ISLFD 2019 – 9th International Symposium on Lyophilization of Pharmaceuticals

September 2-6, 2019

Ghent, Belgium

Presented by the International Society of Lyophilization/Freeze-Drying

Silver Sponsors:

Sponsoring Partners:

Exhibitors:

Process Systems Enterprise (PSE)

Organizing Committee

ISLFD Board of Officers

Kevin Tebrinke, President (USA) - Scientific Protein Laboratories LLC (USA)

Ivette Novoa, Vice-President (Cuba) - National Center of Bioproducts (CU)

Dr. Idania Caballero, Secretary (Cuba) – CIM (CU)

Dr. Zhongping Huang, Treasurer (USA) - West Chester University of Pennsylvania (USA)

ISLFD Board of Directors

Ana Bacaonau, Technical "Gh. Asachi" University of Iasi (RO)

Jos A.W.M Corver, RheaVita (NL)

Enric Jo, Reig Jofre (ES)

Paul Matejtschuk, The National Institute for Biological Standards and Control (UK)

Susan Ríos, Ghent University (BE)

Jorge Sassone, Proquimo Improvement (BR)

ISLFD 2019 Conference - Organizing Committee

Jos A.W.M. Corver, RheaVita (NL) [Chair]

Thomas De Beer, Ghent University (BE) [Venue]

Roberto Pisano, Politecnico di Torino (IT) [Technical Program]

Jorge Sassone, Proquimo Improvement (BR) [Sponsors & Table tops]

Zhongping Huang, West Chester University of Pennsylvania (USA) [Treasurer]

ISLFD 2019 Conference - Scientific Committee

Roberto Pisano, Politecnico di Torino (IT) [Chair]

Antonello Barresi, Politecnico di Torino (IT)

Paul Matejtschuk, The National Institute for Biological Standards and Control (UK)

Ivette Novoa, National Center of Bioproducts (CU)

Susan Rios, Ghent University (BE)

Conference Schedule

Tuesday, September 3

11:45 - 13:30 Registration & Welcome lunch

13:30 - 13:40 Kevin Tebrinke & Jos Corver
Welcoming Comments

13:40 - 14:20 Steven Nail, Baxter BioPharma Solutions
Past, present, and future in lyophilization

SESSION 1. Formulation & Chemistry (Room Refter)

14:20 - 14:45 Elizabeth Topp, Purdue University
Solid-state hydrogen deuterium exchange (ssHDX-MS) in the development of lyophilized protein formulations

14:45 - 15:10 Wolfgang Friess, Ludwig-Maximilians-Universität München
Understanding the freeze-concentrate in lyophilization of Mabs

15:10 - 15:35 Ken-ichi Izutsu, National Institute of Health Sciences
Freeze-drying of protein pharmaceuticals: use of information on component mixing for formulation and process development

15:35 - 15:45 Panel discussion

15:45 - 16:15 Coffee & Networking break (Kapittelzaal)

SESSION 2. Biopharmaceuticals and Biologics (Room Refter)

16:15 - 16:40 Paul Matejtschuk, The National Institute for Biological Standards and Control
Preservation of biological activity during freeze drying – challenges and technological advances

16:40 - 17:05 Victoria Kett, Queen's University of Belfast
Freeze-drying: not just for injectables

17:05 - 17:30 Jorge Sassone, Proquimo Improvement
Lyophilized diagnostic reagent for cancer detection

17:30 - 17:55 Fernanda Fonseca, AgroParisTech INRA
Physical events during cryopreservation: consequences on cells' post-thaw performance and on cryobiological protocols optimisation

17:55 - 18:05 Panel discussion

18:05 - 18:10 Kevin Tebrinke & Jos Corver

Day 1 wrap up

19:00 - … Ghent & "Beer" tour
Beer tasting & fingerfood. Own dinner afterwards

Wednesday, September 4

7:45 – 8:15 Coffee & Networking (Kapittelzaal)
8:15 – 8:20 Kevin Tebrinke & Jos Corver
Reflections on Day 1

SESSION 3. Competing technologies (Part I) (Room Refter)

8:20 - 9:00 Jos Corver, RheaVita
Challenges in continuous lyophilization

9:00 - 9:25 Roberto Pisano, Politecnico di Torino
From batch to continuous: the lyophilization of suspended vials for pharmaceuticals in unit doses

9:25 - 9:50 Israel Borges Sebastiao, Pfizer
Dynamic spray freeze-drying of pharmaceuticals: model validation and product characterization

9:50 - 10:00 Panel discussion

10:00 - 10:30 Coffee & Networking break (Kapittelzaal)

SESSION 4. Competing technologies (Part II) (Room Refter)

10:30 - 10:55 Arnab Ganguly, IMA Life
Accelerating freeze-drying through continuous aseptic spray freeze-drying

10:55 - 11:20 Thomas De Beer, Ghent University
A SMART technology for the continuous manufacturing of lyophilized orally disintegrating tablets

11:20 - 11:45 Kyuya Nakagawa, Kyoto University
Atmospheric freeze-drying of food products: practical modeling and quality assessment

11:45 - 11:55 Panel discussion
11:55 - 12:15 Sandwich lunch (Kapittelzaal)

13:00 – 19:00 Visit to Pfizer`s and GSK`s facilities

19:00 - … Free evening

Thursday, September 5

7:45 – 8:10 Coffee & Networking (Kapittelzaal)
8:10 – 8:15 Kevin Tebrinke & Jos Corver
Reflections on Day 2

SESSION 5. Process development, monitoring & scale-up (Part I) (Room Refter)

8:15 - 8:55 Antonello Barresi, Politecnico di Torino
Model-based tools for cycle development and scale-up

8:55 - 9:20 Andrew Strongrich, Purdue University
IoT PAT for Lyophilization

9:20 - 9:45 Bert Dekens, Hosokawa
Active freeze-drying

9:45 - 10:10 Pavol Rajniak, Slovak Technical University
Integrated use of mechanistic models, targeted experiments and modern analytical tools for development and troubleshooting of lyophilisation cycles: packing of vials approach

10:10- 10:20 Panel discussion

10:20 - 10:50 Coffee & Networking break (Kapittelzaal)

SESSION 6. Process development, monitoring & scale-up (Part II) (Room Refter)

10:50 - 11:15 Geoff Smith, De Montfort University
Electrical Impedance methods for developing a lyophilization cycle

11:15 - 11:40 Henning Gieseler, Friedrich Alexander University Erlangen-Nürnberg
Freeze drying from organic co-solvent systems: thermal analysis and process design

11:40 - 12:05 Yves Mayeresse, GlaxoSmithKline
Specificity of vaccine freeze-drying

12:05 - 12:15 Panel discussion

12:15 - 14:15 Lunch (Room Refter)

SESSION 7. Industrial session (Part I) (Room Refter)

14:15 - 15:15 Yossi Shapira, Teva
Nanoparticles API freeze drying using organic solvents

15:15 - 15:40 Sophie Declomesnil, 4DPharma plc
Lyophilization in bulk of anaerobic bacteria, specificity of the process

15:40 - 15:50 Panel discussion

16:00 - 18:00 POSTER SESSION & COFFEE (Kapittelzaal)

19:00 - … GALA DINNER at Ghent Marriott Hotel

Friday, September 6

7:45 – 8:10 Coffee & Networking (Kapittelzaal)
8:10 – 8:15 Kevin Tebrinke & Jos Corver
Reflections on Day 3

SESSION 8. Industrial session (Part II) (Room Refter)

8:15 - 8:40 Salvatore Carmisciano, Novartis
Freeze dryer recipe process transfer: challenge in heat transfer coefficient between freeze dryers and way-out

8:40 - 9:05 Ali Afnan, Step Change Pharma
Freeze-drying and regulatory aspects

9:05 - 9:30 Kevin Ward, Biopharma
Quantification of the physical robustness of lyophilized biotherapeutics

9:30 - 9:55 Maik Guttzeit, Bayer Pharmaceuticals
Design requirements for shelf temperature control and testing

9:55 - 10:05 Panel discussion

10:05 - 10:35 Coffee & Networking break (Kapittelzaal)

SESSION 9. Young Researchers in Life Sciences & Lyophilization (Room Refter)

10:35 - 10:55 Andrea Arsiccio, Politecnico di Torino
Surface-driven denaturation of proteins during freeze-drying: An insight into the role of surfactants

10:55 - 11:15 Gust Nuytten, Ghent University
Evaluation of cracking and shrinkage of freeze-dried cakes when using a continuous freeze-drying method

11:15 - 11:35 Catalina Alvarez Campuzano, Universidad Nacional de Colombia
Effect of Vacuum Induced Surface Freezing on the physical and chemical characteristics of freeze-dried strawberry pulp

11:35 - 11:55 Anand Vadesa, De Montfort University
Application of a novel impedance-based freeze-drying microscope for the product formulation development

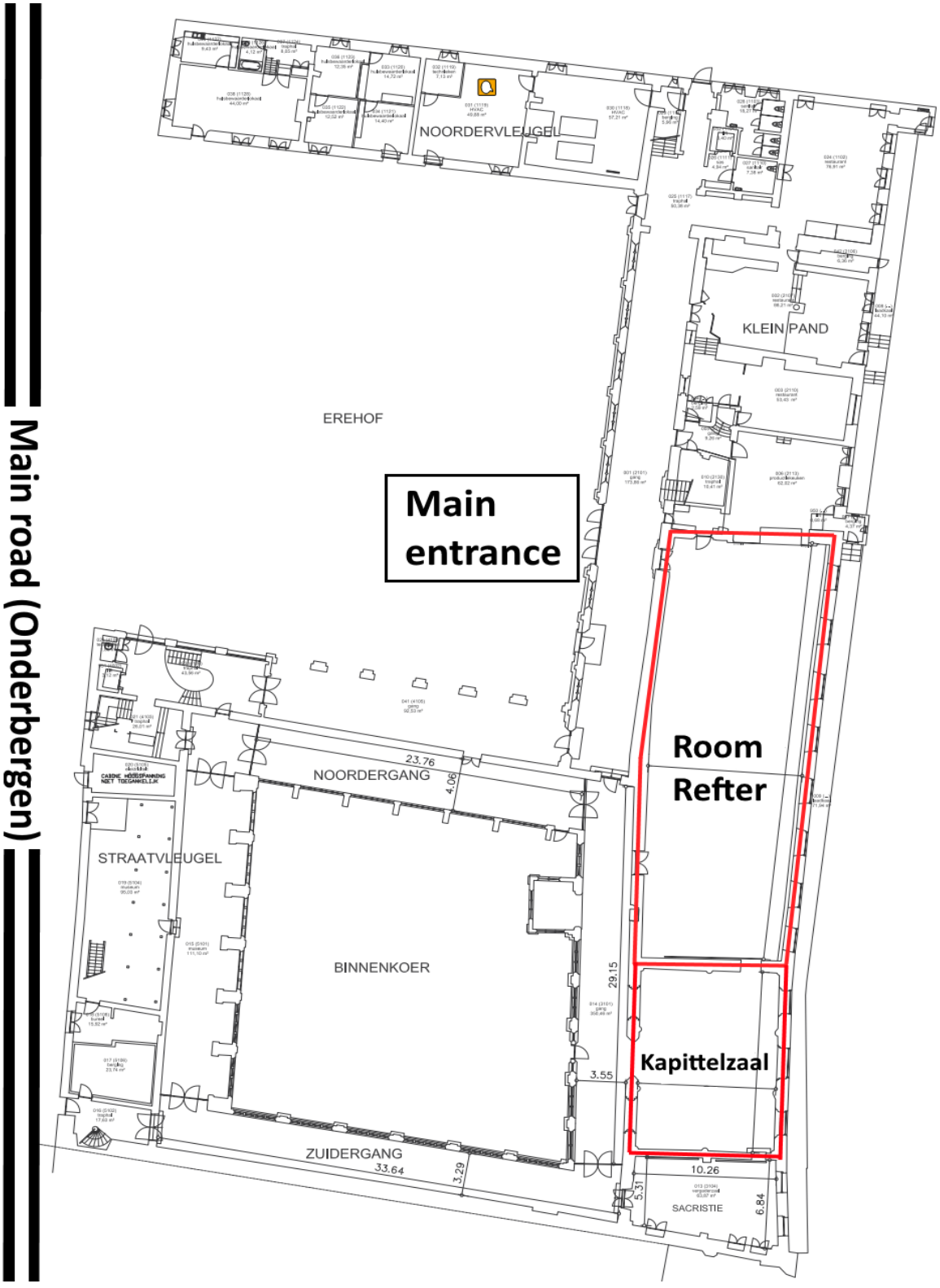
11:55 - 12:15 **Valeria Gervasi**, University College Cork
Formulation strategies and modelling approaches to successfully develop lyophilised high concentration protein formulations

12:15 - 12:25 **Panel discussion**

12:25 -12:40 **Kevin Tebrinke** & **Jos Corver**
Awards & Symposium conclusions & proposed next steps

12:40 - 14:00 **Lunch (Room Refter)**

Conference Venue Floor Plan



Invited Speakers' Biographies

STEVEN NAIL

Steven L. Nail is currently a Principal Scientist in the Pharmaceutical Development organization of Baxter Pharmaceutical Solutions, Bloomington, IN. His undergraduate training is in chemical engineering at Purdue University, and his Ph.D. is in pharmaceutics, also from Purdue. From 1975-1991 he worked for The Upjohn Company, Kalamazoo, MI in various capacities, all related to development and manufacture of parenteral products, with a special interest in the science and technology of freeze-drying. In 1991, he became Associate Professor in the School of Pharmacy at Purdue, and was promoted to Professor in 1999. His research interests at Purdue focused on the physical chemistry of freezing and freeze-drying, characterization of frozen systems and freeze-dried solids, stability of proteins as freeze-dried products, pharmaceutical thermal analysis, and pharmaceutical applications of supercritical fluid technology. His teaching responsibilities have included undergraduate pharmacy courses in parenteral pharmaceutical products and graduate courses in pharmaceutical processing. From 2002 until 2006, he was Research Fellow in the Pharmaceutical Sciences R&D organization at Eli Lilly & Co., Indianapolis, IN. He was formerly Chairman of the USP Committee of Experts in Parenteral Products. He is a Fellow of the American Association of Pharmaceutical Scientists, and received the Research Achievement Award in Pharmaceutical Technology from AAPS in 2007. In 2013, he was recognized as a Distinguished Alumnus by the School of Pharmacy, Purdue University. In 2017, he received the Honored Baxter Career Award from Baxter Healthcare, Inc.

ELIZABETH M. TOPP

Elizabeth M. Topp is a Professor in the Department of Industrial and Physical Pharmacy at Purdue University in West Lafayette, Indiana. She received a bachelor's degree in chemical engineering from the University of Delaware (B.Ch.E.), a master's in chemical and biochemical engineering from the University of Pennsylvania (M.E.) and a Ph.D. in pharmaceutics from the University of Michigan. Dr. Topp's research addresses the chemical and physical stability of protein drugs, with particular emphasis on lyophilized solids. She is a Fellow of the American Association of Pharmaceutical Scientists and a former Associate Editor of the Journal of Pharmaceutical Sciences. With Dr. Alina Alexeenko of Purdue's School of Aeronautics and Astronautics, she co-directs LyoHUB, an industry-led consortium to advance lyophilization technology.

WOLFGANG FRIESS

Wolfgang Frieß holds a position as Professor for Pharmaceutical Technology and Biopharmaceutics at the LMU Munich since 2001. He received his PhD in Pharmaceutical Technology in 1993 and his Pharmacy degree in 1989 from the University of Erlangen. He has worked for several years in academia both in Germany and the US. His primary research goals are protein formulation, drug delivery and biomaterials, in particular new analytical tools for protein formulations, freeze-drying of proteins and different local delivery routes. He is co-editor of the European Journal of Pharmaceutics and Biopharmaceutics and has published over 150 research papers, patents and book chapters.

KEN-ICHI IZUTSU

Dr. Ken-ichi Izutsu is a Director of Drug Division in National Institute of Health Sciences (NIHS), Japan. After obtaining his MSc in pharmaceutical Science in Nagoya City University in 1987, he started working at the NIHS. He got his Ph.D. degree on pharmaceutical sciences at Nagoya City University (Prof. Yotsuyanagi) in 1994 based on Studies on the effect of excipients on the stability of protein pharmaceuticals during freeze-drying and storage. After carrying out research on protein formulations at University of Colorado Health Sciences Center as a visiting scientist (Prof. Carpenter, 1995-1997), he worked at The Health Science Division at Ministry of Health, Labor and Welfare, Japan as a Research planning coordinator (1997-1999). He returned to NIHS in 1999 and studied application of physical analysis on formulation design of pharmaceutical products as a Senior Research Scientist. From 2009, he also studied bioequivalence study methods and characterization of complex generic formulations, engaging in Secretariat of Expert Committee on Quality of Generic Pharmaceutical Products as a Secretariat. He is an expert committee member for Japanese Pharmacopeia and drug reviews at PMDA, Japan.

PAUL MATEJTSCHUK

Paul set up and leads a formulation and freeze-drying team at NIBSC focussed on delivering lyo solutions for biological reference materials, many of them WHO International Reference preparations (Joined NIBSC in 2001). He has wide interests in biologics characterisation, analytical methods, moisture determination and metrological aspects of reference material manufacture.

He has over thirty years postdoctoral experience in downstream processing and analysis of biologics, has co-authored over 40 peer reviewed papers and many other publications. He has collaborated over 13 years with Prof Paul Dalby (Department of Biochemical Engineering, University College London) on microscale freeze drying and structure: function relationships in proteins and has co-supervised three successful PhD students over this time.

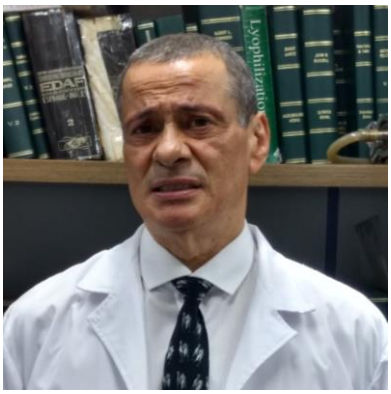
Paul is one of a number of Directors of the International Society for Lyophilization Freeze Drying (www.islyophilization.org) since 2005.

Paul co-edited (2019) with Dr Kevin Ward (Biopharma Technology Ltd, UK) a recent volume on "Lyophilization of Pharmaceuticals & Biopharmaceuticals" for Springer in the Methods in Pharmacology & Toxicology series https://www.springer.com/gp/book/9781493989270

VICTORIA KETT

Vicky Kett is Senior Lecturer in Pharmaceutics in the School of pharmacy, Queen's University Belfast. She has 20 years' experience in development and characterisation of freeze-dried pharmaceuticals. She has a degree in biological chemistry from University of Kent including 1-year study at University of Jena and obtained her PhD on the use of modulated temperature differential scanning calorimetry in the development and characterisation of amorphous pharmaceuticals from the London School of Pharmacy, now UCL School of Pharmacy.

She was Chair of the Royal Society of Chemistry Thermal Methods Group and continues to be committee member of the same. She is past recipient of the RSC Cyril Keattch award for her work in thermal analysis of freeze-dried products and the Northern Ireland Invent Health and Biotech award for the Vaccinetab product. She works closely with colleagues in the development of mucosal drug delivery systems including formulations for nasal, pulmonary and vaginal delivery of a range of therapeutics including antigen, antibiotics and microbicides. Diseases targeted include HIV and AIDS, cystic fibrosis and COPD. She is author of over 50 peer-reviewed publications and has received funding from a range of UK and international government, EU, pharmaceutical industry and charitable institutions .



JORGE SASSONE

Dr. Jorge Sassone, argentine, 55 years old, earned his BA in Chemistry in 1989 as Specialist in Analytical Chemistry, Organic Chemistry and Organic Synthesis at FCEN – UBA- Argentina. Further late, he started his study as Scientific Researcher in the same faculty when was awarded a scholarship for further training in fine Chemistry at Max Planck institute in Germany. In 1992 he obtained a Master specialist in Mechanisms of action of Pesticides at Arizona University, USA and in 1994 he made a Post-graduate in cGLP-GMP at Garndall Associated , USA. In 1999, he obtained his PHD in Chemistry at Harvard, Boston-Mas-USA. He was professor of Qualitative and Quantitative Chemistry, Advanced Quantitative Chemistry, and Radiochemistry and Nuclear Chemistry at FCEN-UBA, Argentina. Since 1993 we worked in industries Pharmaceutical, Veterinary and Agro chemical in several areas as Research and Development, Prodution, and Validation. He was and is consultant for many companies around the world as Bayer, Sandoz, Novartis, Basf, Abbot, Pfizer, Merk S&D, Schering Plough, Sanofi Aventis, GSK, Baxter,Organon, Ourofino, Blau, Eurofarma, União Quimica, E M S , etc. He has got more than 25 years of experience in Lyophilisation Field working with all kind of products like sinthetic drugs, biologicals,

biotechnologicals, and foods; he has worked in Oncologics, Hormonals, Proteins, Polypeptides, Antibiotics, Antigens, Antibodies, Vaccines, Diagnostic reagents, Contrast reagents, etc. He has writen four books: Citostaticos, Hormonios, Antivirais: tecnologia de fabricacion y control; Liofililização-Bases Teoricas, Pratica de processo e Fases da Liofilização; Formulação em Liofilização, Lyophilized protein development.He is continuously invited speaker- professor in events related to Synthetics, Biologics, and Biotechnologics Lyophilization in America and Europe. Several papers published as Formulation Matters in PMPS (UK, may 2010), FD in Pharma in PMPS (UK, aug 2009), PAT in Lyophilization (ISL IC 2007, Dublin, Ireland), and Liposomal in Lyo (ISL IC 2013, SP, Brazil), Theory and Practice on alleged Buffer pH shifting during freezing in Lyophilized protein Formulations (Barcelona 2015, ISL-FD IC) . Currently, he is General Director of PROQUIMO-IMPROVEMENT in São Paulo, Brazil, R&D lab for Pharma, Vet and Agrochemical ; he is also member of the American Chemical Society and Director of the Board of Directors at the ISL-FD. He works as a scientific consultant for ANVISA, the Brazilian Regulatory Agency.

FERNANDA FONSECA

Fernanda Fonseca, received her PhD in Biotechnology and Process Engineering in 2001, from AgroParisTech, France. She is Research Director at the French National Institute for Agricultural Research (INRA), in Versailles-Grignon Centre, France. She leads an EU project (H2020-Marie Skłodowska-Curie research and innovation program) on the Preservation of micro-organisms by understanding the protective mechanisms of oligosaccharides (PREMIUM). She has previously participated to two EU projects concerning freeze-drying of proteins and bacteria. Her research field is biotechnology and process engineering, with particular emphasis in fermentation, formulation and stabilization of biological products by freezing and freeze-drying. The principal models of study have been lactic acid bacteria, but also proteins, mammalian cells and food products. Her research interests focus in the

thermophysical and chemical changes taking place within biomaterials during freezing and freeze-drying. She has collaborated with SOLEIL synchrotron scientists for developing biophysical approaches (FTIR spectroscopy and fluorescence microscopy) for studying bacteria at the single cell level and during process. Recent research has focused on membrane stiffening and vitrification of the intracellular environment during freezing, aiming at relating these events to biological and functional outcomes. The utmost ambition is to find general principles for rationalising the development of new formulated products and optimizing the stabilization process.

JOS CORVER

Jos Corver holds a degree in applied physics in the field of rheology and physical transport phenomena. Leading development and engineering work in various industries enriched his expertise in aerodynamics, powder physics, vacuum deposition techniques, material science and NMR. Having had leading roles in industrialization processes he also improved his expertise in project management and quality and statistical assessment including Design of Experiments, Multiple Regression and FMEA. After 12 years within BOC Edwards / IMA Life, he started his own company since 2011, RheaVita. RheaVita strives for innovative improvements in pharmaceutical processing and specifically in freeze drying and the processes supporting this. In parallel he takes project management roles in semiconductor industry to support his desire to implement cross-over between different industrial fields. Jos Corver has published in various areas like pharmaceutical, optical en business literature. He is author of a range of patents on the application of NMR, improvements in freeze drying, color printing and Extreme UV sources.

Jos Corver is member of the Board of Directors of the ISL-FD.

ROBERTO PISANO

Roberto Pisano is a Professor of Chemical Engineering at Politecnico di Torino (Italy), where he received his Ph.D. in 2009. Professor Pisano's research focuses on the application of both computational and experimental methods to engineering chemical products and processes, with particular emphasis to pharmaceutical processing and formulation of both small molecules and biologics. He has been visitor researcher at Centre de Ressources Technologiques – Institut Technique Agro-Industriel (Strasbourg, France) in 2008, and at the Department of Chemical Engineering of Massachusetts Institute of Technology (Cambridge, USA) in 2016. He has worked with many pharmaceutical companies in research or consulting. He has published more than 80 papers, 11 book chapters and currently has 4 patents issued or pending. Furthermore, he has recently co-edited one book, Freeze-Drying of Pharmaceutical Products (CRC Press).

ISRAEL BORGES SEBASTIÃO

Israel Borges Sebastião has a BSc degree in mechanical engineering from the University of Mogi das Cruzes and a MSc degree in space engineering and technology from the National Institute for Space Research, both in São Paulo, Brazil. In 2017, he received a PhD degree in aerospace engineering from Purdue University (USA) under the guidance of Prof. Alina Alexeenko. After a 1-year postdoc at Purdue, in the fall of 2018, Israel joined Pfizer's BioTherapeutics R&D team in Andover, Massachusetts (USA). His main current task is to help building engineering-level models to assist the development of efficient spray freeze-drying processes and support scale up from laboratory to commercial scale equipment.

ARNAB GANGULY

Arnab received a PhD in the School of Aeronautics & Astronautics at Purdue University in 2014 with a focus on modeling low-pressure water-vapor flows applied to freeze-drying. In 2012, he was awarded the Baxter Young Investigator award for his research during the MS and PhD work. Arnab is currently Technology Manager at IMA Life, North America, leading the R&D effort with focus on emerging technologies for aseptic processing applications.

THOMAS DE BEER

Thomas De Beer graduated in pharmaceutical sciences in 2002 at the Ghent University in Belgium. He obtained his PhD at the same university in 2007. For his PhD research, he examined the suitability of Raman spectroscopy as a Process Analytical Technology tool for pharmaceutical production processes. Within his PhD research period, he worked four months at University of Copenhagen in Denmark, Department of Pharmaceutics and Analytical Chemistry (Prof. Jukka Rantanen). After his PhD, he was an FWO funded post-doctoral fellow at the Ghent University (2007-2010). Within his post-doc mandate, he worked 9 months at the Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics from the Ludwig-Maximilians-University in Munich, Germany (Prof. Winter and Prof. Frieβ). In February 2010, he became professor in Process Analytics & Technology at the Faculty of Pharmaceutical Sciences from the university of Ghent. His research goals include bringing innovation pharmaceutical production processes (freeze-drying, hot-melt extrusion, continuous from-powder-to-tablet processing etc.). More specifically, Prof. De Beer contributes to the development of continuous manufacturing processes for drug products such as solids, semi-solids, liquids and biologicals (continuous

freeze-drying of unit doses). Thomas De Beer is also director of Ghent University's Center of Excellence in Sustainable Pharmaceutical Engineering (CESPE) which is founded in 2016. In 2018, Thomas De Beer became co-founder and CTO of the Ghent University spin-off company RheaVita which provides a continuous freeze-drying technology for the pharmaceutical market.

KYUYA NAKAGAWA

Kyuya Nakagawa is an Associate Professor at the Department of Chemical Engineering, Kyoto University. He received his PhD from the Department of Chemical Engineering, Kyoto University in 2003. Now he specializes in the areas of food drying, freezing, freeze-drying, and microencapsulation. He has published over 100 scientific articles, and these articles have ever been cited more than 1500 times. He is interested in the development of sophisticated-practical processing technologies that allows to design desirable qualities in bio-based products to give various functionalities. He is now motivated to apply QbD approach for food freeze-drying, where numbers of qualities must be realiezed in a cost-effective processing. He recognizes that physicochemical phenomena during freezing and drying are of great tool to design functional products.

ANTONELLO BARRESI

Antonello Barresi is currently full professor of Transport phenomena at Politecnico di Torino, in charge of the course of Process development and design. Currently Italian national delegate in the WP on Drying of the European Federation of Chemical Engineering. His main research interests in drying include: drying and freeze drying of pharmaceuticals and enzymes, modelling and optimization of freeze-drying processes, control of industrial freeze-dryers. Most recent research is focused on process transfer, scale-up and cycle development, and new approaches for process development and quality control in freeze-drying of pharmaceutical and food products. Author of more than 250 papers (of which

about 160 published on international Journals or books) and more than 100 Conference presentations. Recently coedited the book "Freeze-drying of Pharmaceutical Products" writing chapters on "Innovations in freeze-drying control and in-line optimization" and "Use of CFD for the design and optimization of freeze-dryers".

ANDREW STRONGRICH

Andrew Strongrich received his BS and MS in Aeronautical and Astronautical Engineering from Purdue University in 2013 and 2015, respectively. He is currently a doctoral candidate at Purdue where he also holds the role of superuser in the LyoHUB Demonstration Facility. His research interests include closed-loop control of lyophilization processes, wireless sensor networks, cold plasmas, and thermally driven flows in microelectromechanical systems.

HENNING GIESELER

Henning Gieseler studied pharmacy at the University of Würzburg (Germany) and is a licensed pharmacist since 2000. In 2004, he received his PhD from the Department of Pharmaceutics, Friedrich-Alexander-University (FAU) Erlangen-Nürnberg. After a post-doctoral research period at the School of Pharmacy, University of Connecticut, USA (Prof. Michael J. Pikal) until early 2006, he returned to his former department as an assistant professor and formed the "Freeze Drying Focus Group" (FDFG) within the division of pharmaceutics. In 2010, Dr. Gieseler obtained his habilitation and venia legendi in pharmaceutical sciences from the FAU with the thesis "Quality by Design (QbD) in Freeze-Drying Using Advanced Process Analytical Technology (PAT)". In the same year, he also founded the company GILYOS GmbH in Würzburg, a highly specialized CRO in the field of pharmaceutical freeze drying. Today, Dr. Gieseler holds the CSO position within GILYOS, serves as adjunct faculty at the FAU Erlangen-Nürnberg and is head of the FDFG within the department of

pharmaceutics within the FAU. Over the last 15 years, he published numerous articles, book chapters and patents in the field of freeze drying science and related areas.

PAVOL RAJNIAK

Pavol started working on lyophilization after coming to the Merck Research Laboratories (currently Merck Commercialization Technology), West Point, USA in 1998. At Merck, he developed a software package LYOSOFT which has been used at the company for development, scale-up, transfer and optimization of several lyophilization cycles (both, primary and secondary drying) at the company. After retiring from Merck in 2014 and moving back to Europe he worked at Research Centre Pharmaceutical Engineering in Graz, Austria and then since 2016 until now at the Process Systems Enterprise in London, where he was responsible for the development of the gPROMS version of the lyophilization software for both, the single vial approach and the packing of vial approach. Since September 2018 he works again at his 'Alma Mater', the Slovak Technical University in Slovakia and continues working with the PSE London on several lyophilization projects for different pharmaceutical companies. Currently, his main research focus is on the freezing step of the lyophilization cycles.

GEOFF SMITH

Geoff Smith is Professor of Pharmaceutical Process Analytical Technology in the Leicester School of Pharmacy at De Montfort University (UK). His research group focusses on pharmaceutical applications for impedance, dielectric and terahertz spectroscopies alongside optical techniques such as laser speckle and optical flow.

He is responsible for the development of through-vial impedance spectroscopy (TVIS) as a PAT tool for monitoring phase behaviour (ice formation and eutectics), ice interface temperatures, primary drying rates and end points. This development marks the first time that impedance spectroscopy has been used to characterize materials within conventional

glass freeze-drying vials, without having to insert the electrodes into the product (i.e. the solution undergoing freeze-drying). This feature of the technology sets it apart from other in-process impedance measurement systems, in which a bulky electrode assembly is inserted into the solution being freeze-dried, to provide a product-non-invasive technology. In 2018, and in collaboration with Biopharma, he developed Z-FDM - a freeze-drying microscope with an integrated impedance spectrometer, for the objective assessment of the collapse temperature and for the determination of ice nucleation temperatures, solidification times, and drying rates.

BERT DEKENS

Bert Dekens is currently working as Application Manager in the Pharma group of Hosokawa Micron B.V. He joined Hosokawa Micron B.V. 15 years ago and is responsible for sales in the pharma industry concentrating on mixing and drying applications.

YVES MAYERESSE

Yves Mayeresse is director in manufacturing technology inside MSAT by GSK Vaccines. He has more than twenty-five years of experience in the pharmaceutical sector and has worked for different companies.

Yves has managed activities such as parenteral production, set-up of new Freeze-Drying facilities, design of Freeze-Drying cycle and development of new stabilizers for freeze-dried products. Transfer of product towards different internal and external site. He has worked on the industrialization of new freeze-dried products and then in the technical life cycle management. Now, Yves is focusing on different new and established technologies used for the primary and secondary operations. He is an engineer in biochemistry, has written articles about Freeze-Drying science and is a regular speaker for conferences on Freeze-Drying. Since 2016 he is the Leader of the PDA Interest Group Lyophilization and

coordinates the group's activities in Europe. He is a regular committee member for PDA annual meeting Europe and Chairman of the freeze-drying conference.

YOSSI SHAPIRA

Biologist in education BSc degree gained from Tel Aviv University. Main field of expert marine biology.
Working for Teva Pharmaceutical Industries 39 years. Expert in lyophilization products developing, tech transfer, training, problems solving and FD processes optimization
Experienced large variety of lyophilization equipment specifying, qualifying, testing, challenging and operating. From laboratory bench top lyophilizers to the largest – 175sqm bulk API lyophilizer. Major part was done while employing sterile production technologies for parentrals products manufacturing.
Freeze drying experience from small museum marine specimens to vaccines, pharma products through APIs lyophilization. Aqueous solutions based products as organic solutions based products.
Consulting& training lyophilization part reviewer in ANDAs.
In 2002 – ISLFD lyophilization conference chairperson in Amsterdam.
In 2003 – guest speaker in ISLFD Chicago conference.
In 2017 – PDA lyophilization chapter speaker.
In 2019 – Lyotalk - Amsterdam conference invited speaker.

SOPHIE DECLOMESNIL

She holds a degree in Engineering from Agrosup Dijon (France) as well as a PhD degree in biopolymer production from bacterial fermentation from Picardie University (Amiens-France).
She has more than twenty years of experience in Freeze drying process. She is actually Process Development Director at 4DPharma and responsible for the technical operation as Fermentation and Lyophilization at the Lab and pilot scale for a new class of medicines LBP (Live Biotherapeutic Product). Previously, during 21 years, she worked as head of R&D for Lyofal where she designed Freeze-Drying cycle and developed stabilizers for different freeze-dried products such as Medical devices, bacteria, protein…

SALVATORE CARMISCIANO

Salvatore Carmisciano graduated from the Faculty of Chemical Engineering in Roma.
He joined Novartis vaccines in 2010 working as formulation expert in the fields of Drug Product process and formulation development. In 2013, Salvatore joined Sandoz Austria working as Drug Product Leader being responsible on project process characterization and down scale model concepts.
In 2017, he joined the Novartis Drug Product Development Slovenia as Senior Scientist leading technical transfer/validation activities and supporting projects submission. He advised drug product leaders and Scientists inside Novartis Drug Product Development organization for studies, concepts and procedures regarding liquid and freeze-dried sterile products.
In 2018, he moved to the position of Head Project and Process Sciences in the Novartis Drug Product Development organization by managing pharmaceutical (drug product) development projects and leading late phase development team of scientists.

ALI AFNAN

KEVIN WARD

Kevin Ward entered the world of freeze-drying in 1993 when he embarked on a PhD in the lyophilisation of proteins and liposomes. After working in the pharmaceutical industry and in vaccine development, he joined Biopharma in 2000. As R&D Director, he has built up a team of 8 dedicated freeze-drying scientists, worked on over 3000 projects for more than 500 client companies across the globe, developed 3 analytical instruments, run more than 200 training courses, and successfully secured funding for 15 collaborative research projects. He has appeared on BBC television for his team's work on cell preservation and been an advisor to the BBC for one of their popular science shows. Kevin is a Fellow of the Royal Society of Chemistry and from 2000-2014, he was part of a not-for-profit group at the Pharmaceutical and Healthcare Sciences Society that

produced a series of technical monographs to support users of freeze-dryers, and he chaired the group from 2007-2014. Kevin has been industrial supervisor to two doctoral students, and has authored numerous papers, book chapters and patents. He continues to lecture on the worldwide stage and co-edited a textbook on technological advances in freeze-drying that was published in January 2019.

MAIK GUTTZEIT

Maik Guttzeit holds a Dipl.-Ing. degree in process technology. For almost 20 years Maik was Team Leader Validation at GEA which provides customized GMP Lyophilizer systems.

He is member of the GAMP® D-A-CH committee and also member of ASME BPE Subcommittee on System Design.

Since September 2018 he is working for Bayer Pharmaceuticals as the global Technology Manager Aseptic and Sterile.

ANDREA ARSICCIO

Andrea Arsiccio is a Ph.D. student in Chemical Engineering at Politecnico di Torino, where he obtained a Master's Degree in Chemical and Sustainable Process Engineering in 2016. His research focuses on protein stability and formulation development for biopharmaceuticals to be freeze dried. He also has an expertise in Molecular Dynamics simulations for biological systems, and physical chemistry in general. In 2018, he was a visiting researcher for six months in Prof. Shea's group at University of California, Santa Barbara, where he worked in the field of surface-driven denaturation of proteins and aggregation of amyloid-forming peptides. In the same year he also spent two months in Dr. Matejtschuk's group at the National Institute for Biological Standards and Control (Potters Bar, UK), where he investigated the effects of freezing and freeze drying on protein stability. During his Ph.D. he

authored papers in the field of physical chemistry, pharmaceutical sciences and drying technology.

GUST NUYTTEN

Gust Nuytten obtained a master's degree in drug development in 2017 from the university of Ghent, and graduated as an industrial pharmacist in 2018. That same year, he started his doctoral research under supervision of professor Thomas De Beer, in the Laboratory of Pharmaceutical Process Analytical Technology. He is currently doing work on the freeze-drying of bacteria using a continuous freeze-drying concept.

CATALINA ALVAREZ C.

(Manizales, Colombia, Dec 11th) Chemical Engineer, Master in Engineering- Chemical Engineering (2015) and PhD. Candidate in Engineering - Chemical Engineering from the Universidad Nacional de Colombia, at Manizales, with the COLCIENCIAS "Doctorados Nacionales 2015" grant.

She has been assistant researcher at the Jóvenes Investigadores e Innovadores, Colciencias (2013) program. She had worked in different research projects at Biotechnology and Agribusiness Institute of the Universidad Nacional de Colombia, Manizales (Food technology and Microbiology).

M. Eng. Alvarez has been Autor/Coautor of scientific articles and had presented some of her work in different national and international events as oral speaker. She participated as invited student of the BIOPRO World Talent Campus, Denmark (2016). She had recived an Academic Achievement Exaltation form the Universidad Nacional de Colombia, at Manizales (2013). Her main investigation areas are food preservation and transformation, freeze-dying of food, biotechnology, bioproducts and industrial microbiology.

ANAND VADESA

Anand Vadesa is a PhD candidate on a project funded by EPSRC, working with Prof Geoff Smith at De Montfort University's Pharmaceutical Technologies Group and Prof Paul Dalby of Department of Biochemical Engineering at University College London. His research goal is to develop an impedance-based process analytical technology for the development of product formulation screening and manufacturing processes in the freeze-drying of biologics. He received his MChem from the University of Leicester in 2013, and he has experience of working at CDMO for two and a half years supporting quality team on the clinical trial projects. He also worked as an analytical scientist at FMCG for two years leading analytical studies on several over the counter drugs.

VALERIA GERVASI

Valeria Gervasi graduated with a MSc (Pharm) degree in Pharmaceutical Chemistry and Technologies at University of Calabria (UNICAL), Italy in 2015. During her MSc degree, as part of an Erasmus program, she spent six months in the Pharmaceutical Technology Lab of the School of Pharmacy at University College Cork (UCC), Ireland under the supervision of Dr. Abina Crean. In 2016, she started a PhD in lyophilisation of high concentration protein formulations at UCC under the supervision of Dr. Abina Crean and Dr. Sonja Vucen. Her PhD project was part of an academic-industry collaboration funded by Enterprise Ireland and Science Foundation Ireland. During her PhD project, she had experience in teaching and supervising undergraduate students. As part of the PhD project, she undertook research in the Manufacturing Science Department of Sanofi, Waterford, Ireland. During her industrial experience, she was trained on lyophilisation process development and several techniques for product characterization. Since June 2019, she has been employed as a Development Scientist at Sanofi, Waterford. She is currently the author of one peer-reviewed paper and several oral and poster publications presented at national and international conferences.

Abstracts for Invited Lectures

List of Abstracts for Invited Lectures

Past, Present, and Future in Lyophilization

Nail, Steven L.
Baxter Pharmaceutical Solutions, Bloomington, IN
*E-mail of the corresponding author: steven_nail@baxter.com

Abstract

This talk is in many ways a personal tale of my involvement with pharmaceutical freeze drying, starting as a fresh-out-of-graduate school scientist in 1975. I'll discuss then versus now in areas like published literature on freeze drying, the application of heat and mass transfer fundamentals, characterization of formulations intended for freeze drying, freeze drying equipment design and construction, and process monitoring and control. I'll then briefly discuss some current issues in the science and technology of freeze drying, such as the use of a graphical design space for optimization of primary drying and recent experience with vial fogging. The talk will close with a look to the future, where freeze dryers are either re-designed to make them more efficient, or where the traditional approach of freeze drying a solution after filling into a vial is abandoned altogether.

Solid-state hydrogen deuterium exchange (ssHDX-MS) in the development of lyophilized protein formulations

Topp, Elizabeth M.*
Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, Indiana
*E-mail of the corresponding author: topp@purdue.edu

Abstract

Many protein drugs are marketed as lyophilized solid powders. To develop the product, candidate formulations are evaluated in stability studies that can take years to complete. While physicochemical analyses (e.g., FTIR, Tg) provide information about the properties of the solid, they are usually poorly correlated with storage stability. There is a need for a stability-indicating analytical method for proteins in lyophilized powders and other amorphous solids.

Our group has developed solid-state hydrogen deuterium exchange with mass spectrometric analysis (ssHDX-MS) as a novel analytical method for proteins in lyophilized solids, providing high resolution information on protein structure and matrix interactions. We have shown that ssHDX-MS is sensitive to changes in formulation, moisture content and processing method. ssHDX-MS has also been shown to be highly correlated with the storage stability of various formulations of myoglobin (Mb) and of a monoclonal antibody (mAb). ssHDX-MS thus shows promise as a stability-indicating method that could help accelerate the development of lyophilized protein drugs.

This presentation will provide an overview of the ssHDX-MS method and summarize results demonstrating correlation of ssHDX-MS metrics with the storage stability of several lyophilized proteins. Ongoing studies, directed toward establishing a mechanistic model for ssHDX-MS, will also be presented.

Understanding the freeze-concentrate in lyophilization of Mabs

Friess, W.*; Seifert, I.
Ludwig-Maximilians-Universität München, Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Munich, Germany
*E-mail of the corresponding author: wolfgang.friess@cup.uni-muenchen.de

Abstract

Upon freeze drying proteins are exposed to various stresses, one of them is the freeze-concentration. We tried to understand the stability of a mAb in the freeze-concentrate. The composition of the freeze-concentrate could be analyzed by DSC indicating 70-75% water content. Freeze-concentrates were prepared utilizing partial freeze-drying. No aggregate formation was observed over 1 month storage of the freeze-concentrates at -80, -10 and 25 °C. Thus, despite various general concerns, exceeding Tg' for a short time during freeze-drying is not critical for protein stability.

Introduction

Upon freeze drying proteins are exposed to various stresses. One of them is the freeze-concentration. The increase in the concentration of protein and excipients means that one cannot directly tell protein-protein interactions and protein stability based on the liquid starting formulation before freeze drying. We evaluated different methods to analyze the composition of the freeze-concentrate. The water content can be determined using DSC of a concentration series of protein/excipient mixtures. Direct analysis of the individual formation is possible depending on composition and instrument sensitivity. Subsequently, preparation of the freeze-concentrate needs to be performed. Different approaches including direct dissolution, lyophilization or spray-drying and reconstitution as well as partial lyophilization and replenishing were to be tested. The obtained freeze-concentrates were analyzed with respect to protein-protein interactions and mobility by SAXS and viscosity. A 1 month stability study of freeze-concentrates was performed at -80, -10 and 25 °C. The formation of aggregates was analysed by SEC and Light Obscuration.

Materials and Methods

The formulations contained sucrose, a mAb and histidine buffer as main components. Samples were analyzed by DSC (DSC 821^e, Mettler Toledo) for unfrozen water upon heating from -40°C to 20°C at 2 K/min formation [Roos&Karel, 1991], SAXS for protein-protein interaction (SAXSess mc², Anton Paar), viscosity (MCR100, Anton Paar), soluble aggregates by size exclusion chromatography (BEH SEC 200Å, Waters) and sub-visible particles analysis by light obscuration (SVSS-C35, PAMAS).

Results

Three different methods were evaluated to analyze the water content of the freeze-concentrate. A one-point measurement showed inadequately high standard deviation. Analysis of T_g' and the shift of T_m for different concentrations and using the intercept of the melting peak curve and T_g' onset rendered adequate results but comes with the challenges to dissolve very high concentrations of protein and excipient. The most promising method is based on the analysis of the unfrozen water. The quality of the results improves with high ts content measurements but concentrations above 50% can be omitted [Fig.1]. The solid content of different freeze-concentrates Wg' was in the range of 70 to 75%.

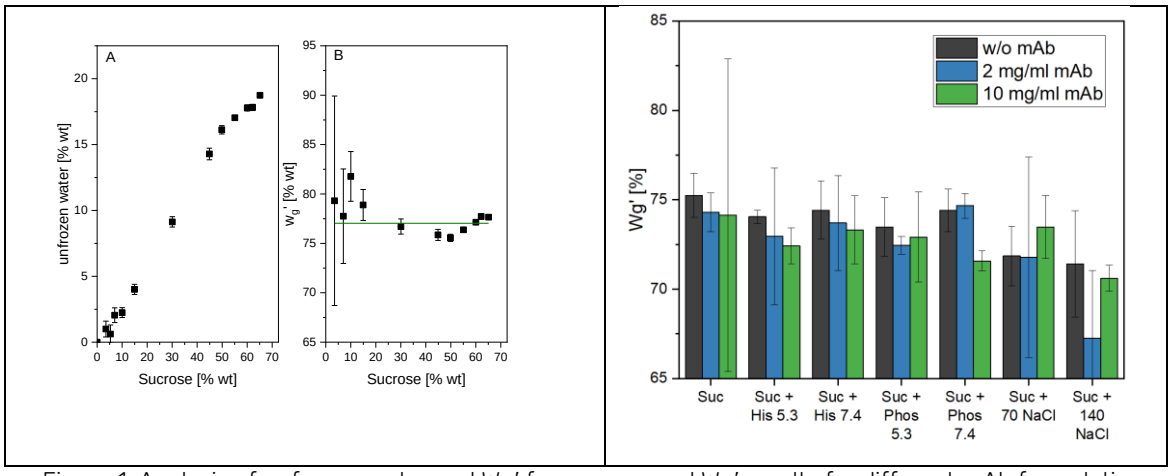


Figure 1. Analysis of unfrozen water and Wg' for sucrose and Wg' results for different mAb formulations.

Different methods to obtain freeze-concentrates for further analysis were evaluated. Direct dissolution of excipients, lyophilized mab as well as lyophilized or spray-dried formulations was not feasible. Finally, partially freeze-dried samples which were replenished on weight base enabled the preparation of freeze-concentrates. Different freeze-concentrates were tested on stability varying the mAb concentration and adding sodium chloride to shield repulsive protein-protein interactions potentially triggering aggregation. No sample showed signs of protein aggregation upon storage at -80°C, -10°C and 25 °C. Thus, aggressive freeze-drying above Tg' can be considered as uncritical. SAXS did not indicate significant changes in protein-protein interactions and rheometry demonstrated substantial reduction in molecular mobility in the freeze-concentrate, both underlining the fact that the mab did not form aggregates upon storage in the freeze-concentrate.

Acknowledgments

The authors thank A. Bregolin and D. Fissore for support in the analysis of the freeze-concentrate composition and C. Porsiel for SAXS analysis.

References

Roos, Y. and Karel, M., Amorphous state and delayed ice formation in sucrose solutions. Int. J. Food Sci. Technol., 1991. **26**(6): 553-566.

Freeze-drying of protein pharmaceuticals: use of information on component mixing for formulation and process development

Izutsu K., Ando, D., Usui, A., Abe, Y., Yamamoto, E., Yoshida H.
National institute of Health Sciences, Division of Drugs, Tonomachi, Kawasaki 210-9501, Japan
*E-mail of the corresponding author: izutsu@nihs.go.jp

Abstract

This study examined relationship between mixing state of freeze-concentrated proteins (gelatin, albumin) and sugar or sugar alcohols (e.g., sucrose, mannitol) on the excipient crystallization. Thermal analysis of frozen solutions showed T_g' (glass transition temperature of maximally freeze-concentrated solutes) and peaks of solute crystallization/crystal form change, in the first scans and second scans after holding of the frozen solutions at different temperature. Use of three sugar alcohols (mannitol, myo-inositol, sorbitol) with different crystallization propensity in the frozen solutions indicated large contribution of the solute mixing state, different depending on the concentration ratios on the solute crystallization crystallization of the solutes in the multi-component frozen solutions. Excipient-rich frozen solutions suggested formation of both excipient-dominant and solute-mixture concentrated phases that allow rapid crystallization of mannitol. Contrarily, freezing of some protein-rich aqueous solutions concentrate the solutes in the mixture state that kinetically prevent crystallization of mannitol.

Introduction

Freeze-dried therapeutic protein formulations contain multiple ingredients (stabilizer, bulking agent, pH modifier, tonicity modifier) beside the protein. Freezing of aqueous solutions concentrates the solutes into narrow phases surrounding ice crystals. Varied mixing and crystallization states of the ingredients in the frozen solutions should affect both the physical stability of dried solids and integrity of protein conformation. Relationship between the possible phase separation in the amorphous freeze-concentrate on the excipient crystallization has not well elucidated.

Materials and Methods

Recombinant human albumin, mannitol and solbitol were obtained from Sigma-Aldrich. (Nippi). Low MW gelatin and *myo*-inositol were purchased from Nippi Inc. and Fujifilm Wako Pure Chemical Co., respectively. Aqueous solutions (20µl) containing the protein, excipient, and 20mM phosphate buffer in hermetic aluminum pan were applied for thermal analysis using DSC Q2000 (TA Instruments). The samples were scanned from -70°C at 5°C/min. Some frozen solutions were hold at -25 to -5°C up to 480 min before the second heating scan to see the effect of heat-treatment.

Results

The frozen low MW gelatin solution showed apparent T_g' at approximately -12°C, which allowed study of the solute mixing states in the protein-excipient combination. Transition temperature profiles (T_g') of frozen gelatin-excipient solutions rich in rarely or slowly crystallizing excipients suggested separation of the solutes to the single-solute and solute-mixture phases. A solute combination frozen solution (70 mg/mL gelatin and 30 mg/mL mannitol) showed T_g' at approximately -26°C. Holding of

the frozen solution at different temperatures (-35 to -5°C) showed slow crystallization of mannitol above the transition temperature. PXRD analysis of freeze-dried solids supported the observations in the frozen solutions.

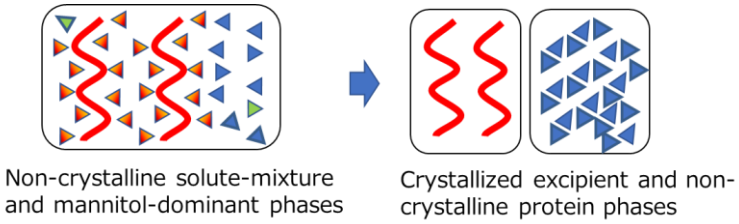


Fig. 1 *Schematic figure of the solute crystallization effect*

References

[1] T. W. Randolph, Phase separation of excipients during lyophilization: effects on protein stability, J. Pharm. Sci. 86 (1997) 1198-1203.

[2] K. Izutsu, H. Shibata, H. Yoshida, Y. Goda, Miscibility as a factor for component crystallization in multisolute frozen solutions, J. Pharm. Sci. 103 (2014) 2139-2146.

Preservation of biological activity during freeze drying – challenges and technological advances

Paul Matejtschuk
Standardisation Science, National Institute for Biological Srandards & Control (NIBSC),
Blanche Lane, South Mimms, Potters Bar. Hertfordshire. EN6 3QG. United Kingdom.
*E-mail of the corresponding author: paul.matejtschuk@nibsc.org

Abstract

Freeze drying is a common means of stabilizing biologicals for long term stability. At NIBSC we develop and stabilize a wide range of biological materials to serve as primary reference materials to assign bioactivity.

This presentatiion will illustrate different bioactivities and the strategies applied to stabilize proteins, polysaccharides, sera and viruses for optimal bioactivity

We will demonstrate how rapid microscale screening can be used to optimize formulations for maximizing bioactivity, using G-CSF as a model system.

Finally, we will focus on recent collaborations with Through-Vial Impedance Sectroscopy as a tool to study and optimize the freeze drying process for model proteins.

Freeze-drying: not just for injectables

Kett, V. a*
School of Pharmacy, Queen's University of Belfast, Belfast BT5 6GX United Kingdom
*E-mail of the corresponding author v.kett@qub.ac.uk

Abstract

In this presentation I shall give an overview of some of the non-injectable freeze-dried formats that we have developed at Queen's University Belfast over the last 15 years for controlled delivery of a range of therapeutics using various mucosal routes of administration. We have also developed formats that include more than one drug while others include particles such as liposomes that encapsulate the active or even nanoparticles in nanoparticles [1-3]. Careful choice of excipients and shape has allowed us to develop formulations that include fast-dissolving formats for immediate release, formats for nasal delivery of vaccine and for vaginal delivery of microbicide by incorporation into a vaginal ring. Rings can include the freeze-dried therapeutic as inserts in the rings [4] or by inclusion in the ring as a core that is exposed to the mucosal environment by windows in the sheath. The latter products are able to release HIV microbocide at a therapeutic dose over 30 days or longer [1, 5, 6].

References

[1] J.W. McBride, R.K. Malcolm, N. Dias, D. Cameron, R.E. Offord, O. Hartley, V.L. Kett, B. Devlin, P. Boyd, Development and pharmacokinetics of a combination vaginal ring for sustained release of dapivirine and the protein microbicide 5P12-RANTES, Int. J. Pharm., 564 (2019) 207-213.

[2] H. Yusuf, A.A. Ali, N. Orr, M.M. Tunney, H.O. McCarthy, V.L. Kett, Novel freeze-dried DDA and TPGS liposomes are suitable for nasal delivery of vaccine, Int. J. Pharm., 533 (2017) 179-186.

[3] A.K. Jain, A. Massey, H. Yusuf, D.M. McDonald, H.O. McCarthy, V.L. Kett, Development of polymeric-cationic peptide composite nanoparticles, a nanoparticle-in-nanoparticle system for controlled gene delivery, International Journal of Nanomedicine, 10 (2015) 7183-7196.

[4] P.F. McKay, J.F.S. Mann, A. Pattani, V. Kett, Y. Aldon, D. King, R.K. Malcolm, R.J. Shattock, Intravaginal immunisation using a novel antigen-releasing ring device elicits robust vaccine antigen-specific systemic and mucosal humoral immune responses, J. Control. Release, 249 (2017) 74-83.

[5] J. McBride, P. Boyd, R. Offord, O. Hartley, V. Kett, K. Malcolm, Novel Vaginal Ring Design for the Controlled Release of the Macromolecule Microbicide 5P12-RANTES, Aids Res. Hum. Retrovir., 34 (2018) 108-108.

[6] J.W. McBride, P. Boyd, N. Dias, D. Cameron, R.E. Offord, O. Hartley, V.L. Kett, R.K. Malcolm, Vaginal rings with exposed cores for sustained delivery of the HIV CCR5 inhibitor 5P12-RANTES, J. Control. Release, 298 (2019) 1-11.

Lyophilized diagnostic reagent for cancer detection

Sassone J. A
Proquimo-Improvement do Brasil; PhD Chemistry, Principal researcher and Director

*E-mail of the corresponding author: jorge@improved.com.br

Abstract

Radiopharmaceuticals for diagnostic are those having radionuclides in its composition capable to emit gamma radiation or positron emission, two sorts of very penetrating emissions which cross tissues and are easily detected externally. There exist Perfusion radiopharmaceuticals (1st generation), non selectives that reach organs in the proportion of blood flow; they are the most used today. A second kind are those called Specific radiopharmaceuticals (2nd generation) still today in the stage of exhaustive development; this kind we treat in our work: they contain a biologically active molecule that specifically binds to cell receptors and maintain biospecificity even after binding to the radionuclide. In this work we show the rationale to formulate the Injectable Lyophilized Specific radiopharmaceuticals using one example (HYNIC-TOC), we also illustrate with tables, photos, graphic representation, schemes, all related to Thermal properties obtained, Lyocycle development process, reconstitution and incubation previous to administrations,tability, etc

Physical events during cryopreservation: consequences on cells' post-thaw performance and on cryobiological protocols optimisation

Fonseca F.[a*]; Meneghel J. [b]; Kilbride P. [b]; Passot S.[a]; Morris G.J.[b]

[a] UMR GMPA, AgroParisTech, INRA, Université Paris Saclay, 78850 Thiverval-Grignon, France

[b] Asymptote - GE Healthcare, Cambridge, UK

*fernanda.fonseca@inra.fr

Abstract

For decades, scientists have looked for the causes of cell cryoinjury so as to identify the optimum conditions for freezing and storing frozen cells. Cell dehydration and intracellular ice formation have been argued as the main mechanisms of cell damage. Maintaining the cells in a vitreous matrix below the glass transition temperature of the surrounding medium is known as a key condition for cell preservation. However, it is only in 2013 that the measurement of the intracellular glass transition of different cell types has been made possible. Moreover, the relevance of intracellular and extracellular vitrification during slow cooling of micro-organisms and mammalian cells has been the subject of recent research. The physical events taking place during freezing are reviewed here, focusing on the role of the physical state of the intracellular and extracellular environments in determining the response of cells to stresses encountered during cryopreservation. The implications on cryoprotectants selection, freezing rates, and controlled cooling endpoint to be set for cell storage are discussed.

Introduction

The widely accepted model of cells' freezing injury is the "two-factor hypothesis" (1). It has been developed with mammalian cells and exhibited in many cases good correlation with survival on thawing. Intracellular ice formation at high cooling rates and osmotic dehydration at slow cooling rates applies to eukaryotic cells, but is is only partly true for prokaryotes. At very high cooling rates, no intracellular ice is formed in bacteria, but injuries are caused by cell plasmolysis occurring during thawing (2). Characterizing the physical state of the intracellular compartment in highly dehydrated cells in which intracellular ice is absent became crucial for delivering performant controlled cooling protocols. The intracellular glass transition temperature was first detected in different unicellular organisms at high subzero temperatures (between -10°C and –26°C) (3) and vitrification was argued to provide a survival strategy. Based on this pioneering work, the recent studies here presented have focused on the characterization of the physical state of thecell membrane, intracellular and extracellular environments and on their implications on cells' responses to cryopreservation.

Materials and Methods

The lipid phase transition temperature of cell membranes as well as the ice nucleation and ice melting temperatures were determined by Fourier Transformed InfraRed (FTIR) spectroscopy. Extracellular (Tg'e) and intracellular (Tg'i) glass transition temperatures were determined by differential canning calorimetry (DSC) on protective medium and cell pellets, respectively. The biological activity of micro-organisms and mammalian cells was quantified before and after freezing in order to relate the cell response to the freezing conditions applied and to the physical events measured.

Results

Physical events taking place during freeze-thawing bacteria and mammalian cells were characterized in the presence of reference cryoprotectants (Fig. 1). It was demonstrated that bacteria with the lowest value of intracellular Tg' survive the freezing process better than cells with a higher intracellular Tg'. Besides, cooling at a slow, controlled rate until Tg'i is reached appears critical for a successful cryopreservation of a wide variety of mammalian cells.

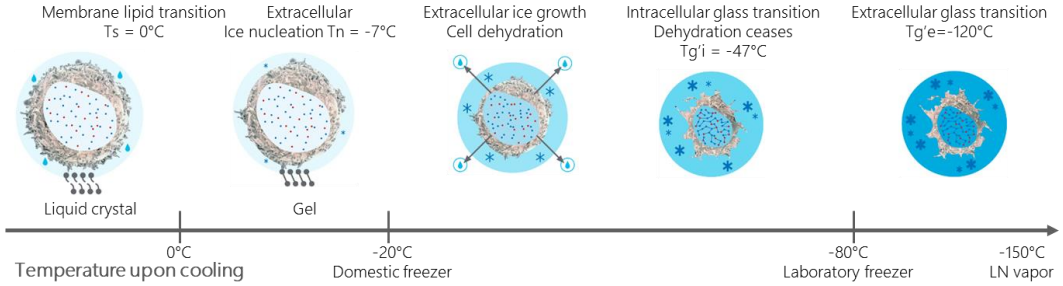


Fig. 1 *Schematic of the physical events occurring during freezing of a Jurkat cell in the presence of dimethyl sulfoxide (adapted from Meneghel et al. 2019 (4)).*

Conclusions

The vitrification of the intracellular environment has an important role in the response of cells to preservation and on the optimization of freezing protocols. However, long-term stability in the frozen state can be achieved below both the Tg'e and the Tg'i. Innovation in the field of protective molecules is required and new oligosaccharides mixtures presenting a great potential are under study (5).

References

[1] Mazur, P. 1984.. Am. J. Physiol. C 16:125–142
[2] Fonseca F, et al. (2006) Appl Environ Microbiol 72:6474–6482. doi:10.1128/AEM.00998-06
[3] Clarke A, et al. (2013). PLoS ONE, 8(6):e66207. doi:10.1371/journal.pone.0066207
[4] Meneghel et al. (2019) PLoS ONE 14(5): e0217304. doi.org/10.1371/journal.pone.0217304
[5] PREMIUM project (H2020 MSCA-RISE, n°777657)

Challenges in Continuous Lyophilization

Corver, J.[a]*; Van Bockstal, P.J. [b]; De Beer, T.[b]
[a] RheaVita, Ghent, Belgium
[b] Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium
*E-mail of the corresponding author: jos.corver@rheavita.com

Abstract

Introduction

In 2003, FDA issued the PAT initiative to enhance drug product quality and reduce variability thereof. Continuous processing was addressed as one of the underlying improvements leading to a better control of critical quality attributes (CQA's) in interaction with the processes. In the development of continuous alternatives for batch-wise pharmaceutical manufacturing processes, the focus till now was mainly on solid dosage forms. However, continuous processing of biopharmaceuticals, both up- and downstream, is also of major importance. Many process steps have been converted in continuous operation, but freeze-drying still is a batch process in manufacturing.

Continuous (bulk) freeze-drying already exists in food industry (see CONRAD, GEA, for producing coffee powder). New developments for pharmaceutical applications are underway, which will be addressed in the remainder of the presentation.

Materials and Methods

In the presentation, first the drawbacks of current batch freeze-drying are highlighted, with a focus on establishing quality. Issues that have to be overcome to make continuous processing applicable are addressed. Applicable PAT tools for process monitoring and control will be shown. Current developments are indicated and compared following two distinct ways of operation: producing bulk (e.g. IMA, Fig. 1, HOSOKAWA , Fig. 2) or producing doses in containers (e.g RHEAVITA, Fig. 3).

Conclusions

There are some promising routes to effectuate continuous freeze-drying which satisfy the inferences made by the PAT initiative. Furthermore, from operational point of view, it can be shown that continuous freeze-drying has additional business potential by reducing time-to-market and sustainable operations.

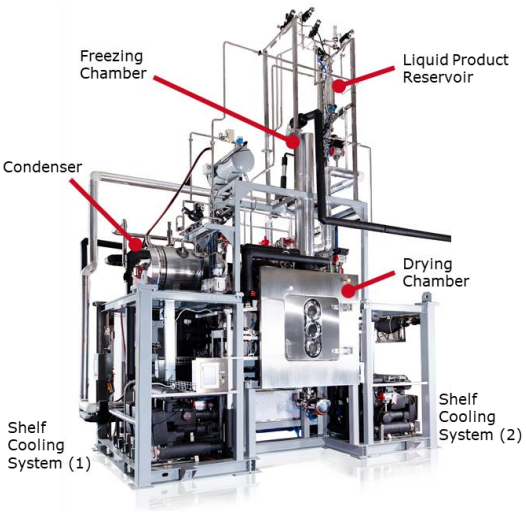


Fig. 1 *IMA's Continuous Spray Freeze-Drying*

Fig. 2 *Hosokawa's Containd Active Freeze-Drying*

Fig. 3 *RheaVita's Continuous Freeze-Dryer*

Modernizing lyophilization of pharmaceuticals in unit doses via continuous manufacturing

Pisano, R.
Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy
*E-mail of the corresponding author: roberto.pisano@polito.it

Abstract

This work show an alternative pharmaceutical freeze-drying concept, which makes it possible to move from batch to continuous manufacturing. The continuous flow of vials is achieved by suspending them over a moving track. The vials move through chambers which have different pressure and temperature conditions and are separated by a load-lock system. Uniformity in freezing conditions is demonstrated by combining the Vacuum Induced Surface Freezing method and convective freezing.

Introduction

In response to the current trend in the pharmaceutical industry [1], a new concept for the lyophilization of pharmaceuticals in unit-doses is presented: the continuous freeze-drying/lyophilisation of suspended vials. This configuration makes it possible to set up a continuous lyophilization process that produces a final product with similar characteristics to those traditionally obtained by means of the batch process, but which avoids the drawbacks of conventional, batch freeze-drying [2]. The feasibility and advantages of this new concept are presented in this work.

Methods

The continuous flow of vials is achieved by suspending them over a moving track. The uniformity in freezing conditions resulted from the combination of convective freezing and Vacuum Induced Surface Freezing method, while heat is transferred substantially through radiation during drying. The vials move through chambers which have different pressure and temperature conditions and are separated by a sluice-gate system. A schematic of the concept is shown in Fig. 1.

Results

In order to obtain a quantitative estimation of the advantages of the proposed continuous strategy, with respect to the batch one, a functional version of the continuous plant has been set up, adapting a batch freeze-dryer. This plant allowed us to simulate the same heat and mass transfer conditions to which vials would be exposed in a continuous freeze-dryer. The performances of the batch and continuous configurations were evaluated in terms of processing time and vial-to-vial variability.
Continuous lyophilization has been found to improve heat transfer uniformity, and a dramatic reduction in the process time (up to 5 times) has been observed. This last result makes it possible to lower the production costs by 50-70%. As far as the structure of lyophilized products is concerned, this technology improves vial-to-vial and intra-vial homogeneity. Overall, this technology results in higher productivities and yields of improved product quality. Additionally, equipment size is greatly reduced (up to 10-15 times) compared to batch lyophilization for the same mass per time throughput.

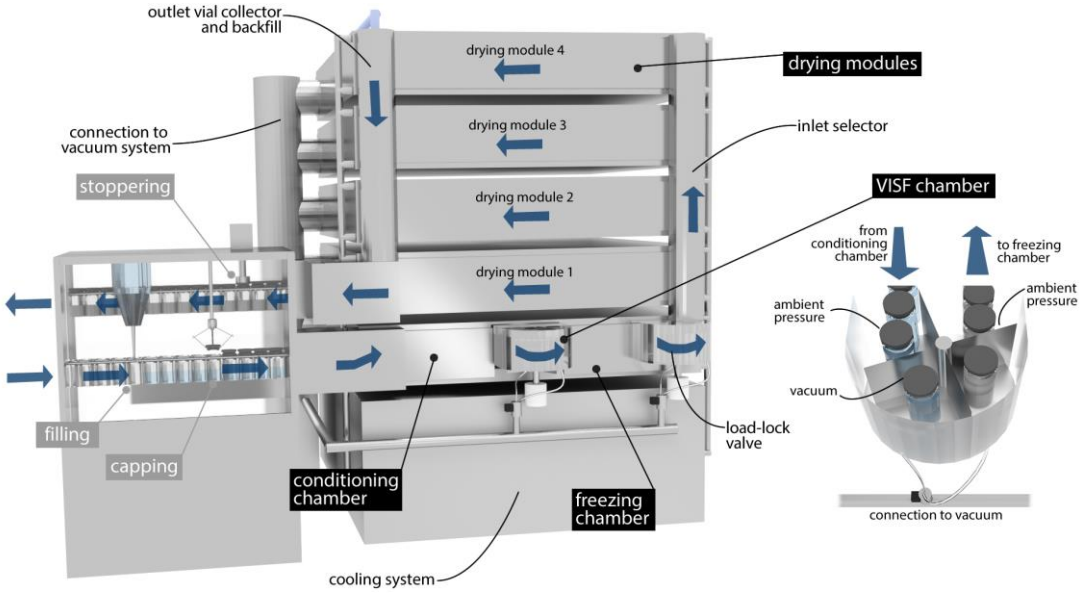


Fig. 1 *Schematic of the continuous lyophilizer*

Conclusions

The obtained results have demonstrated the feasibility of our concept as a valid alternative to conventional batch lyophilisation, which may open up new perspectives and opportunities to completely re-think the production of parenteral products and make freeze-drying more efficient and versatile.

References

[1] R. Pisano, A. Arsiccio, L.C. Capozzi, B.L. Trout, Achieving continuous manufacturing in lyophilization: Technologies and approaches, Eur. J. Pharm. Biopharm. 142 (2019):265– 279.

L.C. Capozzi, B.L. Trout, R. Pisano, From batch to continuous: freeze-drying of suspended vials for pharmaceuticals in unit-doses, Ind. Eng. Chem. Res. 58(2019):1635-1649.

Dynamic spray freeze-drying of pharmaceuticals: model validation and product properties

Borges Sebastião, I.*
Pfizer Inc., BioTherapeutics Pharmaceutical Sciences, Andover, Massachusetts, USA
*E-mail of the corresponding author: israel.b.sebastiao@pfizer.com

Abstract

Spray freeze-drying (SFD) is an evolving technology for fast and efficient manufacturing of bulk lyophilized pharmaceuticals when compared to conventional freeze-drying in trays or vials. In the process of interest, the solution is dispersed into a cold gaseous medium producing frozen millimeter-sized droplets that are subsequently dried via sublimation and desorption in a vacuum rotary drum. In this work, we cover the development and experimental validation of a spray-freezing model and illustrate how it can be utilized to understand the impact of different process parameters on the freezing dynamics. Likewise, we discuss preliminary efforts to model the drying evolution of frozen droplets in a rotary dryer based on canonical heat and mass transfer equations as well as computational fluid dynamics (CFD) techniques. Finally, our currently available database on the characterization of different spray-freeze-dried biologicals is presented.

Introduction

Spray freeze-drying (SFD) is an alternative technology that combines the benefits of spray-drying and conventional lyophilization to produce drug substance and drug product as free-flowing dried powders (Fig. 1). It could eliminate the need to ship frozen drug substance (current state) across manufacturing sites and reduce the challenges associated with the cold chain logistics (desired future state). Because of the large surface-to-volume ratio of the frozen microdroplets, SFD technology can also potentially manufacture bulk lyophilized drugs at higher rates compared with conventional freeze-drying.

Although established heat and mass transfer models are available for prediction of temperature and water content evolutions in vial-based lyophilization, there is no analogous framework dedicated to SFD processes. Development of such models are critical to understand the underlying physics of the SFD technology allowing for improved equipment design, process development and optimization, and scale up.

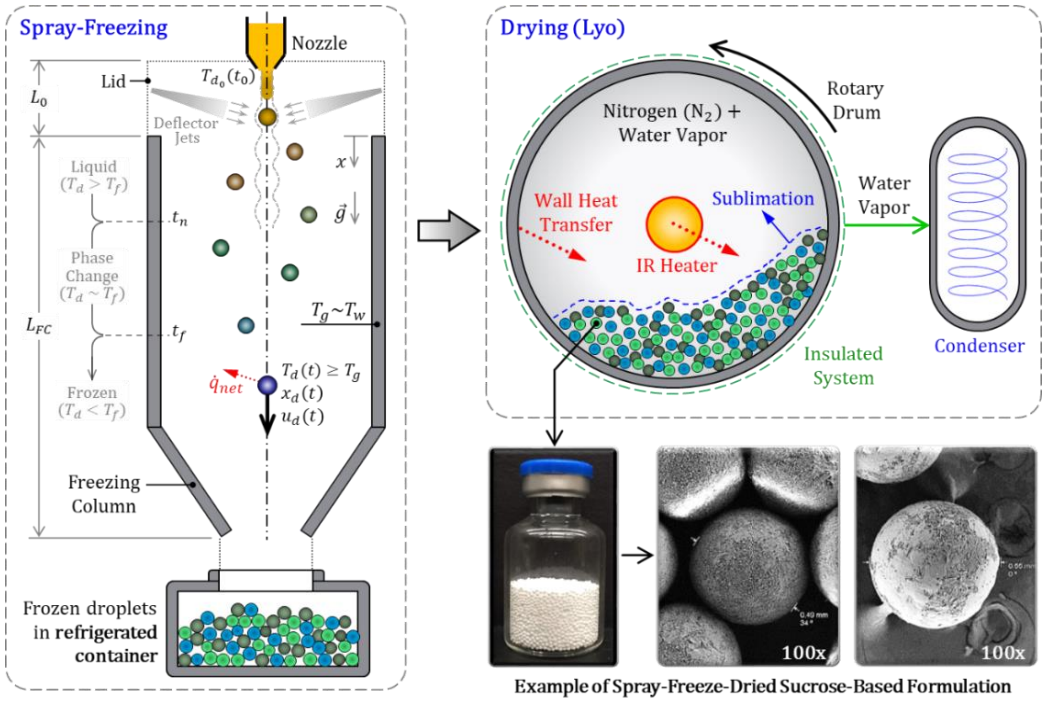


Fig. 1 Schematics of present SFD system: spray-freezing (left) and vacuum rotary-drying (right).

In this context, the main goal of this work is to share our recent efforts to develop and validate an engineering-level spray-freezing model and illustrate its practical applications [1-2]. We also discuss the different modeling approaches under consideration and respective challenges to predict the drying dynamics of frozen pellets in a lab-scale rotary drum. As a secondary goal, we briefly assess product quality attributes of SFD biologics in comparison with vial freeze-drying. Finally, we summarize the lessons learned from modeling and experiments and suggest future directions.

References

[1] I.B. Sebastiao, B. Bhatnagar, S. Tchessalov, S. Ohtake, M. Plitzko, B. Luy, A. Alexeenko, Bulk Dynamic Spray Freeze-Drying Part 1: Modeling of Droplet Cooling and Phase Change, J. Pharm. Sci. 108 (2019) 2063-2074.

[2] I.B. Sebastiao, B. Bhatnagar, S. Tchessalov, S. Ohtake, M. Plitzko, B. Luy, A. Alexeenko, Bulk Dynamic Spray Freeze-Drying Part 2: Model-Based Parametric Study for Spray-Freezing Process Characterization, J. Pharm. Sci. 108 (2019) 2075-2085.

Accelerating Freeze-Drying through Continuous Aseptic Spray Freeze-Drying

Arnab Ganguly, Frank DeMarco and Ernesto Renzi
IMA LIFE North America, Tonawanda, NY, USA

Abstract

Freeze drying has traditionally been a batch process making it both, time consuming and energy intensive (Rambhatla et al., 2003; Liu et al., 2008). While the use of new process analytical technology has aided in- process understanding, most processes are run conservatively, making it inherently inefficient. The conservative approach is partly due to lack of equipment limit knowledge at different scales but mostly driven by product limits. For example, a product with larger concentration of active pharmaceutical ingredient could be manufactured with lower fill volumes, but at the cost of higher product resistance in the cake. In the conventional approach to freeze-drying, the product is placed in vials or bulk containments directly on the shelf. The drying rate is controlled by setting the shelf temperature and chamber pressure in the product chamber. (Nail, 1980) identified poor thermal contact between the shelf and the containment as the rate limiting resistance to heat transfer. Moreover, the drying rate in such a configuration is a function of the coldest front in the product volume, the sublimation front. Recent findings (Ganguly et al., 2013) have demonstrated the importance of radiation in the low-pressure environment which can be efficiently utilized in accelerating the process. With such findings, it becomes apparent that even though our process understanding has improved in leaps and bounds, its application to designing an efficient process-equipment configuration has not. Thus, there is a need to re-think the heat and mass transfer for making the process more efficient.

Spray freeze drying can have a transcending impact on process efficiency related to Biologic drug substance storage, inhalation systems, antibiotics for bulk storage among others with direct powder dosing capabilities directly into product containment systems. The current work focuses on using systematic characterization techniques for developing a robust yet gentle continuous aseptic process for spray freeze-drying with an eye toward achieving high throughput/ high cycle efficiency. For example, understanding the freezing behavior is critical to designing a controlled spray freeze-dried process with uniform product characteristics. Here we utilize a high-speed camera with image processing techniques to first understand the product spray characteristics. Algorithms are written and validated to quantify the product size distribution critical for accurate process scale-up. The final presentation will discuss both the process and equipment used in generating freeze dried particulates in a continuous manner and a review of the limitations of such a process.

References

[1] Ganguly, A., Nail, S.L., and Alexeenko, A. "Experimental Determination of the Key Heat Transfer Mechanisms in Pharmaceutical Freeze Drying", J. Pharm. Sci., 102(5), pp.1610-1625, 2013
[2] Liu, Y., Zhao, Y. and Feng, X. Exergy analysis for a freeze-drying process. Appl. Thermal Engr., 28:675–690, 2008
[3] Nail, S.L. The effect of chamber pressure on heat transfer in the freeze drying of parenteral solutions. J. Parenteral Drug Assoc., 34:358–368, 1980
[4] Rambhatla, S. and Pikal, M.J., "Heat and Mass Transfer Scale-up Issues during Freeze-drying: I. Atypical Radiation and the Edge Vial Effect", AAPS PharmSciTech., 4(2): 14, 2003

A SMART technology for the continuous manufacturing of lyophilized orally disintegrating tablets

De Beer T.*, Vanbillemont B., Cover J.
Department of Pharmaceutical Analysis, Laboratory of Pharmaceutical Process Analytical Technology ,Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium

*E-mail of the corresponding author: thomas.debeer@Rheavita.com

Abstract

Driven by growing target populations for orally disintegrating tablets (ODTs) and by the regulatory pressure, it is our aim to develop, design, assembly and validate a SMART (i.e., self-monitoring and self-controling) proof-of-concept (POC) technical prototype for the continuous lyophilization of ODTs. Such continuous process would allow a more efficient, cheaper, greener and controllable ODT manufacturing method compared to traditional batch production systems, offering competitive advantages and business opportunities.

Pharmaceutical freeze drying (lyophilization) is a low-temperature drying process in which aqueous solutions of the ODT formulations are converted into tablets with desired properties (in terms of disintegration time, solid product structure, residual moisture content and mechanical strength) for distribution, storage and administration to patients. Similar to all manufacturing processes of drug products (solids, semi-solids and liquids), conventional manufacturing of lyophilized ODTs is generally accomplished using batch processing that is considered time-consuming, costly, non-flexible, lacking robust quality control and real-time release, and that has limited ODT formulation applicability.

Three major industrial drivers are demanding a more efficient and better controllable pharmaceutical freeze drying technology for ODT unit doses: cost-cutting, regulatory pressure and need for new types of ODT formulations for different increasing target populations (working and traveling, geriatric, pediatric and institutionalized population).

The in this talk presented SMART continuous freeze drying technology for ODTs offers clear advantages over current batch production such as cost reduction (up to 50%), track-and-trace product quality control, and a significant reduction of processing time (> 10 times faster), a substantial sustainability gain and an opportunity to manufacture new types of ODTs (multilayer and mucoadhesive tablets, high dose and fixed dose combination tablets, oral biologics, personalized products) which cannot be processed using the current batch-wise technology.

Atmospheric freeze-drying of food products: practical modeling and quality assessment

Nakagawa, K. [a*]; Horie, A.[b]; Nakabayashi, M.[b]
[a] Department of Chemical Engineering, Kyoto University, Kyoto, Japan
[b] Division of Food Science and Biotechnology, Kyoto University, Kyoto, Japan

*E-mail of the corresponding author: kyuya@cheme.kyoto-u.ac.jp

Abstract

Freeze-drying of food products under atmospheric conditions was studied. This operation was carried out largely above the glass transition temperature of a product, where product shrinkage and micro-collapse due to the glass-rubber transition must be admitted. By this reason, a glassy matrix with particular vapor pressure was assumed and employed for modeling with classical mass and heat balance equations. Apparent vapor pressure of apple slices was experimentally measured and summarized in a diagram, and the values in this diagram were employed for the simulation. Influence of drying conditions on the resultant product qualities were also investigated.

Introduction

Atmospheric freeze-drying (AFD) is basically equivalent to vacuum freeze-drying (VFD), where the ice sublimation is the major dehydration mechanism. The water vapor pressure difference between the frozen zone and the ambient gas is the driving force of the mass transfer. In order to operate drying at below sub-zero temperature, the air humidity must be low at appropriate level. Drying temperature (air temperature) applied for AFD is usually at around –10 to 0°C. The product temperature during drying, that could be several degrees lower than the drying temperature depending on the drying rate, is much higher than the glass transition temperatures (T'g). At this temperature, the product deformation (i.e. shrinkage, collapse, etc.) occur as a consequence of the glass-rubber/ glass-liquid transitions. In such cases, the dried cake layer does not clearly separate from the frozen zone. In this study, a glassy matrix with particular vapor pressure was assumed and employed for modeling a atmospheric freeze-drying with classical mass and heat balance equations. Apparent vapor pressure of apple slices was experimentally measured by a pressure rise test and summarized in a diagram, and the values in this diagram were employed for the simulation.

Modeling and Simulation

A product placed in the atmospheric freeze-dryer is dried by convective air. Assuming that the system is at a quasi-steady state, the heat flow is balanced with the mass flow.

$$Q/_{\Delta H} = \frac{dm}{dt} = \frac{{}^{P_i}/_{T_i} - {}^{P_{air}}/_{T_{air}}}{{}^{1}/_{(1-\gamma)A_{total}k_{gex}} + {}^{1}/_{\gamma A_{total}k_{gin}}} \frac{M}{R} = \frac{T_i - T_{air}}{{}^{1}/_{(1-\gamma)A_{total}h_{ex}} + {}^{1}/_{\gamma A_{total}h_{in}}} \frac{1}{\Delta H} \quad (1)$$

A product (i.e. a slice of apple) has porous structures derived from cellular microstructures. The external parts of the inner pore surfaces contact to the external air flow, whereas the other parts of the surfaces contribute to the convection with the internal air flow. Here, a coefficient γ is introduced to give these surfaces. The surface that effectively contributes to the external convection (A_{eff}) can be written as:

$$A_{\text{eff}} = (1 - \gamma)A_{\text{total}} \quad (2)$$

The use of the apparent water vapor pressure as a function of the temperature and moisture content was a key feature of this study. The relationship among these parameters was experimentally determined by the pressure rise test as the results shown in Fig. 1. The water vapor pressure values were significantly lower than the equilibrium vapor pressure of water (ice), especially in the range at lower moisture content and lower temperature. These values were used for the drying simulation; the water vapor pressure value (P_i) at temperature of T_i was given from this diagram. The simulation results well predict the freeze-drying runs carried out under atmospheric conditions.

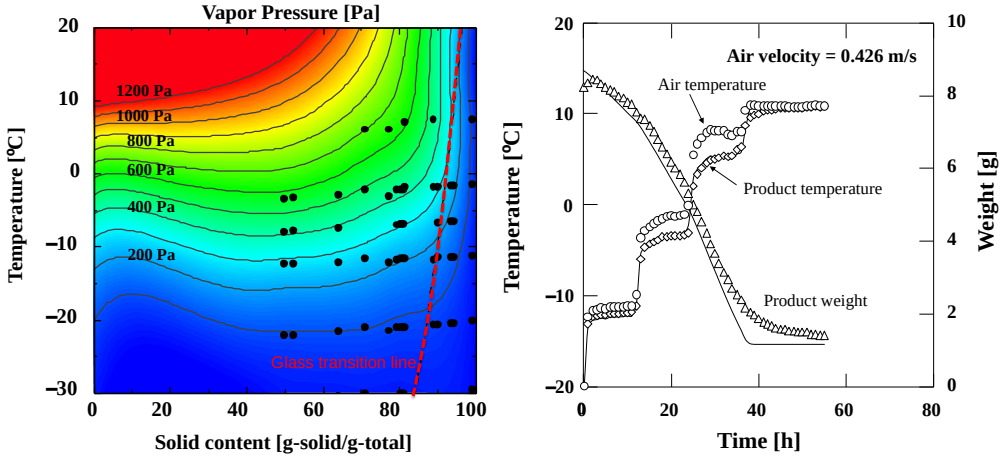


Fig. 1 Apparent water vapor pressure diagram (left) and simulation result (right; solid lines are simulated results).

Model-based tools for cycle development and scale-up

Barresi A. A. [a]

[a] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy

*E-mail of the corresponding author: antonello.pisano@polito.it

Abstract

This contribution discusses how recently developed model-based tools for monitoring and closed-loop control of freeze-drying process can be useful employed for automatic cycle development. Alternatively, they can be used to obtain the parameters needed for off-line optimization and calculation of design space.

Introduction

Tools developed for automatic control can be used for process optimization, process intensification and cycle transfer. In fact, they allow both the determination in-line of base cycles and of reliable heat and mass transfer parameters which can then be used for scale-up and cycle development using off-line approaches.
Different monitoring and control strategies will be considered and commented, showing examples of cycle development.

Automatic cycle development and scale up

An apparatus equipped with an automatic closed-loop control, like LyoDriver, can be used to develop in a few steps (or even with a single run) a close-to-optimal cycle; only the maximum allowable product temperature must be specified, and in additions constrains on the maximum shelf temperature, and on the number and type of steps can be fixed. Examples will be shown of cycles developed for different formulations and for different freezing condition, with controlled and uncontrolled nucleation, to show that differences in the sequence of shelf temperature set points reflects the different thermal characteristics of the excipients and the different matrix structures.
If the industrial apparatus is also equipped with monitoring and control tools, these can also be employed to adapt and transfer the cycle for it, overcoming the well/known scale up issues. It would be sufficient to launch a cycle imposing the proper restrictions on the product temperature.

Off-line cycle optimization and process transfer

The same tools can be adopted also to recover in-line the process parameters, to develop off-line optimization strategies or design spaces; safety margins can be handled in both cases, but in a different way, The design space can be obtained in-line also using a soft sensor, and generally speaking the design space approach can be used to handle the effect of the freezing protocol and of batch non-uniformity.
Alternatively, the same parameters obtained can be used to simulate the drying behavior using a mathematical model of the process; an example will be shown.

Selection of optimal pressure

Most of the methods developed find the optimal shelf temperature sequence for a given pressure, which can be identified by an off-line optimization procedure. Some examples will show how the result depends on the control logic adopted (comparing feedback and model-based controllers).
If LyoDriver is used for cycle development, pressure should be manually adjusted by the user as soon

as there is evidence to suggest that sublimation is rate-controlled by mass transfer. However, this strategy requires that the pressure dependence of the heat transfer coefficient is known at priori, but unfortunately these data are not always available and are both equipment- and container-specific. In case the pressure dependence of the heat transfer coefficient is known, which means that the equipment-vial system has been fully characterized, the manipulation of both shelf temperature and pressure using a MPC (Model Predictive Control) algorithm gives the highest rate of sublimation and hence the shortest drying time.

Optimisation of secondary drying

Coupling the measurement of the desorption rate, obtained by means of the PRT or other devices, with a mathematical model of the process, it is possible to obtain a soft-sensor that estimates in line the desorption constant and the residual amount of solvent in the product at the end of primary drying.
The parameter estimated can be used to obtain the design space also for secondary drying, but the soft sensor can also be integrated inside a control loop which determines the optimal heating strategy for the secondary drying step. In fact, the maximum allowed product temperature of the lyophilized product increases with a decrease in the residual moisture; if this relationship is known, the cycle can be optimized increasing progressively the shelf temperature as far as secondary drying goes on and the residual moisture decreases.

IoT for Lyophilization PAT

Strongrich, A.; Alexeenko, A.
School of Aeronautics and Astronautics, Purdue University, West Lafayette, IN

*E-mail of the corresponding author: alexeenk@purdue.edu

Abstract

The Internet of Things (IoT) framework is applied to pharmaceutical lyophilization to provide an enhancement of existing Process Analytical Technologies (PATs). The IoT network is diverse, consisting of a series of wireless pressure sensors, temperature sensors, and cameras located throughout the process chamber. These measurement and observation capabilities enable a better understanding of the product state during primary and secondary drying and are used to describe several previously unknown primary drying phenomena.

Introduction

Sensor networks based on the Internet of Things (IoT) are proliferating to a broad a range of applications, including personal health monitoring, home automation, defense and agriculture. Industrial processing, representing a substantial portion of the overall IoT market, is projected to experience a CAGR of 48.4% through 2021 [1]. In their simplest form, IoT networks consist of a field of sensor nodes which are able to measure information about their surroundings and transmit the data to either a central host or nearby peer. Their compact size, positioning flexibility, and high power efficiency make them ideal candidates for Process Analytical Technology (PAT) enhancement in lyophilization, specifically for monitoring the process pressure, product temperature and their uniformity throughout the system.

Materials and Methods

The IoT network consists of a diverse array of both sensing and imaging nodes. The sensor nodes integrate directly into the vial pack, providing localized vacuum pressure, gas temperature, and product temperature. Both sensor node types operate on the same 2.45 GHz band and broadcast data in real time to separate hosts. The imaging nodes are installed above the vial pack and are configured to capture and transmit an image over a closed WiFi network. These images are captured by the host in real time and later synchronized with the process data. Synchronization is facilitated by a thermocouple installed in the cameras' fields of view and is carried out through comparison of the measured and observed nucleation event. Experiments applying the sensor nodes are conducted using various bulking agents whereas those using the imaging nodes use WFI exclusively

Results and Conclusions

A lyophilization IoT network uses vacuum gauges, product temperature sensors and two cameras. The IoT network has been installed in a laboratory lyophilizer REVO. The localized vacuum pressure yields the sublimation rate within 9% of the gravimetric method. These date provide a non-invasive measurement of drying progress and product state, e.g. product temperature. The temperature nodes directly quantify cake temperature at several locations in both the axial and radial directions. This enables direct tracking of the sublimation front and provides insight into the heat flow within the cake allowing to estimate the sublimation rate.

The imaging nodes are deployed separately from the sensor nodes to evaluate the true primary drying endpoint as determined by the comparative pressure measurement[2], product temperature measurement method, and Tunable Diode Laser Absorption Spectroscopy (TDLAS)[3]. The comparative scheme is most sensitive to trace water vapor and reveals the presence of bulk water ice in center vials throughout the entire convergence process. TDLAS is able to quantify minute changes in sublimation rate over primary drying, demonstrating a slow decline over the course of primary drying. The camera system correlates this behavior to reduction in water-ice surface area.

We show that the product temperature measurement is the least robust of the endpoint methods. Supplemental tests indicate preferential sublimation along the thermocouple wire, demonstrating the presence of an auxiliary thermal conduction pathway. This effect leads to a separation of the thermocouple from the water-ice early in the primary drying phases, resulting in an erroneously high temperature measurement. It is therefore suggested that the product temperature measurement method be applied for qualitative assessment only.

Application of IoT to lyophilization process monitoring and control offers significant enhancements to existing PAT in the form of localized product state measurement. IoT networks are robust, flexible, secure, and integrate seamlessly with existing lyophilization infrastructure, making them highly attractive both in laboratory and manufacturing setting.

References

[1] Ramamurthy, S. "Wireless Sensors: Technologies and Global Markets." BCC Research (2016).
[2] Patel, S. M., Takayuki D., and M. J. Pikal. "Determination of end point of primary drying in freeze-drying process control." AAPS PharmSciTech 11.1 (2010): 73-84.
[3] Gieseler, H., W. J. Kessler, M. Finson, S. J. Davis, P. A. Mulhall, V. Bons, D. J. Debo, M. J. Pikal. "Evaluation of tunable diode laser absorption spectroscopy for in-process water vapor mass flux measurements during freeze drying." J. of Pharm. Sciences 96.7 (2007): 1776-1793.

Freeze-drying from organic co-solvent systems: thermal analysis and process design

Gieseler, H.*
Department of Pharmaceutics, Friedrich-Alexander-University (FAU) Erlangen-Nürnberg, Germany
*E-mail of the corresponding author: henning.gieseler@fau.de

Abstract

The use of co-solvent systems has been demonstrated to shorten lengthy freeze drying processes and to improve solubility and stability of selected drug molecules. The goal of the present study is to highlight the changes in physico-chemical behavior as well as process related parameters when dealing with co-solvent / water based formulations. Thermal analysis methods provided valuable insight into freezing and nucleation habits of co-solvent and co-solvent excipient mixtures, and the impact of annealing steps. Establishment of evaporation steps during the freezing phase helped to shorten primary drying times, but were found to have little to no impact on final residual solvent concentrations. A study involving gentamicine and insulin proved applicability of the examined properties of co-solvent / water systems in a complex mixture.

Introduction

Water is the most commonly used solvent for freeze drying of pharmaceutical products. However, the design of modern drug molecules has become incredibly complex, and many of these structures are too hydrophobic to allow freeze drying using a conventional water-based formulation. When using co-solvent / water mixtures, attention must be paid to new potential obstacles in both formulation and process development. In particular, the physico-chemical properties of such mixtures can change significantly [1]. Attention must be paid to residual solvent levels in the final drug product which must not exceed toxicological limits defined in the ICH guidelines [2]. Freezing was found as the most critical step in a freeze drying cycle to obtain adequate product quality attributes [3]. The objective of the present work is to follow a multistep approach to explore challenges and opportunities of product development using co-solvent/water based mixtures in freeze drying.

Materials and Methods

Ethanol, dimethyl sulfoxide (DMSO), tert-butanol (TBA), N,N-dimethylacetamide (DMAc), acetone, 1,4-dioxane, mannitol, sucrose and polyvinylpyrrolidone (PVP, 40k MW) were obtained of highest analytical grade (Carl Roth GmbH & Co. KG; Sigma-Aldrich Chemie GmbH). Gentamicin and Insulin were donated by Merck KGaA. Thermal analysis was perfomed using a Mettler Toledo DSC822 and LINKAM FDCS196 freeze drying stage. Freeze drying experiments were performed usind a modified SP Scientific AdVantage Plus freeze dryer. Final product characterization was conducted, among others, by coulometric Karl Fischer residual moisture measurement, gas chromatography (Clarus 850 gas chromate-graph), µ-tomography (phoenix vtomex s), dynamic vapor sorption (DVS Intrinsic) and reverse-phase high-performance liquid chromatography analysis (Waters Alliance 2695).

Results

Thermal analysis revealed that a 70% TBA / water mixture forms a hydrate which resulted in the highest Tg' in the examined 50 mg/mL PVP formulation. Moreover, 40% 1,4-dioxane / water systems were found to change from a single into two eutectic melting points, one of which at lower temperatures than the original melt, In the presence of any excipient. Further, in contrast to well-known principles, fast freezing of 50 mg/mL PVP in 40% 1,4-dioxane / water formed reproducibly

large ice crystals, whereas annealing of the same system resulted in a reproducible small ice crystal formation. Using a standard freezing rate of the same mixture resulted randomly in either small or large crystals. 50 mg/mL mannitol in 50% DMAc / water mixtures showed only ice nucleation during re-heating in the freezing step, the solution could not be nucleated during cooling even at temperatures as low as -80°C. However, annealing supported ice nucleation of the mixture and greatly improved product appearance after freeze drying. Freeze drying experiments showed that some final product quality attributes of low-melting co-solvent / water formulations, such as 50 mg/mL sucrose in 10% ethanol / water, benefit from an interval of moderately reduced chamber pressure during the freezing step. This evaporation step partially removes the co-solvent and elevates the Tg' of the amorphous phase, as confirmed by µ-tomography measurements. However, residual solvent levels did mostly not benefit from this procedure in perspective of toxicological limits. Note that this applied to many of the investigated co-solvents, For example, the freeze drying cycle of 10 mg/mL gentamicin sulfate, 40 mg/mL mannitol in 40% 1,4-dioxan could be shortened by 75%, but the residual soltent level was found to be still 10-times higher than the exposure limit.

Conclusions

Thermal analysis of formulations composed of co-solvent / water systems is of outermost importance to identify suitable co-solvent candidates and to explore the freezing properties of the composition. The design of the freezing step was found to be the most critical phase for successfull processing of such systems. Evaporation steps during freezing and anneal-ing can be highly benefitial to improve product quality attributes and may reduce residual co-solvent levels in the final cake, but typically not down to acceptable toxicological limits.

References

[4] C. Kunz, S. Schuldt-Lieb, H. Gieseler, Freeze-drying from organic co-solvent systems, part 1: thermal analysis of cosolvent-based placebo formulations in the frozen state, J. Pharm. Sci. 107(3), 2018, pp. 887-896.
[5] C. Kunz, H. Gieseler, Factors influencing the retention of organic solvents in products freeze dried from co-solvent systems, J. Pharm. Sci. 107(8), 2018, pp. 2005-2012.
[6] C. Kunz, S. Schuldt-Lieb, H. Gieseler, Freeze-drying from organic co-solvent systems, part 2: process modifcations to reduce residual solvent levels and improve product quality attributes, J. Pharm. Sci. 108(1), 2019, pp. 399-415.

Integrated use of mechanistic models, targeted experiments and modern analytical tools for development and troubleshooting of lyophilisation cycles: Packing of vials approach

Rajniak, P.a,b,*, Moreira, J.b, Bermingham, S.K.b
a Department of Chemical and Biochemical Engineering, Slovak Technical University, Bratislava, Slovakia
b Process Systems Enterprise Ltd., London, United Kingdom
E-mail of the corresponding author: pavol.rajniak@stuba.sk

Abstract

This contribution summarizes results of an approach combining following research techniques for investigation and prediction of a lyophilisation process: a/ mechanistic mathematical models for the packing of vials ; b/ targeted experiments with both, the pure water and the real solution for sequential evaluation of heat and mass transfer coefficients; c/ experiments with and without annealing during the freezing stage of the cycle; d/ monitoring of the process using different PAT tools including a modern TDLAS probe; e/ characterization of the product morphology using the SEM imaging. The combined approach is illustrated by presenting results of two case studies:

- Case Study 1 – experimental and theoretical study of lyophilisation from a packing of vials with and without annealing
- Case Study 2 – theoretical prediction and experimental verification of the choked flow during lyophilization

Methods

A simple gravimetric method was used to evaluate the mass of water sublimed at different locations within a laboratory scale lyophilizer. Unidirectional mathematical model for ice sublimation from a single vial was used for evaluation of heat transfer coefficients between the shelf and bottom of vials at different locations on the shelves. Different cumulative distribution functions were tested for calculation of distribution of evaluated heat transfer coefficients. A mathematical model combining the distribution of heat transfer coefficients with the unidirectional model of primary drying in a vial was developed and employed for calculation of sublimation rates from a packing of vials. A simple optimization procedure was developed for evaluation of the cake resistance parameters (pore size and skin resistance) by fitting the model predictions to the TDLAS mass flux data. SEM imaging was used for visualization of the cake morphology. The methodology was also employed for calculation of the total sublimation rate in experiments with pure water at conditions of the choked flow and for prediction of feasible process conditions for a lyophilisation cycle with a real pharmaceutical formulation. The mathematical models used for the work are available in gPROMS FormulatedProducts [1].

Results

Weibull cumulative distribution function accurately describes the distribution of heat transfer coefficients of an inhomogeneous shelf. Mathematical models for both, the pure ice sublimation and sublimation of water from a pharmaceutical (sucrose-based) formulation satisfactorily simulate the sublimation rates measured experimentally.

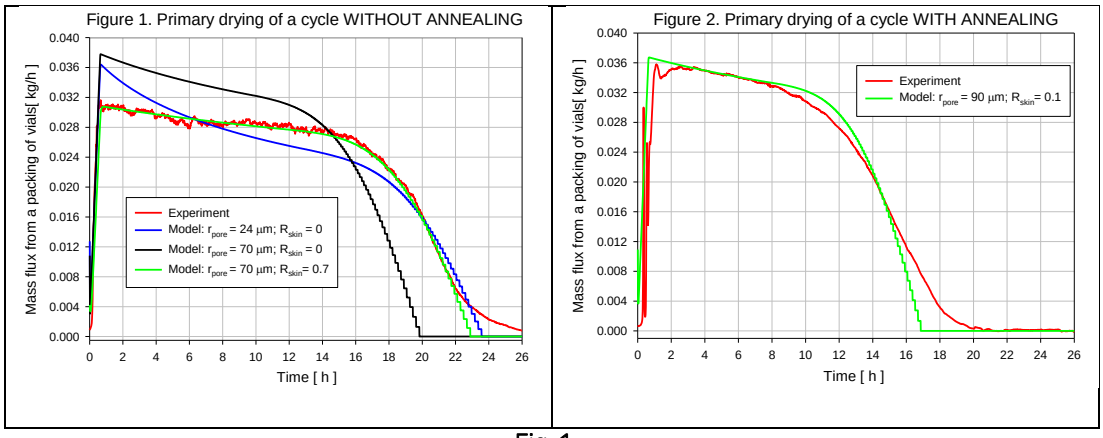


Fig. 1 ...

A model assuming a homogeneous porous cake (black and blue curve in Figure 1) was unable to describe experimental observation (red curve in Figure 1) of a lyophilization cycle without annealing. On the other hand, a model assuming the presence of a skin (skin resistance Rskin = 0.7) at the cake surface followed by a porous structure with pore radii 70 microns (green curve in Figure 1) very well describes experimental observations. A faster sublimation was measured using the TDLAS for a cycle with annealing (Figure 2). The experimental mass flux can be satisfactorily predicted using a cake model with a thinner skin at the surface (skin resistance Rskin = 0.1) followed by a porous structure with pore radii 90 microns (green curve in Figure 2). SEM imaging nicely supports results of theoretical evaluation showing a thicker skin followed by smaller pores for cakes from the cycle without annealing. More details can be found also in [2].

Conclusions

Inhomogeneous lyophilisation is a key challenge for development and scale-up of lyophilisation cycles. Annealing had a positive impact on both, the primary drying acceleration and the product pore structure homogenization for the investigated (sucrose-based) formulation. A multi-dimensional mathematical model of lyophilization from packing of vials in combination with the TDLAS probe is a useful tool for better understanding and faster development, scale-up and trouble-shooting (i.e. the choked flow prediction) of lyophilization processes.

References

[1] gPROMS FormulatedProducts 1.3, Process Systems Enterprise Ltd, December 2018
[2] P. Rajniak, C. Mifkovich, L. Farber. Experimental and Theoretical Study of Lyophilization from a Packing of Vials: Using of Tunable Diode Laser Absorption Spectroscopy (TDLAS) for Process Monitoring and Model Calibration. AIChE Annual Meeting Conference Proceedings, Minneapolis, MN, October 16-21, 2011.

Electrical Impedance Methods for Developing a Lyophilization Cycle

Smith, Geoff
School of Pharmacy, De Motfort University, Leicester, LE1 9BH, UK
*E-mail of the corresponding author: gsmith02@dmu.ac.uk

Abstract

This work describes the theroretical and practical basis for the *in-line* use of impedance spectroscopy in the determination of various critical process parameters (ice nucleation temperature, solidification time, eutectic formation and melt, and the glass transition). A key advantage of the spectroscopy approach, whereby the impedance of the test object (i.e. a glass vial or ampoule containging the product to be freeze-dried) is measured across a range of discreet frequencies (in the range 10 Hz to 1 MHz), is that one can select certain measurement frequencies, and in effect tune the instrument, for the determination of these different facets of the freeze-drying process

Introduction

The electrical impedance of materials has been used for many years in the study of the various processes associated with freeze drying. However, these studies have been restricted to the off-line determination of critical temperatures by laboratory-based analysers that measure at a frequency of 1 kHz[1]. More recently, a new impedance spectrometer has been designed specifically for non-invasive measurements on glass vials used as the product containers for the freeze-drying of injectable drugs. This development has enabled impedance spectroscopy to be implemented as an in-line process analaytical technology (PAT) for product and process development purposes.

Materials and Methods

A bespoke 5 channel impedance analyser was used to measure the 10 Hz and 1 MHz impedance spectrum of a single glass vial (the standard container for the drug solution) which has been modified by conforming two copper-foil electrodes to the outside of the vial, on opposite sides to one another, and soldering two thin/flexible coaxial wires (50 cm in length) that have MCX type coaxial connectors. The cables on the vial are connected to the impedance analyser (located outside the freeze-dryer) via a junction box (located inside the freeze-dryer) via one of the pre-existing ports on the dryer on which is connected a pass-through that has vacuum-tight electrical connections. In separate experiments, water and various solutions of sucrose, lactose and mannitol were either freeze-thawed or freeze-dried inside the impedance measurement vial and, in each experiment, spectra were recorded every 2 min while tracking the temperature using a thermocouple placed in a nearest neighbor vial.

Results & Discussion

Illustrated here (Fig. 1) are those impedance spectra recorded during the re-heating phase of an annealing cycle of frozen water. A number of features of these plots are then extracted for the purpose of demonstrating the applications for this non-invasive impedance technique.

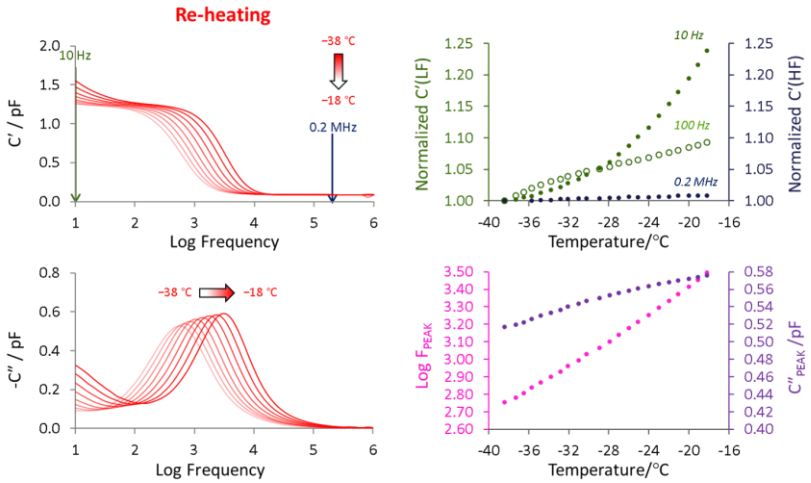


Fig. 1 *(LEFT) Real and imaginary capacitance spectra; (RIGHT) Extracted parameters: TOP real part capacitance C' at selected frequencies, BOTTOM peak frequency (F_{peak}) and peak amplitude (C"peak)*

The conference talk will discuss how: (1) the low frequency region of the real part capacitance spectrum (e.g. 100 Hz) is sensitive to the nucleation temperature and the thermal equilibration time of the vial with the shelf; (2) higher frequencies (e.g. 200 kHz) enables the determination of structural changes inthe frozen solution such as the end of crystallization of water and eutectic solutes, the glass transition and eutectic melting.

Conclusions

This work has demonstrated the importance of the measurement frequency and the choice of real or imaginary capacitance in the determination of critical process parameters.

References

[1] K.R. Ward, P. Matejtschuk, Freeze Drying/Lyophilization of Pharmaceutical and Biological Products, 3 Edn. Informa Healthcare, London, pp. 111-135

Active Freeze Drying – A new technology for contained and aseptic lyophilization

Dekens, A.K.
Hosokawa Micron B.V.
*E-mail of the corresponding author: B.Dekens@hmbv.hosokawa.com

Abstract

A faster and less labor intensive freeze drying process and the possibility of producing loose and free-flowing powder at low temperatures and low pressures, all in one vessel.

Even this short introduction illustrates that the newly developed Active Freeze Drying technology can be seen as a big step in the world of freeze drying and powder technology.

Early experiments with a mixed drying chamber operated at low temperatures and low pressures showed that it is possible to obtain an efficient freeze drying process resulting in a lump-free, free-flowing product. Active Freeze Dryers exhibit a much better heat transfer rate due to the continuous motion of the product. This shortens the drying process because the dried product no longer hinders the sublimation. Finally the freeze drying process is simplified because all process steps can be done in a single processing unit instead of handling trays filled with product between freezing units, drying chambers and crushers. This results in easy handling of the product especially when compared to the traditional tray dryer equipment. Sterile operation is being made easy as the complete process cycle is done in a single contained vessel.

Specificity of vaccine freeze-drying

Mayeresse Yves. [a*]
[a] Manufacturing science and Technology, GSK
*E-mail of the corresponding author: yves.mayeresse@gsk.com

Abstract

The aim of this presentation is to highlight the difference between freeze-drying of classical pharmaceuticals products and vaccine . The measles virus will be used as an example of typical live virus vaccine. In a first step, the classification of virus in term of size, properties and attenuation process will be explained. Then, the typical production process of this virus and the analytical potency test will be highlighted. This will introduce the formulation complexity and the resulting low glass transition temperature for those formulations. An explanation of each contributing element of the formulation will be given. The robustness of freeze-drying cycle in regard of low glass transition will be studied. The implication in term of process validation and equipment specificity will be analyzed. Finally , the amount of vaccine to be produced and the cold chain requirement will highlight the challenge of vaccine industry to supply those live saving vaccines worldwide

Nano Potent Particles API Freeze Drying Using Organic solvent

Shapira Y.*
Lyophilization Expert Teva Pharmaceutical Industries

*E-mail of the corresponding author: Yossi. Shapira@teva.co.il

Abstract

Different approach to freeze drying engineering and process were used in order to supply large quantities of Nano - potent API. Freezing was disconnected from the huge lyophilizer. The traditional tray freeze dryer was increased to 16 m deep, 3x alternatively functioning condensers were designed, fully automatic loading & unloading, CIP, trays bulk filling, trays washing and storage systems were built. High freezing temperature organic volatile solvent is used. Its high quantity, its high freezing temperature presented energetic, operational, and safety advantages and dis advantages which were identified and solved. Bulk API was pelletized and stored in frozen manner. Pellets were automatically filled into large lyophilizers trays, Process is fully automatic. 2 persons: operation and control the process. The combination of high MP solvent, large product quantities, the huge lyophilization plant, determined process different operation. Safety analysis along the filling, loading, freeze drying, unloading, cleaning was done and validated.

Introduction

The amended API was planned for Oral Solid Drug purpose. Low PSD was expected In order to achieve its best efficient pharmaceutical performance, uniform dispersion in the tablet, optimal solubility, .

The required API is a potent compound so micronization, following its dispensing, formulation granulation became dangerous to the operators and to the environment.

A new approach was employed while dedicating a special API plant for the formulation and granulation.

1. Binder was used to trap the API micro particles.
2. Both of them were dissolved in an high melting point solvent.
3. After lyophilization a very porous aqueous soluble granulate was created.

Materials

Crystalline API. Binding material. Mentol , melting point (36- 38°C) solvent.

Method

Ingredients were added into a reactor. Its jacket was heated under nitrogen atmosphere while constant agitating. Full ingredients dissolution in the melted menthol was achieved. The hot solution was dripped into LiN2 forming immediate frozen pellets – (pelletizing).

Discussion

Water (melting temp 0°C) is the most commonly used solvent for lyophilized products. In common used process water solution is frozen even after super cooled (at -15 - -22°C). Following further exposed to low pressure for sublimation. The common Freon lyophilizer's condenser temperature varies from -75°C to -80°C under minimal load, but reaching to -50°C - -40°C at its most efficient

operative point. Thus the temperature difference between the condenser coils temperature to the solvent freezing temperature in water case is about 50°C.

Using solvent having lower freezing point (IE: -46°C, as acetonitrile) will enable smaller temperature difference, less efficient condensation of solvent vapor. Vapor will not freeze on condenser coil surfaces, its liquified droplets will accumulate on condenser's vessel bottom, where high solvent vapor pressure will be achieved back migrating to the product. Menthol has a freezing temperature solvent (+36 to +38°C) was selected, enabling using higher condensation temperature while condensing menthol vapor to rigid layer. Alternative condensers operation removes the condensed volatile menthol layer until full elimination in the product.

Lyophilization in bulk of anaerobic bacteria, specificity of the process

Sophie Declomesnil
4DPharma plc, United Kingdom
*E-mail of the corresponding author: sophie.declomesnil@4dpharmaplc.com

Abstract

The LBP (Live biotherapeutic product) is a new class of medicines. The products are strains of gut commensal bacteria which have been originally isolated from healthy human donors. Each strain is selected for its functionality in a similar way to how a small-molecule or biologic is selected to perform a specific role in combating a disease.

The products are freeze dried, encapsulated and administered orally to the patient. Regarding the freeze drying aspect, the process has to be adapted to maintain alive strict anaerobic bacteria:

- bulk process and not vial
- special container instead of tray
- time of the cycle
- heat transfer to be review
- not only residual moisture but also water activity to be considered.

Freeze dryer recipe process transfer: challenge in heat transfer coefficient between freeze dryers and way-out

Carmisciano, S. [a*]; Buzzi, D.[a]; Apat, A.[a]; Vimpolsek, M.[a] ; Kocar, V.[a] ; Pariš, A.[a]; Stark, C.[b]

[a] Lek d.d., Global Drug Development, Technical Research & Development, Technical Development Biologics Mengeš

[b] Novartis Pharma AG, Global Drug Development, Technical Research & Development, Technical Development Biologics Basel

*E-mail of the corresponding author: salvatore.carmisciano@novartis.com

Abstract

Differences in construction between freeze dryers play a significant role in the lyo recipe development. In particular, this applies to the scale-up from development to commercial freeze dryers or at transfer from one commercial freeze-dryers to another. Said differences fundamentally modify the equipment operating characteristics and with it the temperature product profile during the lyophilization cycle.

The case study elaborates on a product (lyophilized powder, sucrose formulation) with an already developed lyophilization recipe. Obvious differences in the lyophilizer setups between the transferring and receiving site resulted in significantly modified temperature product profiles during the lyophilization cycle (especially during the primary drying phase) when applying the same lyophilization recipe. In some cases the drug product could not even be obtained with the desired drug product quality due to cake collapse.

By measuring the heat transfer coefficient (Kv) (gravimetric measurement) while mimicking the conditions at the receiving and transferring freeze dryer a substantial difference in the values of Kv has been found. Taking into account this difference, and by measuring the Kv in the receiving commercial site freeze dryer a mathematical modelling approach has been applied to re-adjust the original lyophilization recipe with the aim of keeping the same temperature product profile and the same product quality between the receiving and transferring site.

Tech transfer and validation with the re-adjusted lyophilization recipe was executed right first time without any issue/delay.

The mathematical modelling approach was confirmed as a useful tool supporting lyo cycle development, deeper process understanding and successful tech transfer.

Quantification of the Physical Robustness of Lyophilized Biotherapeutics

Ward, K.*; Robinson, J.
Biopharma Process Systems, Biopharma House, Winnall Valley Road, Winchester SO23 0LD, UK
*E-mail of the corresponding author: kward@biopharma.co.uk

Abstract

Lyophilization provides the opportunity to produce material with low moisture content and high surface area, but freeze-dried materials can undergo physical breakup during transportation and handling. To date, little or no quantitative data have been published relating to the mechanical properties of lyophiles, perhaps due in part to the fact that rheometers and tensile testing devices on the market are generally designed for application to higher density and less flexible materials than lyophiles, and with some degree of sample preparation required. Recent studies have demonstrated that acceptable appearance immediately after freeze-drying - typically manifested by lack of obvious defects such as macroscopic collapse - does not necessarily denote acceptable mechanical properties such as cohesiveness or strength [1, 2]. Furthermore, it is apparent that active ingredients and excipients possess certain inherent mechanical properties but that varying process conditions also have an influence. In order to understand such input and output variables in greater detail, we constructed a modified mechanical testing device capable of measuring Young's Modulus (E) and strength (σmax) of a lyophilized cake in situ in a vial. The resulting instrument demonstrated sufficient sensitivity to detect significant differences in E and σmax in samples that would otherwise appear near-identical.

Introduction

For many decades, lyophilization (freeze-drying) has been the method of choice for the stabilization of labile drugs, biotherapeutics and vaccines, as it is considered a gentle process compared with traditional drying methods (such as spray drying, oven drying, fluidized bed drying). Lyophilization also provides an opportunity to produce material with low moisture content and high surface area, allowing the possibility of long-term stability at ambient temperatures and rapid reconstitution prior to use. However, due to their low density, lyophilized products can undergo physical breakup during transportation and handling, sometimes becoming fragmented and powdery, which in turn can have an impact on end user perception of product quality as well as the time taken for reconstitution.

There are a number of standard quantitative tests for critical quality attributes (CQAs) such as residual moisture (or solvent) levels, activity, thermal properties and stability, but while most manufacturers would agree that cosmetic appearance of the product is also important, traditionally, there has been no method to quantify this aspect of the product. Techniques such as scanning electron microscopy (SEM) can provide an idea of morphology and the microscopic level, while gas adsorption methods can go one step further in providing an estimate of specific surface area and mean pore diameter of a lyophile; however, it can legitimately be argued that the sample preparation process itself for either of these measurements can lead to changes to the morphology of the material under test. Rheometers and tensile testing devices on the market are generally designed for application to higher density and less flexible materials than lyophiles, and with some degree of sample preparation required.

Materials and Methods

In this study, we developed a customized testing device comprising a load cell, linear actuator, indenter and modified control software in order to measure the mechanical properties of freeze-dried materials in situ, thus circumventing the need for sampling. Vials of mannitol, sucrose, trehalose, dextran, model proteins and various combinations of these components were lyophilized under different processing conditions (temperature, ramp rate, chamber pressure) and from a series of starting concentrations, to provide a realistic range of samples for testing the sensitivity of the 'MicroPress' instrument and repeatability of the measurements. Sample vials were taken from a wide range of locations within the freeze-dryer so that this parameter could be assessed alongside the formulation and cycle variables.

Results and Conclusions

Data demonstrated that the instrument was sufficiently sensitive to detect statistically significant differences in the mechanical properties of single ingredients when lyophilized individually; that Young's modulus and max stress at failure increased linearly with solute density; that mixing various components to give representative formulations yielded information on the ratios of components that would give the most robust cakes; and that with further optimization of the instrument parameters, differences were even detectible in the mechanical properties of lyophiles in vials taken from different locations across a single lyophilizer shelf. We therefore believe that this method could represent a valuable addition to the existing array of techniques available to provide quantitative measurement of lyophile CQAs.

References

[1] S. McCartney Eng. Doc. thesis "Mechanical Characterisation of Freeze-Dried Biopharmaceuticals", Imperial College London (2014).

[2] S. Hedberg, S. Devi, A. Duralliu and D. Williams, "Mechanical Behavior and Structure of Freeze-Dried Cakes", In: K. Ward and P. Matejtschuk (eds.), Lyophilization of Pharmaceuticals and Biologicals: New Technologies and Approaches, Springer NY (2019).

Design Requirements for Shelf Temperature Control and Testing

Guttzeit M.
Technology Manager Sterile & Aseptic at Bayer AG Pharmaceuticals, Germany
*E-mail of the corresponding author: maik.guttzeit@bayer.com

Abstract

Introduction

Depending of the size of the Lyophilizer there can be very few e.g. vials or more than 100.000 vials on the shelves. Each single vial is valuable for pharmaceuticals companies' business, but furthermore for the patients' health and safety.

A lyophilization process is controlled by time, the vacuum level and the silicone oil temperature (including a lower temperature on the condenser re-sublimation surface). All parameters are pre-defined and set via the control system, assuming that there are stable and uniform conditions within the entire Lyophilizer.

Scope of this presentation is to identify factors for inhomogeneous temperature variance on the shelf and in the chamber, but also to mitigate the influence and define the requirements for proper testing:

- What is the shelf (set) temperature and how to control it within a Lyophilizer?
- Shelf surface temperature: Impact factors for temperature variance and mitigation factors
- What are the design criteria which influence the temperature (pressure) during the process?
- Test set up, acceptance criteria for testing and evaluation of results

Conclusions

Each vial in a lyophilizer has different process conditions. Some of the influencing factors can be mitigated by the set process parameters, others can be actively reduced, but differences cannot be fully avoided. The presentation gives an overview of involved factors and possibilities to reduce the impact.

Surface-driven denaturation of proteins during freeze-drying: An insight into the role of surfactants

Arsiccio, A.[a]; McCarty, J.[b]; Shea, J.-E.[b,c]; Pisano, R.[a*]

[a] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy
[b] Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA, USA
[c] Department of Physics, University of California, Santa Barbara, CA, USA
*E-mail of the corresponding author: roberto.pisano@polito.it

Abstract

Protein-based therapeutics may bind to interfaces during the freeze-drying process, possibly resulting in loss of activity. Here we investigate the mechanism by which surfactant molecules can counteract surface-induced denaturation through a detailed study of the stability of the GB1 peptide at the air-water, ice-water and silica-water interfaces. Using molecular dynamics simulations coupled with metadynamics we show that the amphiphilic nature of surfactants is key to their stabilizing/destabilizing effect, with an orientation-dependent mechanism in which the protein is stabilized when the hydrophilic heads of the surfactant point toward the protein.

Introduction

When proteins bind to interfaces, the resulting changes in protein structure can lead to loss of biological activity. For instance, the air-water, silica (glass)-water and ice-water surfaces are encountered during the freeze-drying process. In the present work, the folding behavior of a model peptide at these surfaces is investigated, in the presence and absence of the surfactant Tween 80. Using a molecular dynamics approach, we show that the extent to which surfactants prevent denaturation is dependent on the nature of the surface.[1]

Materials and Methods

Atomistic molecular dynamics (MD) simulations are coupled with the metadynamics enhanced sampling method [2] to investigate the effect of Tween 80 in preventing surface-induced denaturation of the GB1 peptide (shown in Fig. 1a). A variant of metadynamics called parallel bias metadynamics (PBMetaD) is used.[3]

Results and Discussion

We found that GB1 was destabilized at the air-water and ice-water interfaces, but stabilized at the silica surface. Tween 80 stabilized the protein at the air-water and ice-water surfaces (Fig. 1b), but slightly destabilized the protein at the silica interface. The surfactant molecules bound to the air and silica surface, while they clustered around the protein in the case of ice. An orientation-dependent mechanism of the surfactants was also identified, in which the protein was stabilized when the hydrophilic heads of the surfactant were oriented towards the protein, and destabilized when the hydrophobic tails pointed towards the peptide. The latter orientation stabilized partially unfolded states of the protein, characterized by a larger non-polar surface area. The tails-toward-the-protein configuration is favored in a hydrophilic environment, explaining the mild destabilization observed at the silica-water interface. By contrast, the ice-water surface promotes the heads-toward-the-protein arrangement, that stabilizes the protein native structure. Finally, in the case of the air-water interface, the coating of the interface by the surfactant molecules, and the resulting inhibition of protein adsorption, accounts for the observed stabilization of the protein native structure.

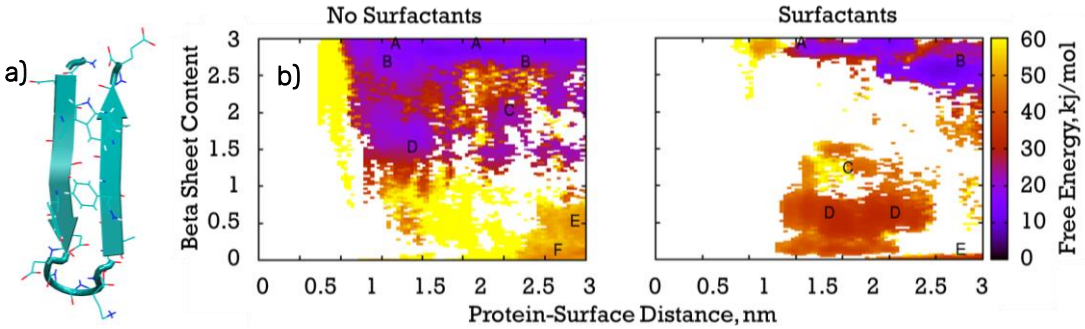


Fig. 1 *a) Cartoon structure of the GB1 hairpin. b) Free energy surface at the ice-water interface, in absence (left) or presence (right) of Tween 80.*

Conclusions

Our simulations suggest that the action of surfactants is complex; the amphiphilic nature of the surfactant, and its relative affinity for the protein and the surface, eventually determines the effect on the protein structure.

References

[1] A. Arsiccio, J. McCarty, R. Pisano, J.-E. Shea, Effect of surfactants on surface-induced denaturation of proteins: Evidence of an orientation-dependent mechanism, J. Phys. Chem. B 122 (2018) 11390-11399.

[2] O. Valsson, P. Tiwary, M. Parrinello, Enhancing important fluctuations: Rare events and metadynamics from a conceptual viewpoint, Annu. Rev. Phys. Chem. 67 (2016) 159-184.

[3] J. Pfaendtner, M. Bonomi, Efficient sampling of high-dimensional free-energy landscapes with parallel bias metadynamics, J. Chem. Theory Comput. 11 (2015), 5062-5067.

Evaluation of cracking and shrinkage of freeze-dried cakes when using a continuous freeze-drying method

Nuytten, G.[a*]; Leys, L.[a], Van Billemont, B.[a], Lammens, J.[b], Van Bockstal, P.[a] , De Beer, T.[a,c], Corver, J.[c]

[a] Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium b Department of Pharmaceutics, Ghent University, Ghent, Belgium

[c] RheaVita, Ghent, Belgium

* E-mail of the corresponding author: Thomas.DeBeer@UGent.be

Abstract

From patients and doctors to regulatory authorities, the appearance of a freeze-dried cake is of importance to a multitude of groups. Therefore, it is of interest to understand the physical origin of defects in freeze-dried cake appearance. For conventional batch-wise freeze-drying, extensive efforts were already undertaken by Ullrich et al. with regards to shrinkage and cracking, which are two common cake defects.[1] In this study, shrinkage and cracking of freeze-dried cakes were investigated while utilizing a continuous freeze- drying approach as developed by Corver et al.[2] It was found that both formulation and freeze-drying process parameters may have an important influence on shrinkage and cracking of the resulting cake structure.

Introduction

Shrinkage and cracking of conventionally freeze-dried cakes were assessed in great detail by Ullrich et al.[1] They were hypothesized to be the result of a release of tension created during the process. Shrinkage was thought to be a release of tension based on viscous flow, and cracking is assumed to occur when viscous flow of the cake is insufficient to release drying tension via shrinkage. Interestingly, this drying tension does not always result in either shrinkage or cracking.

In this study, shrinkage and cracking will be evaluated in a continuous freeze-drying process, as this process has relevant differences compared to conventional freeze-drying, such as process parameters and resulting cake structure.

Materials and Methods

Since Ullrich et al. found that there is an important dependence of shrinkage and cracking on concentration[1], this was evaluated for the continuous freeze-drying method. Also, the influence of secondary drying duration was assessed. For all these experiments, vials were filled with trehalose solutions and freeze-dried according to the continuous method as described by De Meyer et al.[3] Short secondary drying time was 15 minutes, and long secondary drying time was 12 hours. Shrinkage was then measured by taking a picture of the vial bottom and was defined as the area within the outer edge of the cake divided by the inner bottom surface area of the glass vial. Hereafter, cracking was measured by recording a video of a rotating freeze-dried vial. Pixels above a dynamic threshold brightness were considered as part of a crack, and cracking was defined as the amount of “crack pixels” divided by the total amount of pixels.

Results and discussion

In figure 1A, a large amount of cracking is found for the lower concentration of trehalose, and a low amount of cracking is found for the higher concentration. As expected, the opposite is observed for shrinkage.

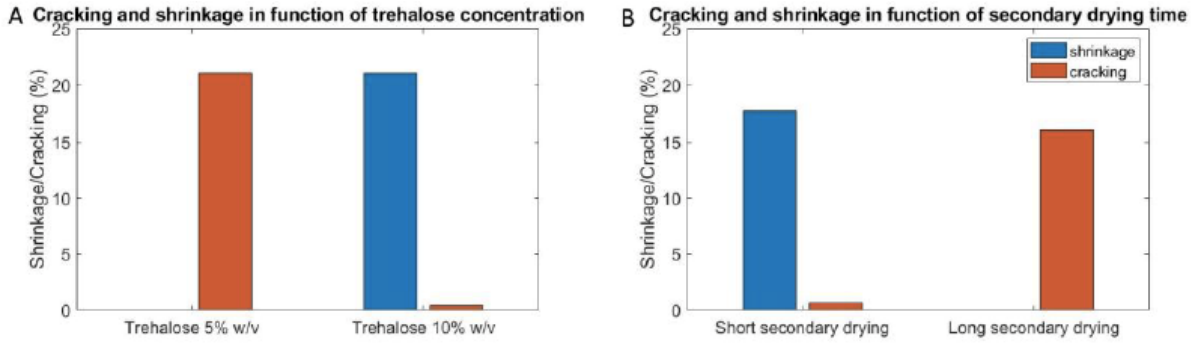


Fig. 1 *A: Shrinkage and cracking expressed as a percentage in function of trehalose concentration B: Shrinkage and cracking expressed as a percentage in function of secondary drying time.*

Fig. 1 A: Shrinkage and cracking expressed as a percentage in function of trehalose concentration B: Shrinkage and cracking expressed as a percentage in function of secondary drying time.

Fig. 1B shows that a long secondary drying phase results in more cracking compared to a short secondary drying phase. A higher residual moisture due to a short secondary drying phase may allow for significant viscous flow through plasticization, but no residual moisture determination was performed for confirmation. Shrinkage may then be a result of the aforementioned viscous flow. Conversely, long secondary drying results in a low residual moisture level, which means, less moisture is available to plasticize the cake structure, and drying tension is more likely to be released via cracking.

Conclusions

Freeze-drying process parameters as well as formulation appear to have a large influence on cracking and shrinkage of freeze-dried cakes in a continuous freeze-drying process. However, additional research is required to elucidate the exact mechanisms behind shrinkage and cracking, In this way, it may be possible to limit or even prevent the two phenomena, resulting in superior cake elegance.

References

[1] Ullrich, S. Measurement of Shrinkage and Cracking in Lyophilized Amorphous Cakes . Part I : Final-Product Assessment. J. Pharm. Sci. 104, 155–164 (2015).

[2] Corver, J. Method and system for freeze-drying injectable compositions, in particular pharmaceutical compositions.(2018).

[3] De Meyer, P. L. Realisation and evaluation of an innovative continuous freeze-drying technology for (bio-)pharmaceutical cylindrical unit doses.

Effect of Vacuum Induced Surface Freezing on the physical and chemical characteristics of freeze-dried strawberry pulp

Alvarez, C.; Orrego, C.E*.

Institute of Biotechnology and Agribusiness, Universidad Nacional de Colombia, Manizales, Colombia
*E-mail of the corresponding author: ceorregoa@unal.edu.co

Abstract

The control of the nucleation step for freeze-drying has been under study in the pharmaceutical field. The aim of this research was to study the effect of vacuum induced surface freezing (VISF) on some physical and chemical quality characteristics of strawberry pulp. The residual moisture content (RMC), CIELab color, Total polyphenol content (TPC) and antioxidant activity (AA) were performed after freeze-drying. The RMC of dried samples varied between 4-6% while their luminosity was reduced when VISF was applied. The TPC and AA were better preserved when the control nucleation technique was applied, compared to the observed for those samples from the experiments where it was not applied. The use of VISF improved the analyzed physical and chemical quality characteristics of the freeze-dried strawberry pulp.

Introduction

Strawberries are an excellent source of antioxidants, which meet an important role in the prevention of chronic and degenerative diseases [1]. Lyophilization is a technique in which most of the nutritional quality, taste, shape and size of dried food products are retained, and, extends the product shelf life. The process begins with the frozen of water, followed by direct sublimation of ice under reduced pressure (primary drying). In the third step (secondary drying), the unfrozen water is released from the sample by desorption and diffusion. Some studies have been published about the effect of different freezing conditions on the final quality characteristics of freeze-dried food [2] and pharmaceutical products[3]. The aim of this research was to study the effect of vacuum induced surface freezing (VISF) on some physical and chemical quality characteristics of freeze-dried strawberry pulp.

Materials and Methods

The four freezing and lyophilization runs of strawberry pulp were conducted in a pilot-scale freeze-drier (LyoBeta 25 by Telstar, Terrassa, Spain) according to table 1. VISF was performed as previously described by Odonne et. al [4]. After freezing, freeze-drying was carried out at 253K until the no-difference between Barathron and Pirani pressure gauges was reached. Then, the shelf temperature was increased and held at 313K for 1 h. The RMC of freeze-dried samples was determined by Karl Fisher titration. Dried pulp color was measured with a portable spectrophotometric colorimeter. Total polyphenols content (TPC) using and antioxidant activity (AA) were measured using Folin-Ciocalteu method and DPPH assay, respectively. All the measurements were done in triplicate.

Table 1. Experimental arrangement

Run	Maltodextrin addition	VISF
1	No	No Applied
2	Up to40 % solids	No Applied
3	No	Applied
4	Up to 40 % solids	Applied

Results

According to table 2 data, the color parameters of the samples with VISF application exhibit superior characteristics to those that were not treated.

Table 2. Dried samples characterization

Run	RMC [%]	Color			TPC [gallic acid mg/ 100g of solids]	AA [Trolox ® µM / 100 g of solids]
		L*	a*	b*		
1	3.78±0.76	65.77±1.88	21.78±0.95	11.58±0.97	1227.31±16.84	2188.94±2.99
2	3.76±0.13	72.87±0.68	14.32±0.99	7.51±0.64	1204.25±0.45	5282.07±1.72
3	4.86±0.28	53.13±1.40	28.16±0.08	15.52±0.34	1320.46±16.11	3656.07±38.02
4	5.82±1.22	69.66±1.12	16.28±0.45	8.61±0.27	1254.93±2.84	4837.23±5.12

The increase in L* and the reduction of a* are undesirable results for a commercial strawberry dehydrated product. Moreover, the VISF treatment also enhance TPC and AA in dried samples, especially those containing MD.

Conclusions

The use of VISF improves significantly the color, total polyphenols content and antioxidant activity of freeze-died strawberry pulp powder.

Acknowledgments

The authors would like to thank the financial support of COLCIENCIAS (Project 727-2015), and FONTAGRO (Project RF16111-RG). C. Alvarez would like to express her gratitude to Professor Roberto Pisano for his support during her stay in DISAT at Politecnico di Torino.

References

[1] A. Petersen, H. Schneider, G. Rau, B. Glasmacher, A new approach for freezing of aqueous solutions under active control of the nucleation temperature., Cryobiology. 53 (2006) 248–57.
[2] F. Giampieri, S. Tulipani, J.M. Alvarez-Suarez, J.L. Quiles, B. Mezzetti, M. Battino, The strawberry: Composition, nutritional quality, and impact on human health, Nutrition. 28 (2012) 9–19.
[3] I. Oddone, R. Pisano, R. Bullich, P. Stewart, Vacuum-induced nucleation as a method for freeze-drying cycle optimization, Ind. Eng. Chem. Res. 53 (2014) 18236–18244.
[4] N.A. Salazar, C. Alvarez, C.E. Orrego, Optimization of freezing parameters for freeze-drying mango (Mangifera indica L.) slices, Dry. Technol. 36 (2018) 192–204.

Application of a novel impedance-based freeze-drying microscope for the product formulation development

Vadesa, A.[a]; Horley, N.[a]; Ward, K.[b]; Dalby, P.[c]; Smith, G. [a*]

[a] DMULyoGroup, School of Pharmacy, De Montfort University, Leicester, UK
[b] Biopharma Process Systems Ltd, Biopharma House, Winchester, UK
[c] Department of Biochemical Engineering, University College London, London, UK
*E-mail of the corresponding author: gsmith02@dmu.ac.uk

Abstract

This work demonstrates the application in freeze-drying of a single analytical instrument (Z-FDM) combining impedance (Z) spectroscopy with freeze-drying microscopy (FDM). The electrical impedance spectrum and visual appearance of a 5% sucrose solution was analysed with an interdigitated micro-electrode array placed on a freeze-drying microscope stage. The critical process parameters of ice growth, sublimation drying rate and end-point were studied using Z-FDM technique in addition to observing the collapse temperature. This early study indicates that by using this combined approach it is possible to obtain further information that can be obtained currently by two separate instruments.

Introduction

Over the past few decades, a number of analytical instruments have been developed for the characterization of product formulations intended for Lyophilization. Freeze-drying microscopy (FDM) is now used routinely to determine the critical temperature at which the product may collapse during primary drying (Tc) whereas a combination of differential thermal analysis (DTA) with electrical impedance analysis has been used to study the critical temperatures of a sample in a frozen state (i.e. the glass transition, ice crystallisation and eutectic melting temperatures) [1]. In this study, a combination of impedance spectroscopy with freeze-drying microscopy has been reported for the first time.

Materials and Methods

A gold interdigitated electrode IDE (Micrux) was integrated into the stage of Lyostat 5 freeze dying microscope using fine copper cable on the inside and a RG316 coaxial cabel on the outside which connects the electrode array to an ISX3-mini impedance analyser (Sciospec). A 5% w/v solution of sucrose (Sigma-Aldrich) was prepared using ultrapure water and a sample of 0.5 µL was placed on the IDE. A glass cover slip was placed on the top of the liquid and then spectra were recorded during freeze-drying.

Results & Conclusions

Fig.1 shows how critical parameters can be recorded by the monitoring the real and imaginary capacitance of the IDE at 1.6 kHz: (A) nucleation onset; (B) solidification endpoint, (B-C) loss of excess heat; (C-D) thermal equilibrium, and the position of the drying front (e.g. E & F) from which the drying rate was determined.

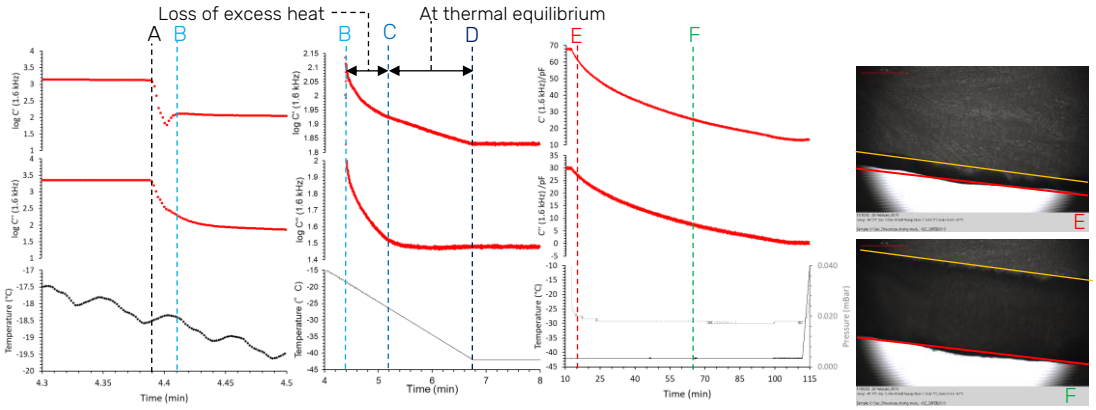


Fig. 1 *Real (C') and imaginary (C") capacitance (at 1.6 kHz) of an IDE on a freeze-drying microscope stage, with a solution of 5% sucrose during freezing and drying*

In a separate experiment, the onset of collapse for the 5% sucrose solution was recorded at -34.5°C by a change in the gradient of the real part capacitance at 1 kHz (Fig.2, see point B).

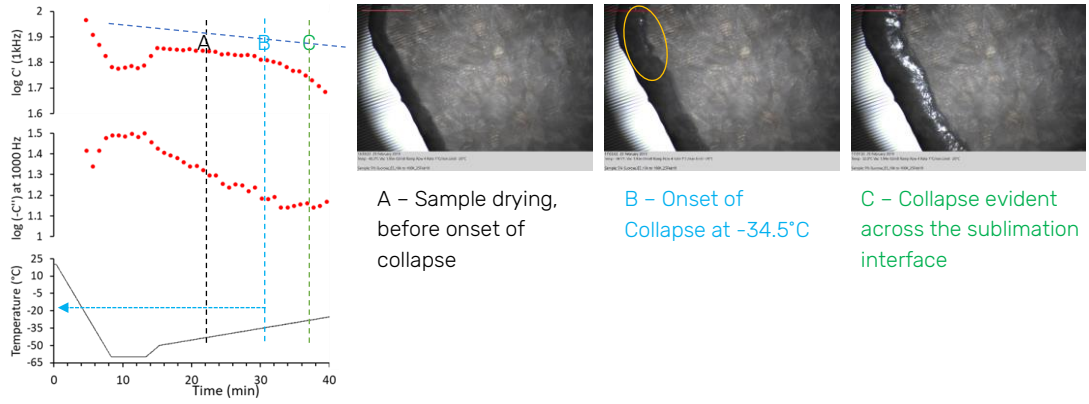


Fig. 2 *The real part capacitance profile shows a discontuinuity with time at the onset of collapse*

References

[1] K.M. Ward, P. Matejtschuk, The use of microscopy, thermal analysis, and impedance measurements to establish critical formulation parameters for freeze-drying cycle development, In: L. Rey, J.C. May, (3rd Eds.), Freeze Drying / Lyophilization of Pharmaceutical & Biological Products,Informa Health care, London, 2010, pp 111-135.

Formulation strategies and modelling approaches to successfully develop lyophilised high concentration protein formulations

Gervasi, V. a,b; Cullen, S. b; McCoy, T. c; Vucen, S. a; Crean A. a

[a]Synthesis and Solid State Pharmaceutical Centre (SSPC), School of Pharmacy, University College Cork, Cork, Ireland

[b]Manufacturing Science Department, Sanofi, Waterford, Ireland

[c]Global Biologics Drug Product Development (BioDPD), Sanofi R&D, Framingham, MA, USA

*E-mail of the corresponding author: valeria-gervasi@hotmail.it Valeria.Gervasi@sanofi.com

Abstract

The current trend in lyophilisation is to formulate antibodies in high concentrations in order to deliver the large therapeutic dose in small volumes subcutaneously. However, several challenges can be encountered when formulating proteins at high concentrations. The aim of this study was to conduct a rational selection of the excipients and a lyophilisation process optimisation, to obtain a stable lyophilised high concentration protein formulation. Formulations selected presented a protein concentration of 100 mg/ml and maximised critical temperatures. The lyophilisation process optimisation was successfully conducted with the aid of a lyomodelling tool. A significant reduction in primary drying time (45%, -11h) was observed. Additionally, an aggressive lyocycle (Tshelf=35 °C) provided un-collapsed products with similar Tg and residual moisture to the products lyophilised at conservative conditions. The possibility to reduce the time of manufacturing for high concentration protein formulations and to store them at ambient conditions without impacting stability, makes their development more convenient for pharmaceutical companies.

Introduction

Antibodies form the main class of therapeutic proteins in commercial products and they are intended for the treatment of several chronic diseases [1]. The current trend is to formulate antibodies in high concentrations in order to deliver the large therapeutic dose in small volumes (1-2ml) subcutaneously. Thus, patients would be enabled to self-administer their medications at home rather than in a hospital setting through IV infusions. However, several challenges can be encountered when formulating proteins at high concentrations. In the liquid state, high concentration protein formulations tend to aggregate and have high viscosity; in the lyophilised state they can have prolonged reconstitution time, high total solute concentrations and cake resistance, that could slow down the drying process and increase the overall time and costs of manufacturing [2]. The aim of this study was to conduct a rational selection of the excipients (type and ratio) and to optimise a lyophilisation process with the aid of QdB approaches in order to obtain a stable lyophilised high concentration protein formulation.

Materials and Methods

BSA and IgG were used as proteins. Arg/Arg-HCl and Sucrose were selected as excipients based on an overview of liquid and lyophilised products approved in the European Union since 1995 [1]. The ratio of protein: excipients relative to the total solute concentration was determined with the aid of DOE. The DOE was also used to generate an empirical model for critical temperatures optimisation and prediction, and further verified with experimental data. The lyophilisation process optimisation was conducted using a lyomodelling tool [3]. Lyocycles were conducted at conservative and

aggressive conditions. Critical quality attributes of the optimised lyophilised products (Tg, residual moisture, product appearance, reconstitution time and biophysical stability) were assessed using a range of techniques and monitoring the products over a 6 months period.

Results

The empirical model generated from the DOE provided formulations with maximised critical temperatures and containing 100 mg/ml of protein. The model was also able to accurately predict both critical temperatures (Tg' and Tc). The lyophilisation process was successfully optimised. A significant reduction in primary drying time (45%, -11h) was observed when shelf temperature (Ts) was increased from -25°C to -15 °C. Additionally, an aggressive lyocycle conducted at a Tshelf of 35 °C provided a short primary drying time (4.75h). The lyophilised products obtained by this lyocycle did not show any collapses and had similar Tg and residual moisture of the products lyophilised at conservative conditions (95 °C and 0.16% respectively). The high Tg makes these products good candidates for the elimination of cold chain during storage. Formulations selected were initially in the amorphous state and observed to maintain their physical solid state after 6 months storage and exposure to drastic humidity conditions. The high concentration of the protein was capable of inhibiting excipients recrystallization, providing a stable amorphous product. The lyophilised formulations were stable over 6 months in presence and absence of cold chain and did not show any presence of irreversible aggregation (SE-HPLC). The challenge of the high reconstitution time for high concentration protein formulations was overcome by lyophilising the product at a low fill volume (1.1 ml= 5 min). Finally, the addition of arg/arg-HCl to the lyophilised formulations showed some advantages reducing the specific surface area and improving the product visual appearance. Furthermore, a reduction of protein aggregation was observed for the liquid product containing arg/arg-HCl once exposed to high intensity light to induce photodegradation.

Conclusions

This study provides a rational approach, insights and strategies that can be applied to overcome some of the challenges encountered during formulation and manufacturing of lyophilised high concentration protein formulations. The use of QbD approaches aid to obtain stable lyophilised high concentration antibody formulations. The possibility to easily reduce the manufacturing time and to store these formulations at ambient conditions makes their development time and costs convenient for pharmaceutical companies.

References

[1] Gervasi V, Dall Agnol R, Cullen S, McCoy T, Vucen S, Crean A. Parenteral protein formulations: An overview of approved products within the European Union. Eur. J. Pharm. Biopharm. 2018;131:8-24.
[2] Kasper JC, Winter G, Friess W. Recent advances and further challenges in lyophilization. Eur. J. Pharm. Biopharm. 2013;85(2):162-9.
[3] Pikal M. Use of laboratory data in freeze drying process design: heat and mass transfer coefficients and the computer simulation of freeze drying. PDA J Pharm Sci Technol. 1985;39(3):115-39.

Abstracts for Posters

List of Abstracts for Posters

Rational design of freeze-drying formulations: A Molecular Dynamics approach

Arsiccio, A.[a]; Pisano, R.[a*]

[a] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy

*E-mail of the corresponding author: roberto.pisano@polito.it

Abstract

Even though the freeze-drying process is often applied to biopharmaceuticals, it may result in protein unfolding or aggregation, and suitable excipients should therefore be added to avoid loss of activity. However, the choice of the formulation is, at present, mostly empirical, due to a lack of knowledge about the phenomena involved. Here, molecular dynamics is used to understand the molecular mechanisms at the basis of protein stabilization, and guide the choice of suitable excipients.

Introduction

Freeze-drying is the most common technique for the storage of protein-based drugs in the solid state. However, several stresses could arise during the process, that may result in loss of therapeutic activity. Some excipients should therefore be added to stabilize the protein and prevent its denaturation.[1] Here we propose an approach, based on molecular dynamics (MD) simulations, capable of identifying the formulation that mostly preserves the biological activity of proteins to be freeze dried. We will show how this in silico approach could be used to clarify the mechanisms of protein stabilization by typical pharmaceutical excipients, including sugars, polyols and amino acids.

Materials and Methods

Molecular dynamics is used to study the interaction between some model proteins, namely, human growth hormon (hGH) and lactate dehydrogenase (LDH) (Fig. 1a), and typical pharmaceutical excipients. A typical simulation box is shown in Fig. 1b. During freezing, the preferential exclusion of excipient molecules from the protein surface [2], which should stabilize the protein against unfolding, can be quantified from the MD trajectory. The strength of the hydrogen-bonding network formed during drying can also be computed, making it possible to evaluate the degree of protein stabilization in the solid state.[3]

Results and Discussion

We observed that the mechanisms of protein stabilization change significantly during the freeze-drying process, mostly as a result of the increase in excipient concentration. Preferential exclusion prevails during freezing, while MD simulations suggest that the mechanisms of lyoprotection should be related to the formation of a dense, compact hydrogen bonding network between the formulation components. It was also observed that not all the excipients are equally effective; for instance, the disaccharides should be better than polyols and amino acids both during freezing and in the dried state (see Fig. 1c). According to our simulations, small differences may exist among the disaccharides, as well, with sucrose and lactose being extremely good cryoprotectants, and trehalose and cellobiose being slightly better for lyoprotection. Some molecular properties seemed to correlate with the protective effect of stabilizers. The higher the molecular volume was, the more the osmolyte was excluded from the protein surface. By contrast, a high hydrogen bonding propensity was linked to the efficiency as a lyoprotectant.

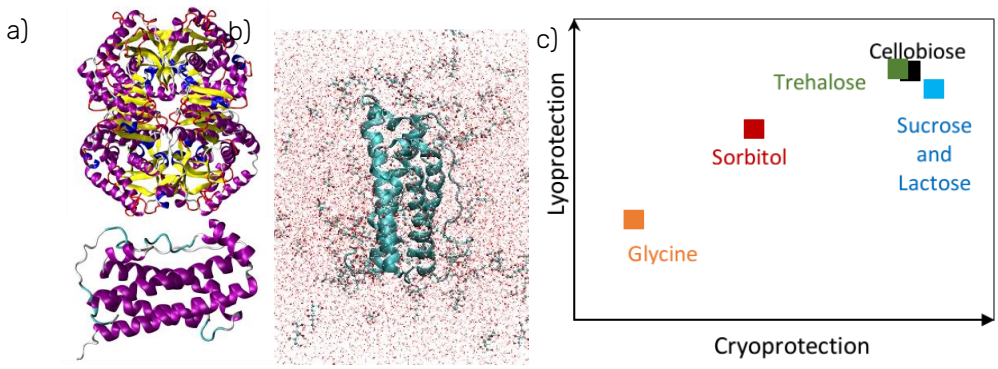


Fig. 1 *a) Cartoon structures of LDH (top) and hGH (bottom). b) Example of an MD simulation box. c) Graphic representation of the efficiency of common pharmaceutical excipients as lyo- (y-axis) or cryo- (x-axis) protectants.*

Conclusions

Our study shows that MD simulations allow the identification of the molecular properties at the basis of protein stabilization, thus simplifying the choice of the formulation. This approach could be combined with experimental techniques to improve the stability of freeze-dried biopharmaceuticals.

References

[1] W. Wang, Lyophilization and development of solid protein pharmaceuticals, Int. J. Pharm. 203 (2000) 1-60.
[2] T. Arakawa, S.N. Timasheff, Stabilization of protein structure by sugars, Biochemistry 21 (1982) 6536-6544.
[3] A. Arsiccio, R. Pisano, Water entrapment and structure ordering as protection mechanisms for protein structural preservation, J. Chem. Phys. 148 (2018) 055102.

Improved Arginine Hydrochloride Formulations for Antibody Lyophilisates

Seifert, I.*; Friess, W.
Ludwig-Maximilians-Universität München, Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Munich, Germany
*E-mail of the corresponding author: Ivonne.seifert@cup.uni-muenchen.de

Abstract

Utilising the advantages of arginine hydrochloride (ArgHCl) in liquid formulations also in freeze dried products, can be problematic due to its low Tg' of -46°C. The objective of this study was to evaluate additional excipients to find an ArgHCl formulation, which provides both pharmaceutically elegant lyophilised products and sufficient protein stability. Pure ArgHCl collapses during freeze drying but shows good protein stability. Adding mannitol results in the best cake appearance but lowest protein stability. A high antibody concentration (50 mg/ml) results in elegant cake appearance but reduced protein stability. Phenylalanine is able to form cakes with minor dents and shows good protein stability. Sucrose renders lyophilisates with minor dents, a rather low Tg and increased particle formation.

Introduction

Various amino acids are known as bulking agents and cryoprotectants for lyophilisation, typically in combination with sugars like sucrose [1]. L-Arginine (Arg) is widely used in liquid for protein solubilisation and stabilisation [2]. In freeze drying, ArgHCl is known for a low T_g' of -46°C [3]. Therefore, collapse during lyophilisation instead of a pharmaceutically elegant cake can result. ArgHCl provides still sufficient protein stability. The combination with different excipients could improve cake appearance: sucrose (Suc) as standard stabiliser [4], mannitol (Man) as classical bulking agent, phenylalanine (Phe) as low concentration bulking agent [5], and a high protein concentration (50 mg/ml) to increase collapse temperature [6].

Materials and Methods

2 mg/ml antibody (mAb) and 50 mg/ml mAb for the high concentration were formulated with ArgHCl at pH 6.0 with about 5% total solid content. Suc, Man, and Phe were added in the following ratios: Suc:Arg 3.5:2, Man:Arg 4:1, and Phe 0.5:4.5 and 1:4.

1.5 ml were freeze-dried (FTS LyoStar III, SP Scientific) in 2R vials: freezing to -50°C at 0.5 K/min; primary drying at 0.06 mbar at -20°C shelf temperature (-32°C product temperature) with end point determination by comparative pressure measurement; secondary drying at 0.06 mbar and 40°C shelf temperature for 8.3 h or 12 h for pure ArgHCl formulation. Lyophilised samples were analysed for visual appearance, residual moisture (RM) by Karl Fischer analysis (AQUA 40.0, Analytik Jena AG), crystallinity by XRD analysis (3000TT, Seifert), Tg by DSC (DSC 821e, Mettler Toledo), and mAb aggregation by size exclusion chromatography (BEH SEC 200Å, Waters; 50 mM Phos 400 mM ClO4- pH 6.0, 0.4 ml/min, UV 280 nm; Agilent 1100, Agilent Technologies) and sub-visible particles analysis (SVSS-C35, PAMAS).

Results

Pure ArgHCl lyophilisates showed major collapse, but a high Tg value, low particle counts and no increase in higher molecular weight species (HMWS) compared to the solution before freeze drying (Figure 1). SucArgHCl lyophilisates showed minor dents and a low Tg value with high RM. The sub-

visible particle count was increased, whereas HMWS did not form. Adding Man resulted in an elegant cake appearance due to δ-Man crystallisation. Protein stability decreased as indicated by the increase in particle count and HMWS. Both Phe concentrations provided good cake appearance, high Tg values, and good protein stability with lower particle counts for Phe:Arg 1:4. 50 mg/ml mAb rendered elegant cakes and an acceptable Tg, but already decreased protein stability with increased particle counts and HMWS.

Table 2. Cake appearance, T_g and RM results, particle count, and HMWS of ArgHCl lyophilisates. n.d.: not detected.

	Cake appearance	T_g [°C]	RM [%]	Particle count ≥1 µm/ml	HMWS [%]
ArgHCl	collapse	74.4 ± 0.01	0.58 ± 0.07	1,446 ± 428	1.72 ± 0.04
SucArgHCl	minor dents	54.7 ± 2.4	1.30 ± 0.03	10,559 ± 1,281	1.77 ± 0.04
ManArgHCl	elegant	n.d.	0.45 ± 0.09	27,774 ± 3,892	4.11 ± 0.36
$Phe_{0.5}Arg_{4.5}HCl$	minor dents	72.8 ± 5.5	0.73 ± 0.10	6,125 ± 949	1.69 ± 0.10
Phe_1Arg_4HCl	minor dents	68.6 ± 0.9	0.94 ± 0.05	1,055 ± 426	1.68 ± 0.04
$ArgHCl_{(50\ mg/ml)}$	elegant	64.0 ± 0.01	0.60 ± 0.23	7,590 ± 4,864	2.94 ± 0.11

Discussion and Conclusion

Pure ArgHCl based mAb formulations tend to collapse upon freeze drying. Good cake appearance was achieved with the bulking agent Man, but protein stability was insufficient. The classical amorphous stabiliser Suc provided good protein stability, but cakes with minor dents and a low T_g. 50 mg/ml mAb showed an elegant cake appearance, but already decreased protein stability. Phe showed only minor dents in cake appearance, high T_g values and good protein stability. Thereby Phe:Arg 1:4 had the lowest particle count. A six months storage stability study at 40°C is ongoing.

References

[1] M. Mattern, et al., Formulation of Proteins in Vacuum-Dried Glasses. II. Process and Storage Stability in Sugar-Free Amino Acid Systems, Pharm. Dev. Technol., 4 (1999) 199-208.
[2] T. Arakawa, et al., Biotechnology applications of amino acids in protein purification and formulations, Amino Acids, 33 (2007) 587-605.
[3] K.I. Izutsu, et al., Amorphous-Amorphous Phase Separation of Freeze-Concentrated Protein and Amino Acid Excipients for Lyophilized Formulations, Chem. Pharm. Bull., 64 (2016) 1674-1680.
[4] P. Stärtzel, Arginine as an Excipient for Protein Freeze-Drying: A Mini Review, J. Pharm. Sci., 107 (2018) 960-967.
[5] J. Horn, et al., Crystallizing amino acids as bulking agents in freeze-drying, Eur. J. Pharm. Biopharm., 132 (2018) 70-82.
[6] J.D. Colandene, et al., Lyophilization cycle development for a high-concentration monoclonal antibody formulation lacking a crystalline bulking agent, J. Pharm. Sci., 96 (2007) 1598-1608.

Heat transfer in vials separated in a rack system during lyophilization

Sarah Daller1; Rudolf Schroeder1; Wolfgang Friess2

[1] AbbVie Deutschland GmbH & Co. KG, Knollstraße 50, 67061 Ludwigshafen, sarah.daller@abbvie.com
[2] Department of Pharmacy; Pharmaceutical Technology and Biopharmaceutics; Ludwig-Maximilians Universität München, Butenandtstrasse 5, D-81377 Muenchen

Abstract

The importance of small batch fill and finish including lyophilization is on the rise in biopharma industry. A Polyetheretherketon (PEEK) rack system has been engineered for use in new flexible pilot scale processing lines. We could show that the rack leads to a reduced edge-vial-effect and shielding against uncontrolled radiation from surrounding components. Thus the rack is a flexible, robust tool for manufacturing of small batches of biopharmaceuticals, which ensures a more controlled heat transfer rendering higher batch uniformity.

Introduction

Vials separated by racks may be used in new semi-automated production lines with lyophilzation implemented. [1] For cycle development and transfer of this new tool a detailed understanding of the heat transfer and its overall effect on the product is necessary. For this purpose the behavior of the rack during freeze drying and the modes of energy transfer as well as the product temperature (Tp) distribution of vials in the rack were analyzed.

Materials and Methods

Freeze drying was performed in a 1.0 m2 lab freeze dryer (HOF, Lohra). A PEEK rack was used to separate 6R vials (Figure 1) filled with 2.5 ml sucrose / histidine placebo. Temperature mapping of product, rack and shelves was performed. Sublimation rates were determined gravimetrically [2] using water at pressures of 50, 100, 150 and 200 mTorr and at a shelf temperature of 5 °C.

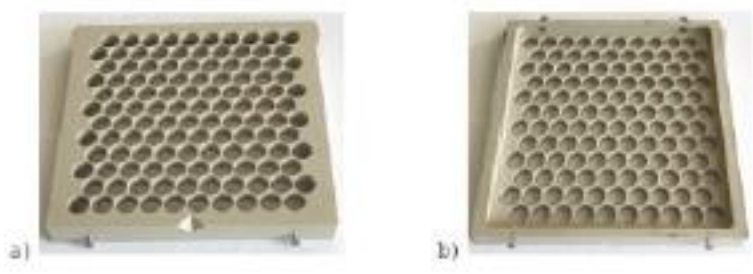


Fig. 1 *Rack, a) top side, b) bottom side*

Results and Discussion

Vials in the rack showed a difference in primary drying time between center and edge vials of about 5 h. Tp at the end of primary drying was 3 °C higher for vials in the rack compared to vials dried in the same arrangement without the rack. Vials without rack showed an earlier beginning of the endpoint of sublimation in primary drying. The tighter range in product temperature between edge and center vials in the rack demonstrated that the rack has a shielding effect, which protects the edge vials against radiation coming from the chamber wall and from upper shelves (Figure 2).

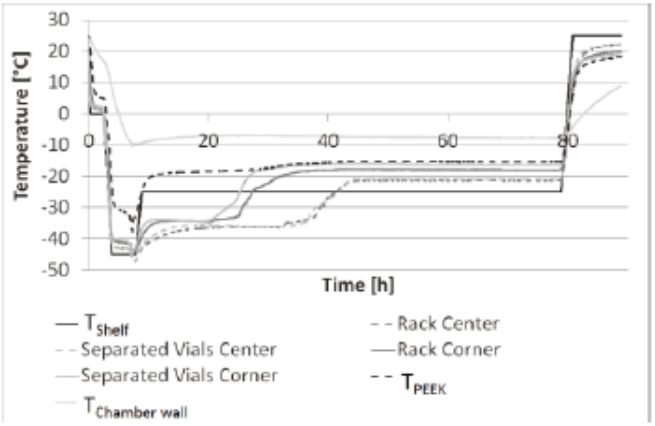


Fig. 2 *Product temperatures of vials separated or in the rack.*

At 50 mTorr sublimation rates in the rack are higher at the corners compared to the centers (Figure 3).

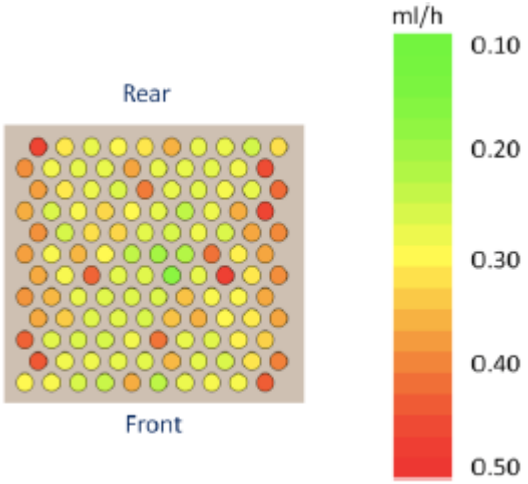


Fig. 3 *Sublimation rates in the rack at 50 mTorr: corners compared to centers.*

Sublimation from vials nested in the rack is dominated by direct contact between vial and shelf, gas conduction and radiation coming from the rack itself, whereas heat transfer via radiation from the chamber wall is minimized.

Disclosures

AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication. Sarah Daller and Rudolf Schroeder are employees of AbbVie and may own AbbVie stock. Wolfgang Friess has served as a consultant to AbbVie. Wolfgang Friess is a Professor at the Ludwig-Maximilians Universität München.

References

[1] Shanley, A., Packaging's Flexible, Patient-Centered Future. BioPharm International, 2017. 30(1): p. 48-50.
[2] Wegiel, L.A., S.J. Ferris, and S.L. Nail, Experimental Aspects of Measuring the Vial Heat Transfer Coefficient in Pharmaceutical Freeze-Drying. AAPS PharmSciTech, 2018. 19(4): p. 1810-1817.

IR thermography for freeze-drying primary stage process monitoring

Harguindeguy, M.[a*]; Colucci, D.[a]; Lietta, E.[a]; Fissore, D.[a]

[a] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy

*E-mail of the corresponding author: maite.harguindeguy@polito.it

Abstract

Freeze-drying monitoring without affecting product safety and dynamics is a recurring issue for process control. This article investigates an infrared (IR) camera specially designed to operate under vacuum freeze-drying conditions as a monitoring device addressing these matters. IR camera temperature profiles were close to the ones obtained by thermocouple measurements. In addition, the estimation of primary drying endpoint, heat transfer coefficient to the product (K_v) and product vapor flux resistance (R_p) obtained from the IR camera temperature measurements were close to the results obtained by the thermocouples. No significant effects were observed on the samples due to the presence of the IR camera in the drying chamber. These results validate the use of the proposed device for vacuum freeze-drying process monitoring.

Introduction

Currently, most freeze-drying monitoring devices require some kind of interaction with the product which may interfere in the process dynamics. Thus, freeze-drying process monitoring without direct interference is a challenging and needful issue. Emteborg *et al.* [1] proposed proposed thermography for process monitoring. However, in that study the camera was placed outside the chamber, measuring vials top temperature only. The aim of this article is to investigate a monitoring device based on thermal imaging for process tracking. This device consists on an infrared camera designed to operate under vacuum freeze-drying conditions, while recording the temperature profile of targeted products.

In a vial process, the camera captures a thermal image of the vial, which should be very close to that of the product inside it. This monitoring should allow estimating the drying stage of the product and thus, the primary drying end point. In addition, with the process temperature profiles, it should be possible to estimate the heat transfer coefficient to the product (K_v) and the product vapor flux resistance (R_p). These parameters can then be used to calculate the heat flux to the product and the sublimation flux and be used for in-line process optimization. [2]

Materials and Methods

All experiments were carried out in a pilot-scale vacuum freeze-drier. To validate the device, process monitoring of primary drying stage was performed simultaneously with T-type thermocouples and the proposed novel thermograpy device. To evaluate the suitability of the infrared camera under different process conditions, freeze-drying was done under different pressure and temperature combinations to lyophilize water-based solutions with different concentrations placed in glass vials.

Results and Discussion

Product temperature profiles measured by the infrared camera were very close to the thermocouple ones. Accordingly, the estimates obtained for the end of primary, Kv and Rp also resulted in similar values for both monitoring systems. These results evidence the validity of the use of thermography for freeze-drying process monitoring. Possible effects from the presence of the infrared camera in the drying chamber were also evaluated. No additional heating was observed in the vials monitored by this device compared to the other vials in the batch.

Conclusions

IR thermography stands as a breakthrough alternative for non-invasive freeze-drying process monitoring. This device allows process control without affecting neither product safety nor its dynamics. In addition, it allows tracking temperature profiles, end of the primary drying stage and estimating K_v and R_p with good precision.

References

[1] H. Emteborg, R. Zeleny, J. Charoud-Got, G. Martos, J. Lüddeke, H. Schellin, K. Teipel, Infrared thermography for monitoring of freeze-drying processes: Instrumental developments and preliminary results, J. Pharm. Sci. 103 (2014) 2088–2097. doi:10.1002/jps.24017.
[2] S.A. Velardi, A.A. Barresi, Development of simplified models for the freeze-drying process and investigation of the optimal operating conditions, Chem. Eng. Res. Des. 86 (2008) 9–22. doi:10.1016/j.cherd.2007.10.007.

New approach for determination and assessment of actual collapse temperature characteristic of freeze-drying process

Jelenko, A.[a*]; Anko, M.[a]; Kočar, V.[b]; Brus, B.[a]

[a]Lek d.d., Global Drug Development, Technical Research & Development, Technical Development Biologics Mengeš, Technical Development Biosimilars, Formulation Development

[b] Lek d.d., Global Drug Development, Technical Research & Development, Technical Development Biologics Mengeš, Biopharmaceutical Process & Product Development, Drug Product Development, Project & Process Sciences

*E-mail of the corresponding author: aljosa.jelenko@novartis.com

Abstract

One of the most critical parameters of an amorphous or partly amorphous formulation intended for freeze-drying within the aggressive cycle is collapse temperature (Tc). By definition, the Tc is maximum allowable product temperature during primary drying phase to avoid collapse and provide acceptable appearance of final product [1].

Since the small change of product temperature can have a significant impact on the duration of primary drying [2], the accurate Tc determination is a key parameter for designing short and efficient lyophilization cycle. The established approach is to determine the Tc using a freeze-drying microscope (FDM), which enables optical observation of a product structure during replication of freeze-drying cycle. However, the freezing and drying dynamics in thin product layer compressed between two glassy surfaces can be different from actual drying process in the vial. Furthermore, as the collapse event is not a single point temperature event when determined by FDM, rather, it's occurrence appear in more or less extended temperature interval defined by onset collapse temperature and full collapse temperature it is also very important to know which value should be taken as critical for the product to visually collapse during process.

We have shown that the primary drying at product temperature above Tc determined with FDM is possible and yields in an elegant and stable product [3]. Furthermore, results of our studies indicate that the difference between FDM determined Tc onset and actual Tc is more pronounced in higher protein concentrated formulations (e.g. above 20 mg/mL). Therefore, actual collapse temperature for specific formulation might be significantly higher.

In order to determine the actual Tc, which is crucial for efficient lyophilization cycle development, the new methodological approach was thoroughly assessed using a small lab freeze-drier equipped with heat-flux sensor and miniature electrical thermocouples. The rationale is that changed drying dynamics characteristic of collapse event reflects upon change in heat flux and product temperature. With the new approach, actual Tc may be determined, which is characteristic of formulation in combination with specific freezing and drying process. Secondly, it is also important to be aware that temperature sensor inserted inside the material may affect the material formation in freezing phase and thus have direct impact on drying characteristics and product temperature. Even more it is also important to know that different types and different sizes of inserted sensors may have impact on temperature readings as they may conduct heat from surrounding and results in falsely higher temperature readings. Therefore, the new approach poses a great potential for further lyophilization process optimization including the validation of temperature reading sensor accuracy, impact of drying above Tc collapse which may result in sub-visible microcollapse with no impact on the stability CQA and the temperature at which actual collapse is observed during lyophilization process.

References

[1] Pikal, M.J., Shah, S. 1990. The collapse temperature in freeze drying: dependence on measurement methodology and rate of water removal from the glassy phase. Int. J. Pharm. 62, 165–186.

[2] Tang X, Pikal MJ. 2004. Design of freeze-drying processes for pharmaceuticals. Practical advice. Pharm Res 21(2):191–200.

[3] Bjelošević M, Seljak KB, Trstenjak U, Logar M, Brus B, Ahlin Grabnar P. 2018. Aggressive conditions during primary drying as a contemporary approach to optimize freeze-drying cycles of biopharmaceuticals. Eur J Pharm Sci. (15)122:292-302.

Application of near-infrared hyperspectral imaging and chemometric calibration to characterize moisture content heterogeneity during freeze-drying of vaccines

Scutellà, B. [a*]; Kapitain, A.[b]; Bourlès, E.[a]

[a] Drug Product Belgium, GSK, Rue de l'Institut 89, 1330 Rixensart, Belgium

[b] CMC Statistical Science, GSK, Rue de l'Institut 89, 1330 Rixensart, Belgium

*E-mail of the corresponding author: bernadette.z.scutella@gsk.com

Abstract

Moisture content is of paramount importance when comes to the quality of freeze-dried vaccines, as it can impact the physical and biological stability of the product. Monitoring the moisture content value and distribution during the freeze-drying cycle and within the final product batch is critical to guarantee the product quality. Usually, time-consuming, destructive methods are employed for the determination of the product moisture content, e.g. the Karl-Fisher titration or the gravimetric balance. The NIR spectroscopy offers a fast and non-destructive alternative to more classical methods, but does not provide informations regarding the spatial distribution of the moisture in the sample. In this work, the NIR hyperspectral imaging method for moisture content determination was developed and successively was employed to monitor moisture content evolution and variability during the secondary drying step of a sugar based vaccine formulation.

Introduction

Freeze-drying (lyophilisation) is a drying process largely employed for stabilization of heat labile food, pharmaceutical and biological products such as vaccines.[1-3] In the freeze- drying process of vaccines, the liquid formulation is firstly filled into glass containers (vials), which are loaded on the shelves of the equipment. Then, the process involves three successive steps: (i) freezing, or solidification of the product, (ii) primary drying, in which the ice crystals are removed by sublimation under vacuum from the poreous interstitial matrix and (iii) secondary drying, in which bound, unfrozen water is removed by desorption. [1-3] The secondary drying step governs the moisture content value and homogeneity in the vaccine unit and lot. Moisture content is considered as one of the most important critical quality attribute of the freeze-dried product as:

- It governs the physical stability of the product. High moisture content value may lead to low value of glass transition temperature for amorphous sugar matrix, which will increase the risk of transition to a rubbery and unstable state at temperature close to ambient temperature;[4,5]
- The active component of the vaccine (e.g., recombinant protein, live attenuated virus) are often sensible to too high/too low levels of moisture content, thus the value of moisture content may significantly impact on the stability in time of the product.[6]

Thus, the monitoring of the value of moisture content and its homogeneity in the vial batch during the freeze-drying cycle can be a powerful tool of quality control. However, the determination of the moisture content can be a challenging process, and it is presently performed by three main methods: (i) Karl-Fisher titration, (ii) thermobalance and (iii) Near- Infrared (NIR) spectroscopy. Karl-Fisher titration is usually considered as the method of reference in pharmaceutical industry for the moisture content determination. However, it is a destructive method, and it requires a long sample preparation, with a limited number of vials analysed per time slot. The thermo-gravimetric balance is used to

perform quick measurement of the moisture content of the product. This measurement is destructive and require a large amount of product (e.g., 2g, equal to about 4 freeze-dried cakes). In contrast, NIR spectroscopy is fast, non-destructive, non-invasive and requires only minimal sample preparation. It is known that classical NIR spectroscopy provides a mean spectrum, and thus an average measurement of the moisture content contained the sample, with the consequent lose of information on the spatial distribution of the components.[7]

Recently, NIR hyperspectral imaging (NIR HSI) has been investigated and imployed to determine the quality of agricultural (especially cereals) and food products, as well as for archeology, paleontology and for medical application.[7] The NIR hyperspectral imaging combines the classical NIR spectroscopy with digital imaging and has the advantage to get information simultaneously on both the spatial distribution of the spectra (and thus of the chemical components) in the sample and on the visual aspect of the sample. Presently, limited use of NIR hyperspectral imaging for the determination of the moisture content in freeze-drying of biologicals and pharmaceuticals has been employed, with no evidence in literature.

The main goal of this work was to develop the NIR HSI method to measure the moisture content and the water activity of sugar-based vaccine formulations. Firstly, chemometric calibration was employed to relate spectral measurements on the product to the moisture content value, and pre-processing of the spectra was perfomed via normalization and derivatives methods before successive multivariate analysis (Principal Component Analysis, PCA) to remove noise. Successively, the NIR HSI was employed to characterize the evolution of the moisture content during the secondary drying step of a case study vaccine by using the sample thief in a pilot scale freeze-dryer, and to assess the moisture content variability in the vial batch at the end of the freeze-drying cycle.

Materials and Methods

Previously freeze-dried vaccine formulations (0.5mL filling volume) were re-equilibrated at a water activity of 12%, 15%, 28% and 35% by using a climatic chamber (Memmert, Schwabach, Germany). The vials were placed without stoppers inside the climatic chamber and maintained at 25°C. At different timepoints (1, 2, 3, 6, 7, 8, 9 and 10 days) stoppers were inserted into the vial necks and the water activity of the vials headspace was checked by using the FSM-water activity (Lighthouse, Charlottesville, VA) to assure that the target value of water activity was reached. The vials were then analysed at the NIR hyperspectral camera and finally at the Karl Fisher.

Fig. 1 *View of NIR hyperspectral camera. The open drawer shows the plate with 50 positions on which the samples needs to be placed for analysis.*

The NIR hyperspectral camera (Indatech, Clapiers, France) is shown in Fig.1. It consisted of a chamber made of stainless steel and provided of a drawer and a plate with 56 positions (holes in Fig.1) for the analysis of 3 ml vials. Before the analysis, the vials were placed onto the selected hole and the drawer was closed. Inside the chamber, a camera Specim FX17 (Specim, Les Ulis, France) collected the hyperspectral data in the near-infrared NIR region (900 to 1700 nm). Each pixel of the digital image recorded contains a NIR spectrum. A halogen lamp is present to illuminate the interior of the chamber. The camera was provided by a pushbroom system, which allows to acquire hyperspectral images from moving samples, and thus for all the lines of vials placed on the plate.

Savitzky-Golay derivative type 1, Standard Normal variate (SNV) and PCA were used to pre- process the hyperspectral images and eliminate unwanted information (such as spectral noise), and successively to relate the spectra to the moisture content measured into the vials via Karl Fischer.

In a second step, 0.5mL of sugar-based vaccine formulation was filled into each of 800 3mL vials (Müller & Müller, Holzminden, Germany) and arranged in a hexagonal configuration on a bottomless tray, sourrounded by a metallic rail. The tray was used to load the vials on the shelf precooled at -52°C. The bottom of the tray was removed immediately after loading, whereas the metallic rail was left to keep the vials in a stable position during the cycle. The REVO Millrock freeze-dryer (Millrock, Kingstone, NY) was used to perform the freeze-drying cycles. The freeze-dryer was equipped with a drying chamber containing 3 shelves, sealed by an acrylic door, and a condenser. On the acrylic door the freeze-dryer presented a sample thief, used to insert the stopper into the vial necks during the cycle without impacting on the value of the operating variables.

During the secondary drying step of the freeze-drying cycle, stoppers were inserted into the vial neck in a number of vials at different time points by using the sample thief. At the end of the cycle, all the vials were stoppered at 825mBar. The moisture content value and variability of the vials stoppered at the different time points was determined by using the NIR hyperspectral imaging and on 3 vials only for each time point using the Karl-Fisher titration. Finally, the desorption kinetics was characterized as classically proposed in literature.[8,9]

Preliminary results: chemometric calibration

Fig. 2 shows an example of the hyperspectral images acquired via the camera of 26 re- equilibrated product cakes containing different moisture content.

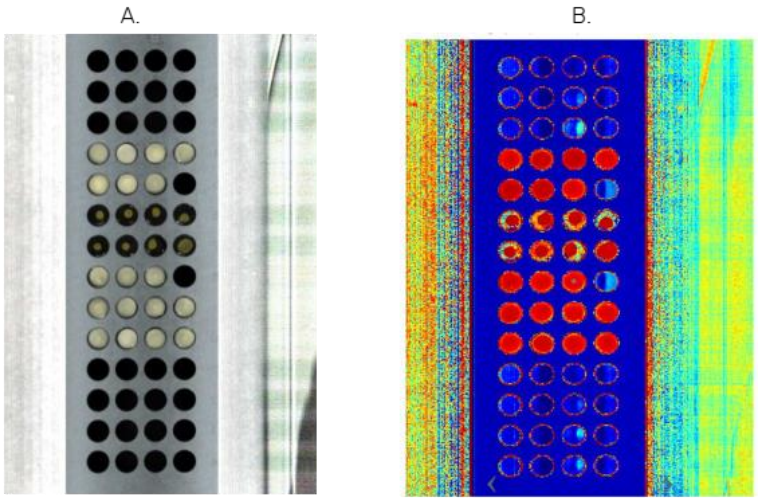


Fig. 2 *Image acquired from the hyperspectral camera and composed by the (A) grey-scale digital image and the (B) hyperspectral NIR image.*

Fig. 2A represents the digital image in grey scale, whereas Fig. 2B represents the 1st principal component scores of hyperspectral NIR image. Pre-processing of spectral data was required to reduce noise and obtain suitable information on moisture content of the product cake. Four kinds of noise were identified: (i) the spectra generated from the unlighted plate, (ii) the spectrum of the plate, (iii) the spectra of the empty holes and (iv) the spectra of the vial glass. A principal component analysis was performed to delete the noise and identify only the cake spectra. Fig. 3 shows the average of the cake spectra (pixels from hyperspectral image) obtained for each analysed vial.

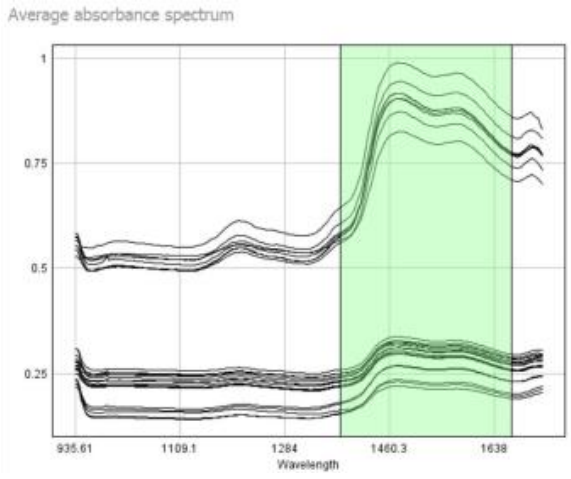


Fig. 3 *Average absorbance spectra vs. wavelenght for the analysed vials. Each speactra is equal the average of all the spectra (pixels in the hyperspectral image) for each vial. Wavelenghts of water region were highlighted in green.*

Finally, partial least square regression (PLS) was used to model the internal relation between the spectra and the Karl Fischer data (Fig. 4), and to find a set of latent variables (components) that maximized the covariance between the calculated and measured moisture content calculated.

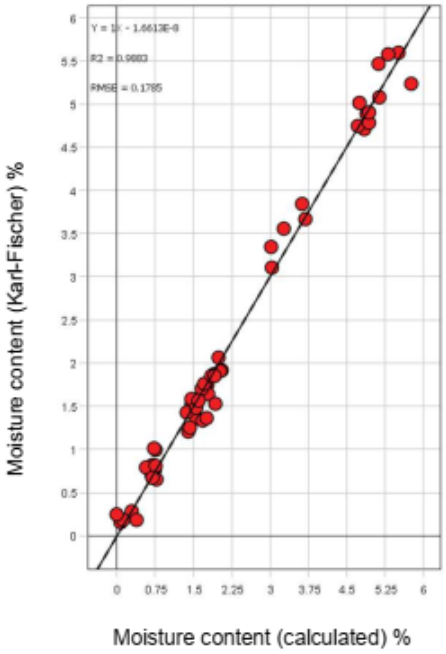


Fig. 4 *Moisture content of samples measured using the Karl-Fisher method vs. Moisture content calculated via the NIR hyperspectral imaging.*

Preliminary conclusions

In this study, previous published works on the importance of moisture content determination of freeze-dried products were reviewed. Successively, a new technology based on NIR hyperspectral imaging was presented. Chemometric calibration and multivariate analysis were used to relate the NIR hyperspectra to the Karl-Fisher analysis. Finally, the NIR hyperspectral imaging was used to characterize the desorption kinetics and develop the secondary drying step of a freeze-drying cycle for a case-study vaccine formulation.

References

[1] T.A. Jennings, Lyophilization: Introduction and Basic Principles, first ed., CRC Press, Englewood, CO, 1999.
[2] C. Ratti, Hot air and freeze-drying of high-value foods: a review, J. Food Eng. 49 (4) (2001) 311–319.
[3] F. Franks, Freeze-drying of bioproducts: putting principles into practice, Eur. J. Pharm. Biopharm. 45(3) (1998) 221-229.
[4] S. Passot, S. Cenard, I. Douania, I. C. Trelea, F. Fonseca, Critical water activity and amorphous state for optimal preservation of lyophilised lactic acid bacteria, Food Chem. 132(4) (2012) 1699-1705.
[5] L. L. Chang, D. Shepherd, J. Sun, X. C. Tang, M. J. Pikal, Effect of sorbitol and residual moisture on the stability of lyophilized antibodies: Implications for the mechanism of protein stabilization in the solid state, J. Pharm. Sci. 94(7) (2005) 1445-1455.
[6] L. J. J. Hansen, R. Daoussi, C. Vervaet, J. P. Remon, T. R. M. De Beer, Freeze-drying of live virus vaccines: a review, Vaccine 33(42) (2015) 5507-5519.
[7] M. Manley, Near-infrared spectroscopy and hyperspectral imaging: non-destructive analysis of

biological materials, Chem. Soc. Rev. 43 (2014) 8200.
[8] I. C. Trelea, S. Passot, F. Fonseca, M. Marin, An interactive tool for the optimization of freeze-drying cycles based on quality criteria, Dry. Technol. 25(5) (2007) 741-751.
[9] R. Pisano, D. Fissore, A. A. Barresi, Quality by design in the secondary drying step of a freeze-drying process, Dry. Technol. 30(11-12) (2012) 1307-1316.

Acknowledgments

The authors would like to thank Olivier Despas, Alain Philippart, Vincent Ronsse (GSK Vaccines) for their help in performing the freeze-drying cycle and the experimental analysis (Karl-Fisher, hyperspectral camera). Furthermore, the authors are grateful to Yves Mayeresse and Benoit Moreau (GSK Vaccines) for reviewing this work.

Conflict of interest

Erwan Bourlès, André Kapitain and Bernadette Scutellà are employees of the GSK group of companies.

Authors contributions

Bernadette Scutellà, André Kapitain and Erwan Bourlès, were involved in the conception and design of the study. Bernadette Scutellà and André Kaptain acquired the data, analysed and interpreted the experimental results. All the authors were involved in drafting the manuscript or revising it critically for important intellectual content. All the authors had full access to the data and approved the manuscript before it was submitted by the corresponding author.

Influence of Vaccuming Strategies on Critical Quality Attributes of Lyophilized Materials

Joshi P., Egan D.
Bernal Research Institute, Pharmaceutical Manufacturing Technology Centre (PMTC), University of Limerick, Ireland

*E-mail of the corresponding author: parth.joshi@ul.ie, david.egan@ul.ie

Abstract

I Drying in a lyophilisation process involves regulating the drying chamber at a certain pressure by utilizing dry Nitrogen gas for extended periods of time. Storing large quantities of nitrogen results in additional logistics and manufacturing capital costs. This research work discusses the comparative influence of utilizing novel, yet industrially feasible chamber pressure regulation strategies on lyophilized Critical Quality Attributes (CQA) such as % residual moisture and visual cake quality of lyophilized milk and trehalose dihydrate - milk suspension in glass vials.

The results indicate no realistic difference in residual moisture cake quality and no change in visual cake quality for the 2 controls. Hence, facilitating alternative vacuum pressure regulation strategies potentially leading to reduced energy footprint and utilisation costs in the industry.

Introduction

Drying is the longest stage in the lyophilisation process with industrial drying times varying from 10 hours to days depending on the nature of the starting molecule and process parameters [1]–[3]. A traditional strategy applied for regulating drying pressure is by purging dry Nitrogen gas in the drying chamber over the span of primary and secondary drying, while the vacuum pump(s) operate at full capacity. This results in high dry nitrogen consumption as well as costs involved with procurement, storage, and transportation.

This study focuses on the drying process of a lyophilisation cycle, where chamber pressure and product temperature are monitored and logged real-time. The aim of this study is to provide alternative techniques for chamber pressure regulation without inducing any practical impact on the CQA of the lyophilized cake. Currently, no literature is available on alternative chamber pressure control methods. Novel, yet easily implemented, vacuum regulation strategies such as automated valve control, and automated pump control as well as manual valve control have been comparatively investigated against the traditional method of purging the drying chamber with nitrogen gas for controlling chamber pressure used by the industry.

Store bought milk and a 15% (w/v) trehalose dihydrate containing milk suspension were used as model substances for this research investigation. 15% trehalose dihydrate suspension was selectively used as an additive model substance based on Ullrich et. Al's observation of a high degree of cake damage during processing [1]. Adding trehalose dihydrate to the milk suspension influenced the quality of the cake for a standard lyophilized process. This acted as good indicator of whether the vacuum strategies affected the CQA of the produced cake.

The influence of fill depths on cake quality with the different chamber pressure control strategies was determined. The results show that the cake quality is independent of the purging of dry nitrogen gas for the 2 model substances involved in this study.

Materials and Methods

Trehalose dihydrate was obtained from Tokyo Chemical Industries and Golden Vale ® whole milk was store-bought. 15% trehalose dihyrdate solution was prepared in deionized water. All experiments were processed in a Severn Science LS40 pilot scale freeze dryer with a batch of 100 vials/ experimental run on a single shelf. In each experimental run, 50 vials had a fill depth of 1 mL and the other 50 vials a fill depth of 3 mL in 10 mL gerreschimer glass vials.

The chamber pressure was monitored at 1000 ms time intervals using an active pirani gauge. The material in 8 vials from each experimental run was logged real time using an in- house developed temperature monitoring system with 0.7 µm thermocouples. All real-time process data was logged using an in-house written LabVIEW® program.

Processed samples were analyzed for their % residual moisture by using a Sartorius MA-37 thermogravimetric moisture detector. Cake quality was visually compared since freezing conditions were unchanged.

Automated valve control involved usage of a solenoid valve controlled using a LabVIEW® program. Similarly, Pump control was accomplished by a LabVIEW program which turned ON-OFF the vacuum pump when a desired pressure setpoint was achieved. Manual valve control was achieved by using a gate valve which was physically regulated. All physical valves were placed on the hose connecting the vacuum pump and the condenser chamber.

Conclusions

This work showcases that alternative vacuum pressure strategies can be used to run lyophilisation cycles without influencing CQA for two model materials. Hence, the potential for the industry to reduce environmental footprint along with improving process economics by mitigating the utilization of nitrogen purging for chamber pressure control during the lyophilisation process.

References

[1] S. Ullrich, S. Seyferth, and G. Lee, “Measurement of shrinkage and cracking in lyophilized amorphous cakes. Part I: Final-product assessment,” J. Pharm. Sci., vol. 104, no. 1, pp. 155–164, 2015.
[2] P. Chouvenc, S. Vessot, J. Andrieu, and P. Vacus, “Optimization of the Freeze-Drying Cycle : A New Model for Pressure Rise Analysis,” vol. 22, no. 7, pp. 1577–1601, 2004.
[3] S. M. Patel, T. Doen, and M. J. Pikal, “Determination of End Point of Primary Drying in Freeze-Drying Process Control,” AAPS PharmSciTech, vol. 11, no. 1, pp. 73–84, 2010.

Proposal for a continuous In-Vial, Lyophilisation process utilising Vacuum-Induced evaporative freezing

Egan D.*; Joshi, P., Casilagan. S
Pharmaceutical Manufacturing Technology Centre, Bernal Research Institute, University of Limerick, Ireland

*E-mail of the corresponding author: david.egan@ul.ie

Abstract

This paper describes a novel approach to the development of a continuous, in-Vial Lyophilisation process. Unlike a conventional process, enclosed chambers are not used, instead each individual vial becomes both freezing and drying chambers; with freezing achieved by vacuum induced evaporation, not by refrigeration. Careful control of the pressure reduction in the vial is first used to de-gas the liquid to prevent excessive bubbling, which would spread material throughout the vial, stopper etc. Then, the vial is plunged further into vacuum to induce freezing; after which lyophilisation begins. Not being encased in a chamber, the externs of the vial surface are available for controlled heating to drive lyophilisation and also video / spectroscopic interrogation during the freezing / drying process. Each individual cake of dried material can be monitored to achieve a desired product response, e.g. % resuidal water content using NIR. A Temperature / Pressure profile during the process is shown which traces the Vapour boundary of the water phase diagram. Examples, including video and pressure profiles, are shown of the process being undertaken on Water, Milk and a Trehalose dihydrate based cake. All showed successful controlled degassing, vacuum induced evaporative freezing and subsequent Lyophilisation into an acceptable cake.

Introduction

Freeze-drying, lyophilisation, lyophilization, or cryodesiccation is a dehydration process typically used to preserve a perishable material or make material more convenient for transport. A conventional freeze dryer consists of two chambers that are joined together using a short large pipe containing a valve. In such a setup, the material to be dried is frozen using refrigeration equipment which cools a silicone fluid and passes this through the shelves on which the material sits, thus freezing the water (or other solvent) in the material. Lyophilisation occurs when the surrounding atmosphere pressure is reduced to allow the frozen liquid in the material to sublimate directly from the solid phase to the gas phase, which subsequently condenses upon a colder surface in the condenser.

In an industrial setting, thousands, maybe even hundreds of thousands of vials can be dried at the same time. The problem with this is that it is a batch process and the rate of drying is not the same all around the chamber. For example, vials tend to dry more quickly adjacent the door and the edge of the shelves. In general, the drying cycles are very conservative and pander to the slowest drying vial, with a large safety margin built in; resulting in very long cycles that are probably far longer than required. Industry accept this, as the alternative can be to risk millions of euro worth of material.

In addition, as the vials are all packed together on the shelves in these industrial batch processes, there is little chance of monitoring individual vials to determine if they are finished drying or have dried correctly. There is certainly no scope to individually monitor each vial or dry the material in the vial to a quality attribute set-point, e.g. % residual water content.

A whole new approach to the lyophilisation of materials in vials is proposed. This process removes the requirement for enclosed chambers, instead using the vial as the chamber. By utilising vacuum induced evaporative freezing, the need for a refrigerated system to freeze the material is also removed. As the vial surface is available for interrogation, the lyophilisation of each individual cake can be controlled and monitored.

Methods and Materials

An example of a conventional drying cycle.

This begins with the material to be dried in vials being charged onto the shelves of the drying chamber. While remaining at atmospheric pressure, or just a little below to keep the door closed, the shelves are cooled to freeze the material to say -30oC. While this is happening, the condenser chamber is cooled to -50oC and the condenser chamber (only) evacuated. Other systems can have a single chamber with the condenser and shelves located together.

When the material is frozen sufficiently, the valve between the two chambers (or in the case of the single chamber, the pumps are activated) is opened to re-join the chambers together and evacuate the drying chamber. The temperature of the shelves in the drying chamber is raised to say -5oC to input energy to drive lyophilisation forward. Although outside the scope of this document, care must be taken not to increase the temperature of the shelves above a "critical temperature" or the material can be destroyed. Because fo the reduced pressure, ice from the frozen material sublimes as a gas and is captured in the condensation chamber on the much colder condenser. Once completed, the shelves compress the stoppers to seal the vials.

Description of the proposed novel In-Vial lyophilisation process

Evacuating, freezing and drying each vial individually outside the confines of an enclosure now allows a genuinely continuous lyophilisation process to be developed. A process can be envisaged where the following steps can be undertaken in series, one after the other continuously:

1) The material to be dried is dispensed into a vial.
2) The rubber stopper is partially fitted to allow evacuation.
3) A connection seals around the top of the vial and that "holder" enters a production line where controlled evacuation occurs to de-gass and subsequent cause freezing of the material.
    a. Monitoring of each vial is possible in terms of the pressure within the vial, external temperature of the vial, audio spectrum reading through the vial, imaging of the material or light spectroscopic analysis through the glass vial.
4) When frozen, the Holder and vial are moved along the line to the next section where the vial is further evacuated to drive lyophilisation. As the external surface of the vial is available, the vial may be heated in a controlled manner to help drive drying forward.
    a. Continued monitoring of each vial is possible in terms of the pressure within the vial, external temperature of the vial, imaging of the material or spectroscopic analysis through the glass vial.
    b. At this stage in the process, the drying cake can be controlled to a quality attribute set-point, e.g. % residual moisture for example; or any other desired attribute as detectable by visual or spectroscopic means.
    c. Secondary drying is also possible by heating the vial, using warm air or IR lamps or immersion in a temperature controlled liquid etc., while continuing to evacuate.

5) When dried to satisfaction, the connection and vial moves to the next stage where the vial can be further evacuated or back filled with an inert gas – whichever is desired. Then the rubber stopper is pressed home to seal the vial.
6) Thisfinalstagereleasesthevialfromtheholderandattachesafoiltopandlabel, prints on the vial etc; then packages the final product with a certificate possible for each individual vial.

Temperature and Pressure monitoring of an example of the proposed method when applied to water within a drying chamber

Although the proposed process is best applied to individual vials, it also works successfully within a conventional drying chamber. This experiment was undertaken on a water sample withing the drying chamber of a a Severn Science LS40 pilot scale freeze dryer. Within this enclosure, the temperature of the water sample and the pressure of the enclosure was monitored using an in-house developed temperature monitoring system and by hardwiring in the pressure signals of the freeze drier. An in-house software system was developed in LabView to continuously monitor the measurements and log the data

The image below is the temperature and pressure profile of a sample of water in a freeze dryer chamber where the pressure was reduced in stages to induce freezing. No cooling of the shelves was used, they were held at about 20oC. The blue line is Temperature Vs Pressure data and traces the sample as the pressure is reduced. This is superimposed upon the phase diagram of water for that region. The following points describe the temperatue and pressure trace of the water as the pressure is reduced as described previously in the proposed process.

- The starting point of the experiment is at about 700mBar and 20 °C. This is the top most Blue point - 1.
    - This slightly reduced pressure was to keep the door closed.
- As the pressure is reduced in steps, while preventing bubbling due to rapid degassing, the temperature mostly remained constant.
    - The different stages of de-gassing can be seen from Blue points 2 – 6 where the temperature does drop slightly at each pressure level.
- The last two stages of degassing, Blue points 7 and 8, show some small reduction in water temperature.
- After de-gassing is completed, the pressure is reduced rapidly (after Blue point 8) until the temperature – pressure trace reaches the liquid / vapour boundary and then it continues along the boundary until freezing occurs where a spike in temperature is measured.
- Pressure and temperature continues to reduce during drying and the trace tends to drift into the vapour region of the phase diagram.

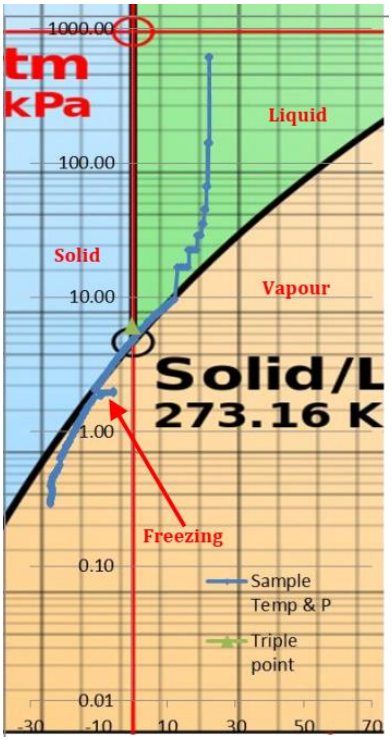


Fig. 1 *Showing the Temperature and Pressure profile of a sample of water evacuated in the drying chamber without any cooling of the shelves super imposed upon the phase diagram of water.*

Pressure monitored example of the In-Vial Lyophilisation of milk using the proposed process

For this example, 2ml of milk was placed in a 25ml vial. The vial was attached directly to an Edwards 12 single phase vacuum pump. Between the pump and the vial, a manual valve was used to control the evacuation of the vial as the vacuum pump worked continuously.

The proposed process as described previously was applied to this vial containing the milk. While undertaking the process, the pressure at the pump was monitored and is shown in the pressure profile below. The following steps were undertaken and are described on the pressure chart.

1) The pump was at a reduced pressure of about 0.04mBar and the pressure increased to about 1mbar when the vial was first attached. The pressure at the pump reduced after again this disruption.
2) The vacuum valve was opened very slightly, and with careful observation of the milk for bubbles.
3) The valve was repeatedly opened slightly & closed until bubbles were just seen to form to degas the milk over time.
4) The valve was allowed to leak a tiny amount which resulted in the vacuum pump pressure slowly reducing, while observing if bubbles formed
5) The valve was opened incrementally a few times to ensure that no further bubbles formed.
6) When we were confident that the milk was completely degassed, the valve was opened fully allowing the milk to freeze by vacuum induced evaporative freezing.
7) Vacuum was continued and the milk freeze dried.

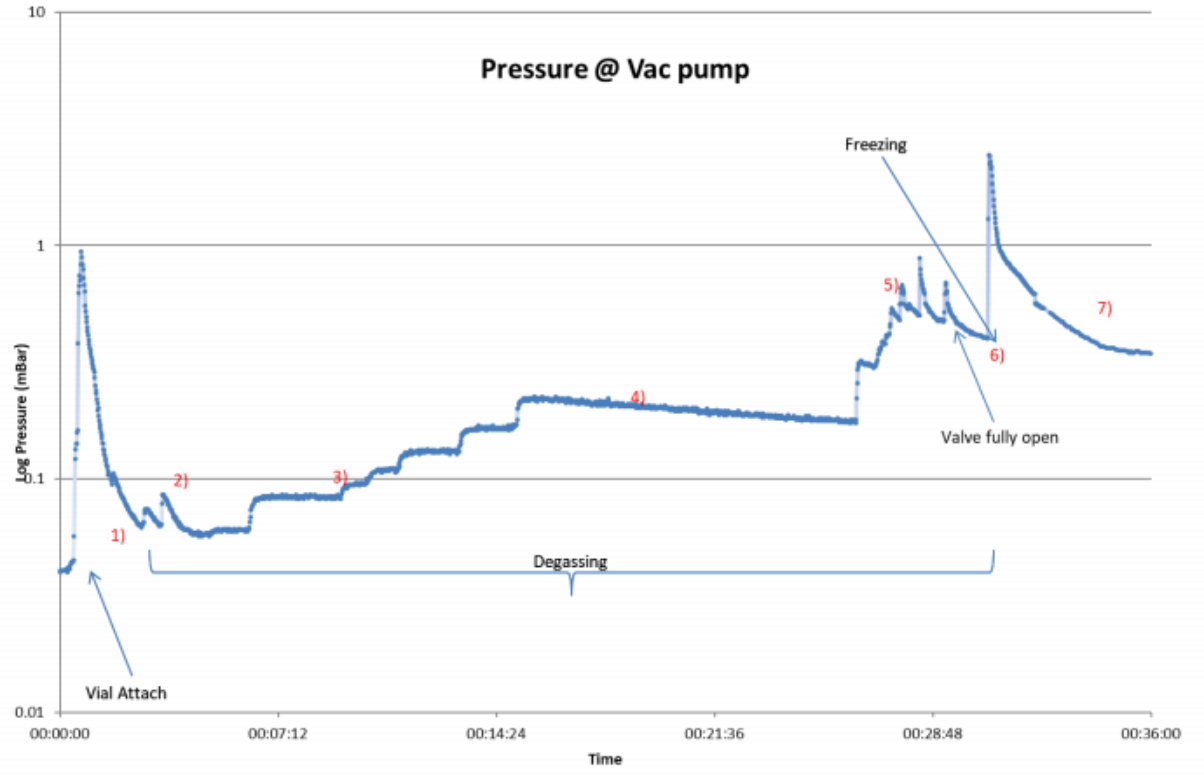


Fig. 2 *Pressure profile at the pump of a sample of milk in a single vile, showing the stages of degassing and vacuum induced freezing.*

Conclusions and Advantages

1) By using evacuation to freeze the material to be dried, the whole refrigeration system conventionally used to freeze the material in a vial is not required.
    a. This alone could drastically reduce the cost of freeze drying equipment.
2) Each vial can be evacuated individually, by connection at the neck to a vacuum setup.
    a. By not being confined to a chamber, the outside surface of each vial is available so that the surface can interrogated to monitor the freezing and drying process.
    b. Each vial will only be in the process until dried, so there could be a significant overall saving in time.
    c. The atmosphere around the vial can also be manipulated as desired on a local level to achieve the desired result in a finished cake.
3) A true continuous lyophilisation process is feasible where material can be dispensed into vials, frozen, dried, sealed and packaged all in a single line.
4) 100% monitoring and certification of each individual vial is possible.

Numerical and analytical methods for contaminant detection in freeze dryers

Kshirsagar V*., Ganguly A.
IMA Life North America, Tonawanda, NY, USA

*E-mail of the corresponding author: Vaibhav.Kshirsagar@imalife.com

Abstract

The introduction of non-sterile substances during an aseptic production batch in a freeze dryer can lead to contamination of the entire batch. The source of these contaminants could be external, through physical vacuum leaks or virtual leaks internal to the freeze dryer. There are numerous experimental methods through which these contaminants may be detected – pressure rise test, visual inspection, mass spectrometers, UV dye. The data presented here will describe some of these methods and demonstrate ways to distinguish between contaminants.

It is important to know the concentration of such contaminants within the freeze dryer. In addition, the port location on which a mass spectrometer is mounted will influence the detection of leaks. Numerical simulations of contaminants in the freeze dryer can be used to obtain a concentration profile of heat transfer fluid that may have leaked. CFD calculations show that the ppm levels for heat transfer fluid leaks measured at the ports depend upon process conditions and port locations. However, for dedicated cycles to measure contaminants, the dryer size rather than the port location has the maximum influence on measured ppm.

These CFD calculations will act as a basis for optimized RGA implementation for process monitoring in freeze dryers.

Introduction

Numerical simulations are set up to replicate an oil leak during a freeze drying process. Transient calculations begin at the time a leak has sprung up in the dryer at a known location. The drop size and the quantity of oil leak is an assumption. For a lab scale dryer with a total shelf area of 0.43 m2, a leak of 250 mg of 1.6 cSt silicone oil, which corresponds to a few drops, was created over less than 0.4 seconds in the corner of a shelf. This location is close to where the shelf hoses connect to the shelves, internally transferring the heat transfer fluid. The heat treatment during freezing and SIP cycles makes the welds in these locations critical spots for fluid leaks. Fig. 1 shows the mass fraction of the silicone oil within the freeze dryer in the plane where the leak is located.

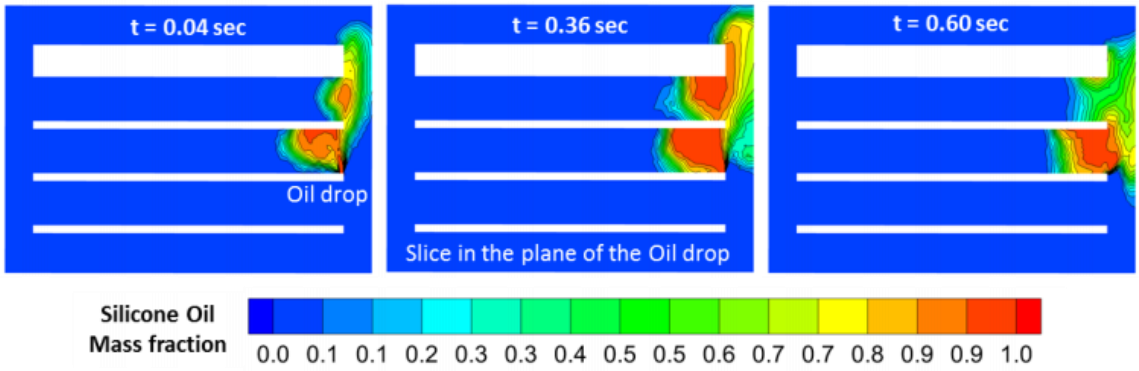


Fig. 1 *Mass fraction of silicone oil in the same slice at different time instances*

This leak, however may not necessarily translate to detection of the silicone oil with a mass spectrometer, or other analytical techniques. For example, the oil is not transported to some of the ports located away from the leak during a process. Fig. 2 shows the mass fraction of silicone oil at a port location suitably chosen for detection. It shows the rapid decay of the mass fraction plot. Thus, a careful selection of the sampling port is required for a mass spectrometer to detect contaminants. It will significantly depend upon freeze dryer design, process conditions and the nature of these contaminants. In this presentation, the authors will focus on experimental techniques and the correlating numerical simulations to demonstrate contaminant detection in freeze dryers.

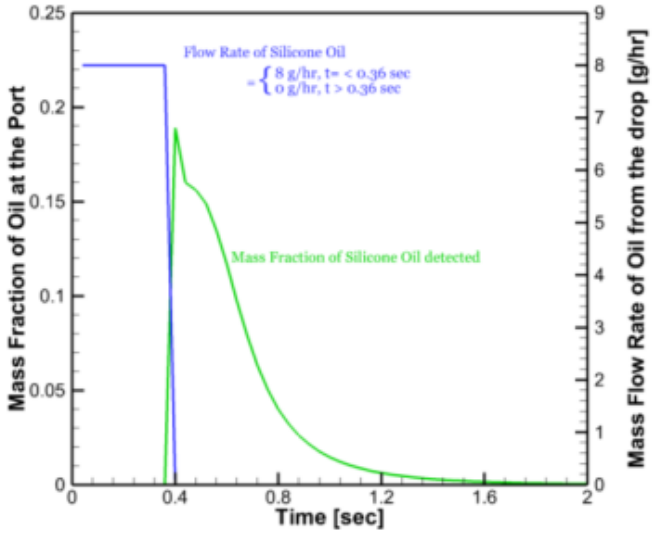


Fig. 2 *Decay of silicone oil mass fraction detected at the port over time*

Materials and Methods

Numerical Simulations are set up to replicate experimental conditions during contaminant detection. ANSYS FLUENT v19 is used for the numerical solutions to solve the Navier Stokes' equation.

For the experimental setup, silicone oils with viscosities of 1.6 cSt and 5 cSt (Dow Chemicals, MI), a petroleum based hydraulic oil (Shell, the Netherlands) and helium from pressurized cylinders (Haun Companies, NY) are used. A mass spectrometer (Atonarp, CA) is used for detection on different freeze dryers and ports.

References

[1] A. Ganguly, J. Stewart, A. Rhoden, M. Volny, N. Saad, Mass spectrometry in freeze-drying: Motivations for using a bespoke PAT for laboratory and production environment, E.J.P.Biopharm.127(2018) 298-308
[2] A. Alexeenko, A. Ganguly, S. Nail, Computational Analysis of Fluid Dynamics in Pharmaceutical FreezeDrying, J. Pharm Sci. 98 (2009) 3483-3494.

Through-Vial Impedance Spectroscopy (TVIS): A New Approach to Characterizing Phase Transition of Sugar-Salt Solutions

Jeeraruangrattana, Y. [a]; Polygalov, E. [a]; Ermolina, I.[a]; Smith, G.[a*]
[a] DMULyoGroup, School of Pharmacy, De Montfort University, UK

*E-mail of the corresponding author: gsmith02@dmu.ac.uk

Abstract

Through-vial impedance spectroscopy (TVIS) which exploits the frequency dependent dielectric properties of sample contained in a glass container has been developed for monitoring a small scale of freeze drying process. In this study, the real part capacitance at two fixed frequencies was used as a new method for identifying phase transitions during freezing and annealing stages. The electrical capacitance of three solutions having different sugar/salt ratios were measured in a 10 mL glass tubing vial over the frequency range of 1 MHz to 10 Hz during a cooling-heating cycle. A discontinuity in the time profile of capacitance at low frequency (i.e. 10 Hz) supported by evidence from a digital camera, indicates the liquid-solid phase transition of ice formation. On the contrary, a measurement frequency above an ice relaxation frequency (i.e. 200 kHz) was used for investigating the unfrozen fraction phase in terms of the ice solidification end point (from which the freezing time can be calculated) and the glass to liquid transition. Two interesting observations were made (i) glass transition temperature (T_g') determined by TVIS agreed with values obtained by differential scanning calorimetry (DSC), (ii) the solidification time increases with increasing salt concentration.

Introduction

The development of robust freeze drying products and processes necessitates a detailed understanding of the in-vial characteristics of the product formulation during processing. However, the majority of techniques used to date for determining critical process parameters are restricted to off-line laboratory techniques. Through vial impedance spectroscopy (TVIS) is a relatively new technique which could assist the development process by measuring the in situ behavior of the product formulation. However, applications to date for the determination of critical process parameters have been restricted to simple solutions of solutes which have relatively low electrolytic conductivities [1].The aim of this work is to further develop the TVIS approach so that is possible to determine these parameters in conductive samples by exploring other facets of the TVIS spectrum

Materials and Methods

Three solutions (containing 5 %w/v sucrose and either 0, 0.26 or 0.55 %w/v sodium chloride (NaCl) were measured to obtain the impedance spectra within the TVIS vial over the frequency of 1 MHz to 10 Hz. The measurement was taken every 2 minutes during a cooling-heating cycle in a VirTis Advantage Plus Freeze-dryer. One of the plain vials close to TVIS vials had a thermocouple placed therein to provide a representative temperature in the TVIS vial. The capacitance spectra were analysed by LyoView™ software. Evidence of ice nucleation was recorded by a digital camera. Differential scanning calorimetry (DSC) was used for analysing glass transition temperature (T_g') of each formulations.

Results and Conclusions

The parameters derived from the TVIS complex capacitance spectra were (i) the peak amplitude (C''_{PEAK}) and the peak frequency (F_{PEAK}), (ii) a real part capacitance at 10 Hz (C'(10 Hz)) and 200 kHz (C'(0.2 MHz)). Inflections in the time profiles of all parameters indicated the onset of ice nucleation (as confirmed by images from digital camera). Whereas at 200 kHz (which is well above the ice relaxation frequency of 1 kHz) the capacitance of ice has almost no temperature dependence and so any changes in C'(0.2 MHz) either with time or temperature, can be associated with the completion of ice formation on freezing or the glass transition on re-heating. In this study we observed that the point at which the DSC thermogram starts to increase is mirrored by an inflection in the high frequency capacitance, and therefore suggests that time profile of (C'(0.2 MHz)) could be applied for determining the onset of glass transition of sample within vial. By using a temperature from neighbouring vial along with TVIS parameter indicating ice formation (i.e. C''_{PEAK}, F_{PEAK}, (C'(10 Hz)), it would be able to predict the ice nucleation temperature (T_n) from capacitance at low frequency, which were -10.5, -11.7 and -11.9 °C respectively for the solutions of 5 %w/v sucrose with 0, 0.26 and 0.55 %w/v NaCl. The result also demonstrated a twofold increase in the solidification time as salt concentration increases from 0 to 0.55 %w/v. In a similar manner, a temperature profile of (C'(0.2 MHz)) could be used for measuring glass transition temperature of a sample filled in vial (T'_g). In this study, T'_g value measured by DSC has found to be similar to T'_g estimated by TVIS. The difference between both methods was ~ 1 °C. This finding highlights a new approach to characterizing phase change during freeze drying cycle.

References

[1] G. Smith, M.S. Arshad, E. Polygalov, I. Ermolina, An application for impedance spectroscopy in the characterisation of the glass transition during the lyophilization cycle: the example of a 10% w/v maltodextrin solution, Eur. J. Pharm. Biopharm 85 (2013) 1130-1140.

Multiplexing Through Vial Impedance Spectroscopy (TVIS) with Comparative Pressure Measurement for the Determination of the Primary Drying Endpoint of Immunoglobulin (IgG)

Pandya, B. [a]; Matejtschuk, P.[b]; Jeeraruangratta, Y. [a], Smith, G.[a*]
[a] DMU LyoGroup, School of Pharmacy, De Montfort University, Leicester, United Kingdom
[b] National Institute for Biological Standards & Control (NIBSC), Potters Bar, United Kingdom
*E-mail of the corresponding author: gsmith02@dmu.ac.uk

Abstract

Two IgG formulations, one with 5% sucrose and the other with 5% Mannitol:Sucrose (ratio 4:1) both in 20mM Histidiine 0.01% Tween 20 pH 6.5, were freeze dried in a Telstar freeze dryer equipped with Pirani and Baratron sensors and a Sciospec® five-channel through vial impedance spectroscopy (TVIS) system. The TVIS parameter C'(100kHz) (i.e. the real part electrical capacitance measured at 100 kHz) is sensitive to the amount of ice in the vial. By following the recovery of its time profile during primary drying, it was possible to determine the primary drying endpoint. The TVIS endpoint of the sucrose edge vial occurred well before the onset of the Pirani endpoint suggesting that Pirani was more sensitive to the core vials. The TVIS endpoint in the core vials also occurred somewhere close to the midpoint of the Pirani endpoint suggesting that other core vials were still in primary drying.

Introduction

Comparative pressure measurement using the Pirani and the Capacitance Manometer (MKS Baratron) sensors is most popular among the batch techniques that have been utilised for the determination of the primary drying endpoint [1]. Through vial impedance spectroscopy (TVIS) is a single vial technique that measures the electrical properties of the glass vial and the contents of the vial. It comprises an electrode system attached on the outside of a standard glass vial, thereby making the measurement non-product invasive. Previously, it has been shown that it is possible to determine the endpoint of a sucrose formulation using the imaginary capacitance at 1 kHz. The aim of this study is to use the time-line of the real capacitance at 100 kHz, i.e. C'(100kHz) to determine the range of primary drying endpoints for a complex protein formulation located at the edge and the core; and to compare the endpoint from TVIS with the endpoint given by the comparative pressure measurement.

Acknowledgements Innovate UK AtlasBio Project Ref. No. 102610

Materials and Methods

A batch of 308 x 5 mL vials (Adelphi VC-010-005) were filled with 3g of 20 mM Histidine Buffer and 0.01% Tween 20 pH 6.5, containing either (i) 4% IgG with 5% sucrose; (ii) 4% IgG with 5% of a 4:1 mannitol:sucrose mixture; or (iii) their placebo equivalents. Two vials from (i) and two vials from (ii) were modified with copper electrodes (19 mm by 10 mm; copper adhesive tape 1181 3M) attached externally to the glass wall at a distance of 3 mm from the vial baseline. One TVIS vial from each IgG containing formulation were placed in the middle of the first row of the edge vials facing the dryer door and the other two TVIS vials were placed in the core. Each TVIS vial was accompanied by two Type T thermocouples placed in the immediate neighbor vials. Freeze drying was carried out in a

Telstar Pilot dryer equipped with Pirani and Capacitance Manometer pressure sensors and a 5-channel TVIS system (Sciospec, Germany). The lyo cycle consisted of a freezing ramp from 20 °C to -50 °C at 0.2 °C/min, two annealing steps (to -15 °C and -28 °C), followed by a 72 h primary drying step at a shelf temperature of -25 °C and finally a secondary drying step at a shelf temperature of 30 °C. The total cycle time for the recipe was approx. 92 h.

Results and Conclusions

Fig. 1(i) shows the time-lines of the Pirani and Baratron sensors and the thermocouple product temperatures. Fig. 1(ii) shows the characteristic dip and recovery of the TVIS parameter C'(100kHz) that we take as the sublimation end-point. While the TVIS endpoint for the sucrose-IgG edge vial occurred 12 h earlier than the onset of the reduction in the Pirani vapour pressure (44 h). In addition, the TVIS endpoint for both core TVIS vials occurred at 62 h and yet the Pirani was still active until 73 h. This may be due to one or both of two factors: (i) other core vials were still drying (most probable) and/or (ii) the Pirani continues to sense water vapour in the dryer even when all the ice has sublimed (least probable).

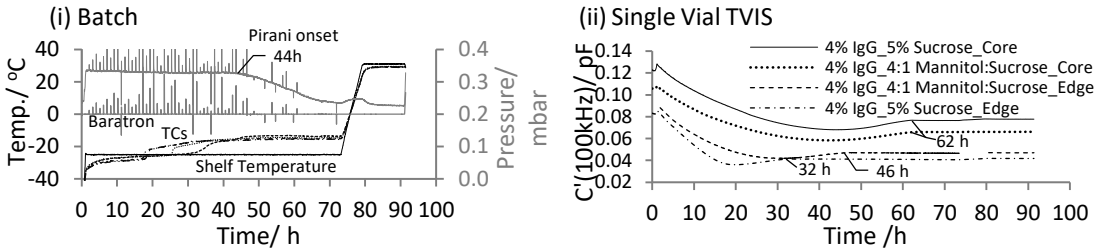


Fig. 1 *Primary drying endpoint determination using (i) comparative pressure measurement (i.e. Pirani and Baratron) and (ii) through vial impedance spectroscopy for two IgG formulations*

References

[1] S.M. Patel, T. Doen, M.J. Pikal, Determination of End Point of Primary Drying in Freeze-Drying Process Control, Aaps Pharmscitech 11 (2010) 73-84.

A model-based comparison of conventional batch freeze drying to a novel continuous spin-freeze-drying concept for single unit vial doses

Leys, L. [a*]; Lammens, J.[a]; Nuytten, G.[a]; Vanbillemont, B [a]; Van Bockstael, P.[a]; De Meyer, L [a] De Beer, T.[a]; Corver, J.[b]

[a] Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium

[b] RheaVita, Ghent, Belgium

*E-mail of the corresponding author: Thomas.DeBeer@ugent.be

Abstract

A new continuous spin-freeze-drying concept was recently developed for single unit doses by our group.[1] For this concept, a mechanistic primary drying model was adapted from a batch freeze-drying model to calculate the optimal dynamic drying trajectory for spin-frozen formulations.[2] In this work, a comparison was made between conventional batch freeze-drying and the new spin-freeze-drying concept by comparing the outputs (i.e. endpoint, optimal shelf/radiator temperature and pressure profile) of both primary drying models. Input parameters such as dried product layer resistance (Rp) were therefore experimentally determined for both freeze-drying methods and compared. It was observed that the Rp profiles for continuous spin-frozen formulations were lower as compared to vials frozen in a conventional batch freeze-dryer. In addition, as spin-freezing drastically increases the surface area of the product and lowers the layer thickness, drying times can significantly be reduced and an excellent cake structure can still be obtained. Spin-freezing-drying proved to be more efficient in reducing primary drying times when an dynamic heating trajectory was used. Predicted results were experimentally verified for both models by validation experiments.

Introduction

Currently, the pharmaceutical industry is shifting from batch-wise production of pharmaceuticals to a more continuous approach. One of the new approaches is the spin-freeze drying concept for single unit vial doses that was proposed by Corver et al. [1] In this concept, a vial is spun along its longitudinal axis and its content is subsequently frozen by a cold gas. Next, the chamber is evacuated and energy for sublimation is provided by IR-radiation. [1] Much progress has been made in the development of this concept and mechanistic models were also obtained to optimize primary drying for spin-frozen formulations. [2]. However, a solid comparison with conventional batch freeze-drying is still lacking. In this project, a comparison is made between conventional batch freeze-drying and the spin-freezing concept by examining the outputs (i.e. endpoint, optimal shelf/radiator temperature and pressure profile) of the primary dyring mechanistic models of both freeze-drying methods .

Materials and Methods

Input model parameters (Tg', Kv and Rp) were experimentally determined for batch and continuous freeze-drying for three model formulations. The heat transfer coefficient (Kv) was calculated by a weight loss experiment and Rp profiles were obtained by product temperature measurements in both batch and spin-frozen formulations. Optimal dynamic drying trajectories were calculated with Matlab. The output results were experimentally verified by performing a validation run for all the formulations.

Results

Despite the high cooling rate, it was observed that spin-freezing in liquid nitrogen lowers the Rp profile of all formulations as compared to batch-frozen vials. This could be attributed to the formation of chimneys in the spin-frozen structure which facilitates vapor escape. This effect, together with the high surface area and low layer thickness of the product, drastically lowers predicted drying times of spin-frozen vials when using an optimal IR-heating trajectory. Using a dynamic IR-heater temperature profile for spin-frozen vials, primary drying times could be reduced by at least 90 % in comparison with a conventional static batch process.. For batch formulations with an optimal heating trajectory, this reduction was approximately 80% for crystalline materials and only 40 % for amorphous formulations. Even though high sublimation rates were simulated, no choked flow was observed at the vial neck. Model predictions could be experimentally verified by a validation experiment.

Conclusions

Spin-freezing significantly reduces primary drying time compared to conventional batch freeze-drying processes. In-house mechanistic primary drying models were able to predict differences in drying times and provide an in depth explanation for the obtained results.

References

[1] J. Corver, "Method and system for freeze-drying injectable compositions , in particular pharmaceutical. tle," WO2013036107, 2013.
[2] P. Van Bockstal et al., "Mechanistic modelling of infrared mediated energy transfer during the primary drying step of a continuous freeze-drying process," Eur. J. Pharm. Biopharm., vol. 114, pp. 11–21, 2017.

A Model Predictive Control strategy of the dynamic primary drying settings for a batch freeze drying process

Vanbillemont, B. [a*]; Nicolaï, N.[b]; Corver, J.[c]; De Beer, T.[a]

[a] Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium
[b] Department of Data Analysis and Mathematical Modelling, Ghent University, Ghent, Belgium
[c] RheaVita, Ghent, Belgium

*E-mail of the corresponding author:brecht.vanbillemont@ugent.be

Abstract

A model predictive control methodology also including uncertainty analysis could help fastening the primary drying phase herewith ensuring a controlled risk of failure. A critical process parameter is the sublimation interface temperature (Ti) that needs to be kept under the collapse temperature, especially during unexpected disturbances, to prevent the loss of structure.

Introduction

Nowadays, the standard way of operating a batch lyophilisator is a protocol-driven method. All freeze-drying phases (i.e. freezing, annealing, primary and secondary drying) are programmed sequentially at fixed time points and within the phase all critical process parameters (CPP) are generally kept constant or varied in a linear way between two setpoints. This way of managing is not the most optimal and efficient way (i.e. process time, quality) to run the process. A model predictive control methodology also including uncertainty analysis could help fastening the primary drying phase herewith ensuring a controlled risk of failure of the CPPs. A key failure to avoid is a sublimation interface temperature (Ti) that exceed the collapse temperature, common during unexpected disturbances, resulting in a loss of structure.

Materials and Methods

A supervisory control for a pilot-scale freeze dryer via Modbus TCP/IP was developed in LABview. The primary drying phase was initialized in respect with the shelf temperature (Ts) and pressure (Pc) machine capabilities. The in-line control method compromised following iterative steps per 120s prediction horizon. At first, the uncertainty range for Ts and Pc were determined from the deviations in the preceding horizon. Next, a finite-horizon optimization was performed in MATLAB by constructing a machine capability grid of Ts and Pc. Followed by an uncertainty analysis of an 8 parameter (including Ts, Pc, dry layer: Ldr) mechanistic primary drying model with 10.000 samples resulting in a design space with a predefined risk of failure (RoF). Subsequently, the combination of Ts and Pc without failures and maximal sublimation rate was selected. Finally, an error propagation on Ldr was performed and used as its uncertainty range in the next prediction horizon. Furthermore, a feedback loop for Ts and Pc was implemented to shift the machine capability grid in case of disturbances such as a vacuum leak. Both the edge and centre vial population was modelled and upon completion of all the edge vials, the centre vial parameters were implemented for process control. The end of primary drying was detected by convergence of pirani and capacitance pressure values.

Results

The lyophilisation process of a 3 ml 3% sucrose solution was shortened significantly due to a reduction in the primary drying time, from 15.3 h with a traditional protocol-driven operating principle (Pc: 10 pa and Ts:-20°C) to 9.8h for a dynamic trajectory with a 0.01 RoF (Fig 1). Furthermore, no signs of substandard quality (i.e. melt-back or collapse) were identified when a deliberate pressure disturbance was introduced due to the periodic update of the model parameters and uncertainty levels.

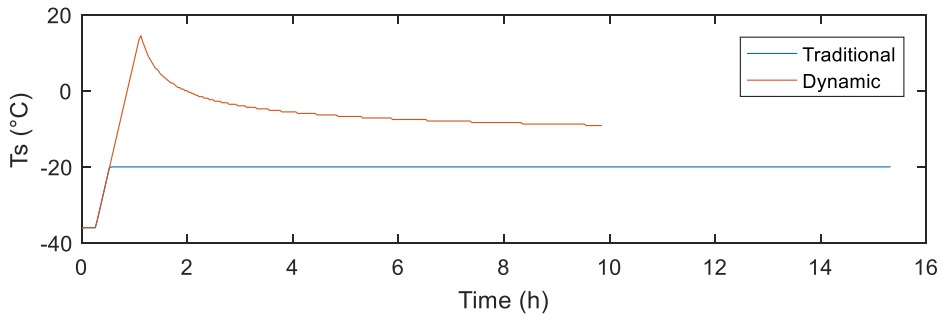


Fig. 1 *Comparison of the shelf temperature for a safe traditional versus dynamic trajectory.*

Conclusion

A model predictive control strategy was successfully applied on a dynamic primary freeze-drying process with incorporation of uncertainty analysis. Based on a receding horizon principle, the uncertainty analysis was updated regularly utilizing the recorded CPPs and the dynamic predictions of Pc and Ts were applied for the next prediction horizon.

Model-based approach to scale-up of freeze-drying processes

Bobba, S. a*; Fissore, D.a; Zinfollino, N.b
[a] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy
[b] Department of Process Development, Merck KGaA, Guidonia Montecelio, Italy

*E-mail of the corresponding author: serena.bobba@polito.it

Abstract

Mathematical modeling is a powerful tool that may be used to scale-up freeze-drying processes developed at lab-scale into commercial scale units. Few tests are needed to determine the heat transfer conditions in the two units, taking also into account the non-uniformity of drying conditions in the batch. In this framework, two industrial freeze-driers were characterized by mean of cheap and easily implementable gravimetric method, with the aid of statistical analysis.

Introduction

During a freeze-drying (FD) cycle the drug product (DP) temperature has to be lower than the critical one, which potentially may cause damages. The DP temperature is a function of process conditions, i.e. shelf temperature (T_S) and chamber pressure (P_C). Therefore, FD cycles are designed by accurately selecting T_S and P_C. FD cycles are developed in lab-scale units, and then transferred to the productions. The lab scale freeze-drier (fd) and the large-scale one have different features, affecting, among the others, the heat transferred to the DP at the same values of T_S and P_C. Hence, for guarantee a similar thermal history of the DP. it may be required to modify the operating conditions. A rational approach is based on the characterization of the fds, focusing, in particular, on the heating rate to the DP. The key parameter for the fd characterization is the overall heat transfer coefficient (K_v) which takes into account the different heating mechanisms of the system[2]. Since vials in different shelf position are influenced by radiation from the chamber differently, it is necessary to evaluate the value of K_v for each vial or for each group of vials experiencing a similar heating path. In lab-scale fd the gravimetric method is used for this purpose, while other technique, like the Tunable Diode Laser Absorption Spectroscopy (TDLAS) was suggested for the manufacturing fd[3]. Although TDLAS is easily implementable in a large-scale unit, it can only provide a mean value of K_v. In this study the gravimetric method was succesfully applied in a large-scale fd, proving it is feasible for production sytes, cheaper to be implemented. Moreover, data from a gravimetric test were statistically elaborated to asses the equivalence of the performance of two machinaries identically designed, as a proof of their consistency in processing the DP similarly.

Materials and Methods

Gravimetric tests were carried out in two fds (21 sqm supplied by IMA) at Merck production site in Italy, designed with the same features to provide same performances. The production line is equipped with a filling machine and an automatic loading/unloading system (ALUS) using isolator technology. Three gravimetric tests were run in one fd, under full load conditions, at 15, 10 and 5 Pa. Vials DIN 2R type filled with pure water were loaded. The gravimetric method requires to monitor the product temperature and to weigh the ice mass sublimated during the test. Hence, some groups of vials were

selected in key shelf zones and on 3 shelves (the first, the last and one in the middle), allowing to investigate the uniformity between shelves. The number of weighted vials was thought to be statistically significant. The product temperature profiles were registered by mean of 16 wireless sensors (TEMPRIS®, IQ Mobil), inserted in some vials placed among the groups of the weighted ones. To deal with the filling machine and the automatic loading system, the vials to be weighted were preventively filled and weighted and, when necessary, the TEMPRIS® sensor was inserted. During the loading step, the line was stopped several times, to substitute in the proper positions the automatically filled vials with the weighted ones. While unloaded, these vials were collected for the reweighting. A single further gravimetric test was needed to be run in the other fd, under same conditions, at 15 Pa. The higher P_C was selected for this test, to verify that no issues linked to the condenser capacity or choking flow would occur with higher sublimation fluxes.

Conclusions

Gravimetric method was applied in an industrial freeze-drier (fd) for characterizing the heat transfer to the product, proving this technique is implementable and effective in large-scale apparatus. Statistical analysis applied to data collected during the gravimetric tests allowed to validate the same characterization for a second fd identically designed.

References

[1] R. Pisano, D. Fissore, A.A. Barresi, Heat transfer in freeze-drying apparatus, in: B. Dos Santos (Ed.), Heat transfer, Rijeka, Croatia, 2011, pp. 91-114.
[2] S. C. Schneid, H. Gieseler, W. J. Kessler, M. J. Pikal, Non-invasive product temperature determination during primary drying using tunable diode laser absorption spectroscopy, Journal of Pharmaceutical Sciences. 98 (2009) 3406-3418.

In-situ micro-CT for in depth characterization of a spin-frozen sample

Lammens, J.[a*]; Vanbillemont, B.[b]; Goethals, W.[c]; Boone, M[c]; Corver, J[d]; De Beer, T[b]; Vervaet, C.[a]

[a] Laboratory of Pharmaceutical Technology, Ghent University, Ghent, Belgium
[b] Laboratory of Process Analytical Technology, Ghent University, Ghent, Belgium
[c] Centre for X-ray Tomography, Ghent University, Ghent, Belgium
[d] RheVita, Ghent, Belgium

*E-mail of the corresponding author: joris.lammens@ugent.be

Abstract

In this study an in-situ X-ray micro computed tomography (µ-CT) technique was developed for in-depth characterization of spin-frozen samples. The method had to be optimized to limit dynamic blurring, while ensuring sufficient resolution. The thickness of the frozen product layer and shape of the sublimation front could be monitored during the entire sublimation process.

Introduction

Recently a continuous freeze-drying technology was developed, in wich spin-freezing is used as freezing step. A mechanistic one-dimensional model was perviously developed to model primary drying of spin-frozen samples[1]. This mechanistic model assumes that there is only one sublimation front moving from the center of the vial towards the vial wall. A second sublimation front moving from the top to the bottom of the vial was assumed to be negligible due to the small frozen product layer thickness. In this study an in-situ µ-CT method was developed and evaluated to validate the assumption of the sublimation front moving from the center of the vial to the vial wall. The shape of the sublimation front in function of time can serve as input for optimization of current models for primary drying of spin-frozen samples.

Materials and Methods

A 10R vial filled with 2,9 mL of a 5% mannitol solution was rotated rapidly around its longitudinal axis. Once the formulation was equally spread over the entire vial wall, the vial was cooled by a flux of cold inert gas. Hence, a thin frozen product layer over the entire vial wall was obtalined. A stainless tube connected to a condensor was installed in the middle of an in-house developed environmental µ -CT scanner. Immediately after spin-freezing the spin-frozen vial was mounted at the top of this stainless tube. Thereafter, the spin-frozen vial was evacuated by starting the vacuum pump connected to the condenser. The energy for sublimation was provided by an electric heating pad, which was wrapped around the spin-frozen vial. The µ-CT scanner rotated around the spin frozen vial with a velocity of 1 rotation per 30 seconds.

Results

Figure 1 represents an image of the frozen product layer after a recording time (T_R) of 12 minutes at a voxel size of 21 µm. As visible in figure 1B some dynamic blurring occurred. This is caused by the moving sublimation front during recording, while the imaging software assumes a non-moving sublimation front. In order to minimize dynamic blurring, T_R was reduced to 6 and 4 minutes. The extent of dynamic blurring was already lower for a T_R of 6 minutes and almost neglectable with a T_R of 4 minutes (Fig. 1 C). However, a shorter recording time is associated with a lower resolution (34

µm) due to a reduced contrast-tonoise ratio. Therefore, a trade-off between image quality and recording time must be found.

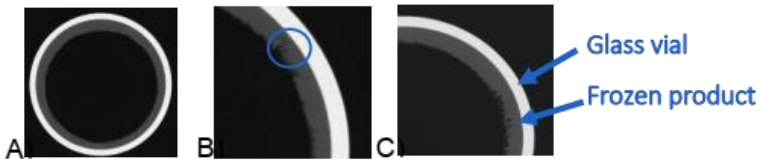


Fig. 1 *A) cross section of a spin frozen vial B) dynamic blurring (T_R =12 min) C) Neglectable amount of dynamic blurring (T_R = 4 min)*

Figure 2 represents an image of the sublimation front at the start of the sublimation process. The thickness of the frozen product layer could be monitored during drying. In addition, the shape of the sublimation front was visualized in detail during the sublimation process, which makes it possible to evaluate the relevance of end-effects. Areas with a thicker frozen product layer are indicated as white regions. Dark regions in the figure correspond with a thinner frozen product layer.

Fig. 2 *Unfolded image of the frozen product layer during primary drying*

Conclusions

The developed in-sity µ-CT method enables visualization of the sublimation front in function of time. The acquired information about the sublimation front in space and time will serve as an input for optimization of the currently available primary drying models. Furthermore, the thickness of the frozen product layer could be evaluated over time at each postion of the vial, enabling the calculation of a Rp-distriubution over the vial in space and time.

References

[1] Van Bockstal et al, Developing a framework to model the primary drying step of a continuous freeze-drying process based on infrared radiation, EJPB.127 (2018)159– 170.

Understanding spin-freezing of aqueous pharmaceutical formulations in the framework of continuous freeze-drying

Rios Revatta, S.[a*]; Vanbillemont B.[a]; Lammens, J.[c]; Nuytten, G.[a]; Leys, L.[a]; Van Bockstal, PJ.[a]; De Meyer, L.[a]; Corver, J.[b]; De Beer ,T.[a]

[a] Laboratory of Pharmaceutical Process Analytical Technology, Ghent University, Ghent, Belgium
[b] RheaVita, Technologiepark-Zwijnaarde 3, Postbus 17, 9052 Ghent-Zwijnaarde
[c] Laboratory of Pharmaceutical Technology, Ghent University, Ghent, Belgium

*E-mail of the corresponding author: Susan.RiosRevatta@UGent.be

Abstract

Spin-freezing is evaluated as a part of an innovative continuous freeze-drying concept for aqueous pharmaceutical unit doses. During spin-freezing, vials filled with an aqueous pharmaceutical solution are rapidly rotated around their longitudinal axis during cooling and freezing. Cooling and freezing are achieved by jetting cold gas around the vial. The temperature of the vial in function of time is monitored using thermal imaging in order to obtain spatial information about the nucleation temperature, the beginning of the crystal growth and the subsequent cooling process. It is our aim to develop and validate a mechanistic model of the spin-freezing process, based on gas flow injection and rotation speed. The thermal imaging non-contact measurement technique is an adequeate tool for validating this model.

Introduction

The aim of this research is to study and evaluate spin freezing as a part of an innovative continuous freeze-drying concept for aqueous pharmaceutical unit doses. Cooling and freezing are achieved using cold gas. The flow and temperature of this cold gas is well controlled. Measurement of the product temperature is part of this closed-loop control. Therefore, a thermal imaging methodology is used as a tool to obtain local temperature measurements. Besides process control, the local thermal information provides specific information about the nucleation and crystallization process. These measurements are relevant in order to identify the temperature of nucleation, i.e. the temperature at the moment when the first ice crystal is formed. The temperature is important since it determines the structure of the product and therefore the behaviour during drying and finally the properties of the final dried product cake. Subsequently, a mechanistic cooling and freezing model is developed and validated, based on a spin-freezing method combining two process aspects: gas flow injection and rotation speed. This model is to be used to describe the behavior of our process and to optimize it as a further step.

Materials and Methods

The vials (DIN 10R), filled with an aqueous pharmaceutical formulation, were rapidly rotated around their longitudinal axis during the period of cooling and freezing. Cooling and freezing was achieved by jetting cold nitrogen gas under different conditions around the vial. Specifically, the flow of the gas and the speed of rotation were modulated. The gas flow was adapted in steps of 10 l/min starting at 20 l/min until 50 l/min; a Dwyer flowmeter model RMA-23-SSV was used for this purpose. The different values used for the rotation speed were: 1200, 1600 and 2000 rpm. For each set of conditions, the experiments were repeated three times. Every spin-freezing experiment was

measured in-line, using thermal imaging techniques in order to measure the temperature of the vial in function of time, with the goal of identifying temperature of nucleation. A FLIR camera, model FLIR A655sc, was used for this purpose. These temperature measurements were processed using Matlab. The mechanistic cooling and freezing model was developed and validated based, on physics equations which explain our process and subsequently, simulated using Matlab.

Results

A thermal image and temperature profile of the spin-frozen aqueous pharmaceutical formulation were obtained. Based on the thermal images, it was possible to follow the product temperature during cooling and freezing (figure 1). The effect of the gas flow, as well as rotation speed on the moment of nucleation, temperature of nucleation, and the plateau phase in figure 1 were analyzed. Temperature of nucleation decreases when the gas flow increases. Additionally, the crystallization phase is shorter when the gas flow increases. In the second part of this study, a mechanistic cooling and freezing model was developed and verified. In one exemplary comparison, we took the flow of the gas at 50 l/min and the speed of rotation at 2000 rpm. We defined our input variables in the model, finding that the flow and the temperature of gas, the geometry of the vial, the geometry of the nozzle and the properties of the gas have a big influence in our simulations. The mechanictic model and the experimental data fit well in the first cooling.

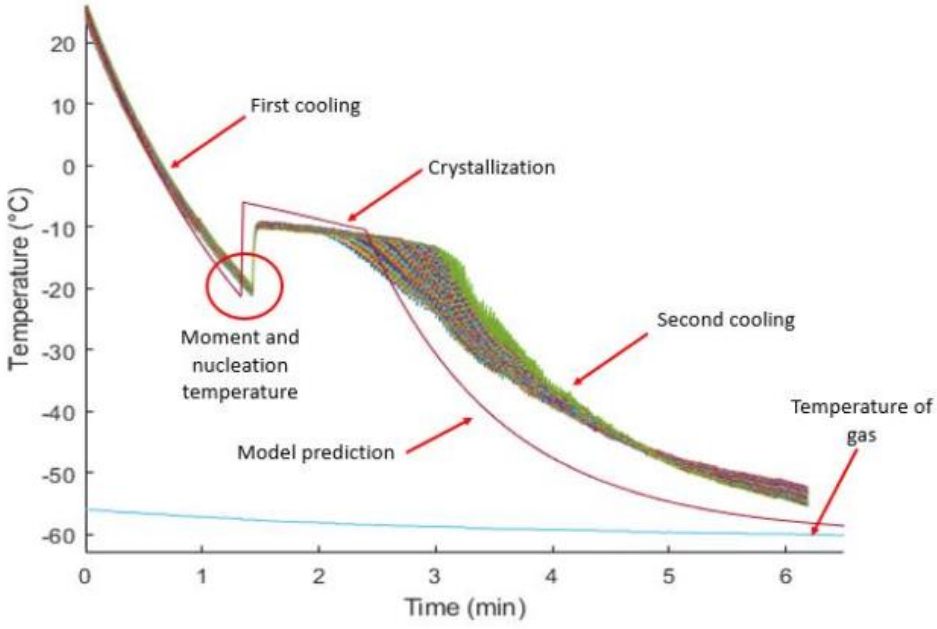


Fig. 1 *Product temperature profile at a gas flow of 50 L/min*

Conclusions

It was possible to closely follow freezing and cooling processes in a non-contact way. Additionally, temperature of nucleation was identified. This temperature assists to understand the evolution of the process and to predict the structure of the final dried product. The effect of the gas flow and the rotation speed were studied. At slower rotation speed, it was not possible to properly identify temperature of nucleation. At higher gas flow, nucleation temperature drops; the moment of nucleation was achieved earlier and the plateau lasted less. It seems possible to model the experimental data with a mechanistic model based on physics equations.

Improving of a lyophilization cycle for Omeprazole 40 mg

Garcia Varona A. [a], Aguila Perez G. [a], Perez Calixto M. [a], Salas Cordoba D. [a]
[a] Pharmaceutical Laboratories AICA+, BioCubaFarma, La Habana, Cuba
*agvarona@nauta.cu

Abstract

The injectable Omeprazole 40 mg has been produced in AICA+ Laboratories since 2003 and has a stability of more than 2 years. Their production is carried out in multipurpose plants and requires maximum utilization of productive capacities, because of which it is needed a modification of the cycle operating parameters in order to reduce costs and environmental impact. Eigth batches of the injectable were lyophilized by using two recipes: original and modified, and they were evaluated according with product quality attributes: organoleptic characteristics, residual moisture content, pH, reconstitution time, potency, sterility, endotoxins and particle content. Lyophilisations were performed in a Lyomega 180 ST, (TELSTAR). As general results, the modifications of the operating parameters allowed the reduction of 8 hours of cycle duration, and a significant decrease of the residual moisture content in the product.

Introduction

At the beginning of 2017, the UEB AICA +, as part of its production strategy, inaugurated a new industrial plant for producing lyophilized injectables. An investment realized by the enterprise in a lyophilizer equipment enabled the technological transfer of some of the freeze-dried products of the UEB LIORAD Laboratories, including the lyophilized injectable Omeprazole 40 mg. Taking into account these antecedents and the new equipment, this work deals with the adaptation of a lyophilization recipe for Omeprazol 40 mg, transferred from the UEB LIORAD Laboratories to the UEB AICA+, in order to decrease the cycle duration and increase production capacity.

Materials and Methods

Eight batches of Omeprazol 40 mg were lyophilized: three with the transferred recipe (UEB LIORAD Laboratories) and the rest with the modified recipe (UEB AICA+). Lyophilizer: industrial freeze dryer LYOMEGA 180 ST DH-RC (TELSTAR, Spain), with 16m^2 and 360 kg of ice. Product quality attributes evaluated: organoleptic characteristics, residual moisture content (Karl Fischer), pH, reconstitution time, sterility, endotoxins and particule content.

Results and Discussion

Table 1. *Comparison of transferred and modified cycles according with cycle parameters..*

Cycle stage	Parameter	Transferred cycle	Modified cycle
Loading	Shelf temperature (in respect of the loading room)	T < T of loading room	T = T of loading room
Freezing	Shelves temperature homogenization	No	-15 °C
	Final freezing temperature	15 °C below T_g`	15 °C below T_g`
Prymary drying	Temperature	5 to 10 °C below T_c	5 to 10 °C below T_c
	Pressure interval	0.080-0.120 mbar	0.130-0.180 mbar

Secondary drying	Temperature Pressure interval	Possible maximum 0 mbar (maximum vacuum)	Possible maximum twice the primary drying pressure

The informations from PIRANI sensors of condenser and chamber were used to determine respectively the endpoint of primary drying and secondary drying.

Table 2. *General results.*

Caracteristic	Transferred cycle	Modified cycle
Total duration	32 hours	24 hours
Residual moisture content	2.2 %	< 1 %

As can be seen in Table 2, the modified recipe has shorter cycle duration and a lesser resisual moisture content; since all batches fulfilled established requirements for the evaluated product quality attributes, the operating parameters can be used as criteria for cycle improvement.

Conclusions

A significant reduction of cycle duration and residual moisture content, for a lyophilization cycle of Omeprazol 40 mg, was achieved by proper modification of the operating parameters from the previous recipe.

References

[1] X. Ch. Tan, M. J. Pikal, Design of freeze-drying processes for pharmaceuticals: practical advice, Pharm. Research. 21 (2004) 191-200.
[2] Sh. Rambhatla, R. Ramot, Ch. Bhugra, M. J. Pikal, Heat and mass-transfer scale-up issues during freeze-drying: II. Control and characterization of the degree of supercooling. AAPS PharmSciTech. 5 (4) article 58 (2004). http://www.aapspharmscitech.com

Application of through vial impedance spectroscopy (TVIS) for the determination of ice nucleation, solidification end point, and mannitol crystallization during freezing and re-heating

Ogugua, L.[a], Orun, A.[a], Oyinlola M.[b], Smith, G.[a]

[a] DMU LyoGroup, School of Pharmacy and [b] Institute of Energy and Sustainable Development, De Montfort University, Leicester, LE1 9BH

*E-mail of the corresponding author: gsmith02@dmu.ac.uk

Abstract

Studies of the freezing behavior of aqueous solutions of mannitol are highly relevant to lyophilization process development given the prevalent use of this excipient. Here, through-vial impedance spectroscopy (TVIS) was used to study the behaviour of an aqueous solution of 5%w/v mannitol during a freezing and re-heating cycle. Temperature calibration of the TVIS parameter $\boldsymbol{F_{PEAK}}$ enabled the determination of the ice nucleation temperature $\boldsymbol{T_n}$ at −13 °C while the ice solidification end point was observed based on the time profile of $\boldsymbol{C'(0.2MHz)}$, i.e. the real part capacitance at 0.2 MHz. A time difference of 20 min between the onset and end point then defines the ice solidification time $\boldsymbol{t_i}$. A later step in $\boldsymbol{C'(0.2MHz)}$ indicated that mannitol crystallized at -30 °C and 20 minutes from end of ice solidification. Upon reheating at 0.2 °C/min, a large increase in $\boldsymbol{C'(0.2MHz)}$was seen at −30 °C indicating the onset of melting of mannitol crystals which then lasted 20 min.

Introduction

The initial freezing step, and any subsequent thermal treatment (annealing) of mannitol-containing formulations are critical to the efficiency and outcomes of a lyo cycle; given that the effective use of mannitol (as a bulking agent that improves the mechanical strength of the cake) necessitates the crystallization of this compound during the initial freezing or subsequent annealing stages. Laboratory studies of aqueous mannitol solutions (DSC, CSM and XRD) observed ice formation and mannitol crystalization at −20 °C and −30 °C respectively, and a melting endotherm of mannitol at −30 °C on re-heating[1]. Whether this behavior is replicated in the larger volume of a freeze-drying vial is the focus of this study.

Method

Through-vial impedance spectroscopy recorded the real and imaginary capacitance spectra of a 10 mL glass tubing vial, with a pair of 10x18 mm electrodes at 3 mm from the base, and containing 3.5 g of a 5% w/v aqueous mannitol solution. Thermocouple temperatures were recorded in a near neighbor vial. Measurements were taken at intervals of 2 min during a 0.5 °C min-1 freezing ramp to -40 °C followed by a 0.2 oC min-1 annealing ramp to -20 °C.

Results and Conclusion

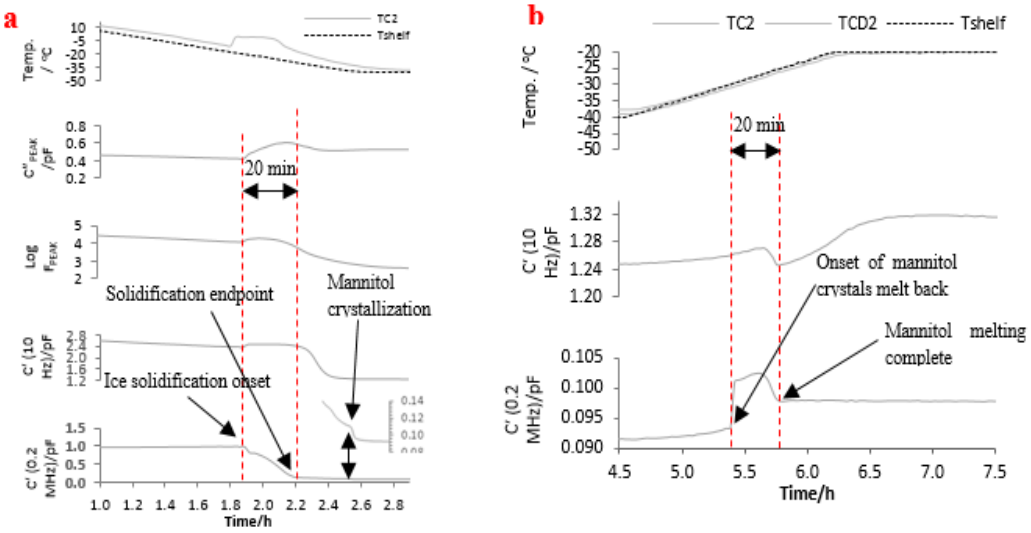


Fig.1 *a) Shows how C"$_{PEAK}$, Log F$_{PEAK}$ and Temperature change with time; events that occur with time. b) Demonstrates C"$_{PEAK}$ response to the melting mannitol crystals during re-heating period.*

Fig. 1a demonstrates that the classic spike in temperature, which is associated with the onset of ice formation, is mimicked by all four TVIS parameters: $\boldsymbol{F_{PEAK}}$, $\boldsymbol{C''_{PEAK}}$, $\boldsymbol{C'(10Hz)}$, and $\boldsymbol{C'(0.2MHz)}$. The explanation is partly due to the temperature spike but mainly due to the dielectric relaxation mechanism changing from the liquid-state Maxwell-Wagner polarization to the solid-state dielectric relaxation of ice. Once ice formation is complete there will be no contribution from the MW process and therefore any further change in temperature (from the loss of the excess latent heat of ice crystallization and the progressive cooling of the shelf) will be reflected in the dielectric parameters of ice and the unfrozen fraction. However, given that the high frequency dielectric propertries of ice are temperature independent then $\boldsymbol{C'(0.2MHz)}$ turns out to be the best TVIS parameter for determining the ice solidification end point (ISE) and any subsequent events, such as mannitol crystallization (observed 20 min after the ISE) and mannitol melt back on re-heating (Fig. 1b). Calibration of the TVIS parameter $\boldsymbol{F_{PEAK}}$ then allows for the temperatures of these events to be recorded, i.e. –13 °C for ice nucleation temperature, and – 30 °C for both the crystallization and the subsequent re-melt of mannitol. $\boldsymbol{F_{PEAK}}$is also a good indicator of the point at which the product achieves thermal equilibrium with the shelf.

References

[1] V.L. Kett, S. Fitzpatrick, B. Cooper, D.Q.M Craig. An Investigation Into the Subambient Behavior of Aqueous Mannitol Solutions Using Differential Scanning Calorimetry, Cold Stage Microscopy, and X-Ray Diffractometry. J Pharm Sci 92 (2003) 1919–1929.

Infrared based real time monitoring of the freezing of pharmaceuticals

Maniaci, R., Colucci, D., Fissore, D.
[a] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy

*E-mail of the corresponding author: domenico.colucci@polito.it

Abstract

In this work a thermal imaging system was successfully applied to the real time monitoring of the freezing of pharmaceutical products. The thermal gradients inside the frozen product and the velocity of the freezing front, were obtained directly from the analysis of the thermal images. Empirical and mechanistic models were used, given these variables, to infer the axial pore distribution, that is the mass transfer resistance inside the product during primary drying. Given the operating conditions, the presented PAT allows a real time estimation of the drying time and product temperature during primary drying, thus the monitoring and optimization of the whole freeze-drying cycle, without any kind of simulation of the freezing process.

Introduction

In the recent years IR imaging has been proposed as a non-invasive technology for real time monitoring and control of a vacuum freeze-drying process [1, 2]. All the application presented in literature so far, discuss the monitoring of the primary drying step, the longest and the most critical step of the whole process. Nevertheless, the freezing step has a dramatic importance on the quality and global economy of the freeze-drying process of pharmaceutical products, since the actual distribution of the crystals formed will determine the pore structure inside the product. The dimension of the pores affects the velocity of sublimation and desorption, that is the mass transfer inside the product in the drying stages. Since the product temperature is a tradeoff between the heat supplied and removed via sublimation, it might also affect the product stability and the preservation of the APIs. A proper design of the freezing might help reduce the heterogeneity and the off-specification product inside the batch. In this work we present a Process Analytical Technology (PAT), based on an infrared camera placed inside the drying chamber, that extracts some vital information on the evolution of the freezing step for several vials of the batch, and infers the product structure.

Materials and Methods

Freezing tests were performed in a lab scale equipment (LyoBeta 25™ Telstar, Spain) and, in each one, four vials 10R (ISO 8362-1) were filled with 5 ml of solution and monitored using the sensor presented by Lietta et al. (2019). 5% and 10% b.w. sucrose, as well as 5% b.w. mannitol solutions were tested. Two full freeze-drying cycles were performed to measure the resulting pore structure from the analysis of the SEM images.

The thermal images were pretreated and segmented to isolate the region corresponding to the product, then three different axial temperature profiles were extracted and averaged. Since both nucleation and crystal growth are exothermic phenomena the axial position of the freezing front can be detected as a maximum of temperature. Given also the temperature at the bottom it is possible to define the thermal gradients inside the frozen layer, G, and the velocity of the freezing front, R. Both an empirical [3] and mechanistic [4] model were used to relate these parameters to the average axial pore distribution. The experimental ending time and maximum temperature along the primary drying

were compared with the values simulated using a simplified model [5] of the process and the inferred pore distribution.

Conclusions

Infrared imaging stands as an effective, non-invasive technology, for monitoring and control of the freeze-drying process. No previous work addressed the possibility to infer, in real time, the pore structure of the frozen product. In all the case studies presented we were able to both, correctly predict the ending time and the maximum temperature experienced by the product during the primary drying phase. The presented PAT could strongly boost the implementation of a real and effective Quality by Design of the vacuum freeze-drying process of pharmaceutical products.

References

[1] E. Lietta. D. Colucci, G. Distefano, D. Fissore. On the use of IR thermography for monitoring a vial freeze-drying process. J. Pharm. Sci.108 (2019) 391-398.
[2] P.J. Van Bockstal, J. Corver, L. De Meyer, C. Vervaet, T. De Beer, T. Thermal imaging as a noncontact inline process analytical tool for product temperature monitoring during continuous freeze-drying of unit doses. Anal. Chem. 90(2018) 13591-13599.
[3] K. Nakagawa A. Hottot S. Vessot, J. Andrieu. Modeling of freezing step during freeze-drying of drugs in vials. AIChE J. 53(2007) 1362-1372.
[4] A. Arsiccio, A.A. Barresi, R. Pisano, Prediction of ice crystal size distribution after freezing of pharmaceutical solutions. Cryst. Growth Des. 17(2017) 4573-4581.
[5] Fissore D. and Pisano R., 2015, Computer-aided framework for the design of freeze-drying cycles: Optimization of the operating conditions of the primary drying stage. Processes, 3(2), 406-421.

Influence of the stoppers on the moisture content of the lyophilized injectable

Aguila, G.*; Nodar, Y.; Peréz, M; Garcia, A
Department of Technology, Technical-Productive Management, Company Laboratories AICA+

*E-mail germana@aica.cu mailto:roberto.pisano@polito.it

Abstract

The stability of a lyophilized product is very sensitive to the effect of residual moisture, for this reason it is important to control it at each stage of the lyophilization process, as well as in the preparation of the packaging material to be used. It was determined the moisture content of 75 bromobutyl stoppers (20 mm). The stoppers were sterilized by steam and dried in oven, and their moisture content was determined by a thermo-gravimetric method. It was evaluated the influence of the moisture content on the weight of the bulbs of omeprazole, vancomycin, acyclovir and chloramphenicol. The moisture content of the industrial lots of omeprazole was evaluated during nine months by the Karl Fischer technique, using sterile stoppers and sterile and dry stoppers, keeping the moisture content within the established limits. It was determined that the weight of the sterilized stoppers increased, as consequence of the moisture absorption of moisture caused by the sterilization.

Introduction

The bromobutyl plugs acquire during the sterilization a measurable amount of water that could be transferred to the lyophilized product. Therefore, drying the corks is an essential process with a view to guarantee the quality of the product. [1] Therefore, we have set ourselves the objective of determining the amount of water acquired by the bromobutyl plugs during sterilization and their influence on the finished product.

Materials and Methods

The bromobutyl stoppers were sterilized in an autoclave and then dried in the oven for five hours at 80 °C, weighings were carried out at different times. The water content was determined by a thermo-gravimetric method. The residual moisture for lyophilisates was determined by the Karl Fischer method, and a statistical analysis of the data obtained was carried out.

Results

The bromobutyl rubber stoppers subjected to the wet heat sterilization process show an increase in weight, due to the moisture acquired during this process as shown in Table 1.

Table 1. *Average mass of water acquired during sterilization and eliminated in drying*

Batch	Weight before sterilizes (g)	Weight esterile stoppers (g)	Acquired water mass (g)	Weight dry stoppers (g)	Water mass removed (g)
47001	2,3774	2,3809	0,0036	2,3721	0,0053
47002	2,3742	2,3778	0,0037	2,3684	0,0058
47003	2,3729	2,3767	0,0038	2,3677	0,0052

The drying of the sterile bromobutyl stoppers allowed the water content to be reduced, the moisture gain of the injectable omeprazole being higher than vancomycin, according to the average weight of its lyophilized masses. According to the specifications, the average weight of the plugs must be between 2-3 g, the values obtained are within the established limits and adequately meet the required characteristics.

Conclusions

The industrial lots of the lyophilized injectable omeprazole, using sterile and dry sterile plugs, keep the moisture content in the limit established during the nine months of study, in cases where only sterilized plugs are used, the humidity values are higher. During the sterilization process the bromobutyl plugs acquire an average of 6 mg of water, which is why it is necessary to carry out the drying operation so that this water is not transferred to the lifelike product.

References

[1] Wang, Brenda A., Frankes I. "Determination of moisture in rubber stoppers: effect of Karl Fischer oven temperatures". PDA Journal ofPharmaceutical Science & Technology. Vol. 55 No.3, pág.12-15, 2014.

Determination of the endpoint of the primary drying in the control of the lyophilization process

Aguila, G. [a*]; Peréz, M.[b]; García, A [c]; Zárate, Y.[d]

[a*, b, c.] Department of Technology, Technical-Productive Management, Company Laboratories AICA+

[d] Center for Genetic Engineering and Biotechnology CIGB

*E-mail of the corresponding author: tamaradg@infomed.sld.cu

Abstract

Freeze drying is an expensive process that involves several auxiliary systems and engineers and requires an extensive processing time. Therefore, the reduction of processing time is a key objective, especially in primary drying. The increase of the programmed temperature for the secondary drying, before the end of the primary drying, will cause the collapse of the product and the rejection of the lot, which will affect both the quality and the economy of the process, for which it is considered very critical to detect the final point. of the primary drying.

Introduction

The automatic loading/unloading systems due to their design are totally incompatible with the methods traditionally used in the industry to determine the end point of the primary drying, since they do not allow the placement of product thermocouples. Therefore, the objectives of this work are the evaluation of a method that allows to determine, in a precise way, the end point of the primary drying and the analysis of the behavior of the residual moisture and the organoleptic characteristics in 3 industrial lots manufactured with the proposed technique.

Materials and Methods

The experiments were performed with mannitol 3,6 % and omeprazole sodium 40 mg as model systems, the results of three methods were compared: 1) comparative pressure measurement (resistive Vs capacitive) 2) dewpoint sensor and 3) thermocouple of the product. The percentage of residual humidity of the product was determined by the Karl Fischer method in the apparent end point of the primary drying for each of them the structure of the tablet and the organoleptic characteristics were evaluated visually.

Results

As shown in Table 1, the pressure comparison and dew point sensor methods showed the lowest levels of residual moisture at the apparent endpoint of primary drying, both techniques are representative of the entire lot. Obtaining the highest values with the thermocouple method of the product, which is a unitary technique. Table 2 shows that there are no significant differences in relation to the homogeneity of drying between 3 industrial batches made with the pressure comparison method as a technique to determine the end point of primary drying. The values of residual moisture obtained are statistically similar, with a reliability of 95 %, since in all cases the value of p is greater than the level of significance of 0,05.

Table 1. *Residual moisture values at the end point of the primary drying*

Number of samples	Hr (%)		
	Pressure comparison	Dew point sensor	Product thermocouple
80	20,53 (%)	21,55 (%)	23,51 (%)

Table 2. *Statistical analysis of drying homogeneity and residual moisture (Hr)*

Variable	Batch	Value of p	Variance	Minimum	Maximum
Hr (%)	04887	0,1792	0,0182	1,21	1,72
	04888	0,9978	0,0178	1,23	1,83
	04889	0,2887	0,0182	1,22	1,72

Conclusions

The comparative measurement of the pressure was the most effective method to determine the endpoint of the primary drying. In addition, it is a technique representative of the whole lot, non-invasive, inexpensive, sterilizable by steam, easy to install without modification to the lyophilizer, not questioned by regulatory authorities and the industrial scale batches manufactured with this determination meet the established quality specifications.

Procedure for the Maintenance of Industrial Freeze Dryers

Castillo L. *
Pharmaceutical Laboratories AICA+, BioCubaFarma, La Habana, Cuba

*E-mail of the corresponding author: *livanc@aica.cu

Abstract
The company AICA Laboratories, belonging to BIOCUBAFARMA, is a company that is dedicated to the production of injectables in the pharmaceutical presentation of liquid injectables, lyophilized and eye drops. Taking into account the economic difficulties faced by Cuba in the last decades, the task of maintenance has become a challenge for Cuban engineers, motivated mainly by the obstacles presented for the timely arrival of parts and supplies for the maintenance of the teams. The objective of this work is to design a monitoring and maintenance procedure for lyophilizers to keep them in a high technical disposition, to ensure the production of medicines that comply with the quality specifications established in the current International Pharmaceutical Regulations. This methodology was based on the realization of a series of controls and actions that must be carried out from the daily frequency to once every two years. The information must be collected and recorded in a precise way so that it is useful for the following maintenance operations. In addition, in the engineering staff, a more specialized knowledge of the lyophilization process, interpretation of the process curves; As well as the study of the freezing curve, rate of descent, time of permanence at low temperature. Study the development of the primary drying, knowing all the lines of the process, condenser, product and trays, as well as vacuum relating them to each other, to know the temperature, time and vacuum of the secondary drying and to master well the problems related to the freeze-dryer, that can occur Inside the sterile room such as: Door closure, rubber seal cleaning, internal cleaning of the freeze dryer, placement of temperature probes, control of probes cables, correct positioning of the vial plugs and movement of the product trays, etc.

Introduction
Maintenance means keeping the equipment in good operation conditions, increasing its working life, avoiding mechanic and electrical failures and making sure that all processes are carried out following the correct way.

A successful maintenance schedule depends mainly on the continuous data acquisition and its adequate analysis, this way the maintenance schedule must be turned into a live document which must be modified depending on the problems and experiences acquired when working with the equipment.

Taking in consideration the economic difficulties faced by Cuba during the last decades, a maintenance procedure has become a challenge to the Cuban engineers, motivated mostly by all the obstacles and scarcity of spare parts when performing the maintenance.

Materials and Methods
This methodology is based on a number of controls and procedures that must be performed from daily controls to one time every two years. The data must be collected and stored in a precise way, in order to use it during the next maintenance operations.

The personnel must have knowledge of the manual content and must be prepared to find the necessary topics when needed such as spare parts, consumption tables, schemes etc. The

technician and the operator have to know the equipment most frequent failures and so the entire freeze-dried process.

Conclusions

1. A successful maintenance schedule depends mainly on the continuous data acquisition and its adequate analysis.
2. The maintenance schedule must be turned into a live document, which must be modified depending on the problems and experiences acquired when working with the equipment.

Evaluation of thermal imaging as a non-contact in-line process analytical tool for product temperature monitoring during continuous freeze-drying of unit doses

Pieter-Jan Van Bockstal[a], Jos Corver[b], Thomas De Beer[a,b]
[a] Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium
[b] RheVita, Ghent, Belgium

*E-mail of the corresponding author: pieterjan.vanbockstal@ugent.be

Abstract

- Continuous freeze-drying based upon spin freezing and IR heat transfer strongly increases process efficiency and improves product quality (uniformity)

- Standard temperature sensors applied in batch freeze-drying are invasive, do not allow proper critical product monitoring and control, and are incompatible with continuous freeze-drying concept

- Thermal imaging allows continuous reliable and precise non-contact critical product temperature (i.e., temperature at sublimation front) determination and control

Introduction

Recently, a continuous freeze-drying concept for unit doses based on spinning the vials during freezing and on optimal energy supply during drying is presented which strongly increases process efficiency and improves product quality (uniformity) compared to traditional batch freeze-drying.[1,2] To obtain an elegant dried product appearance, the product temperature at the sublimation interface T_i should be kept below the critical temperature $T_{i,crit}$ during the entire primary drying step. The invasive standard temperature sensors applied in batch freeze-drying provide unreliable temperature data and are incompatible with the continuous freeze-drying concept.[3] As the sublimation front of the spin frozen vials moves from the inner side of the cake towards the glass wall, thermal imaging offers the opportunity for the correct measurement of T_i during the entire primary drying step.[4]

Materials and Methods

Vials were spin frozen and dried under vacuum. The energy transfer for ice sublimation was provided via non-contact infrared (IR) radiation. The drying progress was monitored via in-line near-infrared (NIR) spectroscopy and thermal imaging, making use of an IR camera.

Results

Via Fourier's law of thermal conduction, the temperature gradient over the vial wall and ice layer was quantified, which allowed the constant monitoring of T_i. Based on these thermal images, the IR energy transfer was computed via the Stefan-Boltzmann law and the dried product mass transfer resistance Rp profile was determined. This procedure allows the determination of the optimal dynamic IR heater temperature profile for the continuous freeze-drying of several products, maintaining T_i below $T_{i,crit}$. In addition, the endpoint of primary drying was detected via thermal imaging and confirmed by in-line NIR spectroscopy.

Conclusions

Thermal imaging allows for the first time the correct continuous measurement of one of the most critical parameters during the primary drying step, i.e., the product temperature at the sublimation front T_i.

References

[1] L. De Meyer, P.J. Van Bockstal, J. Corver, C. Vervaet, J.P. Remon, T. De Beer, Evaluation of spin freezing versus conventional freezing as part of a continuous pharmaceutical freeze-drying concept for unit doses. Int. J. Pharm. 496 (2015) 75-85.

[2] P.J. Van Bockstal, L. De Meyer, J. Corver, C. Vervaet, T. De Beer, Noncontact infrared-mediated heat transfer during continuous freeze-drying of unit doses. J. Pharm. Sci. 106 (2017) 71-82.

[3] S. Nail, S. Tchessalov, E. Shalaev, A. Ganguly, E. Renzi, F. Dimarco, L. Wegiel, S. Ferris, W. Kessler, M. Pikal, G. Sacha, A. Alexeenko, T.N. Thompson, C. Reiter, J. Searles, P. Coiteux, Recommended Best Practices for Process Monitoring Instrumentation in Pharmaceutical Freeze Drying—2017. AAPS PharmSciTech 18 (2017) 2379–2393.

[4] P.J. Van Bockstal, J. Corver, L. De Meyer, C. Vervaet, T. De Beer, Thermal Imaging as a Noncontact Inline Process Analytical Tool for Product Temperature Monitoring during Continuous Freeze-Drying of Unit Doses, Anal. Chem. 90 (2018) 13591–13599.

Freeze-Drying in Atypical Vials: How to Model Heat Transfer During Sublimation by Using 3D Mathematical Modelling

Buceta, J. [a*]; Passot S.[a]; Scutellà B.[b]; Fonseca F.[a]; Bourlès E.[b]; Trelea, I.C.[a]

[a] UMR GMPA, AgroParisTech, INRA, Université Paris Saclay, 78850 Thiverval-Grignon, France

[b] Technical R&D Drug Product GSK, 1330 Rixensart, Belgium

*juan.buceta-correa-de-borba@inra.fr

Abstract

In pharmaceutical freeze-drying, different types of product container can be used: glass tubing vials, syringe, ampoules, micro well-plates, etc. By changing the heat and mass transfers, the choice of container can significantly influence the final product quality. The objective of this work was to investigate the heat transfer during ice sublimation in a novel container specifically designed for accelerating formulation development. A 3D mechanistic model was proposed to describe heat transfer phenomena. Model predictions of temperatures and heat flows were validated with experimental measurements performed in a wide range of operating conditions. The developed model made it possible to quantify the different heat transfer mechanisms and to assess the effect of container's geometry.

Introduction

Vaccines are usually freeze-dried to preserve their quality while being shipped and stored. However, because of the successive steps of freezing and drying, the active ingredient can suffer irreversible damages. An appropriate choice of stabilizing excipients is thus required to protect the active ingredient not only during the process but also throughout long term storage. Defining an optimal formulation ensuring vaccines' stability while reducing freeze-drying cycle time remains a time-consuming and labour-intensive work. Recently, specific devices combining 96 well-plates and really small glass vials (less than 1 mL filled volume) were proposed to improve the efficiency of formulation screening process. Such devices make it possible to increase the number of formulations tested while reducing the amount of active ingredient needed. Very few research has been done on modelling freeze-drying with this kind of device [1]. The present work uses 3D mathematical modelling to understand the heat transfer mechanisms during sublimation in atypical containers based on the work of Scutellà et al. (2017) for serum vials [2]. The VirTis® 96 Well Freeze Drying System (SP Scientific, Stone Ridge, NY, USA), consisting in glass vials and aluminium well plate was selected for our study.

Materials and Methods

1536 vials (500 and 1000 µL type) were filled with distilled water (400 and 600 µL, respectively) and placed in 16 well-plates (WPs). Sublimation mass flows were gravimetrically determined during tests carried out in an Epsilon 2-25D Martin Christ freeze-dryer (Osterode am Harz, DE). WP temperatures were measured using 16 thermocouples in a Millrock freeze-dryer (Kingstone, NY, USA). Operating conditions were chamber pressure from 4 to 65 Pa and shelf temperature from - 40 to - 15 °C. Heat flows received by the vials were calculated based on the mass flows. A 3D model of the heat transfer was developed using COMSOL® Multiphysics 5.3a (Burlington, USA) considering radiation and conduction.

Results

Predicted heat flows and WP temperatures were validated with experimental data presenting a difference smaller than 6 %. For each WP, heat flows received by 1000 µL vials in the outermost positions were from 10 to 34 % higher than those received by other positions. Such difference was not observed in 500 µL vials. WP temperatures were considerably lower than shelf temperatures (up to 20 °C). Fig. 1 shows the geometry of the model and the predicted heat flux directions and temperature field.

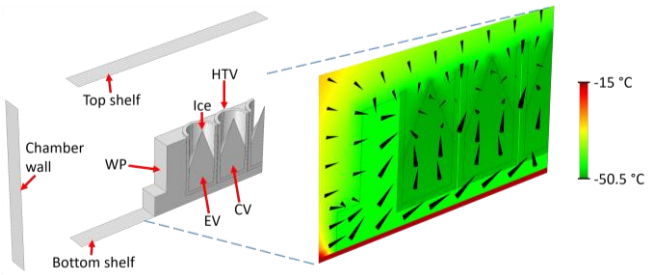


Fig. 1 *Heat fluxes and temperature field. Heat fluxes are represented as black triangles, the temperature field is shown according to the colour scale. 4 Pa, -15 °C, 500 µL vials.*

Conclusions

3D modelling proved to be an effective tool to predict heat flows and temperatures during sublimation in an atypical product container, providing information of the relative contribution of the heat transfer mechanisms. This model could be included in a future heat and mass transfer model of freeze-drying in atypical vials.

References

[1] S. von Graberg. Freeze Drying from Small Containers: Heat and Mass Transfer and Implications on Process Design (2011). Dissertation, Erlangen-Nuremberg Univ., DE.
[2] Scutellà, B. et al. 3D mathematical modelling to understand atypical heat transfer observed in vial freeze-drying. Applied thermal engineering 126, 226–236 (2017).

Scaling up of pharmaceutical freeze drying processes using mathematical modeling

van Bockxmeer, E., de Ruiter, R., Eppink, M.
Synthon Biopharmaceuticals BV, Nijmegen, The Netherlands [b] Department of Pharmaceutical Analysis, Ghent
*E-mail of the corresponding author: Ernst.vanBockxmeer@synthon.com

Abstract

Purpose

Scaling up freeze drying processes from R&D to large scale GMP manufacturing for biopharmaceuticals can be tedious and costly. Often the freeze drying parameters developed in R&D cannot be transferred one-to-one to large scale GMP facilities, which is mainly related to radiation effects of small scale versus large scale freeze dryers. Especially, primary drying (sublimation) phase parameters are critical. Mathematical modeling and design space calculations can aid in the transfer and scale up of freeze drying cycles. Applying simple tools and a simple strategy, only limited cycles need to be performed, saving costs, time and material.

Methods

Heat transfer coefficients (Kv) determination by using water filled batches in final drug product containers using 3-4 short cycles in R&D and 1 short cycle at full scale in GMP facility. Mass difference and temperature in containers is determined at several critical positions from which Kv can be calculated for each position in the freeze dryer.

Mass transfer coefficients (Rp, porosity) determination, design space calculations and model validation can be obtained by limited R&D tests using thermocouples placed in limited product filled vials (properly shielded) and by using a simple PAT tool (Pirani sensor).

Results

Kv measurements visualize the heat distribution over the shelves, from which cold and hot spots were derived. Kv of cold and hot spots in R&D were different from GMP. Rp measurements was obtained from R&D cycles performed at target and outer ranges, indicating porosity did not change within that range. Mathematical model was built based on Rp and Kv, and was validated by comparing theoretical to practical data. Design space was made based on model and the maximum product temperature (Tg' or Tcollapse), providing most target values for the critical parameters (Tshelf and Pchamber) during primary drying. GMP data from a single shelf loaded product confirmed the model calculations were correct.

Results

Mathematical modeling of primary drying phase is a powerful tool to aid in development, scale up and transfer of freeze drying cycles, and can reduce time and costs, and material needs. It also provides the design space for the critical primary drying process parameters (Tshelf and Pchamber).

Acknowledgements

R. Pisano, D. Fissore, Department of Applied Science and Technology, Politecnico di Torino, Italy

B. Zaludek, Synthon BV, Czech Republic

List of Attendees

Ali Afnan
Step Change Pharma, USA

Germàn Aguila Pérez
Pharmaceutical Laboratories AICA, CU

Noor Al-Rifai
Johnson & Johnson, UK

Catalina Alvarez Campuzano
Universidad Nacional de Colombia, CO

Andrea Arsiccio
Politecnico di Torino, IT

Ana Bacaonau
Technical "Gh. Asachi" University of Iasi, RO

Richard Bafour Asare
Hamta and Sons, Gana

Antonello Barresi
Politecnico di Torino, IT

Serena Bobba
Merck Serono, IT

Israel Borges Sebastiao
Pfizer, USA

Erwan Bourlès
GSK Vaccines, BE

Juan Patricio Buceta
INRA, FR

Idania Caballero
CIM, CU

Salvatore Carmisciano
Novartis, SI

Mattia Cassanelli
Biopharma Process Systems Limited, UK

Livan Catillo Gonzalez
Pharmaceuticals Laboratories AICA, CU

Du Hyung Choi
Inje University, KR

Gloria Clua Palua
Laboratorio Reig Jofre, ES

Domenico Colucci
Politecnico di Torino, IT

Jos Corver
RheaVita, NL

Sean Cullen
Sanofi, IE

Sarah Daller
AbbVie Deutschland GmbH&Co.KG, DE

Thomas De Beer
Ghent University, BE

Ann De Saeger
Janssen Pharmaceutica, BE

Sophie Declomesnil
4DPharma plc, UK

BertDekens
Hosokawa, NL

David Egan
University of Limerick, IE

Sevinc Ferrari
National Veterinary Institute (SVA), SE

Fernanda Fonseca
AgroParisTech INRA, FR

Wolfgang Friess
Ludwig-Maximilians-Universität München, DE

Arnab Ganguly
IMA Life, USA

Alejandro Garcia Varona
Pharmaceutical Laboratories AICA, CU

Valeria Gervasi
University College Cork, IE

Henning Gieseler
Friedrich Alexander University Erlangen-Nürnberg, DE

Helene Greige
Lebanese University, LB

Maik Guttzeit
Bayer Pharmaceuticals, DE

Eun-Sol Ha
Pusan National University, KR

Maite Harguindeguy
Politecnico di Torino, IT

Frank Harms
Martin Christ Gefriertrocknungsanlagen GmbH, DE

Zhongping Huang
West Chester University of Pennsylvania, USA

Olukayode Imole
Hosokawa, NL

Ken-ichi Izutsu
National Institute of Health Sciences, JP

Vyankat Jadhav
Tridiagonal Solutions, IND

Eunha Jang
Inje University, KR

Yowwares Jeeraruangrattana
De Montfort University, UK

Ji-Su Jeong
Pusan National University, KR

Seonghoon Jeong
Dongguk University, KR

Parth Joshi
University of Limerick, IE

Tuomas Kaukanen
Orion Diagnostica Oy, FI

Victoria Kett
Queen's University of Belfast, UK

Min-Soo Kim
Pusan National University, KR

Seong Jun Kim
Dongguk University, KR

Petra Knerr
Martin Christ Gefriertrocknungsanlagen GmbH, DE

Joris Lammens
Ghent University, BE

Benjamin Ledermann
GEA, DE

Seon Kwang Lee
Pusan National University, KR

Jaechul Lee
Dongguk University, KR

Laurens Leys
Ghent University, BE

Xiangliang Lin
Esco Aster PTE, SGP

Christian Lotz
Pfanstiehl, CH

Anička Matejčikovà
Slovak Technical University, SK

Paul Matejtschuk
The National Institute for Biological Standards and Control, UK

Yves Mayeresse
GlaxoSmithKline, BE

Ruth McNamara
Sanofi, IR

Joao Moreira
Process Systems Enterprise, UK

Steven Nail
Baxter BioPharma Solutions, USA

Kyuya Nakagawa
Kyoto University, JP

Ivette Novoa
National Center of Bioproducts, CU

Gust Nuytten
Ghent University, BE

Miseo Park
Pusan National University, KR

Roberto Pisano
Politecnico di Torino, IT

Fabian Plaum
HOF Sonderanlagenbau, DE

Pavol Rajniak
Slovak Technical University, SK

Susan Ríos
Ghent University, BE

Monica Rosa
Coriolis Pharma, DE

Jorge Sassone
Proquimo Improvement, UK

Gerhard Schilder
HOF Sonderanlagenbau GmbH, DE

Bernadette Scutellà
GSK Vaccines, BE

Ivonne Seifert
Ludwig-Maximilians-Universität München, DE

Yossi Shapira
Teva, IL

Woo-Yong Sim
Pusan National University, KR

Geoff Smith
De Montfort University, UK

Stephan Soyka
Bayer AG, DE

Andrew Strongrich
Purdue University, USA

Nachal Subramanian
Biopharma Process Systems, UK

Alexander Tambovzev
Optima pharma GmbH, DE

Serguei Tchessalov
Pfizer, USA

Kevin Tebrinke
Scientific Protein Laboratories LLC, USA

Elizabeth Topp
Purdue University, USA

Anand Vadesa
De Montfort University, UK

Alessandra Valone
BSP Pharmaceuticals, IT

Pieter-Jan Van Bockstal
Ghent University, BE

Ernst Van Bockxmeer
Synthon Biopharmaceuticals, NL

Pascal Van Der Cruyssen
Baxter R&D Europe, BE

Brecht Vanbillemont
Ghent University, BE

Kevin Ward
Biopharma, UK

Nunzio Zinfollino
Merck Sorono, IT