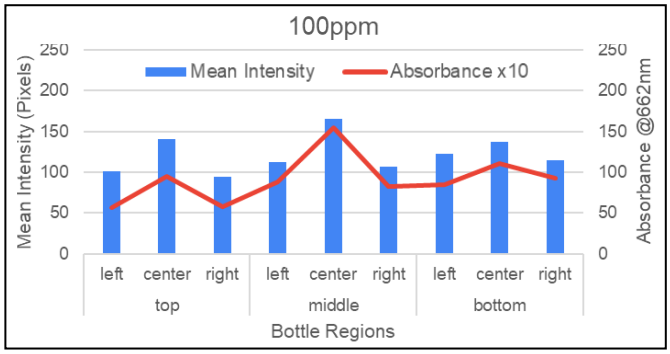


Figure 3: *Mean Intensity vs. Absorbance for 9 different sections of 60mL sample of 100ppm Methylene Blue/ Water Solution.*

Conclusion: To estimate the concentration of the cryoconcentrated region, image analysis and mathematical methods were used as alternatives to the time-consuming process of cutting frozen bottles, thawing, and measuring the absorbance. To compare the data, concentrations from the absorbance calibration curve were used, and it was observed that the mean pixel intensity values from ImageJ analysis followed along with the values from the absorbance calibration curve.

Comparative Evaluation of S–γ Model and AMUSIG Model in Predicting Droplet Size Distributions in Narrow-Gap Homogenizers

Investigators: *Chun Yuan Kung (Purdue PMP student), Thomas Eppinger (Siemens), Petr Kazarin (Purdue Post Doc, now at IMA Life)*

In the dynamic working field of emulsion processing, the accurate prediction of droplet size distribution is an important factor in enhancing and improving efficiency and product quality in various industries. This project resulted in a comparative analysis of the performance of two models, the S-γ model and the Adaptive Multiple Size Group (AMUSIG) model under various conditions by using STAR-CCM+ simulation software, allowing the provided analysis to methodically verify hypotheses, designs, and models in a controlled environment.

Field functions were analyzed across different operational conditions to enhance understanding of fluid dynamics and energy efficiency, important for system performance optimization. The cumulative volume fraction was examined against droplet diameter to better align model predictions with experimental data and gain insights into the breakup and coalescence parameters' effect on the model's droplet size performance. Additionally, a spatial comparison across system regions provided information about the model's feature set and its predictive accuracy for droplet behavior under varying parameter conditions, including a deeper investigation into flow dynamics' impact on droplet size.

In field analysis, high-velocity profiles and turbulent dissipation rates confirmed model stability across high-shear and laminar flows (**Figures 1, 2**). As for Cumulative volume fraction analysis, by comparing models against experimental data, the S-γ exhibited a feature that is conservative for small droplets, while AMUSIG aligns better across sizes, particularly in the mid to larger ranges. While doing models tuning for breakup and coalescence rates, theoretical predictions showed that lower breakup rates and higher coalescence rates resulted in larger droplets and identified a critical point in parameter space indicating the dominance of coalescence, pivotal for creating larger droplets (**Figure 3**). Within the Analysis of Spatial Comparison, the S-γ model features in single peak performance provided stable scenarios where droplets predominantly coalesce and result in a uniform droplet size distribution characterized by a log-normal pattern (**Figure 4**). It was also observed that different conditions impact to the S-γ model droplet size, such as higher surface tension maintains moderate, uniform droplet sizes, lower flow rates resulted in larger droplets, and increased viscosity leads to smaller, more uniform droplets.

Refining the S-γ and AMUSIG models for broader industrial use, including advanced calibration and exploring parameters beyond viscosity, flow rate, and surface tension would be the next step to continue this work. Further research into breakup and coalescence rates will deepen understanding, leading to a comprehensive hybrid model with both models' advantages, which can be used to enhance emulsion process efficiency and a wide range of applications.

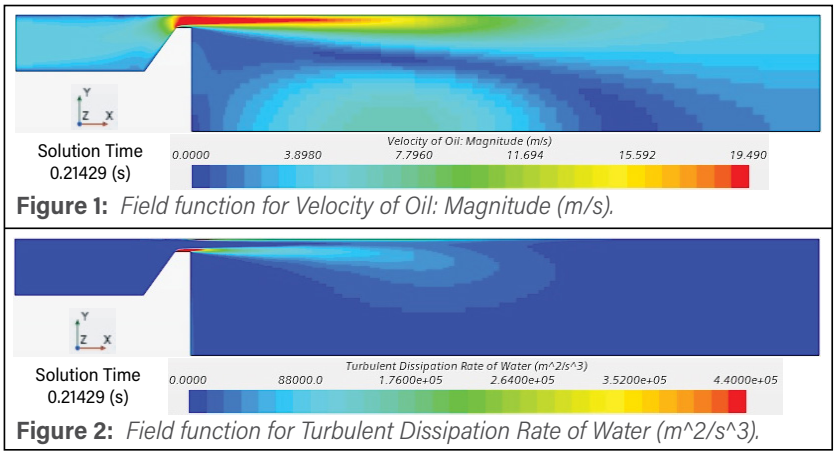


Figure 1: *Field function for Velocity of Oil: Magnitude (m/s).*

Figure 2: *Field function for Turbulent Dissipation Rate of Water (m^2/s^3).*

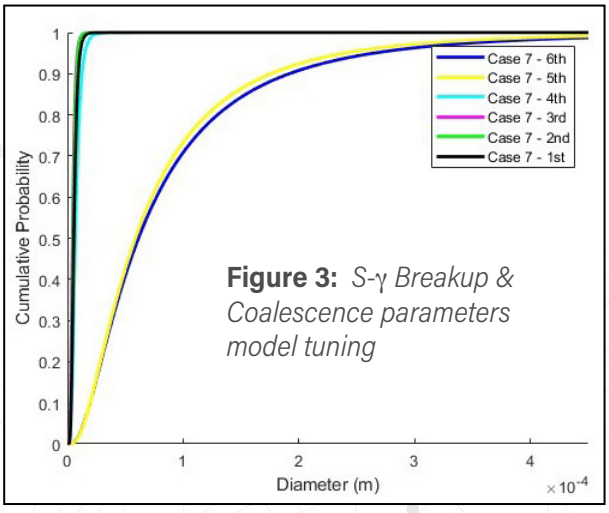


Figure 3: *S-γ Breakup & Coalescence parameters model tuning*

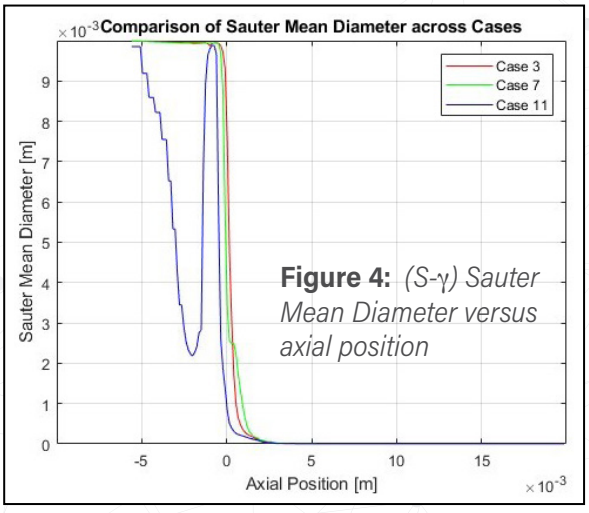


Figure 4: *(S-γ) Sauter Mean Diameter versus axial position*

Purdue Faculty LyoLaunchPad Projects 2017-2024

The LyoLaunchPad program, started in 2017, allows researchers on campus to conduct short-term projects on novel applications of lyophilization using resources at the LyoHUB demo facility at no charge.

Research teams receive training on the state-of-the-art lyophilization equipment and methods. The PIs engage with LyoHUB industry members while sharing research outcomes after completion of the projects.

Faculty/Department	Project Name
 **Lia Stanciu** Materials Engineering	Lyophilized BPA Antigen for Lateral Flow Assay
 **Linda Lee** Agronomy	Freeze Drying of Biosolids for the Analysis of PFAS Compounds, Preserved to Enable Extended Research
 **Brian Dilkes** Biochemistry	Freeze Drying of Maize Root Tissue Samples to be Used for Metabolite Extractions
 **Mukerrem Cakmak** Materials and Mechanical Engineering	Freeze Drying of Tough Multiple-Network Hydrogels
 **David Thompson** Chemistry	Lyophilization of HP-ß-CD/ SBE-ß-CD Poly-Rotaxaanes
 **Andrea Liceaga** Food Sciences	Lyophilization of Cricket Protein for Freeze Drying Cricket Protein Hydrolysates (CPH)
 **Clint Chapple** Agriculture	Investigation of Soluble Metabolites in Arabidopsis and Sorghum Leaves and Stems
 **Wenzhuo Wu** Industrial Engineering	Transforming Hydrogel into Aerogel Using Freeze-Drying

Faculty/Department	Project Name
 **Tony Zhou** Industrial and Molecular Pharmaceutics	Dry Powder Inhalable Formulations of Antibacterial Agenda
 **You-Yeon Won** Chemical Engineering	Lyophilization of PEGylated Block Copolymer Micelles Feasibility Study of Lyophilizing Aqueous Block Copolymer Nano-Assemblies for Room Temperature Storage and Handling
 **Tian Li** Mechanical Engineering	Thermal Insulation Cellulose Foam Freeze Dry Cellulose Solution Made of Methyl Cellulose and Water to Fit for SEM Microscopy
 **Ajay Malshe** Mechanical Engineering	Tunability of Pore Morphology in Edible Scaffolds
 **Sherry Harbin** Biomedical Engineering	Characterization and Scale-up of Collagen Freeze Drying
 **Senay Simsek** Food Science	Cake Starch Gelatinization
 **Jacqueline Linnes** Biomedical Engineering	Lyophilization of Small-Volume Reagents on a Microfluidic Chip
 **Yoon Yeo** Industrial and Molecular Pharmaceutics	Lyophilization Process Optimization of RNAi-Based Therapeutic Carriers, Optimizing the Nanoparticle Formulation for Cold-Chain-Free Storage and Transport

LyoLaunchPad Industry Projects for 2023-2024

2023: Quantitative Analysis of Vial Breakage During Lyophilization Using Wireless Strain Sensors (AbbVie/Pfizer)

2024: Thermal Characterization, Optimization of qPCR and ATP Reagents (LuminUltra)

LYO Launch PAD Projects

Lyophilization of Small Volume Reagents on a Microfluidic Chip

Investigators: *Mary Wanduka (Purdue BME), Drew Strongrich (Purdue LyoHUB), Kyu Yoon (Purdue ChemE), Ian Flynn (Purdue LyoHUB), Jackie Linnes (Purdue BME), Tamara Kinzer-Ursem (Purdue BME)*

As part of a LyoLaunchPad project, LyoHUB facilities were used to optimize the lyophilization conditions of nucleic acid amplification reagents for both in-tube and onto microfluidic devices. The original formulations and lyophilization protocol were unable to produce an efficient, and stable product. Common stabilization excipients (trehalose, dextran, sucrose, and mannitol) were screened in various concentrations. The reagent cocktail of nucleic acids, salts, enzymes, proteins, and nanobeads had complex interactions with the stabilization excipients resulting in three main formulations proving reasonable after undergoing testing in LyoHUB's freeze dry microscope. Stabilization excipients of 10% w/v trehalose, sucrose, and dextran with the reagent cocktail were found to have acceptable collapse temperatures to develop a cycle.

Due to low collapse temperatures and the use of polystyrene PCR tubes suspended above the shelf, producing a pharmaceutically elegant cake proved challenging. The low load of the samples in LyoHUB's Millrock MicroFD resulting in reduced temperature control leading to sample collapse. **Figure 1** shows the initial experiments with trehalose and dextran which resulted in collapse during shelf stability testing. Moving to LyoHUB's SP Scientific LyoStar 3 these issues were resolved and a stable pharmaceutically elegant cake was produced, which can be seen in **Figure 2**.

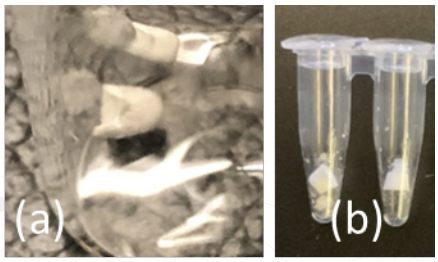


Figure 1: *Collapsed lyophilized cake for (a) trehalose and (b) dextran with reagent cocktail.*

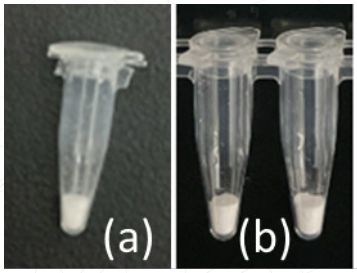


Figure 2: *Lyophilized cake for (a) trehalose and (b) dextran with reagent cocktail.*

Characterization and Scale up of Collagen Freeze Drying

Investigators: *Drew Strongrich (Purdue LyoHUB), Sherry Harbin (Purdue Biomedical)*

GeniPhys, Inc., a Purdue-based start-up, is developing and commercializing its patented Collymer technology—a first-of-a-kind polymerizable (scaffold-forming) collagen that supports custom fabrication of implantable materials that exhibit unprecedented immune tolerance and tissue restoration outcomes. The base collagen formulation is manufactured in a highly stable lyophilized form, with laboratory-scale production involving a manifold freeze dryer. The goal of this LyoLaunchPad project is to transition GeniPhys from a manifold freeze dryer to a bulk-fill, shelf lyophilization format to support scaling of its collagen manufacturing process. Work conducted in collaboration with LyoHUB aimed to inform and optimize lyophilization parameters for GeniPhys' collagen solution. First, freeze-drying microscopy was performed on multiple collagen solution concentrations to predict the collapse temperature. The heat transfer coefficient (Kv) of the bulk-fill stainless tray also was determined by applying experimental lyophilization cycles to water at different chamber pressures. After developing an initial recipe, a lyophilization cycle was performed on the collagen solution in a bulk-fill tray format, allowing determination of the product resistance (Rp). To further optimize the process and verify the Rp, a second cycle was conducted using a higher primary drying temperature. Initial analysis of the resulting collagen (**Figure 1**) showed maintenance of molecular integrity as well as solubilization and polymerization (scaffold-forming) properties. Follow-up studies are planned to determine how LyoHUB's "microwave" technology affects heat transfer characteristics of the collagen solution during lyophilization.

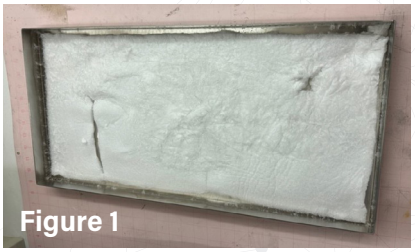


Figure 1

GeniPhys team meets with Drs. Strongrich and Alexeenko to discuss LyoLaunchPad project.

LYO Launch PAD Projects

Cake Starch Gelatinization

Investigators: *Ian Flynn (Purdue LyoHUB), Drew Strongrich (Purdue LyoHUB), Senay Simsek (Purdue Food Science), Yusuf Durmus (Purdue Food Science)*

Gelatinization of starch granules during the cake baking process strongly impacts the final cake volume and texture. Gelatinization occurs by starch granules swelling with water while being heated until they burst and increase the viscosity of solution. The solvent retention capacity (SRC) test is a common quality test done for soft wheat flour, but it is not effective as a tool to select flour for cake production. When looking for a quality soft wheat flour for cake production, low absorption, high peak viscosity, and later gelatinization is desirable. It has been determined that cake expansion is restricted when peak viscosity is low, and gelatinization occurs early. LyoHUB's Freeze Dry Microscope can be utilized in its high temperature range to heat the starch in water or sucrose solutions to visualize physical changes to starch granules during gelatinization. This technique can be used to determine differences in the time and degree of gelatinization for starch from cake flour of different cake quality. **Figure 1** shows examples of different cake qualities.

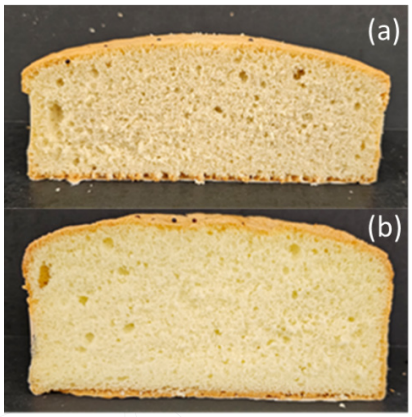


Figure 1: *Comparison of cake quality (a) is poor cake quality and (b) is good cake quality.*

As starch is the main component of the flour, soft red winter (SRW) and soft white (SW) wheat flour were used to determine gelatinization for these specific starch granules. **Figure 2** shows how the high concentrations of sucrose delayed gelatinization for both starches. At 70°C, the starch granules have not begun to swell at high sucrose concentrations but have already completely swelled and burst in only water. High ratio cakes utilize high concentration of sucrose in the batter.

This study showed that sucrose concentrations are crucial for final cake texture. Low sugar, or no sugar, alternatives are a point of interest in the food industry, but without other modifications the early gelatinization of the starches will drastically change the product.

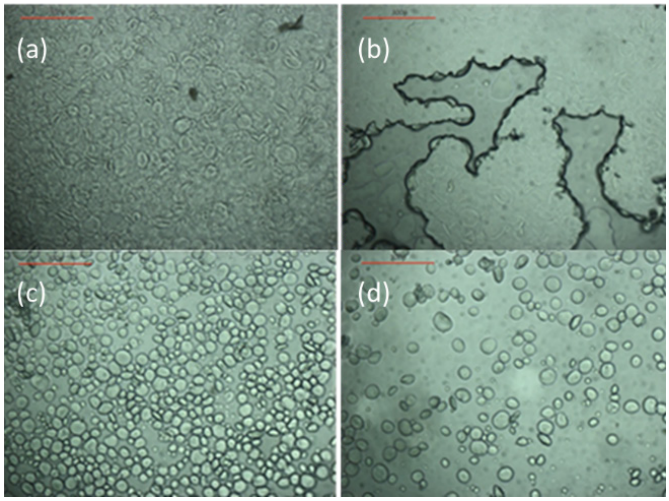


Figure 2: *Comparison of starch gelatinization for SRW wheat in 0% sucrose (a) and 50% sucrose (c), and SW wheat in 0% sucrose (b) and 50% sucrose (d) at the same temperature.*

Freeze Drying of an Alternative Protein Food-by-Design System

Investigators: *Drew Strongrich (Purdue, LyoHUB), Ajay Malshe (Purdue Mechanical Engineering)*

LyoHUB collaborated with the Purdue Professor Ajay Malshe's group in Mechanical Engineering to investigate freeze drying as a manufacturing process for soy-based edible scaffolds. These systems are currently under investigation by food sciences as a means of producing, for example, laboratory-grown meat products. The scaffolds were cast and freeze dried in silicone molds using LyoHUB's REVO freeze dryer under typical process conditions. A range of sample thicknesses were tested and indicate that an overall thickness of 1-2mm provide the most optimal results in terms of pore size, appearance, and mechanical strength. Dry product morphology measurements are currently being conducted using micro-CT.

LYO Launch PAD Projects

Optimization of RNA Nanosac Formulation for Cold-Chain Free Storage and Transportation

Investigators: *Dr. Yoon Yeo (Purdue Industrial and Molecular Pharmaceutics), Yongzhe Li (Purdue Industrial and Molecular Pharmaceutics), Drew Strongrich (Purdue LyoHUB)*

Background: This project studies the lyophilization of Nanosac, a hollow nanocapsule previously developed in Dr. Yeo's laboratory for systemic delivery of siRNA. Nanosac is produced by coating cationized mesoporous silica nanoparticles (MSNs) with siRNA and polydopamine sequentially, followed by the removal of the MSN core [1]. The final product is polydopamine nanocapsules encapsulating siRNA inside. Preliminary experiments conducted with a Labconco FreeZone Benchtop Freeze Dryer showed that samples (Nanosac + cryoprotectants) dried at 4.5 wt% had large particle sizes upon reconstitution. This observation suggests that the uncontrolled shelf temperature during the lyophilization process may have influenced the size of the reconstituted particles, especially when the samples were prepared in relatively high solid concentrations.

Objectives: Dr. Yeo's lab reached out to LyoHUB in order to assist with optimizing the lyophilization of Nanosac for cold-chain-free storage and transportation. To accomplish this, various cryoprotectants such as sucrose, trehalose, and mannitol mixtures were used at different freezing rates. The study compared the uncontrolled shelf temperature of the Labconco benchtop manifold-style freeze dryer with the controlled shelf temperature settings of the MicroFD with the goal of preserving the size of Nanosac by ensuring homogeneous drying. The affect of the drying process on the cake appearance and moisture content as well as the reconstitution time and the colloidal properties of Nanosac were also studied.

Results: Sucrose and mannitol as the cryoprotectant combination and liquid nitrogen quench freezing were selected as optimal conditions in preliminary experiments with the Labconco Benchtop Freeze dryer. Under these conditions, the impact of shelf temperature on Nanosac samples was examined using the MicroFD. Nanosac was prepared in 1.5 w/v% and 4.5 w/v%. At 1.5 w/v%. There was no significant difference in the Z-average upon reconstitution between samples dried with the Labconco and the MicroFD (Fig.1). Both samples showed particle sizes comparable to the fresh sample (approximately 180 nm). At 4.5% solid content, there was a significant difference (Fig.2). Drying without temperature control (Labconco) resulted in the particle size increase to 400 nm after reconstitution, significantly larger than fresh Nanosac, whereas the sample dried with the MicroFD was comparable to the fresh Nanosac in size. This result suggests that the heterogeneous drying due to the lack of temperature control was particularly detrimental to higher solid content samples. The uniform drying by the MicroFD reduced the variability of the cake appearance and moisture content and facilitated the reconstitution of Nanosac with minimal aggregation. The lyophilized formulation in optimal conditions was reconstituted by manual shaking in 20 seconds, with a Z-average comparable to that of freshly prepared samples. In the future, Dr. Yeo's lab aims to determine the shelf-life of the optimally lyophilized Nanosac through an accelerated stability study.

Reference:

[1] Kim, Hyungjun, et al. "Nanosac, a noncationic and soft polyphenol nanocapsule, enables systemic delivery of siRNA to solid tumors." Acs Nano 15.3 (2021): 4576-4593.

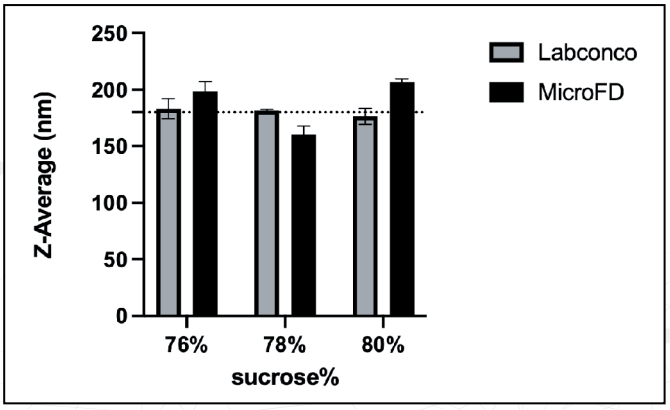


Figure 1: *Z-Average of reconstituted Nanosac with 1.5% solid content across different sucrose percentages (n=3 measurements).*

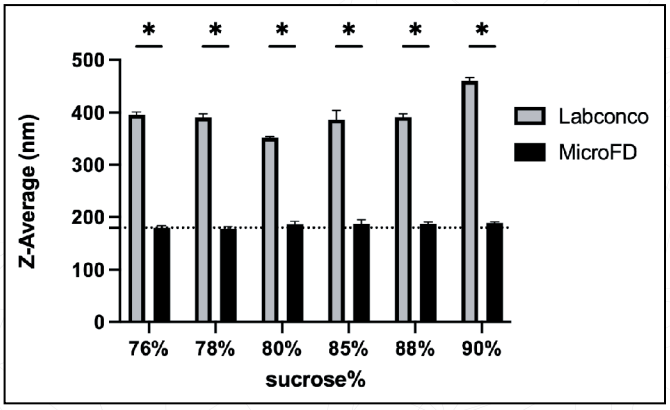


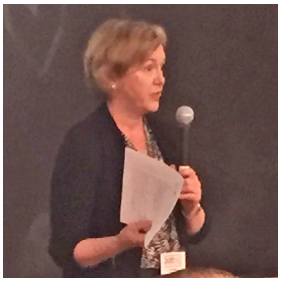
Figure 2: *Z-Average of reconstituted Nanosac with 4.5% solid content across different sucrose percentages (n=3 measurements, *: p < 0.00001).*

Liz Topp to Lead Young Institute for Advanced Manufacturing of Pharmaceuticals

Liz Topp, Co-Founder and Co-Director of LyoHUB, became the inaugural director of the William D. and Sherry L. Young Advanced Manufacturing of Pharmaceuticals Institute at Purdue University in August, 2023. Liz previously served as chief scientific officer of the National Institute for Bioprocessing Research and Training (NIBRT) in Dublin, Ireland and continues as principal investigator for the formulation and stability research group at NIBRT. She is widely recognized as an accomplished researcher, innovator, and educator in advanced pharmaceutical manufacturing and brings a wealth of research, teaching, and leadership experience to this new role.

The Young Institute was established in June 2022 through a donation from Purdue alumnus William D. Young, a pioneer in pharmaceutical and biotechnology manufacturing, and his wife, Sherry L. Young. The institute's mission is to reduce costs and expand access to innovative drugs emerging from biotechnology research.

The institute leverages the collective and individual expertise of more than 30 founding faculty to create innovative business models that attract large-scale funding with the capacity to facilitate advanced research and foster workforce development. The Young Institute, which is home to the Lilly Scholars at Purdue, has also developed an undergraduate certificate in pharmaceutical manufacturing which is available to Purdue students.



Liz will be stepping down this year as Co-Director of LyoHUB in order to focus on this new role as Director of the Young Institute of Advanced Manufacturing of Pharmaceuticals. Fortunately, we will be able to continue to benefit from her brilliance as LyoHUB is an active partner with the Young Institute. We are so grateful to Liz for her dedication, hard work and especially for her special way of making lyophilization pure poetry.

Thank you, Liz. You are an inspiration!

www.purdue.edu/research/features/stories/topp-named-to-lead-institute-for-advanced-pharmaceutical-manufacturing-at-purdue/

Past Meetings

LYO 10 hub.org
Celebrating 10 years
EST. 2014

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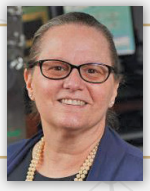

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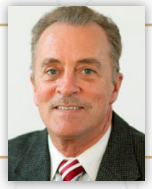

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