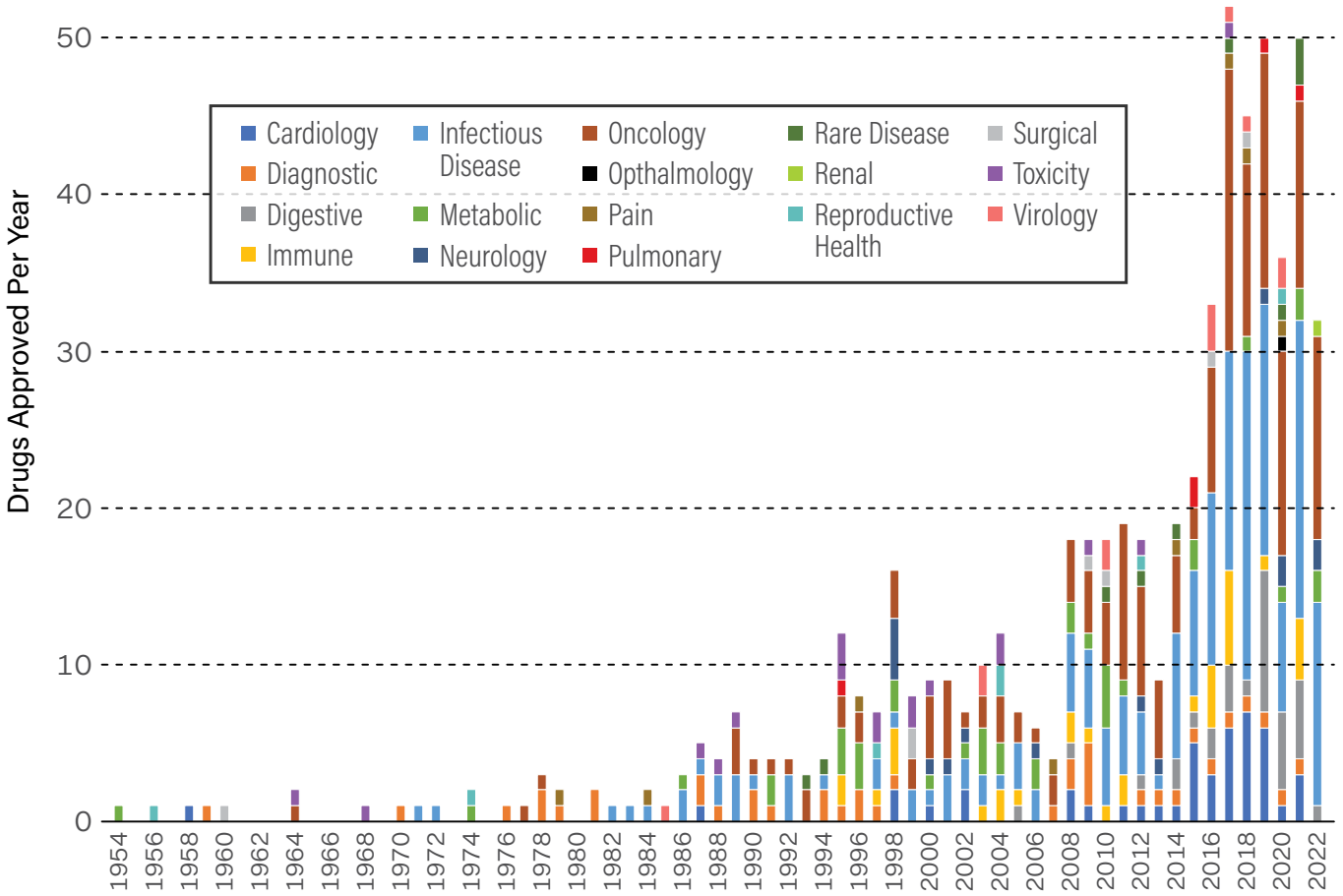
ANNUAL REPORT

ADVANCED LYOPHILIZATION TECHNOLOGY CONSORTIUM

2023

Yearly FDA-Approved Lyophilized Drugs by Indication



LYOhub.org

Front Cover

The graph on the front cover shows the new lyophilized products which have been approved by the FDA since 1954. It is interesting to note:

Approvals of New Lyophilized Products

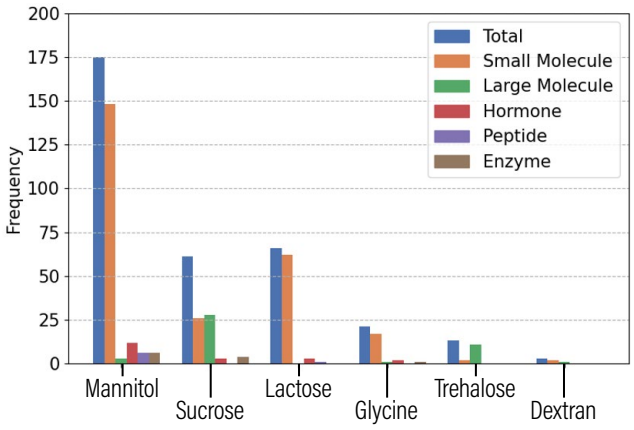
- Prior to 1985, one or two new products per year
- Increase in new products beginning around 2010 for a single small molecule API

The collection of this data was expanded into a much larger study resulting in the LyoHUB Database of Lyophilized Drug Products, now available for use by LyoHUB members at https://pharmahub.org/resources/lyodrugdatabase.

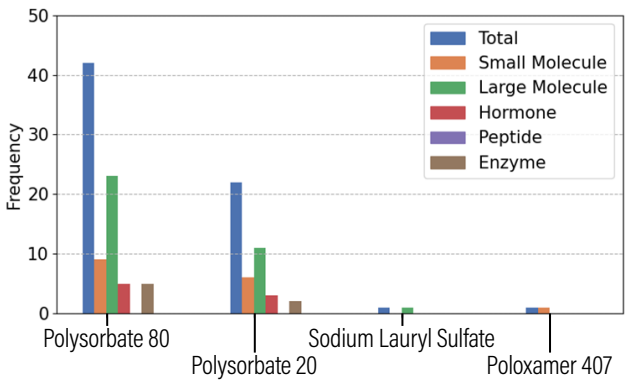
The database includes all FDA-approved lyophilized drugs from the years 1955 to 2022. The lack of a consolidated resource for these drugs motivated the creation of this database, which is the first of its kind. The database encompasses all the FDA approved lyophilized drugs along with the active and inactive ingredients used in their respective formulation. A software application was created to generate this database. The application employed an iterative process, where it scanned the complete list of FDA-approved drugs and cross-referenced each drug with the DailyMed website to determine if the drug was developed through the process of lyophilization. If a drug was found to have been produced through lyophilization, the application utilized DailyMed to extract information on the active and inactive ingredients used in its formulation. This information was then systematically recorded and added to the database. It revealed the presence of over 600 unique submissions filed to the FDA for lyophilized drug approvals to date.

The database provides a comprehensive platform for the review of excipient usage in various lyophilized drugs, including a quantitative analysis of the excipients. The drugs in this database have been further categorized into distinct groups based on molecular type, therapeutic use, and indication, which enhances the overall comprehensiveness of the resource and facilitates a deeper understanding of lyophilized drugs.

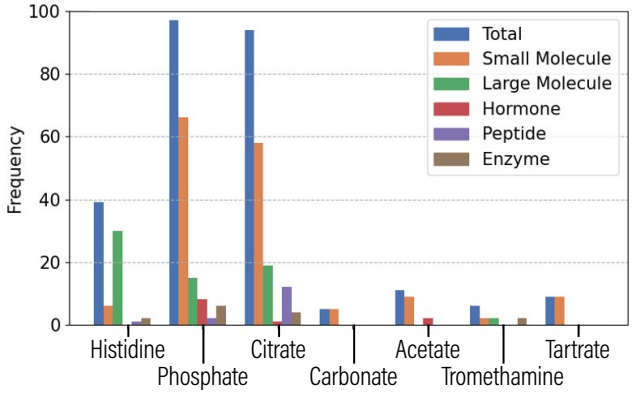
Bulking Agent/Lyo-Protectant Use by API Type, 1985-2021



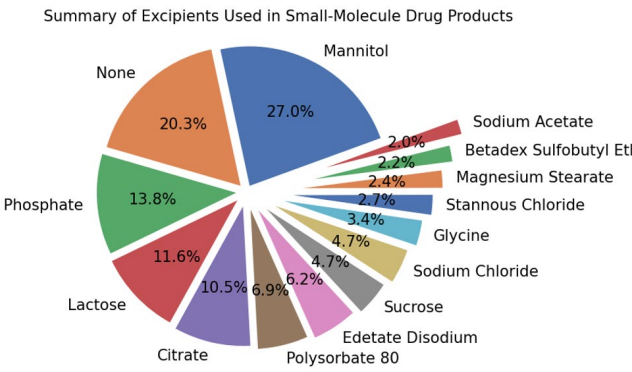
Surfactant Use by API Type, 1985-2021



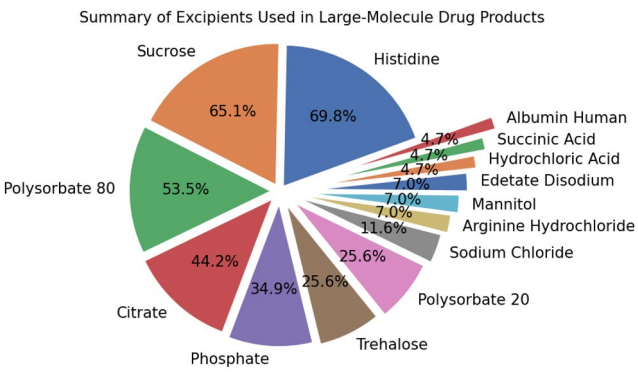
Buffer Use by API Type, 1985-2021



Small Molecule Drug Products, 1985-2021



Large Molecule Drug Products, 1985-2021



DIRECTORS' MESSAGE

LyoHUB, a renowned consortium of experts in lyophilization technology, is pleased to present its annual report for the year 2022. This report highlights the consortium's significant achievements and progress towards its goals in advancing the science and technology of lyophilization. Lyophilization is a crucial process used in the pharmaceutical and biotechnology industries to preserve and stabilize sensitive drugs and biologics, ensuring their efficacy and safety for patients. With a focus on collaboration, innovation, and excellence, LyoHUB has made significant strides in advancing the understanding of lyophilization processes and developing new and improved technologies. This report provides an overview of the consortium's research and development efforts, scientific publications, and collaboration with industry partners, as well as its financial performance and future strategic initiatives. Through this report, you will gain a deeper understanding of LyoHUB's mission, vision, and commitment to improving the science of lyophilization for the betterment of human health.

At LyoHUB, we're committed to advancing the science and technology of lyophilization on all fronts. The paragraph to the left was written by ChatGPT in response to the request, "Write an introduction to this report" when the AI-bot was provided with a draft. The thanks below are real and written by us, mere humans.

We are grateful to the thirty member companies who are the core of LyoHUB, and to Purdue's Colleges of Engineering and Pharmacy for their ongoing support. Our lyophilization pilot laboratories (Demonstration Facility) are housed in Purdue's Birck Nanotechnology Center; we appreciate their partnership and their commitment to the LyoHUB consortium. We continue to be inspired by the students and postdocs who learn about lyophilization through LyoHUB, and whose energy and enthusiasm help to drive new technologies. As always, we are ever grateful to our Operations Manager Jen Gray, whose optimism and cheerful professionalism are the heart and soul of LyoHUB.

Thank you for your interest in LyoHUB and in this annual report. Please reach out to us if you'd like more information about lyophilization and the LyoHUB consortium—you'll encounter amazing (and real) humans and great cutting-edge technology.

All the best,

Alina Alexeenko and Liz Topp

MEMBERSHIP

IMA LIFE Aseptic Processing & Freeze Drying Solutions	Pfizer	Janssen Pharmaceutical Companies of Johnson & Johnson	MILLROCK TECHNOLOGY millrocktech.com
Member Since 2014	Member Since 2014	Member Since 2015	Member Since 2015
INFICON	Baxter	SP	Allergan
Member Since 2015	Member Since 2016	Member Since 2016	Member Since 2016
abbvie	Roche	SIEMENS	FRESENIUS KABI
Member Since 2016	Member Since 2016	Member Since 2017	Member Since 2017
Bristol Myers Squibb™	OPTIMA EXCELLENCE IN PHARMA	AMGEN	Merck KGaA EMD SERONO
Member Since 2017	Member Since 2018	Member Since 2018	Member Since 2019
AstraZeneca	EVONIK POWER TO CREATE	Daiichi-Sankyo	COOK® BIOTECH
Member Since 2019	Member Since 2019	Member Since 2019	Member Since 2019
CORNING	SANOFI	MERCK	FUJIFILM
Member Since 2020	Member Since 2020	Member Since 2021	Member Since 2021
Elanco	Jazz Pharmaceuticals	Metrohm	PSI Physical Sciences Inc.
Member Since 2021	Member Since 2021	Member Since 2022	Member Since 2022
REGENERON	West	tempris®	
Member Since 2022	Member Since 2022	Member Since 2023	

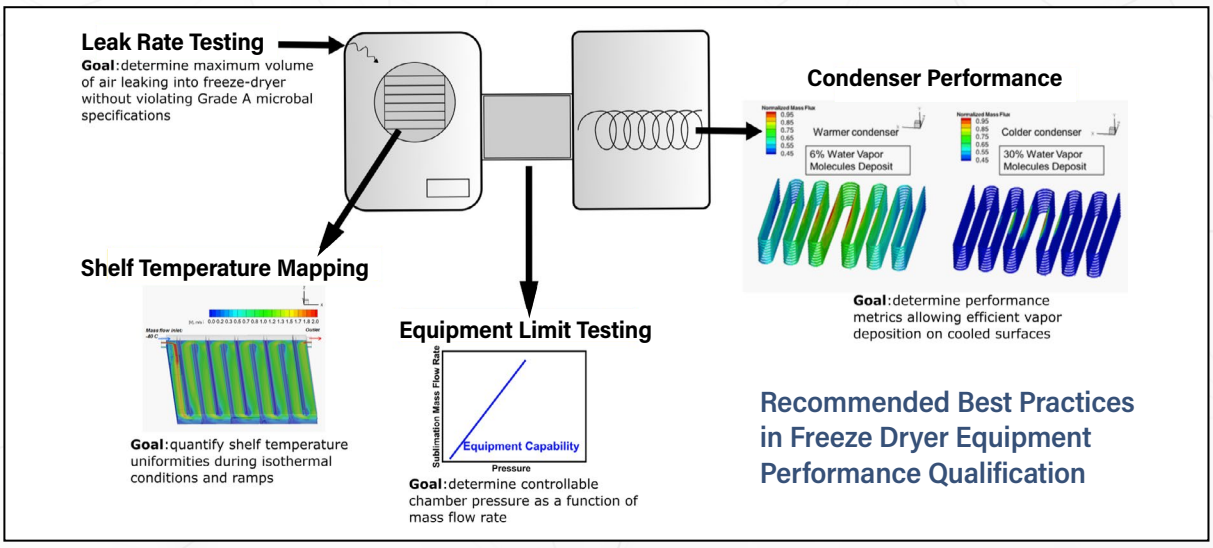
BEST PRACTICES PAPERS

All LyoHUB best practice papers are available in open source, with free 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):

- *Recommended Best Practices in Instrumentation Process Monitoring in Pharmaceutical Freeze Drying*
  Number of Accesses to Date: 17,000+ (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: 6,300+ (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: 4,600+ (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 Transfer
  This paper was developed in collaboration with BioPhorum (BPOG)
  Number of Accesses to Date: 900+ (https://link.springer.com/article/10.1208/s12249-022-02463-x)
- *Recommended Best Practices in Freeze Dryer Equipment Performance Qualification*
  Published in January, 2023 (https://link.springer.com/article/10.1208/s12249-023-02506-x)

In this paper, best practices for performing freeze dryer equipment qualification are recommended, focusing on identifying methods to quantify shelf thermal uniformity (also known as "shelf surface uniformity"), equipment capability, and performance metrics of the freeze dryer essential to the pharmaceutical Quality by Design paradigm. Specific guidelines for performing shelf temperature mapping, freeze dryer equipment limit testing (the capability curve), and condenser performance metrics have been provided. Concerning shelf temperature mapping and equipment capability measurements, the importance of paying attention to the test setup and the use of appropriate testing tools are stressed. In all the guidelines provided, much attention has been paid to identifying the balance between obtaining useful process knowledge, logistical challenges associated with testing in the production environment vs that at laboratory scale, and the frequency of the testing necessary to obtain such useful information. Furthermore, merits and demerits of thermal conditions maintained on the cooled surfaces of the freeze dryer condenser have been discussed identifying the specific influence of the condenser surface temperature on the process conditions using experimental data to support the guidelines. Finally, guidelines for systematic leak rate testing criteria for a freeze dryer are presented. These specific procedural recommendations are based on calculations, measurements, and experience to provide useful process and equipment knowledge.

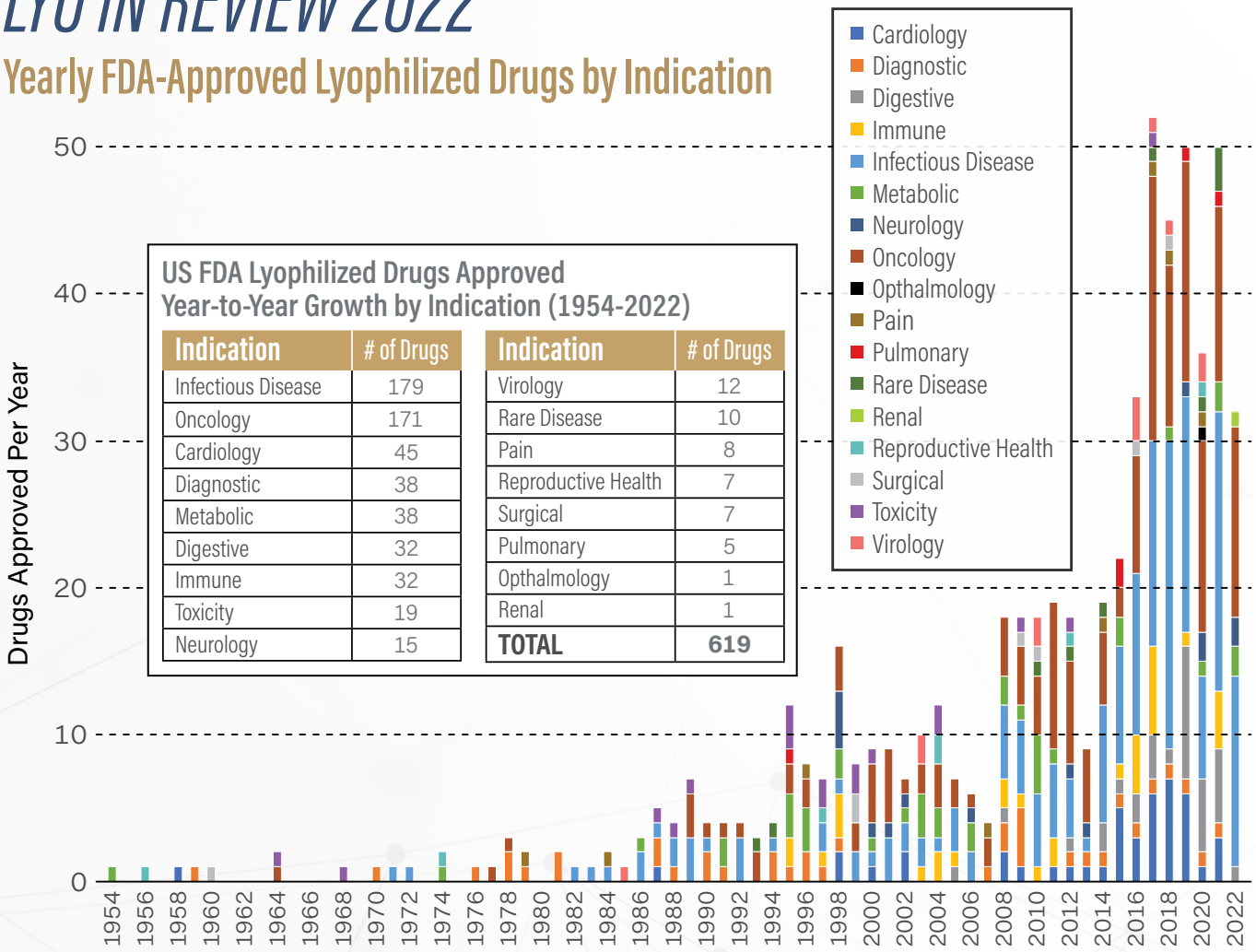


Work also continues on →

- *Best Practices in Scale up and Tech Transfer for Freeze Drying, Paper 2: Challenges, Past Practices, Current Best Practices, Recommendations and Mitigation Plans, led by Serguei Tchessalov (Pfizer)*
  This paper is being developed in collaboration with BioPhorum (BPOG)
- *Best Practices in the Development of Lyophilized Formulations, led by Dr. Elizabeth Topp (Purdue/ NIBRT) and Dr. Greg Sacha (Baxter Healthcare)*
  Paper 1: Excipients for Lyophilized Pharmaceuticals, **Paper 2:** Quality Assessment for Lyophilized Pharmaceuticals, **Paper 3:** Formulation of Lyophilized Pharmaceuticals

LYO IN REVIEW 2022

Yearly FDA-Approved Lyophilized Drugs by Indication



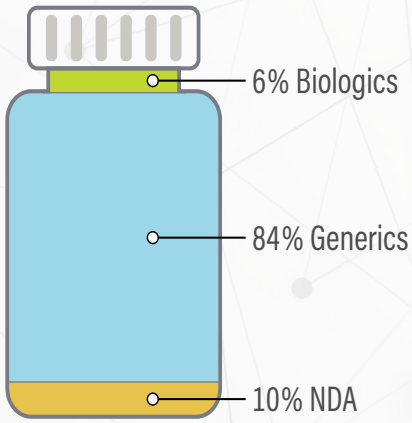
US FDA Lyophilized Drugs Approved Year-to-Year Growth by Indication (1954-2022)

Indication	# of Drugs
Infectious Disease	179
Oncology	171
Cardiology	45
Diagnostic	38
Metabolic	38
Digestive	32
Immune	32
Toxicity	19
Neurology	15

Indication	# of Drugs
Virology	12
Rare Disease	10
Pain	8
Reproductive Health	7
Surgical	7
Pulmonary	5
Opthalmology	1
Renal	1
TOTAL	**619**

In 2022, the US FDA approved 32 lyophilized drugs submitted by a total of 18 companies, not including drugs listed with any of the following conditions: discontinued, solution dosage form, routes of inhalation, oral, spinal, and intrathecal administration. In general, 27 (~84%) of the drugs approved by this agency are generic products, continued by 3 (~10%) new drug applications (NDA), and 2 (~6%) new biologics. By indication, Oncology and Infectious Disease comprised the two largest categories of approved lyophilized drugs with 41% each, followed by Metabolic (6%), Neurology (6%), Digestive (3%), and Renal (3%). Likewise, in 2022 EMA approved 6 lyophilized drugs for Oncology (2), Hematology (1), Metabolic (2), and Neurology (1). Information has been compiled from US Food and Drug Administration database and European Medicines Agency website.

FDA-Approved Lyophilized → Drugs in 2022 by Drug Type



Company List (FDA)	# of Drugs
Meitheal Pharmaceuticals	8
Camber Pharmaceuticals	3
Eugia Pharma	3
Amneal Pharmaceuticals	2
Baxter Healthcare Corp	2
Gland Pharma Ltd	2
Advanced Accelerator Applications	1
Biocon	1
Genzyme Corp	1
Hainan Poly Pharm Co	1
Hikma Pharmaceuticals	1
Mallinckrodt Pharmaceuticals	1
Nexus Pharmaceuticals	1
Northstar Rx	1
Novadoz Pharmaceuticals	1
Revance Therapeutics Inc	1
Slate Run Pharmaceuticals	1
Zydus Pharmaceuticals	1
TOTAL (18 companies)	**32**

LYO IN REVIEW 2022

US FDA Center for Drugs Evaluation and Research (CDER)

Table 1. Lyophilized Drugs Approved by US FDA in 2022 (CDER) by Proprietary Names, Applicants, and Drug Types

Approved Date (MM/DD/YY)	Proprietary Name (Active Ingredients, if different)	Applicant	Drug Type
01/26/2022	ALLOPURINOL	Gland Pharma Ltd	Generic
11/17/2022	AMPHOTERICIN B	Eugia Pharma	Generic
02/03/2022	AZACITIDINE*	Amneal Pharmaceuticals	Generic
12/30/2022	AZACITIDINE*	Eugia Pharma	Generic
05/02/2022	BORTEZOMIB	Zydus Pharmaceuticals	Generic
08/18/2022	DAPTOMYCIN*	Slate Run Pharmaceuticals	Generic
09/23/2022	DAPTOMYCIN*	Camber Pharmaceuticals	Generic
12/23/2022	DAPTOMYCIN*	Camber Pharmaceuticals	Generic
01/25/2022	DAPZURA RT [DAPTOMYCIN]	Baxter Healthcare Corp	NDA
04/15/2022	DECITABINE	Hikma Pharmaceuticals	Generic
02/14/2022	ERYTHROMYCIN LACTOBIONATE	Nexus Pharmaceuticals	Generic
02/10/2022	ESOMEPRAZOLE SODIUM	Hainan Poly Pharm Co	Generic
03/23/2022	LOCAMETZ [GOZETOTIDE]	Advanced Accelerator Applications	NDA
10/24/2022	MICAFUNGIN SODIUM	Northstar Rx	Generic
09/06/2022	MITOMYCIN*	Meitheal Pharmaceuticals	Generic
09/08/2022	MITOMYCIN*	Meitheal Pharmaceuticals	Generic
11/22/2022	MITOMYCIN*	Gland Pharma Ltd	Generic
05/02/2022	PEMETREXED DISODIUM*	Eugia Pharma	Generic
05/25/2022	PEMETREXED DISODIUM*	Biocon	Generic
08/04/2022	PEMETREXED DISODIUM*	Amneal Pharmaceuticals	Generic
08/18/2022	PEMETREXED DISODIUM*	Baxter Healthcare Corp	Generic
12/13/2022	PEMETREXED DISODIUM*	Meitheal Pharmaceuticals	Generic
09/14/2022	TERLIVAZ [TERLIPRESSIN]	Mallinckrodt Pharmaceuticals	NDA
07/06/2022	VANCOMYCIN HYDROCHLORIDE*	Camber Pharmaceuticals	Generic
07/27/2022	VANCOMYCIN HYDROCHLORIDE*	Meitheal Pharmaceuticals	Generic
07/28/2022	VANCOMYCIN HYDROCHLORIDE*	Meitheal Pharmaceuticals	Generic
09/15/2022	VANCOMYCIN HYDROCHLORIDE*	Meitheal Pharmaceuticals	Generic
05/09/2022	VORICONAZALE	Meitheal Pharmaceuticals	Generic
09/15/2022	ZIPRASIDONE MESYLATE	Novadoz Pharmaceuticals	Generic

* Some active ingredients are repeated for same generic drugs with distinctive filled NDCs.

US FDA Center for Drugs Evaluation and Research (CDER) *(Cont'd)*

Table 2. Lyophilized Drugs Approved by US FDA in 2022 (CDER) by Proprietary Names, Excipients, and Indications

Proprietary Name	Excipients	Indications
ALLOPURINOL	Not found	For the management of adult and pediatric patients with leukemia, lymphoma, and solid tumor malignancies who are receiving cancer therapy which causes elevations of serum and urinary uric acid levels and who cannot tolerate oral therapy
AMPHOTERICIN B	• α tocopherol • Sucrose • Sodium succinate hexahydrate • Hydrogenated soybean lecithin • Water • Cholesterol • 1,2-Distearoyl-sn-glycero-3-(phospho-rac-(1-glycerol))	Indicated for empirical therapy for presumed fungal infection in febrile, neutropenic patients, treatment of Cryptococcal Meningitis in HIV-infected patients, treatment of patients with Aspergillus species, Candida species and/or Cryptococcus species infections (see above for the treatment of Cryptococcal Meningitis) refractory to amphotericin B deoxycholate, or in patients where renal impairment or unacceptable toxicity precludes the use of amphotericin B deoxycholate; and treatment of visceral leishmaniasis. In immunocompromised patients with visceral leishmaniasis treated with amphotericin B liposome for injection, relapse rates were high following initial clearance of parasites
AZACITIDINE (2)*	• Mannitol	For the treatment of adult patients with the following FAB myelodysplastic syndrome (MDS) subtypes: Refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS) (if accompanied by neutropenia or thrombocytopenia or requiring transfusions), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-T), and chronic myelomonocytic leukemia (CMMoL)
BORTEZOMIB	• Mannitol	For treatment of adult patients with multiple myeloma, and treatment of adult patients with mantle cell lymphoma
DAPTOMYCIN (3)*	Not found	For the treatment of complicated skin and skin structure infections (cSSSI) in adult and pediatric patients (1 to 17 years of age), Staphylococcus aureus bloodstream infections (bacteremia), in adult patients including those with right-sided infective endocarditis, Staphylococcus aureus bloodstream infections (bacteremia) in pediatric patients (1 to 17 years of age)
DAPZURA RT	• Hydrochloric acid • Mannitol • Sorbitol • Sodium hydroxide	For the treatment of complicated skin and skin structure infections (cSSSI) in adult and pediatric patients (1 to 17 years of age), Staphylococcus aureus bloodstream infections (bacteremia), in adult patients including those with right-sided infective endocarditis, Staphylococcus aureus bloodstream infections (bacteremia) in pediatric patients (1 to 17 years of age)
DECITABINE	• Sodium hydroxide • Potassium phosphate, monobasic • Hydrochloric acid	For treatment of adult patients with myelodysplastic syndromes (MDS) including previously treated and untreated, de novo and secondary MDS of all French American British subtypes (refractory anemia, refractory anemia with ringed side oblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia) and intermediate-1, intermediate-2, and high-risk International Prognostic Scoring System groups
ERYTHROMYCIN LACTOBIONATE	• Water	For the treatment of infections caused by susceptible strains of the designated organisms in the diseases listed below when oral administration is not possible or when the severity of the infection requires immediate high serum levels of erythromycin. Intravenous therapy should be replaced by oral administration at the appropriate time. 1. Upper respiratory tract infections of mild to moderate degree caused by Streptococcus pyogenes (Group A beta-hemolytic streptococci); Streptococcus pneumoniae (Diplococcus pneumoniae); Hemophilus influenzae (when used concomitantly with adequate doses of sulfonamides, since many strains of H. influenzae are not susceptible to the erythromycin concentrations ordinarily achieved). 2. Lower respiratory tract infections of mild to moderate severity caused by Streptococcus pyogenes (Group A beta-hemolytic streptococci); Streptococcus pneumoniae (Diplococcus pneumoniae). 3. Respiratory tract infections due to Mycoplasma pneumoniae. 4. Skin and skin structure infections of mild to moderate severity caused by Streptococcus pyogenes and Staphylococcus aureus (resistant staphylococci may emerge during treatment). 5. Diphtheria: As an adjunct to antitoxin infections due to Corynebacterium diphtheriae to prevent establishment of carriers and to eradicate the organism in carriers. 6. Erythrasma: In the treatment of infections due to Corynebacterium minutissimum. 7. Acute pelvic inflammatory disease caused by Neisseria gonorrhoeae: Erythromycin lactobionate for injection, USP, followed by erythromycin stearate or erythromycin base orally, as an alternative drug in treatment of acute pelvic inflammatory disease caused by N. gonorrhoeae in female patients with a history of sensitivity to penicillin

LYO IN REVIEW 2022

US FDA Center for Drugs Evaluation and Research (CDER) *(Cont'd)*

Table 2 *(cont'd)*

Proprietary Name	Excipients	Indications
ESOMEPRAZOLE SODIUM	• Edetate disodium • Sodium hydroxide	For the Short-term treatment of Gastroesophageal Reflux Disease (GERD) with erosive esophagitis (EE) in adults and pediatric patients 1 month to 17 years of age, as an alternative to oral therapy when oral esomeprazole is not possible or appropriate, and risk reduction of rebleeding of gastric or duodenal ulcers following therapeutic endoscopy for acute bleeding gastric or duodenal ulcers in adults
LOCAMETZ	• Sodium chloride • Water • Sodium acetate • Gentisic acid • Nitrogen	For positron emission tomography (PET) of prostate-specific membrane antigen (PSMA)-positive lesions in men with prostate cancer with suspected metastasis who are candidates for initial definitive therapy, with suspected recurrence based on elevated serum prostate-specific antigen (PSA) level For selection of patients with metastatic prostate cancer, for whom lutetium Lu 177 vipivotide tetraxetan PSMA-directed therapy is indicated
MICAFUNGIN SODIUM (2)*	• Sodium hydroxide • Lactose monohydrate • Anhydrous citric acid	For treatment of candidemia, acute disseminated candidiasis, candida peritonitis and abscesses in adult and pediatric patients 4 months of age and older, treatment of esophageal candidiasis in adult and pediatric patients 4 months of age and older, and prophylaxis of candida infections in adult and pediatric patients 4 months of age and older undergoing Hematopoietic Stem Cell Transplantation (HSCT)
MITOMYCIN (3)*	• Mannitol	Not recommended as single-agent, primary therapy. It has been shown to be useful in the therapy of disseminated adenocarcinoma of the stomach or pancreas in proven combinations with other approved chemotherapeutic agents and as palliative treatment when other modalities have failed
PEMETREXED DISODIUM (5)*	• Sodium hydroxide • Mannitol • Hydrochloric acid	Can be used in combination with pembrolizumab and platinum chemotherapy, for the initial treatment of patients with metastatic non-squamous non-small cell lung cancer (NSCLC), with no EGFR or ALK genomic tumor aberrations, or in combination with cisplatin for the initial treatment of patients with locally advanced or metastatic, non-squamous NSCLC. Can also be used as a single agent for the maintenance treatment of patients with locally advanced or metastatic, non-squamous NSCLC whose disease has not progressed after four cycles of platinum-based first-line chemotherapy, and as a single agent for the treatment of patients with recurrent, metastatic non-squamous, NSCLC after prior chemotherapy
TERLIVAZ	Not found	Indicated to improve kidney function in adults with hepatorenal syndrome with rapid reduction in kidney function
VANCOMYCIN HYDROCHLORIDE (4)*	Not found	Indicated in adult and pediatric patients (neonates and older) for the treatment of septicemia, infective endocarditis, skin and skin structure, infections, bone Infections, and lower respiratory tract Infections
VORICONAZALE	• Betadex sulfobutyl ether sodium	For the treatment of adults and pediatric patients 2 years of age and older with invasive aspergillosis, candidemia in non-neutropenics and other deep tissue Candida infections, esophageal candidiasis, and serious fungal infections caused by Scedosporium apiospermum and Fusarium species including Fusarium solani, in patients intolerant of or refractory to, other therapy
ZIPRASIDONE MESYLATE	• Methanesulfonic acid • Sulfobutylether-β-cyclodextrin	For the acute treatment of agitation in schizophrenic patients

*As commented in Table 1, these generic drugs with different NDCs have similar excipients and are used for the same treatment. In parenthesis the total number of fillings for such drug in year 2022 is included.

LYO IN REVIEW 2022

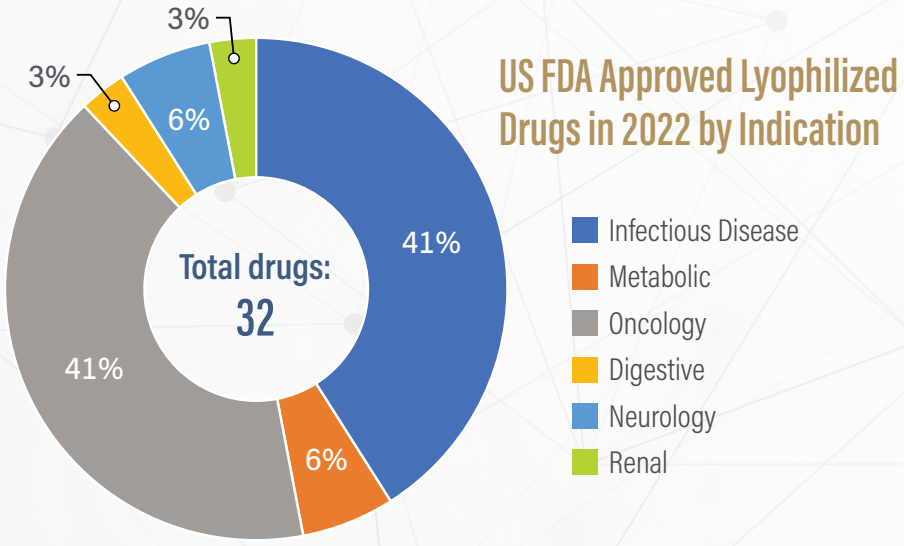
US FDA Center for Biologics Evaluation and Research (CBER)

Table 3. Lyophilized Biologics Approved by US FDA in 2022 (CBER) by Proprietary Names, Applicants, and Drug Types

Approved Date (MM/DD/YY)	Proprietary Name	Applicant	Drug Type
09/07/2022	DAXXIFY	Revance Therapeutics Inc	Biologic
08/31/2022	XENPOZYME	Genzyme Corp	Biologic

Table 4. Lyophilized Biologics Approved by US FDA in 2022 (CBER) by Proprietary Names, Active Ingredients, Excipients, and Indications

Proprietary Name	Active Ingredients	Excipients	Indications
DAXXIFY	BOTULINUM TOXIN TYPE A	• Trehalose dihydrate • Polysorbate 20 • Histidine	For the treatment or improvement of temporary improvement in the appearance of moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity in adult patients, cervical dystonia in adults
XENPOZYME	OLIPUDASE ALFA	• Sodium phosphate, monobasic, monohydrate • Sodium phosphate, dibasic, heptahydrate • Sucrose • Methionine	For treatment of non-central nervous system manifestations of acid sphingomyelinase deficiency (ASMD) in adult and pediatric patients



It is worth noting that in the past decade submissions for lyophilized drugs approvals have increased by an average 15%. From 2012 to 2022, a total of 336 lyophilized drugs were approved which constitute 59% of the total fillings since 1954. The two most relevant categories by indication are treatments for Infectious diseases and Oncology that together comprised ~69% of approvals for the last ten years. The demand for this type of formulations is still expected to rise due to the benefits of the freeze-drying process such us providing longer shelf life and higher stability, and the current increment of chronic diseases. Likewise, an accelerated growth for the lyophilized injectable market is foreseen due to the COVID-19 pandemic.

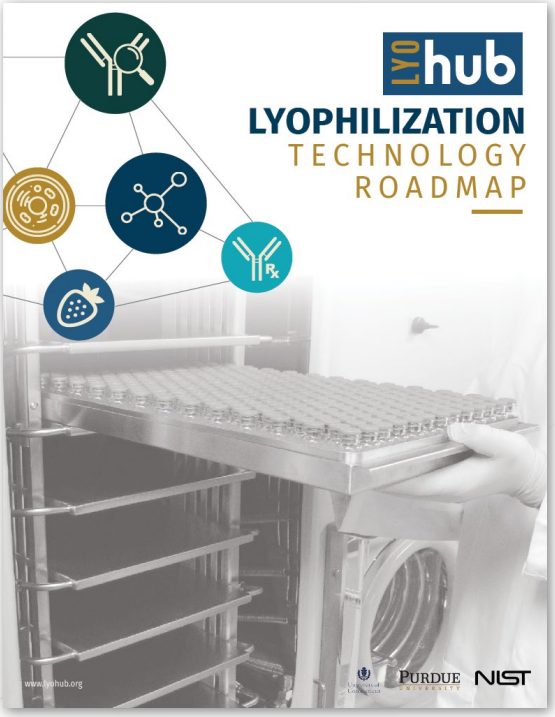
LYO IN REVIEW 2022

European Medicines Agency (EMA)

Table 5. Lyophilized Drugs Approved by EMA in 2022 by Propriety Names, Applicants, Excipients, and Indications

Approved Date (MM/DD/YY)	Propriety Name (Active Ingredients, if different)	Applicant	Excipients	Indications
07/15/2022	CEVENFACTA [EPTACOG BETA]	LFB Biotechnologies	• Arginine hydrochloride • Isoleucine • Trisodium citrate dihydrate • Glycine • Lysine hydrochloride • Polysorbate 80 • Hydrochloric acid	Indicated in adults and adolescents (12 years of age and older) for the treatment of bleeding episodes and for the prevention of bleeding in those undergoing surgery or invasive procedures in the following patient groups: in patients with congenital hemophilia with high-responding inhibitors to coagulation factors VIII or IX (i.e. ≥5 Bethesda Units (BU)); and in patients with congenital hemophilia with low titer inhibitors (BU <5), but expected to have a high anamnestic response to factor VIII or factor IX administration or expected to be refractory to increased dosing of FVIII or FIX
09/15/2022	NULIBRY [FOSDENOPTERIN HYDROBROMIDE]	Zydus	• Mannitol • Sucrose • Ascorbic acid • Hydrochloric acid • Sodium Hydroxide	Indicated to reduce the risk of mortality in patients with molybdenum cofactor deficiency (MoCD) Type A
08/17/2022	PEPAXTI [MELPHALAN FLUFENAMIDE]	Oncopeptides AB	• Sucrose	Indicated, in combination with dexamethasone, for the treatment of adult patients with multiple myeloma who have received at least three prior lines of therapies, whose disease is refractory to at least one proteasome inhibitor, one immunomodulatory agent, and one anti-CD38 monoclonal antibody, and who have demonstrated disease progression on or after the last therapy
04/25/2022	VYDURA [RIMEGEPANT]	Pfizer	• Gelatin • Mannitol (E421) • Mint flavor • Sucralose	Indicated for the acute treatment of migraine with or without aura in adults, and preventive treatment of episodic migraine in adults who have at least 4 migraine attacks per month
06/24/2022	XENPOZYME [OLIPUDASE ALFA]	Genzyme	• Sodium phosphate, dibasic, heptahydrate • Sodium phosphate, monobasic, monohydrate • Sucrose • Methionine	Indicated for treatment of non–central nervous system manifestations of acid sphingomyelinase deficiency (ASMD) in adult and pediatric patients
12/20/2022	ZYNLONTA [LONCATUXIMAB TESIRINE]	ADC Therapeutics	• Histidine • Polysorbate 20 • Sucrose	Indicated for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, DLBCL arising from low-grade lymphoma, and high-grade B-cell lymphoma

ROADMAP



April 2022: The National Institute of Standards and Technology (NIST) Advanced Manufacturing Technology Roadmap Program (MfgTech) selected LyoHUB to expand the 2017 Lyophilization Technology Roadmap to include:

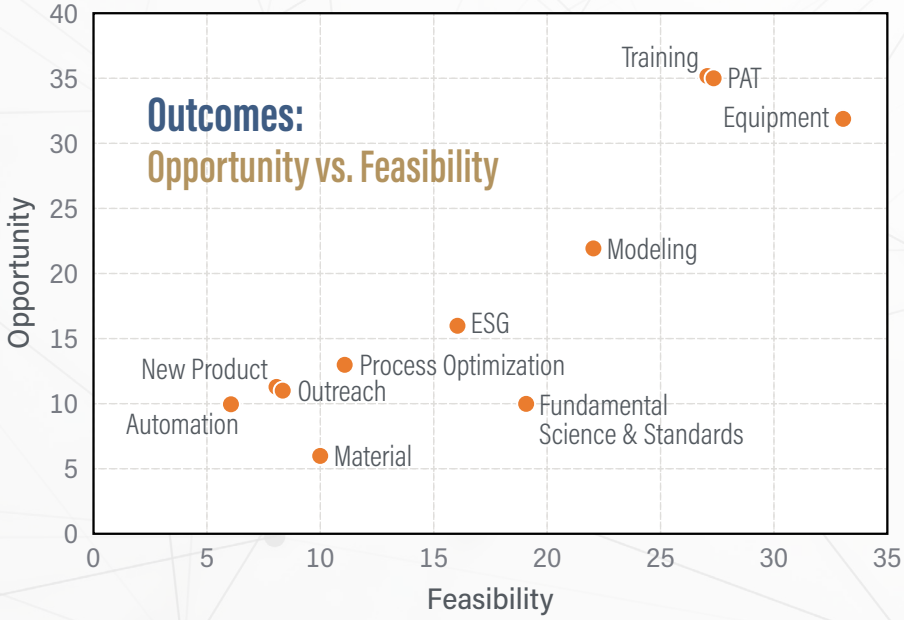
- Novel drying technologies including aseptic spray drying
- Alternatives to drying/lyophilization such as freeze/thaw technologies
- New product modalities including cell and gene therapies, DNA and RNA-based vaccines and therapeutics

The goal of the Lyophilization and Aseptic Drying Technology Roadmap development is to identify drying/preservation technologies that are needed to advance the safety, quality, and profitability of the U.S. pharmaceutical and life sciences industries. Here, "drying/preservation technologies" includes, but is not limited to:

- Improvements to existing lyophilization processes and products, or to supporting instruments, devices, and computational models or analytical methods
- Innovative new lyophilization, drying, preservation processes and products, devices/products combinations or new supporting instruments, computational models or analytical methods focusing on aseptic manufacturing
- Educational, informational, and workforce training materials and programs (including PhD and MS training)
- Identify projects and project areas that address these needs and could be funded by federal agencies, by LyoHUB, by industry or by other groups
- Identify the supporting infrastructure and collaborators needed to accomplish these projects (e.g., websites, pilot facilities)

Workshop 1

A Strategic Landscaping Workshop was held at Purdue University from September 27-28, 2022. At this workshop, participants collected, aggregated, and ranked input to the Trends & Drivers topics, which were defined to capture the diverse market and business factors expected to influence commercial activities over the next ten years. Participants submitted 181 responses. In turn, these were aggregated and ranked by Workshop 1 participants' votes. The process was then repeated to build the roadmap Value Streams/Outcomes layer (i.e., What should we do?) where 110 responses were aggregated into categories and prioritized using predefined feasibility and opportunity metrics, as shown in the figure below.



ROADMAP

Workshop 1 *(Cont'd)*

Industry Participants	Government Participants	University Participants
30	3	20

**This figure includes feedback from separate Workshop 1 virtual meetings as well.*

Participants of the Roadmapping Workshop 1 held at Purdue University.

Workshop 2A

Industry Participants	Government Participants	University Participants
36	5	11

A Topic Roadmapping Workshop was held at the National Academies in Washington D.C. on February 17, 2023 and virtually on February 21, 2023. During this workshop, 36 industry, 5 government and 11 university participants worked in small groups to develop TOPICS #2-5 below in more detail, using a common template, to generate 'first-cut' topic-level roadmaps for review and discussion, to agree on a set of priorities, the way forward and the corresponding action items.

Out of the work done in Workshop 1, five topics were identified for topical roadmapping during Workshop 2A. Those topics are:

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

Participants of the Roadmapping Workshop 2A held at the National Academies, Washington D.C.

Workshop 2B—Topic Roadmapping on the topic of "Training/Workforce Development" will be held during the LyoHUB Annual Meeting in Chicago on April 19, 2023.

Following Workshop 2B, there will be a **Roadmap Synthesis** in late Spring, 2023, followed by Key Performance Indicator (KPI) Development during early summer, 2023. The roadmap will be published and released in Fall, 2023.

SPECIAL PRESENTATIONS TO LYOHUB

April 2022

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

  Blowing the Lid off of Rapid Depressurization Controlled Ice Nucleation in Pharmaceutical Lyophilization

May 2022

- **Kevin Lomasney** | Senior Bioprocessing Trainer at NIBRT (Ireland)

  Lyophilization Excipient Heat Map/Table Trends and Observations

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

  Introduction to LyoHUB Excipient Database

June 2022

- **Davide Fissore** | Professor of Process Control and of Food Processing Technologie at Politecnico di Torino (Italy)

  Use of NIR for Process Monitoring/Control

July 2022

- **Alina Alexeenko** | Co-Director of LyoHUB
- **Serguei Tchessalov** | Research Fellow, Pfizer

  NIST LyoHUB Roadmapping Award

August 2022

- **John Saiz** | Industrial Associate, IfM Engage, University of Cambridge

  Strategic Landscaping Workshop: Perspectives Preparation—Lyophilization and Aseptic Drying Technology Roadmapping for Biotechnology and Pharmaceutical Manufacturing

September 2022

- **Nick Warne** | Vice President of Pharmaceutical Research and Development, Pfizer

  Lessons Learned from the Pandemic: mRNA-LNP Vaccine Development

October 2022

- **Elizabeth Topp** | Co-Director of LyoHUB
- **Greg Sacha** | Associate Director of Research and Development, Baxter

  Progress Update on the Best Practices Papers on Lyophilized Formulations

November 2022

- **Bernard Luy** | COE of Meridion Technologies, Germany

  Spray Freeze Drying—Product Innovation Potential, Manufacturing Logistics and Supply Chain Flexibility

December 2022

- **LyoHUB Roadmapping Leadership Team**

  LyoHUB Roadmapping Project, Results of Workshop #1, Introduction of Topics for Workshop #2

January 2023

- **Joseph Pekny** | Professor of Chemical Engineering at Purdue, CEO of Advanced Process Combinatorics, Inc. (APC)

  The Role of VirtECS in Achieving High Performance Manufacturing Processes: The Case of Assessing Lyophilization Technology Advances

February 2023

- **Sandro Matosevic** | Assistant Professor, College of Pharmacy, Purdue

  Natural Killer (NK) Cell-Based Immunotherapies of Cancer

March 2023

- **LyoHUB Business Meeting**

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

Lyophilization and Aseptic Drying Technology Roadmap for Biotechnology and Pharmaceutical Manufacturing

NIST

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

Tunable RF/Microwave Drying of Biologics

NIIMBL MERCK IMA LIFE

- Funded by NIIMBL (National Institute for Innovation in Manufacturing Biopharmaceuticals)
- $2,000,000 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.

LYOHUB DEMONSTRATION FACILITY

In 2022, LyoHUB received a donation from member company, Metrohm, of a **Metrohm 874 Karl Fischer Oven with 851 Coulometer** (**Figure 1**).

The 874 Oven Sample Processor is used for automatic thermal sample preparation in Karl Fischer titration. The oven method is particularly suitable for samples that do not release their water until higher temperatures have been reached, for sparingly soluble samples, or those that react with the KF reagent.

Basler ACE 2 Pro high-resolution camera.

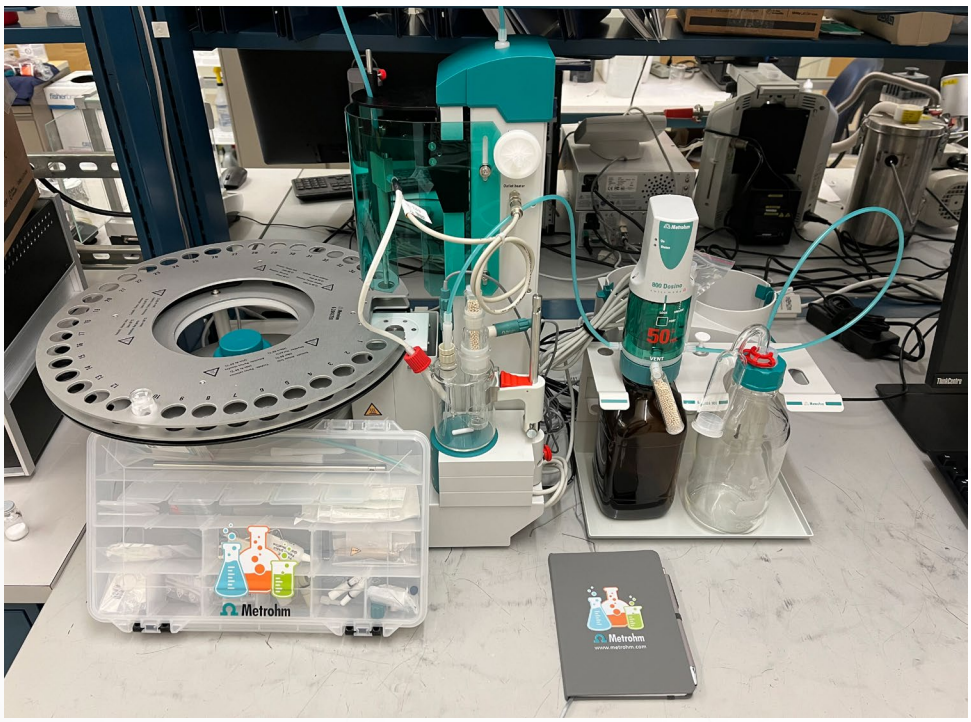


Figure 1: *The Karl Fischer system will be used to determine moisture content of freeze-dried formulations in both 2mL and 6mL glass tubing vials. The unit is equipped with an autosampler that simultaneously increases sampling rate and decreases operator workload.*

An HTC Vive Pro 2 virtual reality system was purchased for the LyoHUB Demonstration Facility to provide a new and exciting visualization experience for users and visitors to the laboratory. An image of the setup being used by Ahmad Darwish is shown in **Figure 1**. The tool was showcased at the NIST roadmapping reception dinner during laboratory tours and included a demonstration of results from simulations of the Rapid Depressurization Controlled Ice Nucleation (RD-CIN) process. Guests used the tool to interact with the data and develop a better understanding of the key mechanisms driving the nucleation process. Capabilities of this system will soon be extended to include computer aided design (CAD) software. 3D modeling in virtual reality is extremely powerful and enables the user to quickly explore and prototype new ideas. Models created using the virtual reality system will be directly linked to our growing portfolio of 3D printing tools to quickly transfer from the digital to the physical spaces.

Figure 1: *HTC Vive Pro 2 virtual reality system being used to visualize the vial headspace environment during rapid depressurization controlled ice nucleation.*

2022 PURDUE PROFESSIONAL MASTER'S CHEMICAL ENGINEERING PROGRAM

Summer Capstone Projects with LyoHUB Members

This program, offered through the Davidson School of Chemical Engineering at Purdue University, is a non-thesis Master's program with students taking both engineering-based and management-based courses. During the summer, students complete a capstone project where they dedicate 40+ hours per week to a 12 week project.

Allergan Pfizer

Process Analytical Technologies in Freeze-drying: Evaluation of Risks of Vial Breakage and Freeze-induced Protein Destabilization Using a Thin-film Strain Sensor

abbvie

Surrogate Solution Design and Preparation for Use in Biological Drug Process Development

Baxter

Co-Solvent Lyophilization: Residual Gas Analysis

COOK BIOTECH

Process Development of the Manufacture of ECM Based Vascular Graft Devices

EVONIK POWER TO CREATE

Optimization of a Recovered Process Solvent Stream at Evonik Tippecanoe Labs

SIEMENS

Modeling Lyophilizers in Transient 3D CFD Using STAR-CCM+

Discrete Modeling of Particles for Pharmaceutical Applications

LyoLaunchPad PROJECTS

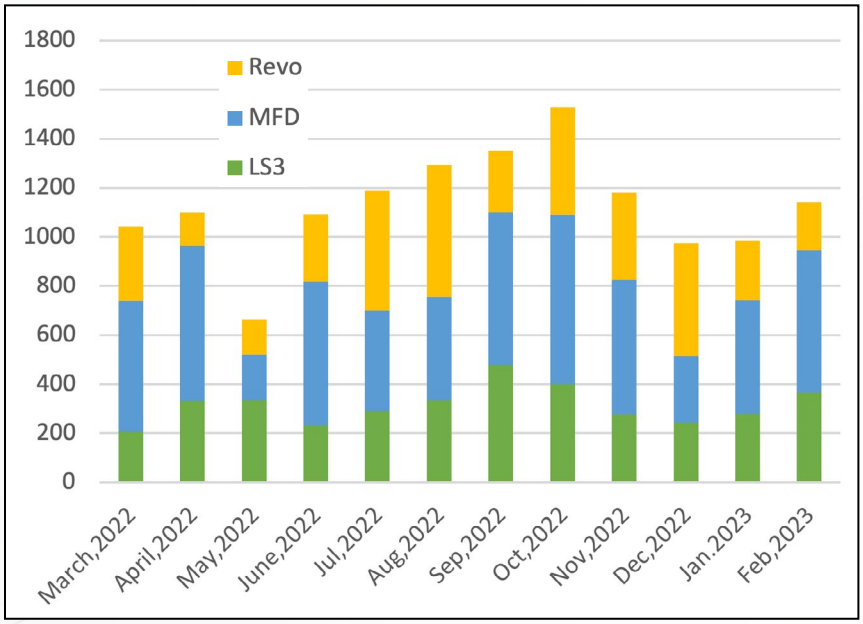
- Freeze dry a cellulose solution made of methyl cellulose and water to make it fit for SEM microscopy (Tian Li lab, Mechanical Engineering)
- Lyophilization of PEGylated Block Copolymer Micelles (You-Yeon Won lab, Chemical Engineering)
- Dry Powder Inhalable Formulations of Antibacterial Agents (Tony Zhou lab, Industrial and Physical Pharmacy)
- Optimized Lyophilization of Gelatin Solutions (Cook Biotech)

INDUSTRY-SPONSORED PROJECTS

- Solid-State Stability of mRNA Vaccines (Pfizer)
- AOM Hardware Performance Validation (ACME)
- Rapid Depressurization Controlled Ice Nucleation Phase 2: Design Space Determination for Production Scale Conditions (Genentech)

DEMONSTRATION FACILITY

Summary of Lyophilizer Use (February 2022 – February 2023)



Years	Total Number of Lyophilization Runs	Total Run Time
2016-2017	87	2971.4
2017-2018	178	9227.2
2018-2019	190	13944
2019-2020	421	16624
2020-2021	184	8505
2021-2022	217*	10614
2022-2023	266*	13542

*Please note that these do not include runs on LyoStar3.

Superusers

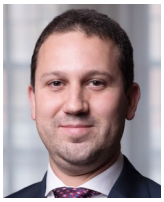
Ashwani Agarwal
Computer Science Graduate Student

Tony Cofer
Aeronautics and Astronautics Engineer

Ahmad Darwish
Electrical and Computer Engineering Postdoctoral Associate

Ian Flynn
LyoHUB Engineering Trainee

Petr Kazarin
Aeronautics and Astronautics Postdoctoral Associate



Andres Roman
Engineering Management Masters Student

Isaac Wheeler
Chemical Engineering Ph.D. Student

Kyu Yoon
Chemical Engineering Research Scientist

LyoSummerSchool 2022

36 participants

15 organizations represented

2 days of lyophilization training with topics:

- Introduction and Lyophilization Process Overview
- Lyophilization Design Space
- Lyophilization Process Modeling
- Laboratory Exercises and Demonstrations
- Post-Lyo Characterization
- Quality Attributes and Lyophilization Equipment
- Process Qualification and Continuous Verification and Regulatory Considerations
- Advanced/Next Generation Lyo Technologies: PAT and Closed Loop Control
- Advanced/Next Generation Lyo Technologies: RF Lyophilization

Instructors

- Alina Alexeenko
- Ahmad Darwish
- Petr Kazarin
- Steve Nail
- Drew Strongrich

Demos

- Material Characterization: Freeze Dry Microscopy, Differential Scanning Calorimetry
- Lyo Setup
- Lab Exercise
- LyoPRONTO
- PAT: Comparative Pressure Measurement, Manometric Temperature Measurement (MTM), Pressure Rise Test (PRT), Residual Gas Analysis, Heat Flux Sensors, Tunable Diode Laser Absorption Spectroscopy (TDLAS), Wireless Sensors
- Post-Lyo Characterization: Differential Scanning Calorimetry, Residual Moisture Analysis, X-Ray Powder Diffraction, Thermogravimetric Analysis, FTIR Spectroscopy

Photos from top to bottom:

1. *LyoSummerSchool 2022 Instructors and Participants.*
2. *LyoHUB Co-Director, Dr. Alina Alexeenko provides tour of the LyoHUB demonstration facility.*
3. *LyoHUB Scientific Advisory Board member, Dr. Steve Nail provides instruction during LyoSummerSchool.*
4. *LyoHUB Research Scientist, Dr. Drew Strongrich provides freeze dry microscopy demo*

MODEL PREDICTIVE CONTROL FOR THE PRIMARY DRYING STAGE OF LYOPHILIZATION

Investigator: *Petr Kazarin (Purdue/AAE)*

Lyophilization, commonly referred to as freeze-drying, is a widely used method of preserving biomaterials and pharmaceuticals. The longest and most time-consuming stage of lyophilization, primary drying, sublimates ice from the frozen product to produce a dry and stable product. The dynamics of the drying process are complex, making it difficult to control and optimize while being essential for the output's quality and stability. In this work, we investigate the application of nonlinear model predictive control (MPC) in the first drying stage of lyophilization. The MPC control approach uses a mathematical model of the primary drying process to forecast future behavior and optimize the operation (significantly reducing the primary drying time while maintaining product quality). In this paper, we demonstrate how MPC improves the efficiency of the lyophilization process.

The nonlinear model predictive control (MPC) technique [1] developed in the GEKKO environment [2] was used to regulate the product temperature and reduce the main drying time. In our situation, the method is based on the outputs of the model (LyoPRONTO primary drying calculator [3]), which predicts the product temperature across the particular prediction horizon at every time step of the process. Based on these predictions, the computer alters the controlled variables (shelf temperature, chamber pressure, or mixed) to make the product temperature match the projected trajectory as closely as possible. To show the efficacy of the MPC control strategy, the experiments were carried out on a Millrock REVO lyophilizer for two formulations: 3 ml of 5% sucrose and 5% mannitol in SCHOTT 6R vials. The temperature was measured using conventional thermocouples inserted into the vial. When the drying procedure was over, the cake's visual quality was demonstrated.

The nonlinear MPC control method has been incorporated into the Millrock REVO lyophilizer as a control technique. The Python OpenOPC module is utilized to bypass the control software and directly connect with the Programmable Logic Controller (PLC). All read and write operations can be queued and done asynchronously due to the multithreaded design of the control system. Directly from the lyophilizer, the model-predictive controller receives product temperature information. Here, the optimal chamber pressure and shelf temperature are determined (while adhering to equipment capability restrictions) and returned to the PLC as setpoints. The controller computes and writes setpoint data at 30-second intervals in order to maintain the product's temperature at its critical temperature during the period of primary drying. However, extra resilience is still required to accommodate both anticipated (e.g., loss of thermocouple contact with ice) and unforeseen events.

Figure 1 depicts the chamber pressure control of the primary drying process for 5% mannitol to illustrate the performance of the algorithm. In this instance, the temperature of the shelf is held constant at 25 °C, but the chamber pressure is varied. After 50 minutes of primary drying, the product temperature approaches the critical threshold of -5 °C. In the following 3 hours, the nonlinear control algorithm manipulates the chamber press to maintain the product temperature within 0.1 °C of the critical value. Four hours after the start of the primary drying process, the pressure rapidly decreases to zero in an attempt to accomplish the control objective, but the product temperature continues to rise. This means that the sublimation front reached the tip of the thermocouple and the sublimation process in the vial is close to the end. At this point, the control algorithm brings the chamber pressure back to where it started and keeps it there until the primary drying step is done. **Figure 2** demonstrates the mannitol 5% in a vial after the PD cycle was finished. No visible signs of collapse were observed.

Other control strategies: shelf temperature control and mixed control (chamber pressure plus shelf temperature control), were also applied to mannitol 5% and sucrose 5% solutions, and the absence of product collapse was demonstrated after visual inspection of the samples.

References:

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

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

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

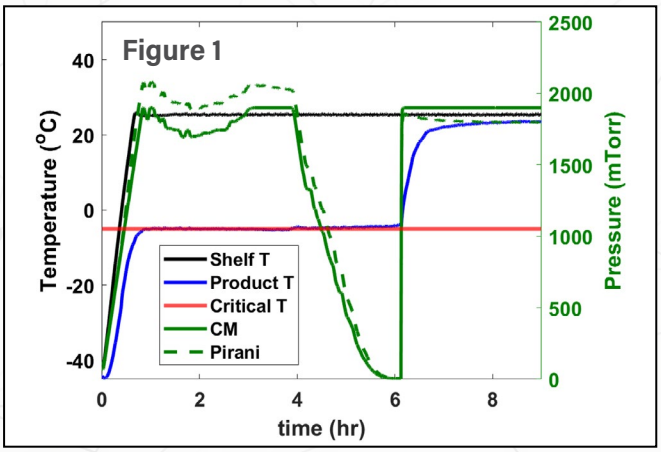


Figure 2

RADIO-FREQUENCY-ASSISTED FREEZE DRYING OF PHARMACEUTICAL PRODUCTS: Influence of Applied Power on Drying Time

Investigators: *Dimitri Peroulis (Purdue/ECE), Ahmad Darwish (Purdue/ECE)*

Because of the rising demand for lyophilized injectable medicines over the last few decades, particularly in light of the present COVID-19 pandemic, freeze-drying or lyophilization technique has recently received a lot of attention. Freeze-drying is widely used in the pharmaceutical industry because it permits the processing of thermolabile products in sterile conditions, even though it is one of the most time-consuming industrial processes with an efficiency of <10%. To that purpose, RF/microwave-based lyophilization is being pursued because it significantly accelerates such processes. LyoHUB is currently developing a product adaptive RF heating approach to shorten the primary drying process while simultaneously improving the overall batch homogeneity compared with conventional freeze-drying.

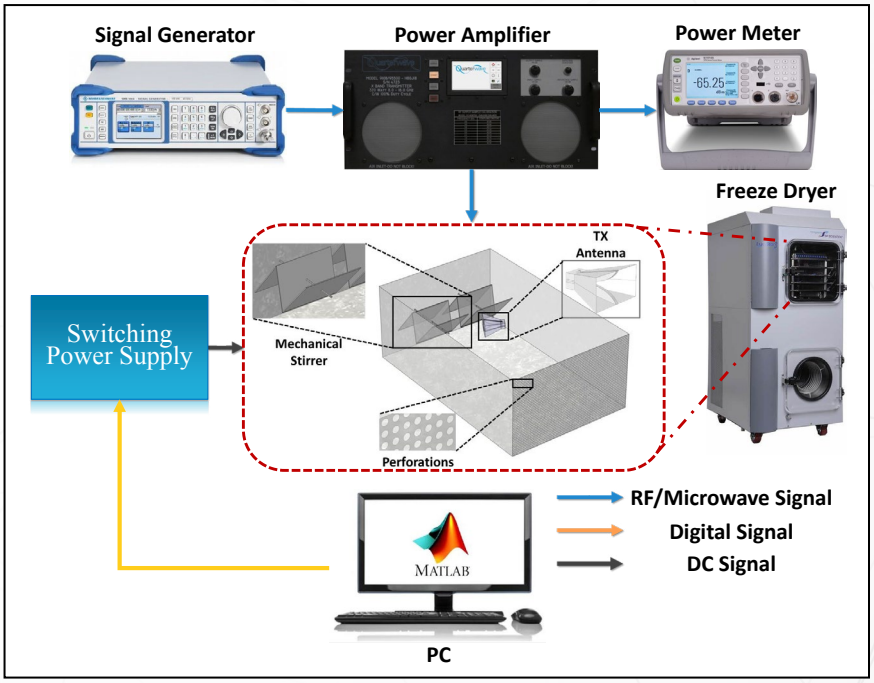


Figure 1: *Block diagram of microwave-assisted lyophilization system.*

Figure 1 depicts the microwave-assisted lyophilization block diagram integrated with a lab-scale lyophilizer. An auxiliary or reverberation chamber (RC), a network analyzer, a power amplifier, an antenna placed inside the RC, and two stepper motors coupled to stirrers inside the RC compose the experimental setup. The RC is a metallic box that houses a transmitting antenna, which serves as a source of RF power inside the chamber. The antenna is connected to a power amplifier to amplify high-frequency (18GHz) electromagnetic signals generated by a signal generator. When the antenna emits electromagnetic waves into the RC, the waves encounter various reflections, resulting in numerous resonances within the chamber. The number of resonances, and thus field uniformity, increases as frequency increases. If the stirrers inside the chamber are fixed, the field distribution stays unchanged, resulting in hot and cold regions inside the RC (highly nonuniform electromagnetic field). To solve this problem, we continually rotate the two stirrers inside the RC to vary the field distribution at different time instants.

Figure 2 displays the Capacitance Manometer (CM) and Pirani gauge pressure measurements against primary drying time for conventional and RF-assisted lyophilization cycles (BSA with sucrose as excipient).

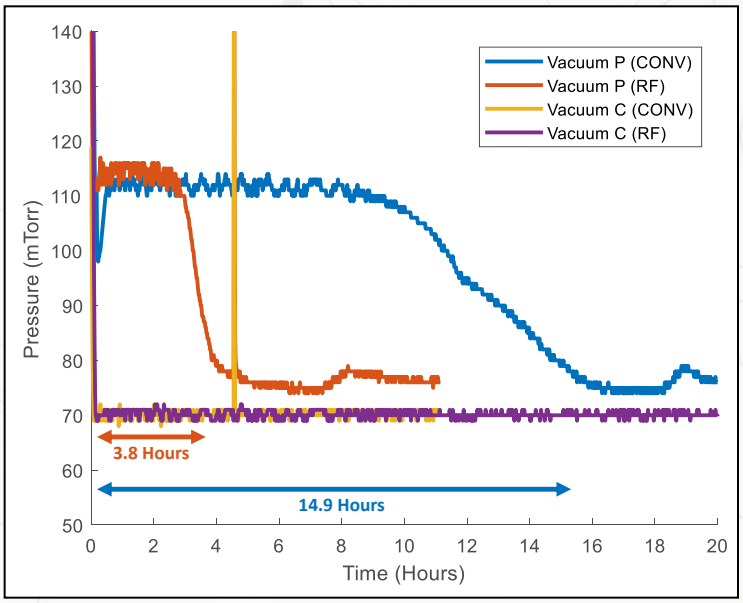


Figure 2: *Capacitance Manometer (CM) and Pirani gauge pressure measurements versus primary drying time for conventional and microwave-assisted lyophilization cycles of sucrose (5% Solids/70% BSA/30% Sucrose), 2R SCHOTT Vial, 0.5 ml fill volume, 60 vials).*

The primary drying time is reduced from 14.93 hours to 3.9 hours using 40W output RF power (a speedup of 3.8x has been perceived). We have not observed any collapses in the Conventional or the RF-assisted cycles.

IN-SITU VIAL STRAIN MEASUREMENTS DURING FREEZE/THAW PROCESSES USING AMORPHOUS EXCIPIENTS

Investigators: *Ian Flynn (Purdue/LyoHUB), Drew Strongrich (Purdue/LyoHUB)*

The effects of mechanical stresses developed between a frozen formulation and glass vial during freezing and thawing operations are not well understood. In many cases, the interfacial forces can become significant, leading to catastrophic failure of the primary packaging (i.e. breakage). To investigate these effects, an experimental campaign using wireless strain gauges was carried out as part of the Purdue's Chemical Engineering Professional Master's Program. The sensors measured strain output using foil gauges and vial wall temperature via a resistance temperature detector (RTD). A 6R vial illustrating the location of the RTD and strain gauge is provided in **Figure 1**. The study focused on freeze/thaw cycles with different concentrations of amorphous excipients, specifically sucrose and trehalose.

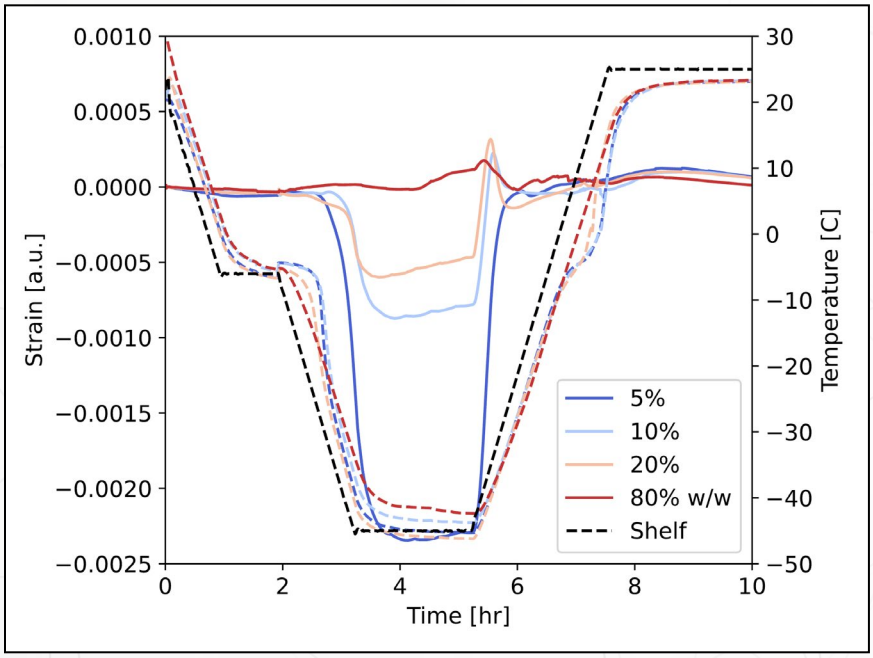


Figure 1: *Image of gauged vial used to characterize thermomechanical response of borosilicate vials during freezing and thawing.*

The strain response during freezing and thawing for sucrose concentrations between 5% w/v and 80% w/w is provided in **Figure 2**. Results clearly indicate a dependence on excipient concentration and frozen matrix temperature. Additionally, local maxima appear near a product temperature of around -32C for concentrations of 10% and 20% w/v. This behavior was attributed to a glass transition in the freeze-concentrated solute and subsequent stress relaxation within the frozen matrix. Results using trehalose were qualitatively similar but exhibited larger gauge output magnitudes, especially at the end of the freezing step. The ability to quantify strain throughout the freezing and thawing process suggest the strain sensors may have utility as a new process analytical technology for cycle design, especially when operating in conditions that are known to produce breakage. Computational studies were also performed as part of this work to develop a better understanding of the principal stresses and strains during the experiments.

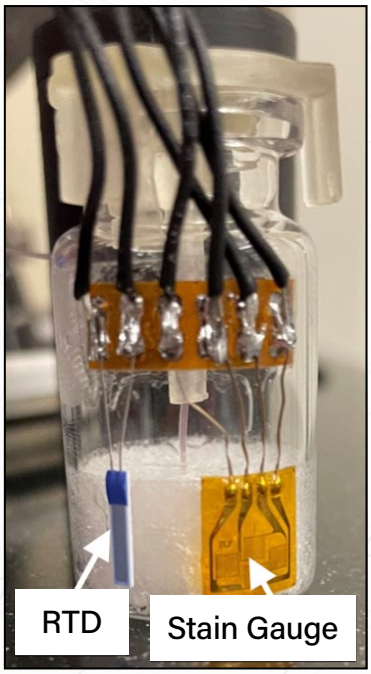


Figure 2: *Measured gauge output during freezing and thawing for different concentrations of sucrose (%w/v unless indicated). The strain response varies with concentration and temperature, response of borosilicate vials during freezing and thawing.*

CHARACTERIZATION OF HEAT TRANSFER THROUGH GAS AND ITS IMPACT ON VIAL HEAT TRANSFER DURING PRIMARY AND SECONDARY DRYINGS

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

The importance of heat transfer during the secondary drying stage cannot be overstated as it greatly influences the temperature profile of the material, which has a direct impact on the removal of bound water. Historically, it has been assumed that the heat transfer coefficient (K_v) during secondary drying is the same as during primary drying and that most of the heat goes towards drying the material. However, recent studies have challenged these assumptions and have suggested that K_v should be a function of the moisture content in the chamber, implying that it should be different in primary and secondary drying [1].

Our recent work [2,3] was the first to experimentally test these claims through the lyophilization of various excipients under different operating conditions in primary and secondary drying using laboratory-scale freeze dryers. To estimate the impact of gas conduction on the overall vial heat transfer, we calculated the theoretical heat transfer coefficient of gas conduction (K_g) based on scanned images of the vial bottom as shown in **Figure 1**.

Our findings showed that the heat transfer coefficient during primary drying increases significantly with increasing chamber pressure, regardless of vial type. This trend is likely due to the high thermal conductivity of water vapor, which depends heavily on pressure. During secondary drying, the vial heat transfer coefficient ($K_{v,\,sec}$) showed a weak dependence on chamber pressure and was found to be 20-60% of K_v, in primary drying, depending on the chamber pressure [3]. Theoretical calculations of K_g support these experimental observations, showing a monotonic increase in K_g with increasing chamber pressure during both primary and secondary drying as shown in **Figure 2**. However, K_g during secondary drying was noticeably different from that during primary drying due to the lower thermal conductivity, resulting in a smaller magnitude change in K_g with chamber pressure compared to water vapor.

Based on these findings and ongoing measurements of moisture content, a simple-to-use calculator for secondary drying is under development. Further investigation will establish a molecular understanding of desorption kinetics in lyophilization.

References:

[1] Sahni, E.K & Pikal, M. (2017). J. Pharm. Sci., 106(3) 779-791.

[2] Yoon, K. & Narsimhan, V. (2022). J. Pharm. Sci., 111(2), 368-381.

[3] Yoon, K. & Narsimhan, V. (2023) Int. J. Pharm., 635, 122746.

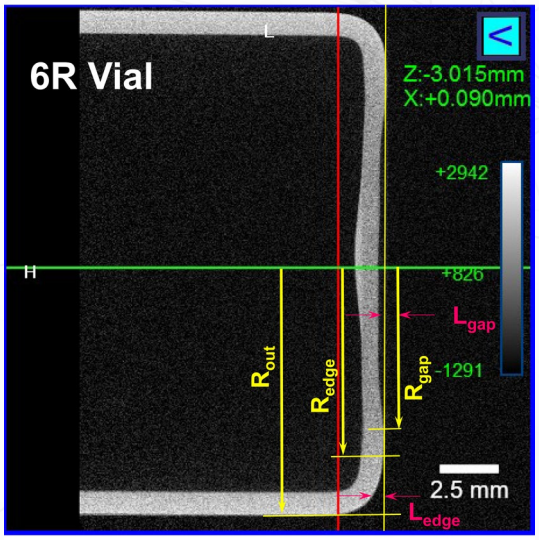


Figure 1: *Schematics of scanned geometric parameters for a vial bottom. Image captured with Purdue Imaging Facility instrument.*

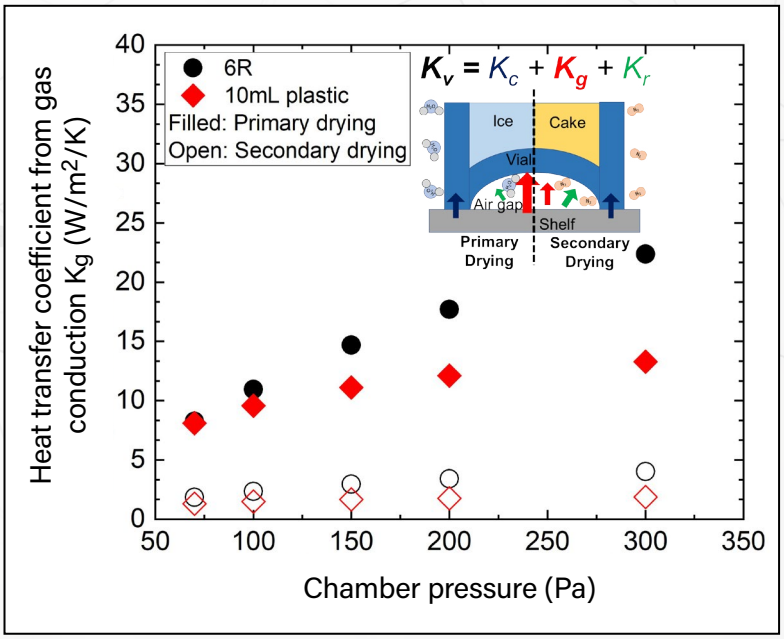


Figure 2: *Theoretical heat transfer through gas conduction of 6R and 10ml SiO2 vial during primary and secondary drying stages at the different chamber pressure (70 mTorr < P_{ch} < 300 mTorr).*

EXCIPIENT DATABASE

Investigators: *Ashwani Agarwal (Purdue/AAE), Drew Strongrich (Purdue/LyoHUB)*

Excipients are inactive substances that play an important role in the formulation and development of drugs. The study and analysis of excipients used in lyophilized drugs is important as it helps in ensuring the quality and efficacy of such drugs. However, to review the usage of excipients in lyophilized drugs there was a lack of a consolidated resource that documented all inactive ingredients (excipients) used in every FDA-approved lyophilized drug. To address this gap, we created a unique and comprehensive database of all FDA-approved lyophilized drugs from 1954 to 2022. This database holds information about every FDA-approved lyophilized drug, including the application number filed with the FDA, the date of application filing, the product name, and the company. It also includes information about the active and inactive ingredients used in each drug, including the name and strength of each ingredient used in the drug's formulation.

An application was developed to create this database. The application first gathered a list of more than 23000 FDA approved drugs using FDA's Application Programming Interface (API). This list was then cross-referenced with DailyMed to isolate around 600 lyophilized drugs from the original 23000 drugs. The application stored these lyophilized drugs in a persistent database. For each of these lyophilized drugs, DailyMed's API endpoints were used to fetch the ingredients information which were then stored in the database. This is represented in **Figure 1**.

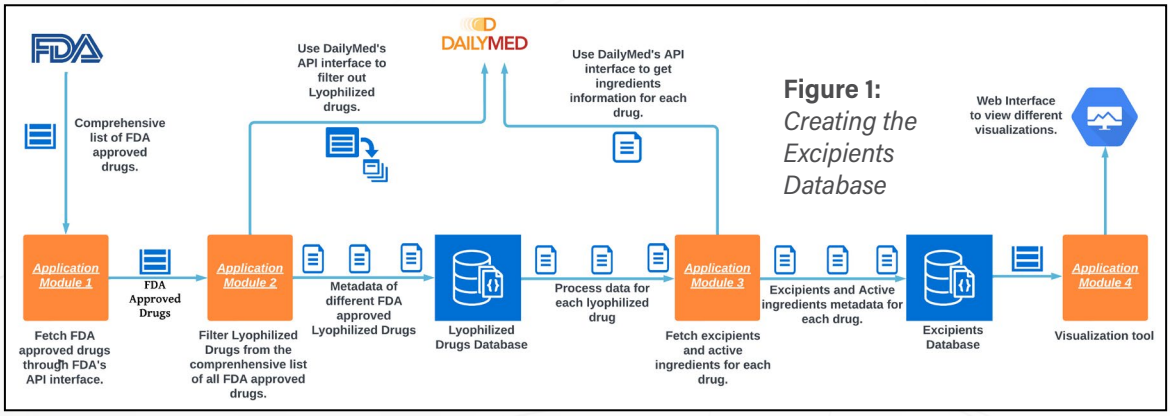


Figure 1: *Creating the Excipients Database*

The results from the application's database showed that, as of 2022, there have been 622 unique FDA-approved lyophilized drug applications. This number continues to grow each year, with the highest recorded number of applications taking place in 2017, as shown in **Figure 2**.

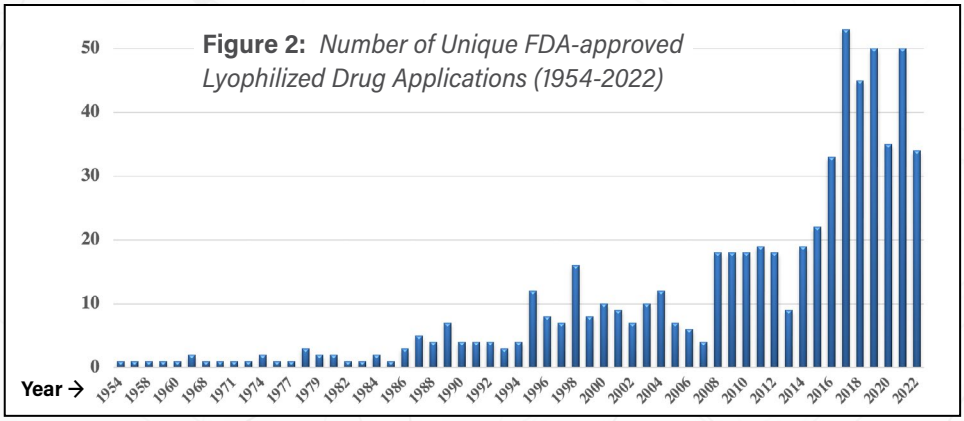


Figure 2: *Number of Unique FDA-approved Lyophilized Drug Applications (1954-2022)*

Furthermore, there is a broad range of excipients utilized in these lyophilized drugs, with over 180 different excipients in use. The most frequently used excipients include mannitol and sodium hydroxide, appearing in over 100 different lyophilized drug formulations, as illustrated in **Figure 3**.

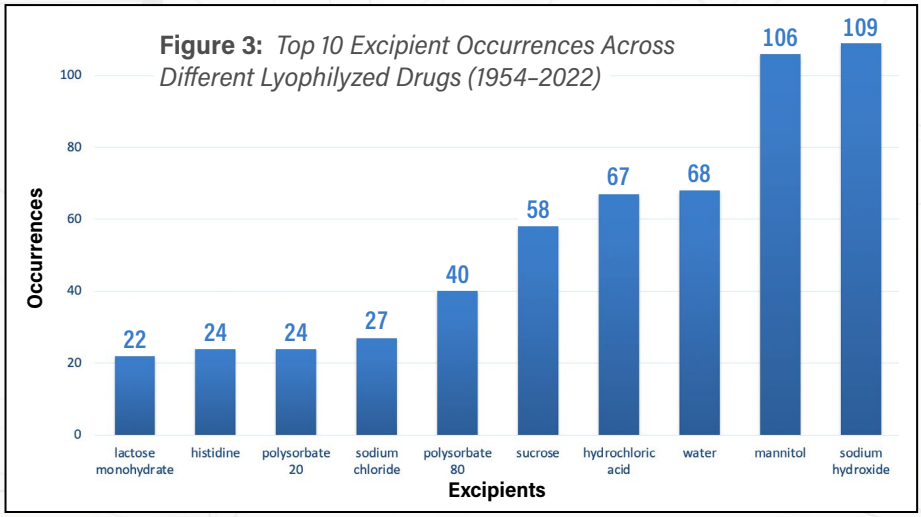


Figure 3: *Top 10 Excipient Occurrences Across Different Lyophilyzed Drugs (1954–2022)*

The database not only records the excipients but also the active ingredients used in each drug formulation. The findings indicate that there are approximately 242 different active ingredients utilized among all the lyophilized drugs, with somatropin being the most common, appearing in ten different drug formulations.

MEASURING LYOPHILIZED POROUS STRUCTURES WITH MICRO-CT SCANNING

Investigators: *Vivek Narsimhan (Purdue/ChemE), Isaac Wheeler (Purdue/ChemE)*

The mass transfer resistance R_p is an essential parameter in models of the primary drying step of lyophilization. This value quantifies how much dried material obstructs the flow of water vapor, and is known to depend on many factors (including formulation, freezing conditions, and drying conditions). By investigating the porous structure of lyophilized material, we hope to improve estimates and understanding of R_p.

Micro-CT scanning is a technique for imaging the insides of structures (analogous to medical CT scans), using X-ray images of a material captured from many angles (a typical example shown in **Figure 1**). This method yields images which each represent a thin slice of the material (such as the horizontal slice in **Figure 2**), which can be assembled into a full volumetric image of the structure. This method has some limitations, such as poor image contrast when using glass vials, as well as requiring robust image processing procedures. However, it is nondestructive in nature, and can provide 3-dimensional information which 2D imaging techniques like SEM inherently cannot; for example, pore connectivity and pore size cannot be rigorously estimated from only 2D information.

Using plastic vials (manufactured by SiO2) and a Bruker SkyScan 1272 (owned by CP3 at Purdue), micro-CT scans of lyophilized mannitol and sucrose have been captured at varying resolutions. A software tool written in the Julia language is under development for computing features like porosity, surface area, and spatially varying pore size distribution. **Figure 3** shows the average pore size as a function of vertical and radial position for a 0.5mm-tall region (corresponding to the region boxed in red on Figure 1). Porosity and surface area estimates in this region are within 10% of the estimates in the top and bottom regions of the cake, but pore sizes vary significantly. Since pore size is known to relate to R_p, this can yield estimates of how mass transfer resistance varies within a single cake and enable detailed models.

Figure 1: *X-ray image of lyophilized mannitol in a 10mL plastic vial.*

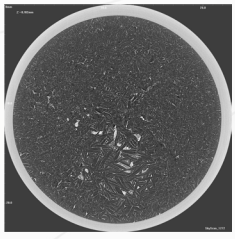


Figure 2: *Reconstructed slice of cake shown in Figure 1, taken from region marked in red.*

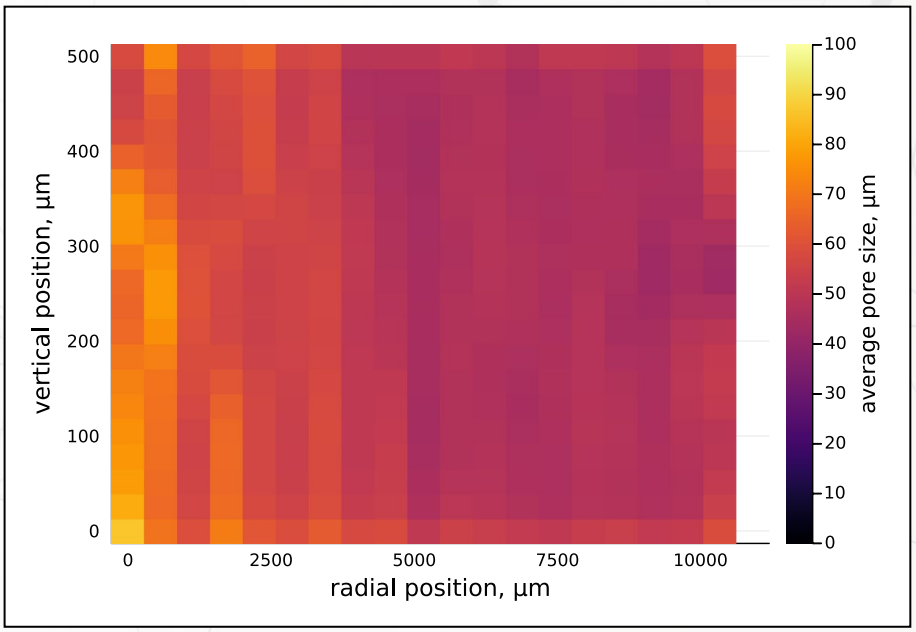


Figure 3: *Average pore size as a function of vertical and radial position for the region of Figure 1 marked in red. Note the distinctly larger pores in the center of the cake (left side of this figure).*

LYOPHILIZATION CYCLE DESIGN AND OPTIMIZATION OF AQUEOUS CO-SOLVENT FORMULATIONS USING RESIDUAL GAS ANALYSIS

Lipid Nano Particle (LNP) encapsulated mRNA vaccines are generally unstable at room temperature and require storage under cryogenic temperature prior to administration. Although freezing is simple, proven, and effective, the cold chain dependency of these substances is unsustainable in the long term. Preparation of LNP-based vaccines typically requires high concentrations of ethanol to prevent agglomeration prior to encapsulation. This makes lyophilization highly difficult due to the severe freezing point depression and large differences in vapor pressure between water and organic solvents. Additional dialysis steps are therefore required to adjust solvent concentrations to levels that are feasible for freeze-drying. As part of NIIMBL American Rescue Plan grant 11, **Advanced Characterization and Manufacturing Methods for mRNA Vaccine Development**, we applied in-situ residual gas analysis using an Inficon Transpector CPM3 on a Millrock REVO lyophilizer to quantify the relative concentrations of the co-solvents in vapor phase during lyophilization of LNP encapsulated yeast RNA vaccine placebos. Time lapse imaging was also used to identify abnormal behaviors throughout the cycle. The spectral data is shown in figure 1 and an image of the product during primary drying in **Figure 2**. The RGA is clearly able to identify the principal peaks of water and ethanol in the formulation as well as the nitrogen ballast gas. This capability allows lyophilization cycles to be developed that selectively target solvents of different vapor pressures throughout the process. In this case, ethanol was removed using a series of setpoints that lie between ice sublimation and ethanol evaporation thresholds. Once extracted, a more aggressive and standard set of pressures and temperatures was used to remove bulk ice, the time lapse images indicated cake lifting near the beginning of primary drying. However, both lifting height and lifted duration were significantly reduced when compared to a reference single-step lyophilization process. The study is ongoing but has already demonstrated the utility of using in-situ residual gas analysis and unconventional imaging techniques for lyophilization cycle development.

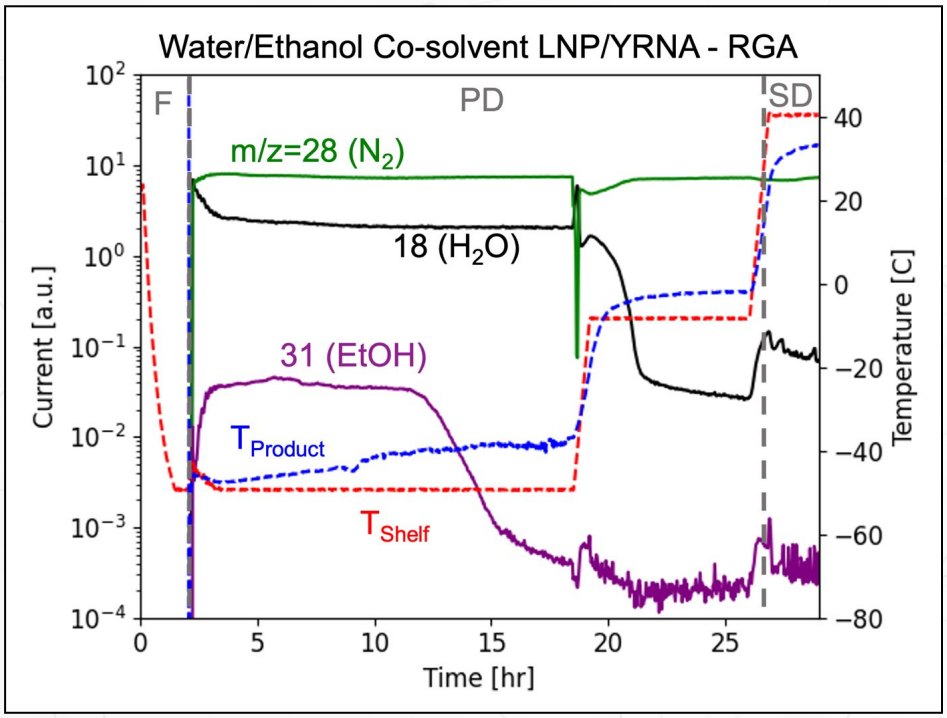


Figure 1: *RGA process data for an LNP encapsulated yeast RNA formulation containing 1% ethanol.*

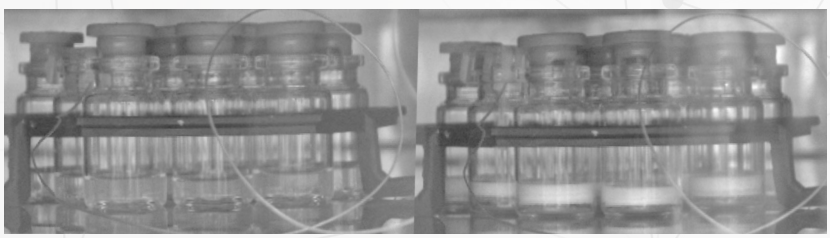


Figure 2: *Time lapse images from the lyophilization of LNP-encapsulated yeast RNA formulation containing 1% ethanol prior to freezing (left) and during primary drying (right).*

LYOLAUNCHPAD PROJECT

Lyophilization of PEGylated Block Copolymer Micelles

With Prof. You-Yeon Won's Group (Purdue ChemE)

Background: Dr. Won's group is developing polymer nanoparticle-based therapeutics, termed "Polymer Lung Surfactant (PLS)", for treatment of acute respiratory distress syndrome (ARDS). Their model PLS consists of hydrophobic polystyrene "core" and water-soluble polyethylene glycol (PEG) "brush" which coat the surface of the core. The pulmonary administration of PLS can boost the surface pressure of alveolar fluid when the natural lung surfactant is compromised by various lung injuries and following edema. The pharmaceutical efficacy of this formulation [1] and the bioavailability of PLS administration based on pharyngeal aspiration [2] has been proven in recent publications.

Objectives: In this project, they aimed to determine the feasibility of lyophilizing PLS formulations for an elongated room temperature storage and easier handling without losing the pharmaceutical effect. They screened structural parameters of nanoparticles (e.g., PEG grafting density) and varied lyophilization process parameters (e.g., temperature ramp rates) to find out the optimal condition for the preservation of the surface activity (i.e., the ability to produce very low surface tension). Because the saccharide or polymeric excipients can reduce the surface activity, they conducted lyophilization of the formulations without any excipient.

Results: They were able to pick up 3 main factors in successful excipient-free lyophilization of the PLS, which is PEG-grafted polymer nanoparticles with enormous surface activity. (i) Faster cooling at the freezing stage resulted in less severe freezing stress applied on the particulate cargos, leading to superior re-dispersibility of PLS formulation. (ii) Higher PEG grafting density provided stronger stabilization of PLS during the lyophilization cycle. (iii) Hydrophilic endgroup of the PEG brush also provided stronger stabilization effect. Therefore, the optimum excipient-free lyophilization can be performed using a ramp rate of ±2°C/min, a normalized PEG grafting density higher than 10, and grafted PEG brushes with hydroxyl end group. Also, the lyophilized particles could produce high surface activity when spread on water from a solid form without a reconstitution step, which shed light on the potential of inhalation delivery method which is more patient-friendly. An example of the best lyophilization results is shown as **Figure 1**.

References:

[1] Kim et al., Polymer Lung Surfactants. ACS Appl. Bio Mater. 2018, 1 (3), 581–592.

[2] Kim et al., Pulmonary Pharmacokinetics of Polymer Lung Surfactants Following Pharyngeal Administration in Mice. Biomacromolecules 2022, 23 (6), 2471–2484.

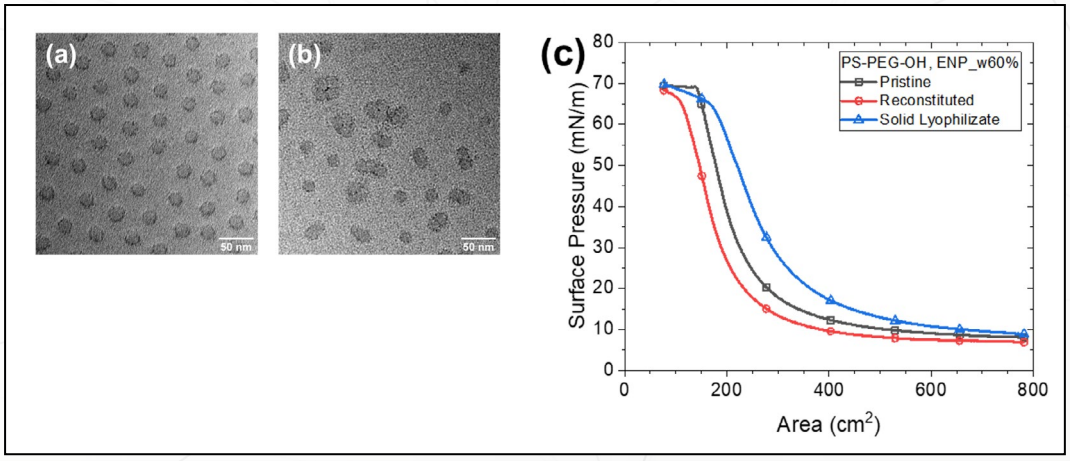


Figure 3: *TEM images of PLS particles (a) prior to lyophilization and (b) reconstituted after lyophilization. (c) Highly reproducible surface activity of reconstituted PLS and solid-spread PLS after lyophilization. PLS particles had normalized PEG grafting density of 11.3 and hydroxy end group at the PEG brush.*

LYOLAUNCHPAD PROJECT

Optimized Lyophilization of Gelatin Solutions

With Cook Biotech

Background: Cook Biotech frequently lyophilizes solid sheets of animal-derived biomaterials as part of the manufacturing process to transform them into commercialized, clinical-grade medical devices. While they have years of experience drying biomaterial sheets, they have recently started to develop a new manufacturing process that requires the lyophilization of solutions of customized gelatin inside large trays. While they were able to develop a drying protocol for these solutions in house, it required leaving the material in the Millrock RD53 lyophilizer for multiple days. The project team, therefore, had 2 aims for improving this process, 1) to optimize the drying protocol for these solutions, and 2) to determine if off-the-shelf commercial gelatin could be substituted for the more expensive, quantity limited custom gelatin during process development and process validation activities. If this substitution was found to be feasible, it would save over $68,000 in costs and accelerate the timeline for critical validation activities.

Objectives: The goal of this project was to determine the critical temperatures of provided gelatin solutions by performing freeze dry microscopy on <10 µL of the liquid low molecular weight gelatin, native high molecular weight gelatin, and modified high molecular weight Gelatin solutions (gelatins dissolved in salt buffers). FDM results of the gelatin solutions were to be compared to determine if the low molecular weight or native high molecular weight solution can be used as a substitute in further testing.

Results: The lyophilization behaviors of the custom gelatin and 2 commercial gelatins (A and B) were characterized and compared to evaluate commercial gelatins A and B as potential substitutes for the custom gelatin. The LyoHUB team performed freeze-drying microscopy to identify the critical temperatures of the frozen gelatin solutions. They also helped to analyze in-house tray lyophilization data from current drying protocol by comparing product temperature responses between commercial gelatin B and the custom gelatin. The LyoHUB team then performed a single-step vial lyophilization to characterize the mass transfer resistance of each gelatin type, and multi-step vial lyophilization to assess the accuracy of the mass transfer resistance data in a simulated trial compared to experimental results. All gelatin types had similar qualitative drying characteristics and mass transfer resistance. The data and expertise provided by the LyoHUB team have shown that either commercial gelatin should be an appropriate substitute for the customized gelatin during process validation activities and that they should be able to significantly speed up the gelatin drying process.[2] Kim et al., Pulmonary Pharmacokinetics of Polymer Lung Surfactants Following Pharyngeal Administration in Mice. Biomacromolecules 2022, 23 (6), 2471–2484.

LYOLAUNCHPAD PROJECT

Inhalable Dry Powder Combination Formulation of Colistin and Bacteriophage Prepared Using Spray Freeze Drying

With Prof. Tony Zhou Group (Purdue Industrial and Physical Pharmacy)

Background: Antibiotic resistance is causing existing antibiotics to become ineffective in treating bacterial infections. Polymyxins are a class of last-line antibiotics that are efficacious in treating multi-resistant Gram-negative infections [1]. However, it is critical to employ this antibiotic such that the chance of resistance development and the toxicity to the patient is minimized. Dr. Zhou's lab is therefore interested in developing a combination formulation of colistin and a bacteriophage to treat infections. Bacteriophages are viruses that target and kill specific host bacteria while multiplying itself. Some advantages of using bacteriophages as a treatment strategy include non-toxicity, target-specificity and an ability to evolve with the host bacteria [2].

To treat lung infections caused by multi-resistant bacteria, parenteral route of colistin leads to low distribution to the lungs and causes dose-dependent nephrotoxicity [3]. Therefore, they aim to develop inhalable dry powders that can deliver the drug directly to the site of infection in high dose. In this project, they employed spray freeze drying (SFD) to manufacture a combination of colistin and a Pseudomonas aeruginosa bacteriophage. The goal of the project is to determine the feasibility of using SFD to produce the dry powder formulation of colistin and bacteriophage.

Objectives: The goal of this project in the LyoHUB Demonstration Facility was to manufacture and characterize dry powder formulations that contain two antibacterial agents: colistin and bacteriophage. This project is expected to proceed as per the following objectives:

1. Determine manufacturing parameters for making viable inhalable powders using spray freeze drying.
2. Study the effect of excipients such as trehalose, mannitol, and leucine on aerosol performance and bacteriophage stabilization after preparation and during formulation storage.
3. Determine the effect of excipients on key solid-state properties of formulations that affect aerosol performance and phage stability.

Results: Dr. Zhou's lab has produced powder formulations using SFD. It is interesting that the residual moisture was higher for certain formulations when a longer secondary drying time was used. The particle sizes of all formulations were also higher with the longer drying time. SEM images of particles containing trehalose showed higher level of agglomeration. When only mannitol was included as an excipient, crystals were observed, and the particles appeared like shredded sheets of paper. Further characterizations of aerosol performance and process optimization are warranted in future studies.

References:

[1] Nation RL, Li J, Cars O, Couet W, Dudley MN, Kaye KS, et al. Framework for optimisation of the clinical use of colistin and polymyxin b: The prato polymyxin consensus. The Lancet Infectious Diseases. 2015;15(2):225-34. https://doi.org/10.1016/s1473-3099(14)70850-3.

[2] Wittebole X, Roock SD, Opal SM. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence. 2014;5(1).

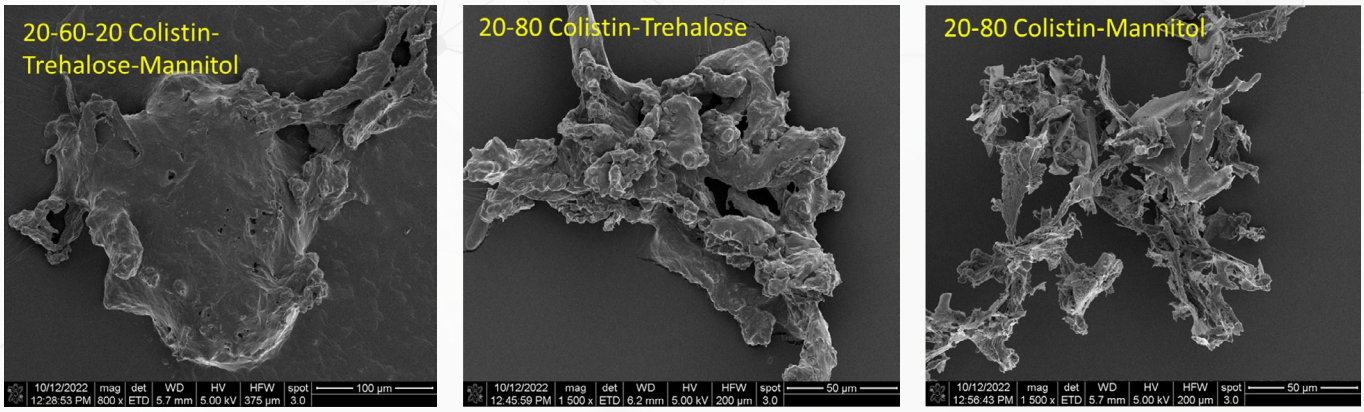


Figure 1: *Scanning electron microscopy images of spray freeze dried formulation particles*

WEBSITE RESOURCES & TRAINING

LYO101 COURSE

Open Access Online Introduction to Lyophilization Course

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

Current enrollment: Over 600

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

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

New Users Trained on Lyophilization Equipment from March 2022–2023

Kinnari Arte | Purdue IPPH Graduate Student

Aswathy Balakrishnan | NIBRT

Jacob Brejcha | Purdue ChemE Graduate Student

Kuo-Tao Fan | Purdue ChemE Graduate Student

Ian Flynn | Purdue ChemE Graduate Student

Chi-Sheng Hung | Purdue ChemE Graduate Student

Shruti Irap | Purdue ChemE Student

Bo Ruei Lai | Purdue ChemE Graduate Student

Shanbo Mu | Purdue ChemE Graduate Student

Vaibhav Pathak | Purdue IPPH Graduate Student

Chanakya Patil | Purdue IPPH Graduate Student

Rachana Sapkota | Purdue IPPH Graduate Student

Abdullah Shamim | Purdue IPPH Graduate Student

Ruei-Yang Tsao | Purdue ChemE Graduate Student

Nicha Vorrasanpisut | Purdue ChemE Student

Yibo Zhang | Purdue ChemE Graduate Student

ASTM LYOPHILIZATION STANDARDS

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

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

www.astm.org/COMMITTEE/E55.html

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.

2022 Annual Meeting at Purdue

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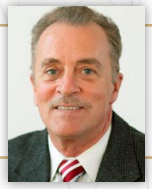

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