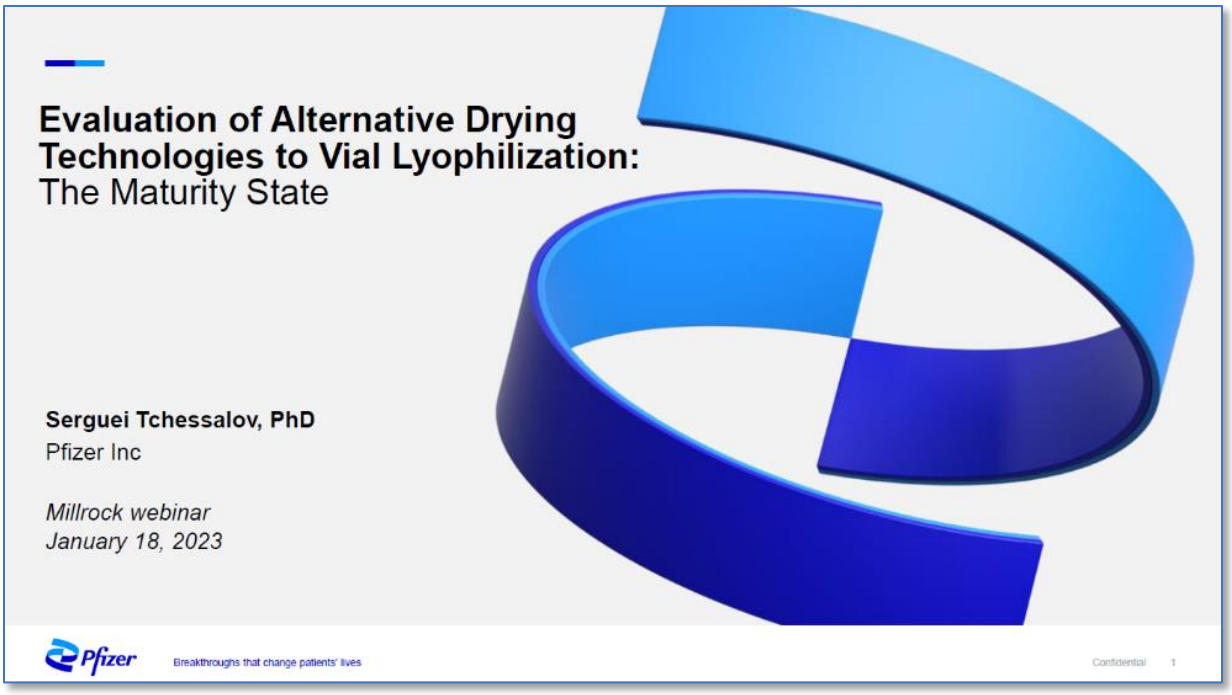
Evaluation of Alternative Drying Technologies to Vial Lyophilization: The Maturity State

Edited Webinar Transcript 1/22/2024

Presented by Serguei Tchessalov, PhD., Pfizer Inc.

Today we will be talking about some advances in spray freeze-drying, touch a little bit on spin freeze-drying and a little bit on foam drying. Spray freeze-drying. I'm talking on behalf of the team, some team members listed there are from Pfizer as well as from Meridian technology.



Let’s take a look at both conventional vial freeze-drying and process and spray freeze-drying. In vial freeze-drying process what we do, we fill relatively accurately vials with liquid product, we move the vials off the shelf, cool it, freeze it, apply vacuum, dry it, stopper, and seal these vials. In spray freeze-drying process, you actually kind of do reverse operations. You first spray freeze it, particles, then you dry them. Ending up with very nice flowable powder, which you can actually, later, fill in any type of container as you can see, vials, dual chamber, bottles, bags, whatever.

So, if you compare those two technologies, for example, freezing time, it takes only seconds to freeze small particles. And it's very useful for products that require very fast freezing. Drying, because of small particles, you can dry about 40 times faster than you do the same product in the vial.

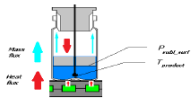
Conventional Vial Freeze-Drying (VFD) vs. Spray Freeze-Drying (SFD):

Vial freeze-drying

Liquid fill

Freezing

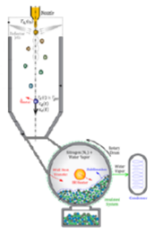
Drying



Potential benefits of Spray Freeze-Drying

Factor	VFD	SFD	Benefits/ application
Freezing time	Hours	Seconds	Temperature/freeze-concentration sensitive products
Drying time	Days	Hours	Up to 40-time faster drying (single layer), drying of bulk is longer
Low collapse products	Long cycles	Short cycles	Extremely low Tc (<-40°C) can be produced by SFD
Reconstitution time	Minutes	Seconds	Up to 7-time faster recon time
Accuracy of fill	<0.5% Liquid	<1.6% Pellets	No significant difference in accuracy of dose
Aseptic	Yes	Yes	Both technologies can produce sterile product
Flexibility in dose	No	Yes	SFD pellets can be filled in different types of containers

Spray Freeze-Drying



Pellets Fill

Containers

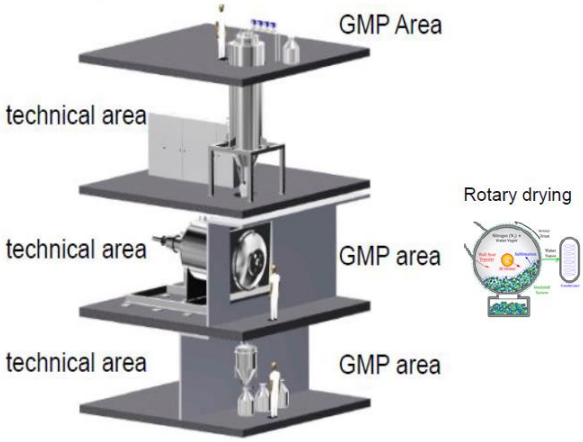
- Vials
- Dual chamber containers (syringes/cartridges/vials)
- Bottles
- Bags etc

Pfizer

Confidential 4

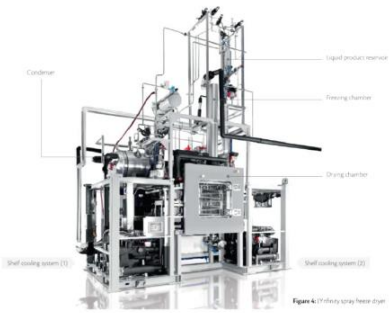
Meridion Technologies vs. IMA Life (Commercial Scales)

Meridion Rotary Drying (available to date)

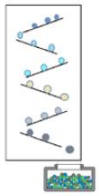


Rotary drying

IMA Life Conveyer (continuous) Drying (in development)



Drying chamber: Vibrating shelves/or conveyer belts



2 condensers allow continuous freeze-drying

Pfizer

- Bernhard Luy, Breckenridge 2012

ima.it/pharma/lab4life/publications/

Confidential 5

And actually, you can successfully do very localized product, even below -40 degrees Celsius, it can be done by spray freeze-drying. In the reconstitution, we demonstrated

that you could do as fast as seven times faster in spray freeze-drying compared to vial freeze-dried product.

Yes, accuracy is a little bit less than liquid product, but it's still good enough to deliver a dose, a required dose. Aseptic, yes, with both technology you can deliver aseptic product. In terms of flexibility, yes, spray freeze-drying definitely provides many more options as compared to vial freeze-drying.

So, what we have currently is, from a commercial point of view, we have two companies that can make commercial scale equipment. One of them is Meridion. And the Meridion technology, what you have, you have large column, dual column, in which you spray liquid product, it freezes, then it's collected in a drum. Frozen pellets, once the spraying process completed, valve is closed and vacuum applied you will be surprised, you can get up to five liter.

And then you provide heat through infrared radiation and contact at very low vacuum conditions. And then the process is complete, which you can use pressurized test, Pirani vs. Capacitance Manometer, you will basically remove product into a container which you can move anywhere to fill inside. So, in IMA Life, they have essentially the same freezing process. It's a low temperature column. But what they have, they have two condensers, and that's allowed to do continuous spray freeze-drying process. You freeze particles, you collect them in intermediate chamber, and then you dump them into a drying chamber, in which you have some sort of conveyor that you move frozen pellets until they actually dry. I will suggest you look at a link there and some details you can grab from their website.

Summary of Spray Freeze-Drying Lessons at Laboratory Scale

Study	Materials Tested	Observations	Challenges / Mitigation
Low collapse products	Materials with Tc<-35°C	Residual moisture <0.5% (allows RT stability) Turbidity of reconstituted solutions	Turbidity can be reduced by addition of surfactant
High concentration proteins	50 mg/ml mAb in 2% sucrose	After reconstitution, DP protein concentration above 200 mg/ml can be achieved, recon time can be reduced by factor of 7 (compared to VFD)	Above 200 mg/ml viscosity can exceed 60 cPs
Impact of surfactant~~PS-80~~ on turbid solutions	50 mg/ml mAb in 2% sucrose	By increasing concentration of surfactant to 2%, particles above 10µm can be reduced by 99%	Solution remains slightly turbid
Impact of particle size	50 mg/ml mAb in 2% sucrose	No difference in product quality (moisture, packing density) in the particle size range between 300 and 700 µm (throughput increases by at least 4-fold)	Reconstitution time for 300 µm particles was slightly longer
Stability of SFD proteins	3 mAbs in sugar-based formulations	2 years stability – comparable to VFD	Turbidity remains high for one mab
Stability of LNP-based products	LNP-based product in sucrose	At least 1 year stability – comparable to VFD	Initial increase in LNP size can be mitigated by annealing
SFD of small molecule entity	Commercially available antibiotic	Reconstitution time reduced by factor of 2	Ongoing stability study

No surfactant Surfactant

Summary: SFD was applied to different modalities demonstrating improvement in reconstitution time and comparable stability to vial freeze-drying

Pfizer

So, what we've done so far in terms of spray freeze-drying. And we did low collapse products. Collapse temperature was below -35 degrees Celsius, and we get moisture below half percent. And with some products, we see some turbidity of the reconstitution, but if you add some surfactant, it typically goes away. With spray freeze-drying, you get very high concentration. After lyophilizing you can reconstitute with less volume.

A vial versus the same amount of spray freeze dry powder, can be done seven times faster, which could be important. We tested because we see impact of ... Surfactant, we tested to degrees that works and we increased concentration up to 2%, which in the allowed to increase particle number ... particles above 10 microns decreased by factor of 100. But still the solution remains slightly turbid, so keep in mind that it can work, surfactant, but not for products.

Particle size. If you, for example, have certain micron particles versus 700 micron, for column it really doesn't matter. And by having less particle size larger, you can increase by doing that. And we tested what impact of this particle size on flowability, and we found there is at least in the range between 300 and 700 micron was no impact. Just a little bit longer recon time for 300 micron particles, but that was not significant. So stability, product, tested with three molecules antibody. Product was stable for three years. Very comparable to vial freeze-drying process.

LNP. We also tried LNP. It's also a comparable product to the vial freeze-dried material and it's stable for at least one year. Yes, it does increase particle size after spray freeze-drying, but if you do annealing steps that will go away. We also tried small molecules, a few of them, and we demonstrated that it's a good quality, but recon time can be significantly decreased. So, as you can see, we applied this technology to different modalities.

Summary of Experiments at Commercial scale (Meridion)

Spray freezing column

Rotary dryer

Products tested	Scale	Observations	Challenges / Mitigation
50-150 mg/mL mAb in trehalose	50-150L, particle size 0.6-0.8 mm	Yield 83-93%	Column clogging – avoided by temperature control and mechanical adaptations. Stickiness - reduced by lowering pressure Attrition - could be reduced by minimizing residence time and optimization of process parameters
Sucrose-NaCl-surfactant	100L	Initial yield: 64% (Garmish 2018) After modifications: >97%	Fluidization - managed by cycle optimization with implementation of PAT tool and modification of drum Electrostatic - could be managed by implementation of antistatic device
Dried pellets (Sucrose-NaCl) were filled with accuracy better than 1.7% in a range 10-57 mg by two independent vendors			

Summary: Different modalities can be produced by SFD at commercial scale (50-150L) with yield >97% and filled with the accuracy better than 2%

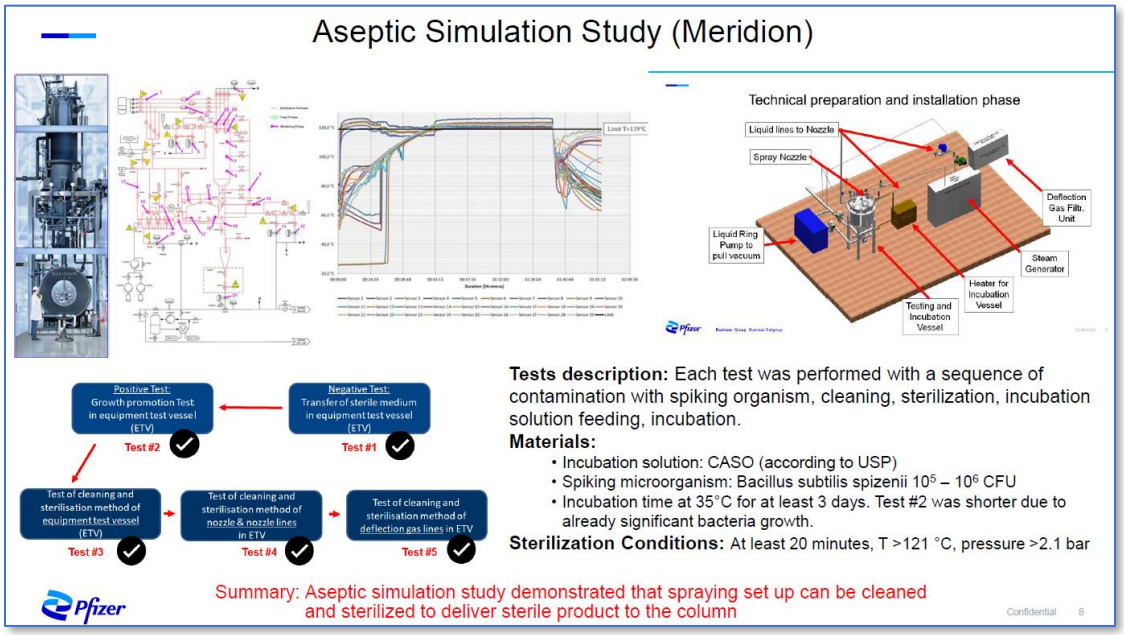
Pfizer

We also tried spray freeze-drying some of our product at the lab scale. We tested product in the concentration range, 50 to 15. And the particle size range, 0.6, 0.8 millimeters. And we got about 80, the lowest about 83% of yield. The reason we lost the product was some clogging in the column. Initially at least in the spraying process. But that can be eliminated by just lowering the temperature of this intermediate chamber between column and the drying chamber.

Stickiness. If you dry at high temperature, it seems like a product exceeds slightly glass transition temperature then it can stick to the wall. So, if you reduce pressure and they reduce high product temperature, that goes away. Attrition. Yes, particles remain in the drum and some friction occurs, and that can result in small particle dust, so the smallest time you hold particle in the chamber, the less impact.

So, we also tried very challenging, I would say, materials, sucrose sodium chloride. Some of those products can be very hard to freeze dry in the vial. When we started doing this work, we just got initial yield about 64%. And that was mainly due fluidization toward end of drying, so you can see the sublimation rate is so high that particles become so light, so it just carries out to condenser, so that's why we were losing so much product. But by implementation of some PT tools and modification of drum and mixing process, we were able to reduce this impact.

Electrostatic can be also removed by implementing antistatic device. With all this implementation, we are able to get consistently a yield more than 97%. And we tried this dry pellet with two companies that make powder filling equipment, and we get accuracy better than 1.7% in the range of between 10 and 57 milligrams. So, you can see that we demonstrated also that this different modality can be produced at large scale with high yield and filled accurately in any type of container.



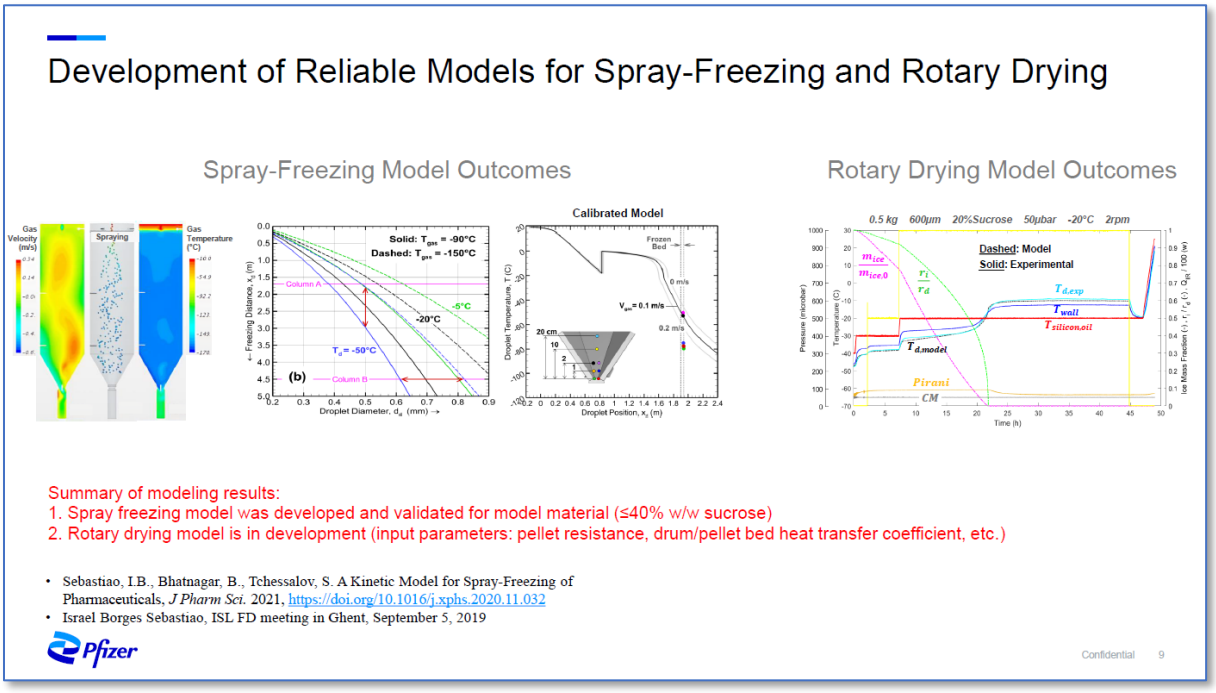
Another work that we did is to demonstrate that this technology can produce aseptic material. So back to 2018, I presented my first work from Meridion when they attached about 29 thermocouples in different locations with the spray freeze dryer. And we were able to demonstrate that you actually can reach the desired temperature, about 120 degrees Celsius, for at least half an hour. So basically, successfully sterilize internal surfaces of a dryer.

The challenge was that and the question came out, yes, you can do all of this, but the nozzle diameter looks small. It's about 0.3 millimeter. Then how can you control that? So, the next step was to demonstrate that you can actually aseptically deliver solutions through this nozzle and steam sterilize clean it, and that could be done in a robust manner. So, what we did is, you can see on the right, is that we took nozzle from the column, put in a vessel which had some jacket, and we can control temperature of this vessel. It has also had a steam generator, deflection gas filter unit, and it also has a liquid pump to remove product from that vessel.

So, what we did, we performed a few tests. The first test, we just simply add the incubation solution, CASO, through the filter into this vessel, wait for three days, at 35 degrees Celsius, it hasn't happened, so we demonstrate that it can be sterilized and kept sterile in the vessel. Then the second test, we intentionally contaminated with microorganisms and then after that it was basically added as an incubation solution, incubated. You can visually see the growth demonstrated. Yes, you can promote the growth. In test number three, we did all of this, plus after it was incubated, we steam sterilize it, clean it, and added an incubation solution. Wait for three days, nothing happened. So, we demonstrated that it can be cleaned and sterilized.

Those three tests were done without a nozzle. So, test number four when we added the nozzle and contaminated through the nozzle, we perform all of this testing again, and demonstrate that, "Okay. Even through the nozzle, we can contaminate, clean, steam sterilize, and the product will be sterile." In the test number five, last one, in addition to that, we also contaminated deflection lines and all the process was done exactly as the old test previously, except that we hold for about, I think, seven or eight days at 35 degrees Celsius, to make sure that nothing grows at all. So, we demonstrated that the product can be done in an aseptic way.

All right. So you need to also understand your process very well. And you can do numerous experiments. And at that time, we already purchased large scale equipment from Meridion. And you can do a lot of experimentation to understand your design space, or you can, in parallel, do some modeling. So first, we did some modeling of the column, and you can see some results of this. And we demonstrated that if you just sprayed without spreading those particles, the particles would move down toward the bottom of column. When the solution crystallizes, it needs heat. And that heat could impact how particles are moving and they can just coalesce ... so get to each other, form larger particles.

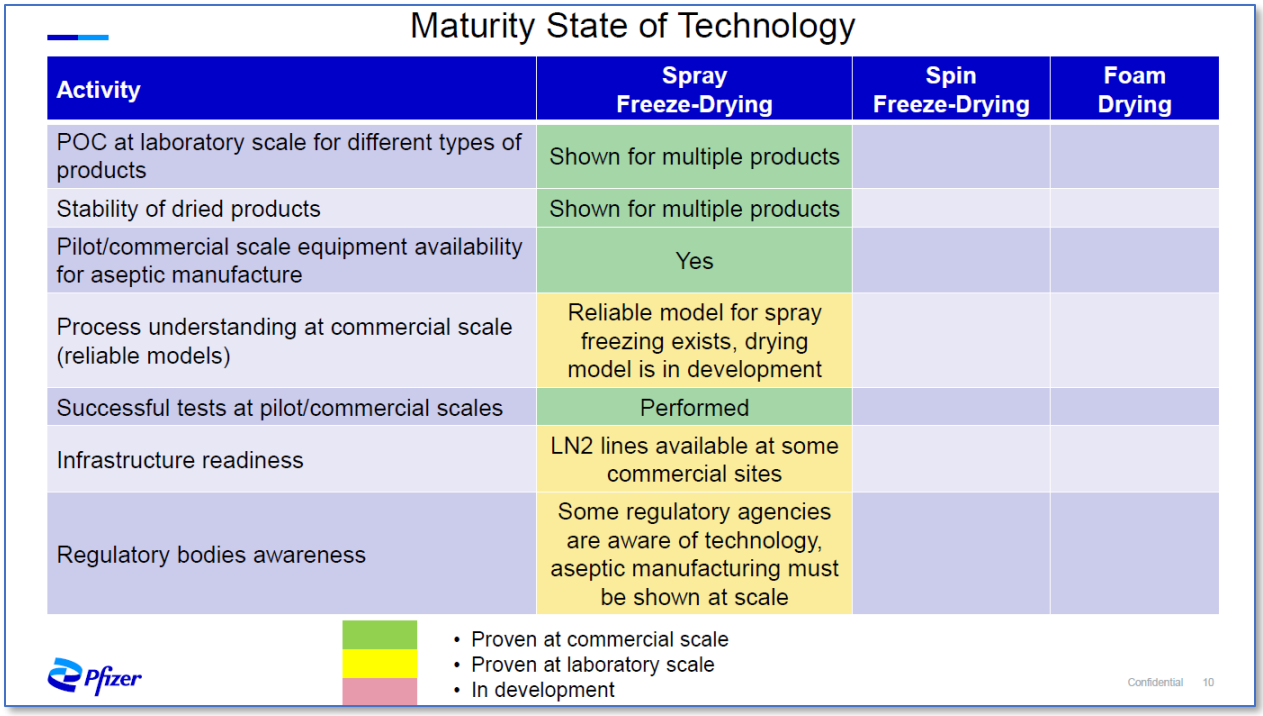


And to eliminate that, or minimize that, there was a deflection applied to this stream of liquid. And you can see that these particles are spread within the column, which A, improved freezing, and B, allowed less possibility for them to stick to each other. You do see some flow from the bottom to the top that could also impact particles moving within with the column. All of this was included as parameters into the model.

And you can see that that's how this modeling, where you can see impact of, for example in this particular case, temperature, column temperature. If y-axis is the freezing distance at the column, and on x-axis is droplet diameter, in millimeters. For example, for half millimeter pellets, in order to freeze product below -50 degrees Celsius, you need to have a column above three meters. But if you decrease column temperature to -150 degrees Celsius, you only need 1.6.

So, it helps you to design proper diameter and proper lengths of column. So, to confirm this model, we did some experimentations and we just simply put some cone made of Styrofoam inside a column at a certain distance, and we collect the frozen pellets, measure the temperature of those pellets, and compare to the results of modeling. And you can see the yellow line quite well. So, we were confident that we have very robust model of spray freezing. In terms of drying, we are working on generating good parameters, like for example, resistance within small pellets, as well as transfer coefficient from a heating surface to the pellets itself.

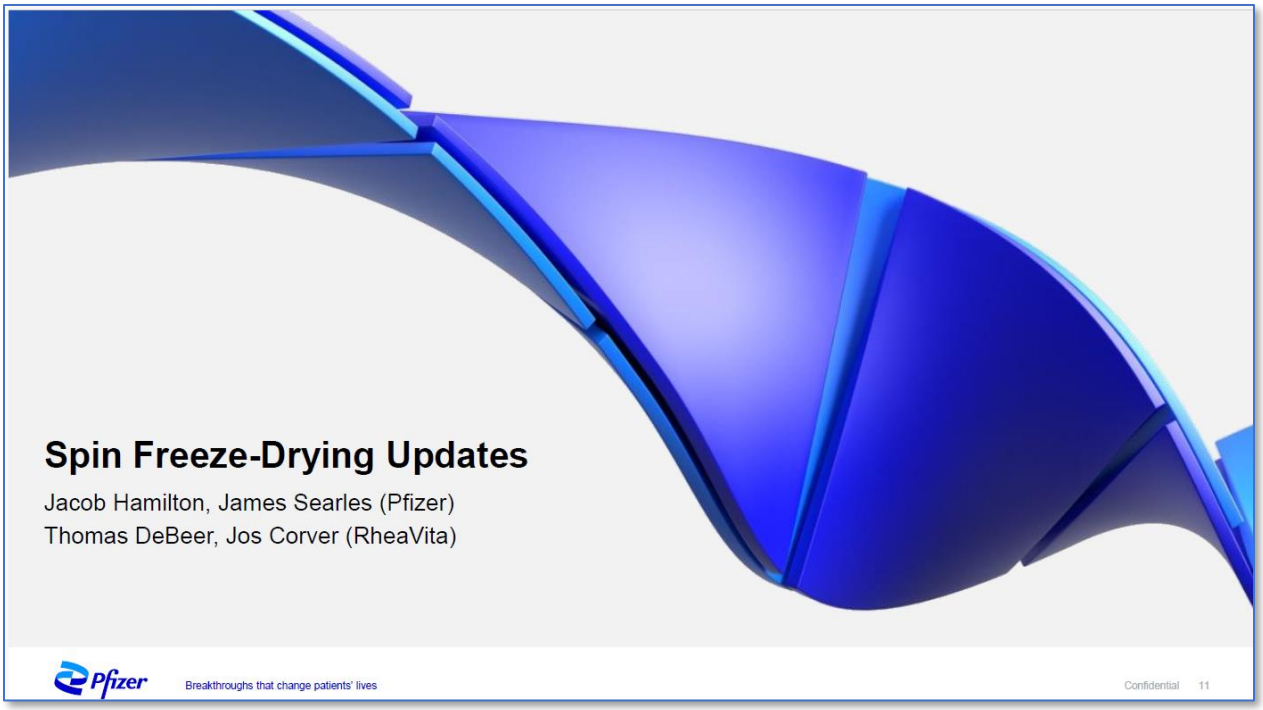
And for that, we use a Millrock Micro Freeze Dryer, which has heat flux sensors, so hopefully we'll get some results on the input parameters that we can put in the model. Okay, that's modeling.



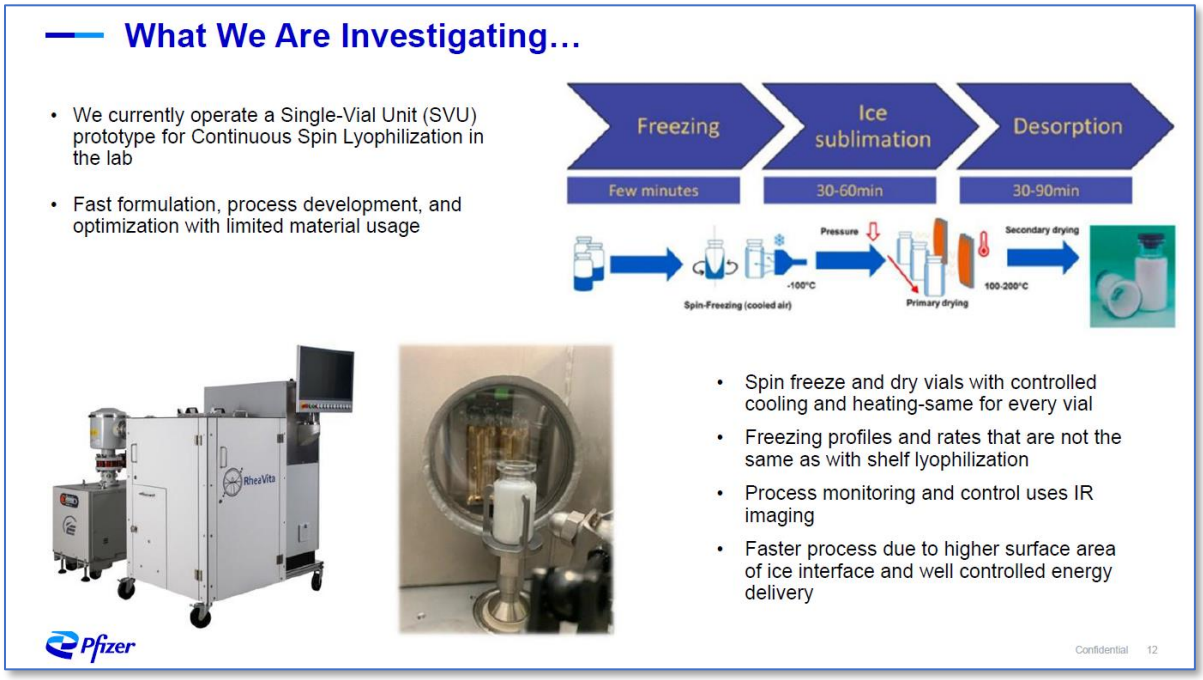
And that brings me to a summary, a slide, on what we have done with spray freeze-drying and what is the maturity state. And as you can see, we've done a lot of work at lab scale. So, stability is good. We have commercial equipment. We do understand, very well, spray freezing process, and drying process we're still working on that. We've done some tests of commercial freeze-drying.

In terms of infrastructure, you know the most sites do have liquid nitrogen supply, but of course it's also required to build additional infrastructure, plus some lines. We presented at least three times to the FDA as this technology was Meridion and IMA, and I think they were very happy with these results.

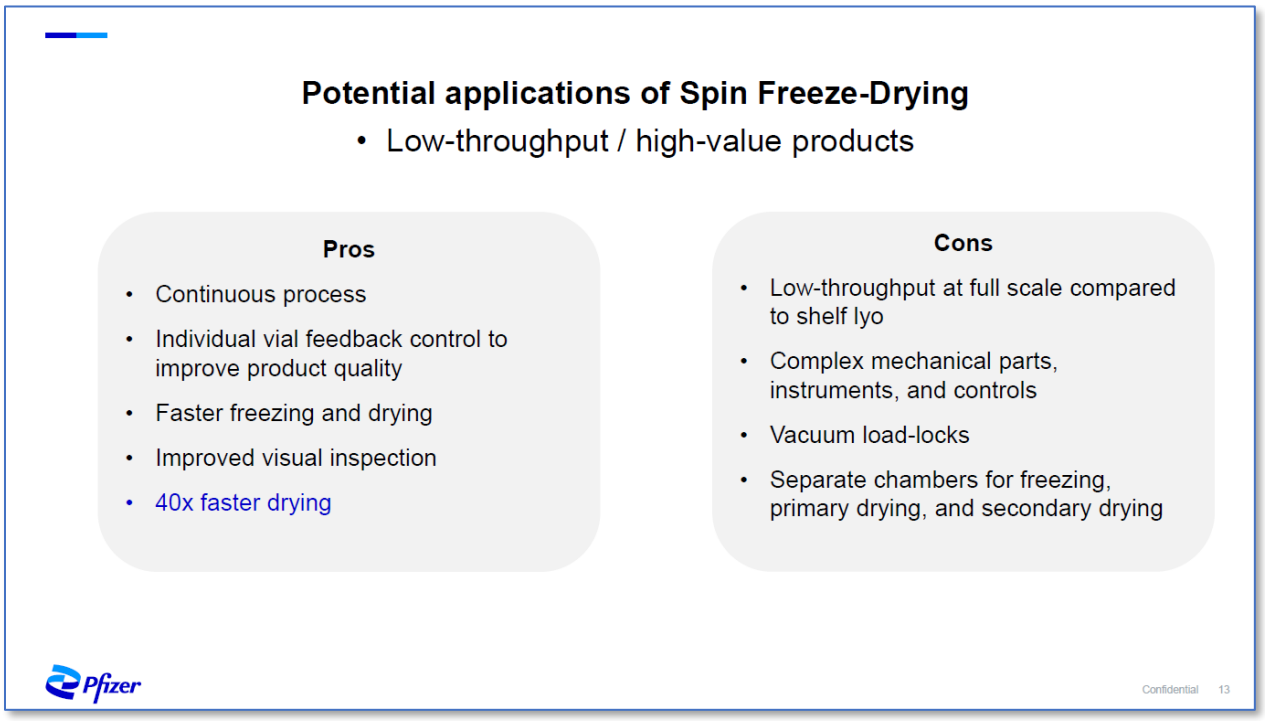
Okay. That's spray freeze-drying. Let's move to spin freeze freeze-drying process. And I'm speaking again on behalf of our large team at Pfizer and RheaVita.



So, in spin freeze-drying process, what you do is you take your vial, same vial that we use in the vial freeze-drying process, you spin it. In our case, it's about 4,000 RPM. Then you apply cold nitrogen to the wall, and you freeze it at a rate you really want. You can do a very wide range of freezing rates. Then you have an infrared source of heat. So, again, allow some very homogeneous drying process. And once primary drying is done you can raise the temperature to secondary drying and remove water.



And you can see through the bottom of this vial which helps to have a visual inspection. So, what's a benefit of this is, of course, you have control of freezing. Every single vial is essentially done the same way. That's important. Freezing profiles are very different. You can have a very wide range of freezing rates. You can control every single vial, and because of the high surface and thin layer, you can dry very fast, about 40 times.



And so, we put pros and cons of this technology on this slide. And first, it's a continuous process, which minimizes some errors.

It's vial feedback control during process. So, again, every single vial can be controlled. It's fast to dry, visual inspection is improved, 40 times faster. The problem is, I don't know if it's a problem or not, it's low throughput compared to a large freeze dryer. But that may not be a problem if you have, let's say, a very expensive product which you don't need to have a high throughput. Let's say you need to make only 500 vials for gene therapy or even for some expensive products, so that could be equipment to use. However, it has some complexity of course. There are a lot of mechanical parts, there are moving parts, some vacuum load-locks, so that creates some possible challenges.

Summary of Work – Experiment and Formulations

- **Study 1: Lipid Nanoparticles**
  - Formulation: LNP in sucrose-buffer matrix, pH 7.4
  - Objective: To investigate the effect of conventional FD vs. Spin FD on product attributes
  - Does fast freezing translate to a less decrease in %Encapsulation efficiency typically associated with LNPs post freeze-drying and reconstitution?

- **Study 2: Adeno Associated Virus (AAV)**
  - *Formulation*: AAV in sucrose-buffer-surfactant matrix, pH 7.4
  - Objective: Investigate the effect of conventional vial FD vs. spin lyo (fast freezing) AAV
  - Critical to evaluate the benefits of fast freezing for low volume, high value products (viral vectors)

- **Study 3: Spin Lyo Protein**
  - Formulation: Low concentration protein in mannitol-sucrose-isotonicity agent matrix, pH 7.4
  - Objective: Test a Mannitol-Sucrose-Protein formulation with Spin FD and assess mannitol hemihydrate.
  - Assessing claim that annealing may not be needed for formulations containing mannitol.

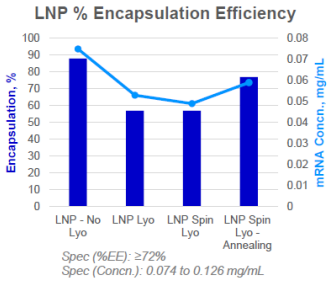
- **Business case evaluation in progress**
  - Commercial - Ability to process 250 vials/day GMP grade DP

Pfizer

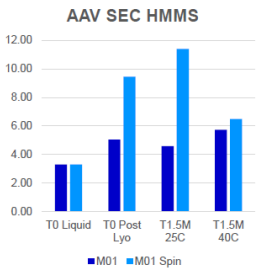
So, we are trying this technology on a few products. It's LNPs, trying to understand if we can by changing ... freezing rate can we increase percent of encapsulation. And with AAVs, associated virus, we want to make sure that we can provide faster cooling rates that we hope can help to stabilize AAV. And then we also tried this for some of protein, for Mannitol contained formulation. So, there's some results shown on this slide. Let's focus first on LNPs.

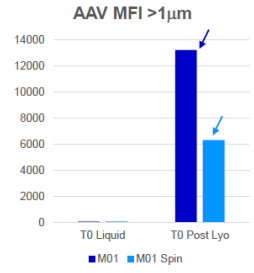
Summary of Work – Results

- **Study 1: Lipid Nanoparticles**
  LNP %encapsulation better on samples with spin freeze + annealing



- **Study 2: Adeno Associated Virus (AAV)**
  AAV link between SEC and MFI
  Higher aggregation in spin lyo samples





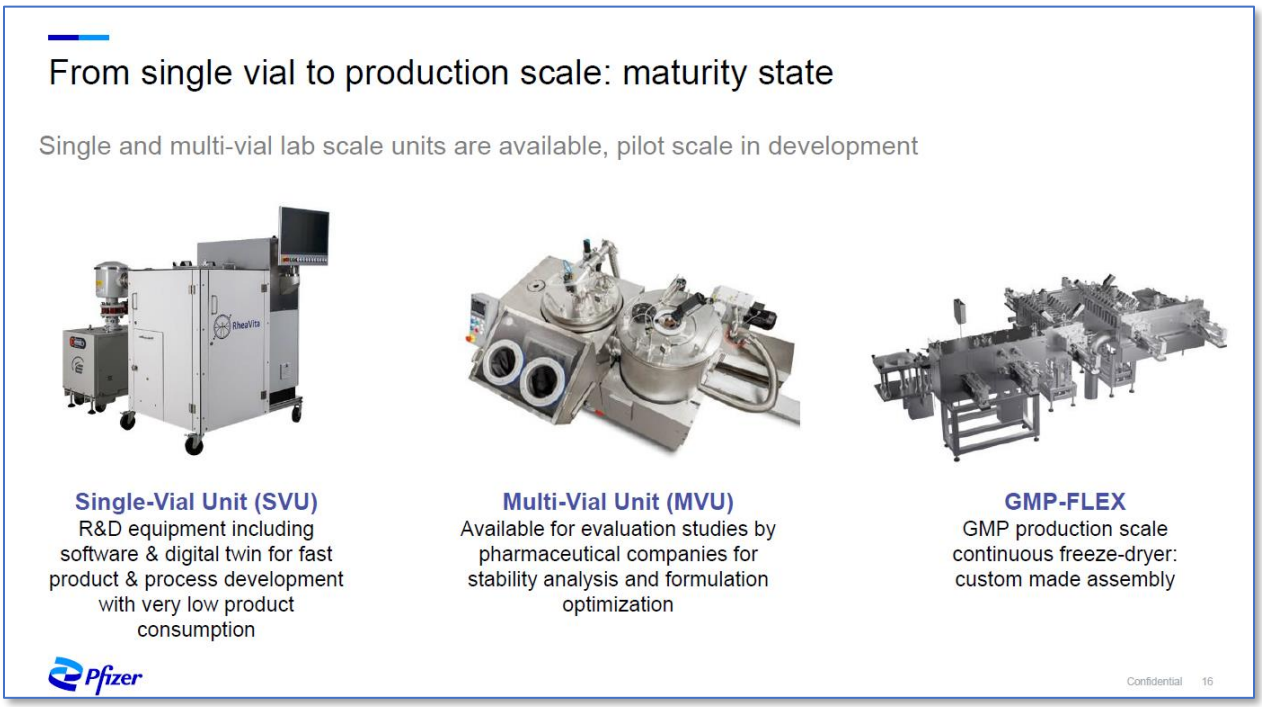
- **Study 3: Spin Lyo Protein**
  High moisture cakes, mannitol hemihydrate present. Will need to further optimize cycle - most likely annealing needs to be implemented or high secondary drying temperature needs to be used

- In summary: Further evaluation of Spin freeze-drying needs to be performed. Modifications to software allowing annealing, slow ramps and wall temperature controls are needed

Pfizer

You can see that on the percent of encapsulation on the left and concentration on the right, after lyophilization we can see the drop of percent of encapsulation, which also was similar with spin freeze-drying, so we didn't really benefit faster freezing. But if you add annealing, you can see there's some improvement.

So, if you look at virus then you can see that ... what was a surprise for us, so for some reason we see some increase in high molecular weight as compared to vial freeze-drying process. Particle size, the number of particles was decreased, but we still see some negative impact, at least on this AAV that we used. So, the conclusion was further is needed on this technology and application to this type of product. We also tried, again, protein, see if we don't need annealing.



Okay. So, what is available now, it's lyophilized from RheaVita, so single-vial unit that we have now, and it's available. Same as the multi-vial unit, I think we can make about 100 vials in this unit at the University of Ghent]. I've seen with my own eyes it's working very well. I heard that they build already a commercial scale GMP-grade line. It's probably just in process of validating at this point. Here's a couple publications they

shared. So go ahead and take a look at those publications, where they demonstrated that spin freeze-drying can be used for making LNPs, which are stable.

Application of Continuous Spin Freeze-Drying for Preservation of LNPs (RheaVita)


Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Continuous freeze-drying of messenger RNA lipid nanoparticles enables storage at higher temperatures

Sofie Meulewaeter[a,b], Gust Nuytten[c], Miffy H.Y. Cheng[d], Stefaan C. De Smedt[a,b], Pieter R. Cullis[d], Thomas De Beer[c], Ine Lentacker[a,b,*,1], Rein Verbeke[a,b,*,1]


European Journal of Pharmaceutics and Biopharmaceutics

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Lyophilization and nebulization of pulmonary surfactant-coated nanogels for siRNA inhalation therapy

Pieterjan Merckx[a], Joris Lammens[b], Gust Nuytten[c], Bram Bogaert[a], Roberta Guagliardo[a], Tania Maes[d], Chris Vervaet[b], Thomas De Beer[c], Stefaan C. De Smedt[a], Koen Raemdonck[a,*]

"*We demonstrated that lyophilization of mRNA LNPs is an attractive strategy to enhance the stability of mRNA vaccines at higher temperatures, as lyophilized mRNA LNPs preserved their functionality when stored at 4°C, 22°C and even at 37°C for a period of 12 weeks*"

Case study RheaVita technology > batch freeze-drying (higher encapsulation efficiency)

- Wider range of process settings possible with RheaVita technology
- e.g., wider range of very controlled cooling and freezing rates
- Better control of residual moisture content
- Much shorter time under vacuum

Pfizer

So, there is a benefit. They mentioned that A, it's continuous, it's very fast process. It's efficient, safe, whereas at least you have no batch rejections because if you sense a problem, you reject the vial, not the entire batch.

Benefits of Continuous Process (RheaVita)

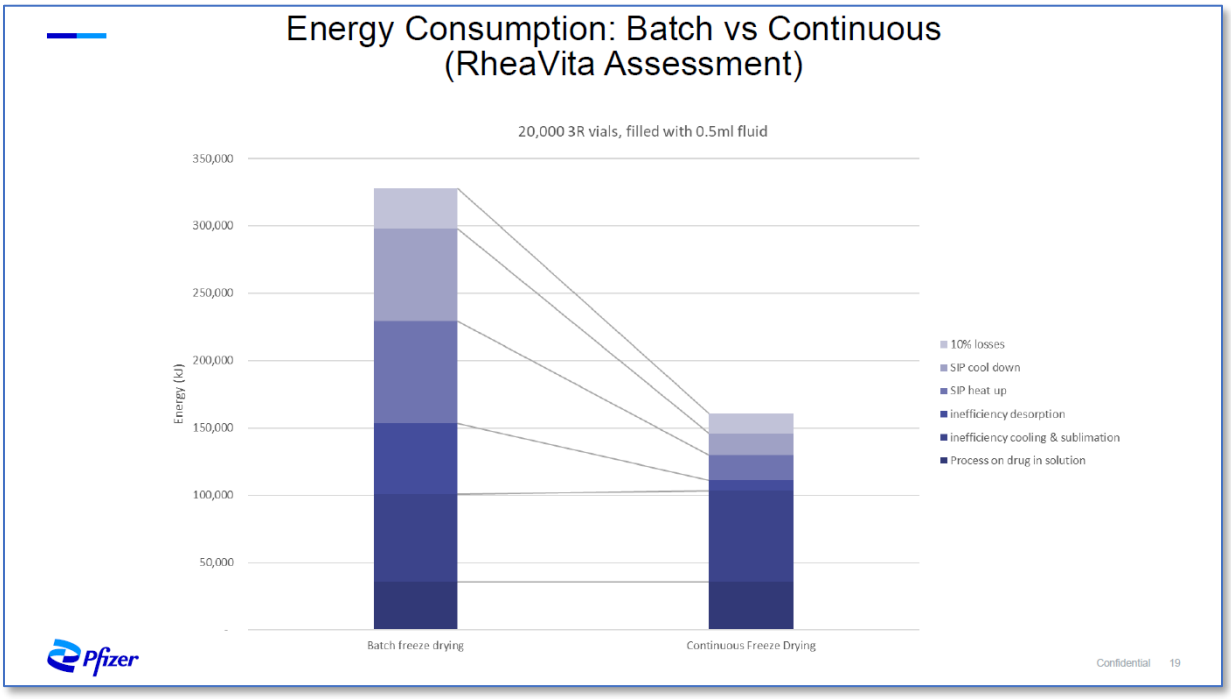
- **Continuous and controlled** freeze-drying technology for **unit doses** with unique features addressing all challenges associated with batch freeze-drying
- **Very fast** process & throughput – hours instead of days
- **Improved Quality Assurance** – decreased defect levels – approaching zero
  - Identical process conditions for each vial
  - Process visualization methods **(PAT)** provide 100% unit monitoring, control, inspection
  - Same **quality** from pre-clinical to production – no scale-up issues
- **Inherent high potential for RTR from process understanding, control and 100% inspection**
- Equivalent **efficacy and safety**
- No large batch rejections
- Proven **process understanding** via validated mechanistic models and **digital twins (model-based design)**
- **Fast** formulation and process **development** with limited material needs
- **Flexibility/production efficiency:** rapid change-over, short CIP/SIP times, flexible volumes
- **Faster time-to-market** for biopharmaceuticals (reduction > 1 year estimated)
- Reduced **ecological footprint** and **operational costs**
- **Enabler for products**
  - Wide controlled cooling & freezing rate
  - Low Tg' & Tc products
  - Speeds up reconstitution

Pfizer

They have very good models, so they understand that process very well. Using this unit, you can do very fast formulation process development. It's very flexible. You can change between different type of vials. And according to this company, you can get faster to market, at least reduction of this time, it's one year according to them.

And it reduced ecological footprint and operation cost.

And if you go to the next slide, you can see that according to RheaVita calculations that the cost-energy would be twice less compared to vial freeze-drying process.



Maturity State of Technology

Activity	Spray Freeze-Drying	Spin Freeze-Drying	Foam Drying
POC at laboratory scale for different types of products	Shown for multiple products	Shown for multiple products	
Stability of dried products	Shown for multiple products	In evaluation	
Pilot/commercial scale equipment availability for aseptic manufacture	Yes	In development	
Process understanding at commercial scale (reliable models)	Reliable model for spray freezing exists, drying model is in development	Model was developed and validated at laboratory scale	
Successful tests at pilot/commercial scales	Performed	Not available	
Infrastructure readiness	LN2 lines available at some commercial sites	LN2 lines available at some commercial sites	
Regulatory bodies awareness	Some regulatory agencies are aware of technology, aseptic manufacturing must be shown at scale	Some regulatory agencies are aware of technology, aseptic manufacturing must be shown at scale	

- Proven at commercial scale
- Proven at laboratory scale
- In development

Pfizer

While they claim they built commercial scale equipment, I don't think it's operational yet, but hopefully this will change to yellow very soon, or green. Process understanding, yes, models are developed and validated at a laboratory scale need to be confirmed at a commercial scale. Because equipment wasn't available, nothing was done at commercial scale yet. In terms of infrastructure, yes, liquid nitrogen lines available at site, so it can be relatively easily implemented.

Foam Drying

Armando Cardenas, Alex Langford, James Searles, Kate Torres, Satoshi Ohtake (all Pfizer)

So, with that, let me move to foam drying. So, again, I speak on behalf of our team from Saint Louis, Armando, Alex, James Searles, Kate and Satoshi Ohtake.

Foam Drying

- Foam drying is a drying technology, similar to freeze drying and spray drying, that has potential to improve storage stability of pharmaceutical products at increased temperatures

Advantages:
- Drying under ambient temperatures, with the **ability to avoid ice formation**
- Drying cycles can be much faster than spray/freeze drying cycles
- Better preservation of biologic activity compared to spray/freeze drying

Disadvantages:
- Nonhomogeneous foam between vials in the same cycle
- Difficult to replicate with large batch size

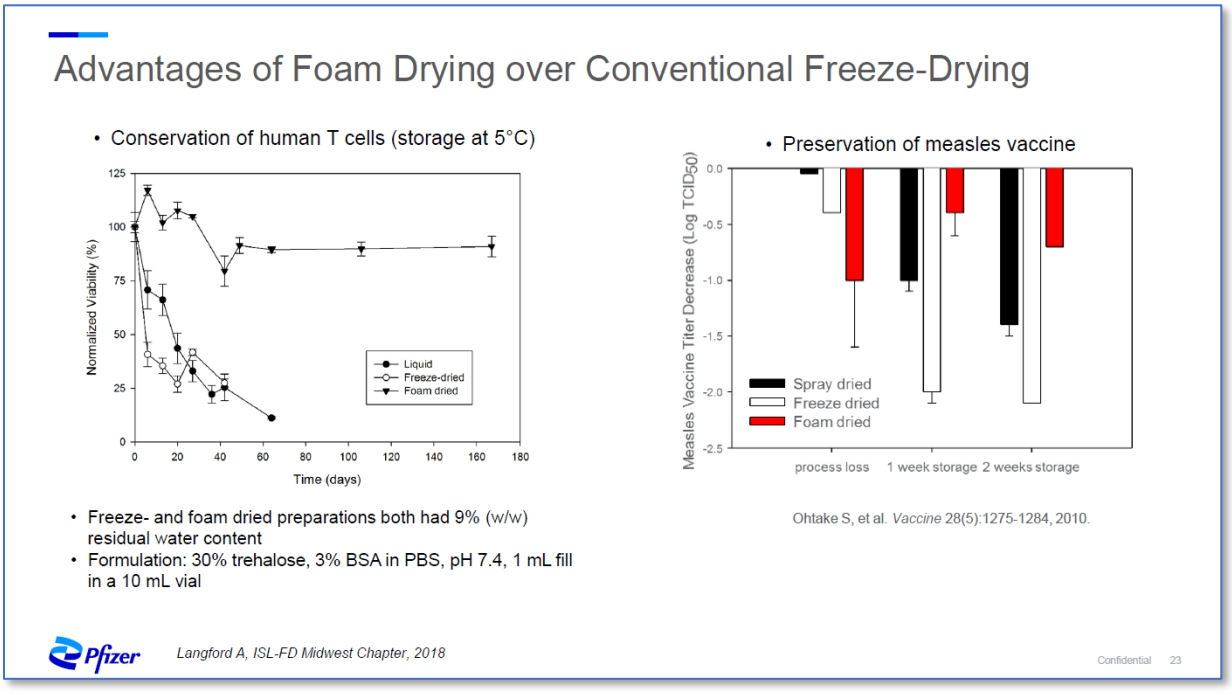
- There has been limited work studying the design space of foam drying with an understanding of the process of foam formation in relation to formulation and cycle development

Ohtake, Satoshi, Ken-ichi Izutsu, and David Lechuga-Ballesteros, eds. *Drying Technologies for Biotechnology and Pharmaceutical Applications*. John Wiley & Sons, 2020.

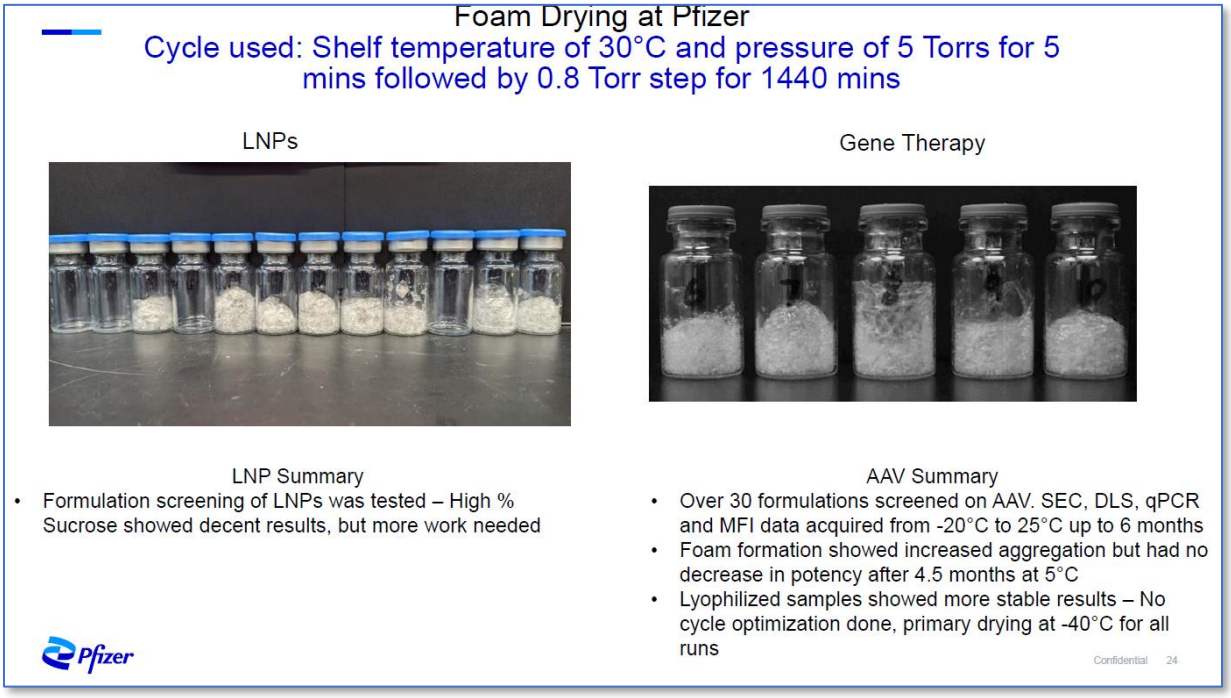
So, foam drying. Foam drying was known forever, and I think the from the middle of the 50's and it has always been sort of negative, for example, with the formation of foam during lyophilization was kind of considered as a negative. Until I believe in 2000 was patented, where they demonstrated that this technology can be widely used for different types of product, and they have a lot of patents on that.

So, we tried to evaluate this technology for the product that is really sensitive to freezing. That was essentially the idea for this technology I believe. What you can do also, it's very fast dryer, by foaming, you create a lot of operational surface. And the primary drying, if you consider it as a primary drying, because it's never frozen, then the most sort of initial evaporation step is very fast. And then once you remove the majority of water, then you start to remove the moisture during secondary drying.

And it's even with that it takes longer time than normal secondary drying, but even with that, it's still shorter process, okay? The challenge is foam formation is not homogeneous, unfortunately, and I will show this later, and it's very difficult to replicate this foam at a large scale. At least I have not seen it. So, there was also limited works done in studying design space, at least in terms of ... it wasn't published a lot. However, if you look at some impact of this foam drying on different modalities, we can see some positive impact, for example, on T cells.



You can see that foam drying stays relatively stable, stable compared to initial, as opposed to regular vial freeze-drying process, which down to the moisture content is similar to the foam drying. So, yes, some of products don't like freezing. Same, for example, measles vaccine, you can see data that foam-dried measles vaccine was much more stable than lyophilized vaccine.



Again, foam drying, unfortunately, it's not really reproducible. In old vials, you can see that every single vial looks differently. We tried with LNPs and we also tried gene therapy products, so about 30 formulations that we tried, and you can see that it's really not the same. With that said, we get some positive results on some products, as I mentioned, but with LNP and gene therapy we didn’t have a lot of lyophilized samples, for example, for gene therapy product was more stable than foam drying product.

Okay. So, if you compare this technology to spray freeze-drying and spin freeze-drying, yes, it was shown that some proof of concept for some of products. Not for all of them, but for some. Stability, we are still evaluating that. And the key point of that that you need to get to low moisture in order to have longterm stability, so the secondary drying must be long.

Maturity State of Technology

Activity	Spray Freeze-Drying	Spin Freeze-Drying	Foam Drying
POC at laboratory scale for different types of products	Shown for multiple products	Shown for multiple products	Shown for some products
Stability of dried products	Shown for multiple products	In evaluation	In evaluation
Pilot/commercial scale equipment availability for aseptic manufacture	Yes	In development	Not yet available but only minor modifications are needed
Process understanding at commercial scale (reliable models)	Reliable model for spray freezing exists, drying model is in development	Model was developed and validated at laboratory scale	Not available
Successful tests at pilot/commercial scales	Performed	Not available	Not available
Infrastructure readiness	LN2 lines available at some commercial sites	LN2 lines available at some commercial sites	Infrastructure is available
Regulatory bodies awareness	Some regulatory agencies are aware of technology, aseptic manufacturing must be shown at scale	Some regulatory agencies are aware of technology, aseptic manufacturing must be shown at scale	No information

- Proven at commercial scale
- Proven at laboratory scale
- In development

Pfizer

In terms of infrastructure, so we do have actually existing equipment can be easily converted into foam drying. All you need is to have additional valve for example, of course some filter and you need to adjust the software that allows you to control pressure between one and 10 torrs. That's the pressure range which you make foam. So that's relatively easy to convert any existing freeze dryers to foam drying, as long as you demonstrate for some of your products that's really good technology.

Acknowledgements

Pfizer

- Fawziya Ali, Hieu La, Agota Csiki Fejer, Dean Ly, Vito Maglio, Nathan Wilson, Tony Gudinas, Nicole Bundy, Eda Fenercioglu, Jacob Pantazis, Michelle Lisowski, Aadithya Krishnan, Heer Sethi
- Ramin Darvari, Louane Hann, Nicholas Warne

Purdue University:
- Alina Alexeenko

Meridion Technologies:
- Alexander Lukas

RheaVita:
- Jan Van Hauwermeiren
- Mathieu Massart

Pfizer

And in terms of regulatory, I presented it last year at and there was a lot of FDA people. They were interested in that, but I think they have some questions about reproducibility of this process. And, again, as I mentioned, that's really challenging at this point. So, I would like to acknowledge a lot of people that contributed to this work, from Pfizer, Purdue, Meridion and RheaVita. And with that, I'm ready to answer questions.