Assess Product Heterogeneity and Scale-up Risks in Pharmaceutical Freeze Drying via Mathematical Modeling

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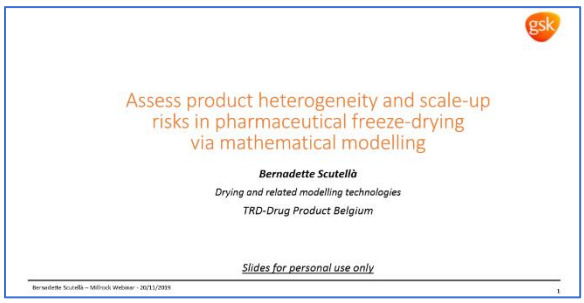
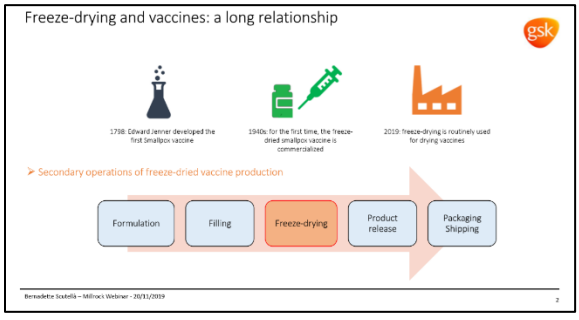
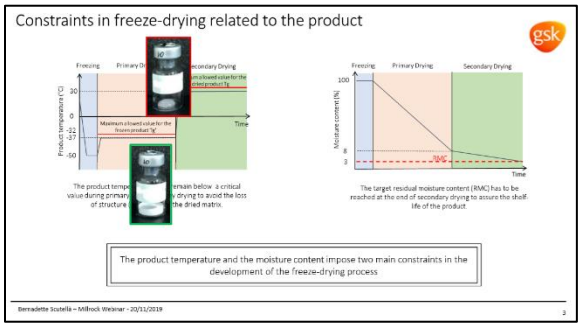
Today, I will discuss with you one of the main challenges that we need to face when we develop and scale-up a freeze drying cycle, meaning the assessment and possibly the prediction of the product heterogeneity. Slide 1



I work in GSK for vaccine research and development. Freeze-drying and vaccines have a very long relationship. Slide 2 Some of you may know that the first vaccine to be developed was developed by Edward Jenner and was the smallpox vaccine. However, this was still in liquid form and it took almost two centuries in order to have the first freeze-dried vaccine to be commercialized. Nowadays, freeze-drying is, perhaps, the main drying process to be used in order to dry vaccines and many pharmaceuticals. When it comes to freeze drying, we refer to it as a "secondary operation." In vaccine production we have, first, the production of the drug substance, which is the "primary operation."



In the drug product, we receive the drug system. We add to the drug system the excipient in order to stabilize it. We fill it into doses, in single vials. The vials are then freeze-dried and checked after processing and, finally, they are packed and shipped to the markets. Freeze drying is a process, which is very related to the product. It's very product dependent.

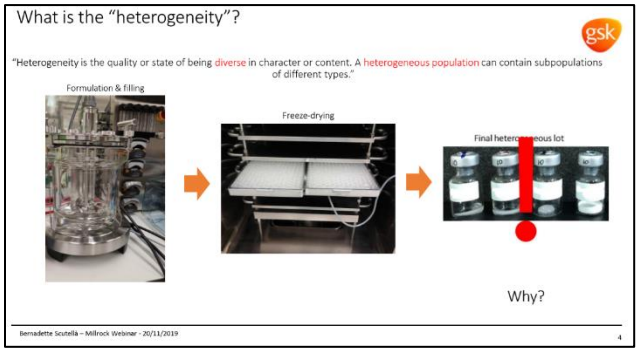


In particular, the product poses two main constraints to the freeze-drying process in terms of product temperature and moisture content.

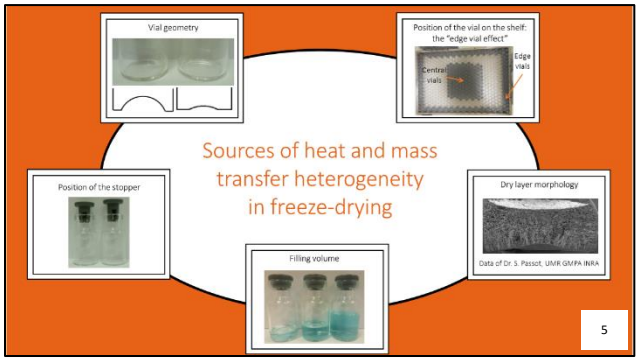
In the first graph on the left, you can see the evolution of the product temperature during the three main phases of freeze drying. Slide 3 We have the freezing, the primary drying and the secondary drying or desorption. During primary drying and secondary drying, it's important that the product temperature remains below a critical value in order to have a nice and elegant cake, so that it maintains the same volume and shape of the initial liquid. If we go above the critical value that can be considered the “glass transition temperature,” the collapse temperature, we will have collapse of the cake and it needs to be rejected. This is the first constraint that we need to consider.

The second constraint is related to the moisture content. The moisture content decreases throughout the process. You can see in the second graph the evolution of this parameter, especially during primary drying. We have a sharp decrease because of the sublimation of the ice crystal, which is almost 80% of the total water in the product. Then we have the final removal of the moisture content during the desorption of secondary drying until we reach the target moisture content. The target moisture content depends on the product. For example, for vaccine it is no more than 3% and most of the time we go much, much lower than 3%. We need to ensure to maintain the constraint of moisture content.

Another concept with which many of you may be familiar in the field of freeze drying, is heterogeneity.



Slide 4 We may say that heterogeneity is the state of being different in some character or in some content. A heterogeneous population, for example, may be divided in subpopulations of different types. Often, at the end of a lyophilization cycle the batch may have elegant cake but also retracted cake or totally collapsed cake. Why is this so? The problem with freeze drying is the variability of the heat and mass transfer phenomenon that takes place during the process.

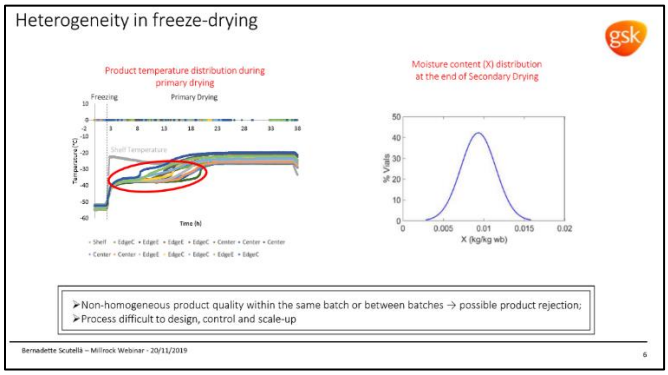


This variability of heat and mass transfer is due to some sources of heterogeneity, which are the variability of the vial geometry. Basically, in big lots of vials we cannot pretend that all the vials are identical, one to the other. There will be some variability in the contact area with the shelf and the vial bottom, and this may impact the heat transfer. The heat transfer is also impacted by the known edge vial effect, where edge vials will receive a higher heat flow rate compared to center vials.

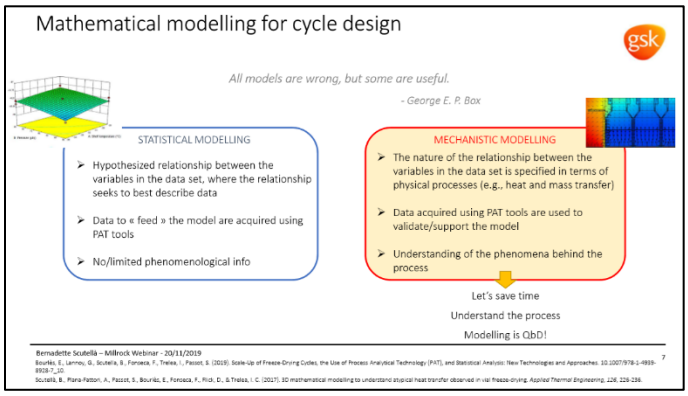
The mass transfer will also be impacted by the variability or the difference in terms of dry layer morphology that we can have between one vial and the other. Two other minor sources of variability are the filling volume and the position of the stopper. Slide 5 The images here are exaggerated to just to give an idea. These sources can be considered minor as the precision of the filling machine and the stoppering machine is quite appreciable. Therefore, with respect to the other three sources mentioned, the vial fill and stopper position are negligible.

The presence of the sources of heterogeneity, the difference of heat and mass transfer phenomenon that we can have during the process, directly reflect on the product temperature during primary drying and the moisture content during secondary drying.

Slide 6 On the left, you can see the evolution of the shelf temperature and the product temperature profile during freezing and primary drying. You can see that the product, which was recorded by thermocouples in the freeze dryer presents different profiles. Depending on the position of the vial on the shelf we can have a different thermal history for the vial, and, different sublimation points, which correspond to the moment at which the product temperature reaches or goes above the shelf temperature.

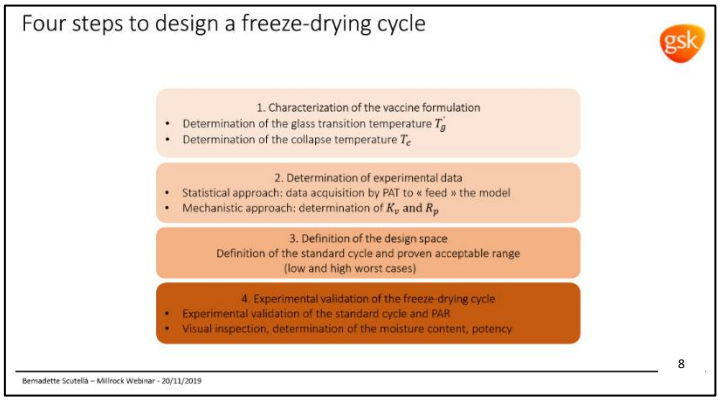


We can also observe distribution of the moisture content in a lot of vials at the end of secondary drying. We cannot expect that all the vials contained in a lot have the same moisture content. There will be a range of values. In this case, for example, if the target moisture content is 1% there will be some vials with a higher moisture content. Therefore, the secondary drying parameters will need to be redesigned, which is the consequence. The consequence of having this heterogenic freeze drying is that we obtain a non-homogeneous product quality. Vials may be rejected if the parameters are not well defined, and for the freeze-drying scientist, it means that the process is quite challenging to develop and to be scaled-up.



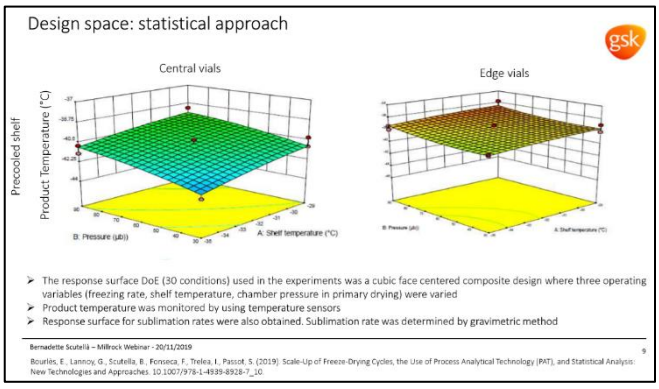
Slide 7 What is the solution? The solution that we like to use is mathematical modeling to define the cycle and for cycle scale up. We can source between two big families when it comes to mathematical modeling. Statistical modeling consists in feeding hypothetic relationship between different variables with experimental data that usually comes by a design of experiments. For example, we can derive the relationship between the chamber pressure, the shelf temperature and the product temperature by monitoring the product temperature under different conditions and by using thermal calculations. But this kind of approach is very experimental, expensive, and it doesn't give you any phenomenological info regarding the process.

Instead, we use more of a mechanistic approach, which is based on equations. Equations describing heat and mass transfer during primary and secondary drying, and these equations validate the data, experimentally obtained, and are used to validate the model and not to create the model itself. Mechanistic modeling has three main advantages. First, we save time because running a simulation once the model is validated takes much less (time) than performing a freeze-drying cycle to verify the parameters. We can understand the process. We acquire the information that can be applied also in other situations during freeze drying and development of scale-up. Also, mechanistic modeling is QbD because we are allowed to create and understand the relationship between the product and the process.



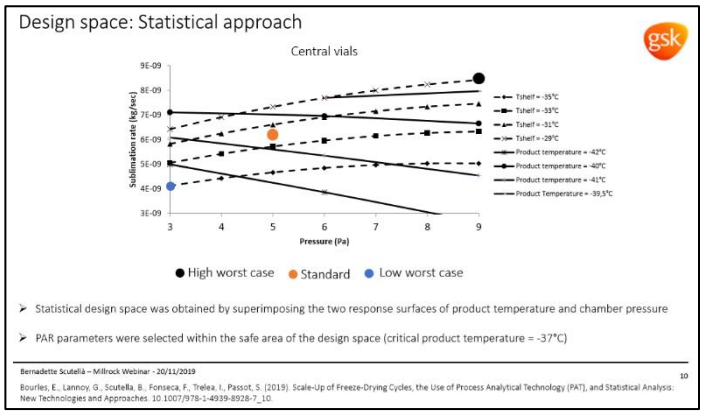
Slide 8 In order to design a freeze-drying cycle, we perform four main steps. The first step is the characterization of the vaccine formulation, meaning the definition of the critical temperature, the collapse temperature, the glass transition temperature. Then we determine experimental data, in this case we use the mechanistical approach, the data acquisition will be performed in a DoE (design of experiments) by using Process Analytical Tools. If we use the mechanistic approach, we need to determine the modeling parameters, such as Kv and Rp.

Once we have the experimental data we can then define our design space, also create or run our model to define the design space, and into the design space, we define our standard cycle parameters and approve an acceptable range, which is constituted by two worst cases, a low and high one, which present more or less aggressive parameters than the standard cycle. Finally, we experimentally validate our standard cycle and our PAR (proven acceptable range), and we do some cake characterization after, such as visual inspection, moisture content determination, and potency of the product.



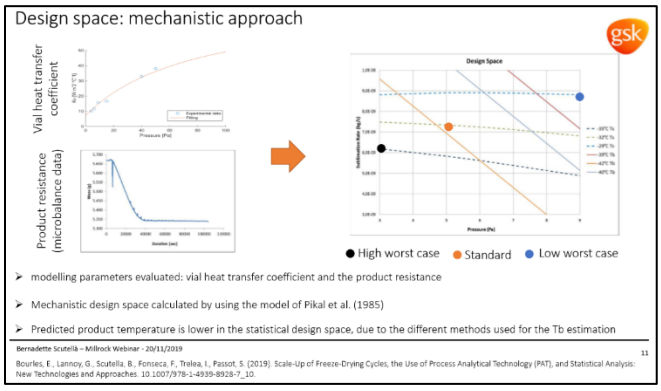
Slide 9 Here is an example of both the same space for statistical approach and mechanistic approach. We used the statistical approach to determine the statistical design space for one of our products. We ran DoE of 30 conditions, in which we evaluate the relation of the three operating variables, meaning the freezing rate, the shelf temperature and the chamber pressure during primary drying. And we monitored the product temperature, which is here shown in terms of response surface, and the sublimation rate. On the right, you can find, respectively, the surface response for central vials and for edge vials. As these are experimental data, we expect to have a certain trend. For example, when shelf temperature increases, we can see an increase in the product temperature and the edge vial results to be higher of about three degree compared to central vial.

Once we have obtained the response surface from the experimental data of the DoE, we can superimpose the response surface of product temperature and sublimation rate in order to determine the design space.

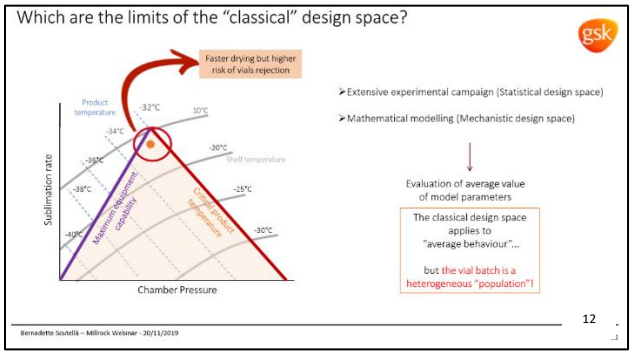


Slide 10 Here is shown the design space for central vials obtained for the same product. The design space is here expressed in terms of sublimation rate against the pressure. The dotted line are the shelf temperatures and the straight line corresponded to the product temperature. The design space here represents the space error because the critical temperature of our product was -37, which is much higher in respect to the -39 that we have here. This can be all considered the safe area of the design space.

Here we selected the operating variables of our process, the standard process, which is the orange circle here, which were -32 and five Pascal, and then we selected our proven accessible range, which is the highest case with more aggressive parameters and the lowest case with less aggressive parameters.

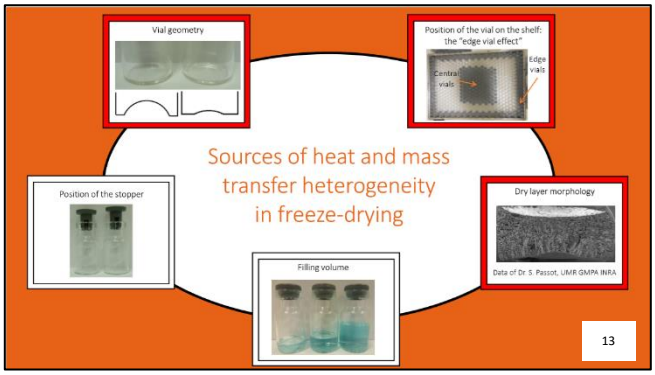


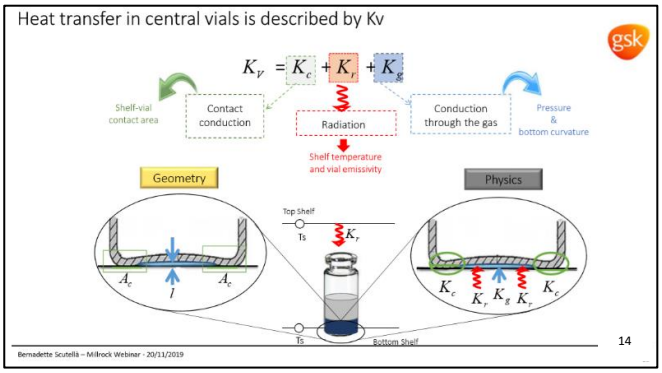
Slide 11 For the same product we also had determined the mechanistic design space. In this case we had to evaluate the vial heat transfer coefficient. The vial heat transfer coefficient depends only on the vial, so we don't need to do that for every product. And the product resistance, which, instead, depends on the matrix. We need to repeat this experiment every time there is a need to determine a cycle for a different product. From these two data, by using the model of Professor Pikal, we were able to estimate the mechanistic design space, which is expressed in the same terms of the statistical one. We have sublimation rates, pressure of the chamber, the dotted line of the shelf temperature, and the straight line are the product temperatures. Here we can find also the operating variable representing the PAR.



Slide 12 Now that we have understood which methodology to determine and how to use the design space, we will briefly look at the limit of the classical design space. A good practice, when we design our freeze-drying cycle, is to select the parameter as close as possible to the edge of failure, to the critical product temperature, in order to maximize the sublimation rate and to reduce the drying time. However, it must be said that when we determine the design space, we determine an average design space. In theory, every vial in our process may have its own design space. Of course, this is not feasible so we need to somehow find a compromise between the average information that we find in the design space by calculating the design space, and the variability, which cannot be eliminated from our process.

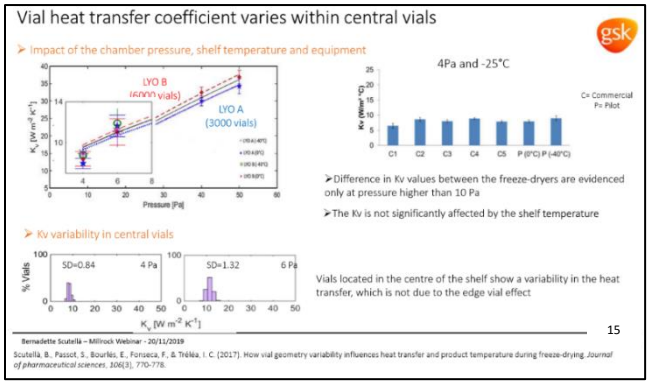
Slide 13 What we usually do is to use some tools in order to integrate the variability of the vial geometry, the edge vial effect and variability of dry layer morphology in total when we design the scale-up cycle.



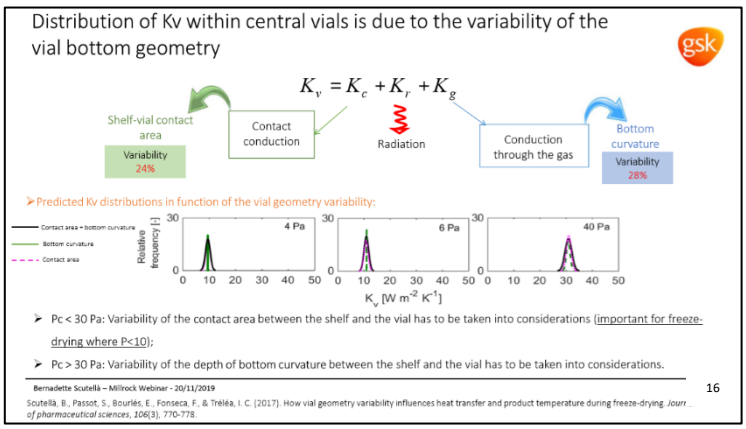


Slide 14 As you may know, the heat transfer between the shelf and the central vial may be expressed in terms of the vial heat transfer coefficient Kv. Kv is composed of three main contributions. One is the contact conduction, KC, which is given by the contact between the vial and the shelf, and this contribution is proportional to the contact area that we have between the vial and the shelf itself. Then we have the radiation, which is a contribution from both the bottom shelf and the top shelf, and this depends on the shelf's temperature and vial emissivity. And finally, we have the conduction through the gas, which depends on the pressure and the bottom curvature. It's the conduction through the gas entrapped in the curvature of the vial bottom.

Slide 15 When it comes to dependence of Kv on operating variables and equipment, we have performed induction analysis to evaluate the impact of chamber pressure, shelf temperature, and of the equipment itself. In the first graph on the left, you can see the evolution of the vial heat transfer coefficient in function of the pressure for two pilot scale freeze-dryers. LYO A, 3000 vials, we speak about the vials of 3mL, so quite small vials, and LYO B of 6000 vials and for two kind of shelf temperatures, zero degrees and -40.



The main conclusion was that the shelf temperature, at least at low chamber pressure, lower than 10 Pascal is not a significant impact on Kv. Whereas we can observe that with increasing the chamber pressure, our Kv significantly increases. Between 4 and 50 Pascal we have a Kv of four times higher. When it comes to the equipment, we also have observed that at low chamber pressure, lower than 10 Pascal, which is actually the range of pressure in which we work for vaccine production, we don't observe any significant difference between the two pilot scale freeze-dryers. We have also determined the Kv in different equipment, so not only pilot scale but also commercial scale, as you can see is represented in the second graph by C1 to C5 and



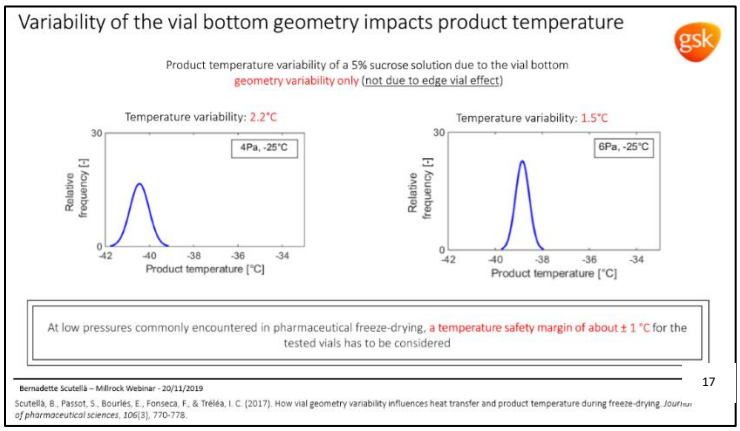
except for the first freeze-dryer, we did not observe any difference among the other, which can help when we need to perform the scale-up.

However, when you perform the experimental determination of Kv, you still can observe some variability in the Kv of central vials, and this variability is very important in respect to the error measurements that can be evaluated.

Slide 16 Therefore, we decided to investigate the impact of the vial geometry on the variability of the vial heat transfer coefficient. We have performed a dimensional analysis on a lot of 120 vials and we have defined, and we have calculated our coefficient of variability of 24% for the shelf vial contact area and 28% for the bottom curvature. This value was then used to reproduce a theoretical distribution of the Kv by considering only the bottom curvature variability, which is the green distribution, only the shelf vial contact area variability, which is the pink distribution, and by considering both variabilities.

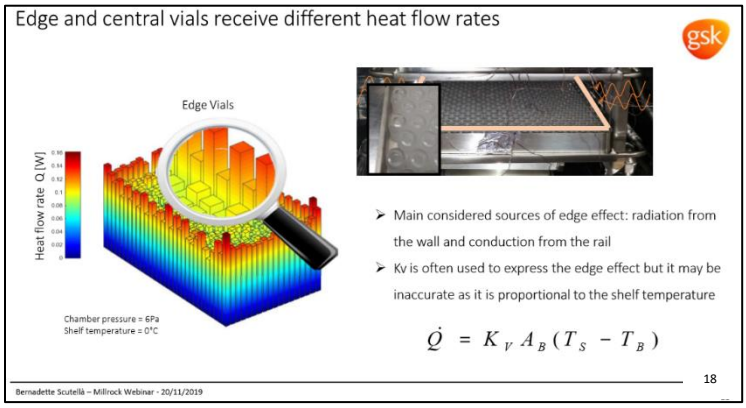
What we have observed is that at low chamber pressure, lower than 30 Pascal, the curvature variability does not impact on the variability of Kv. And almost the whole variability of Kv for central vials can be considered due to the variability of the contact area.

This is important to note because if you work in a range of pressure, as we do, lower than 10 Pascal, you actually need to really take care when you choose your shelf-vial contact area variability much less than bottom curvature. In contrast, if you work at higher pressure you will need to consider both. This can be a guideline when you choose your type of vial and your supplier.



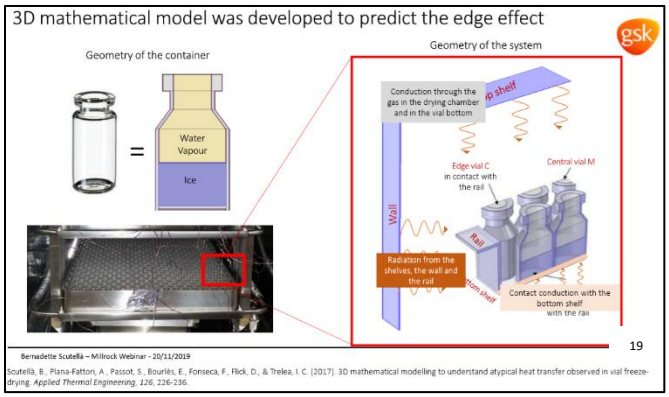
Slide 17 The theoretical distribution that we obtained by determining the variability of the vial geometry was also used to estimate the distribution of the product temperature at different operating parameters, especially at different pressures, four Pascal and six Pascal. We have concluded that the geometry can impact the product temperature variability and potentially on the product quality for about two degrees. So, when we select the parameter for our freeze-drying cycle, we need to consider a temperature safety margin of plus / minus one degree.

Slide 18 Regarding the edge vial and especially “the edge vial effect” you can see in this slide a presentation of the heat transfer in the vials located on the shelf.



Basically, every bar here represents one vial. The higher the bar, the higher the he at transfer received by the vial itself. You can readily note that edge vials present a higher transfer rate compared to central vials. Central vials here as well, we've shown variability in the heat transfer due to the vial geometry variability.

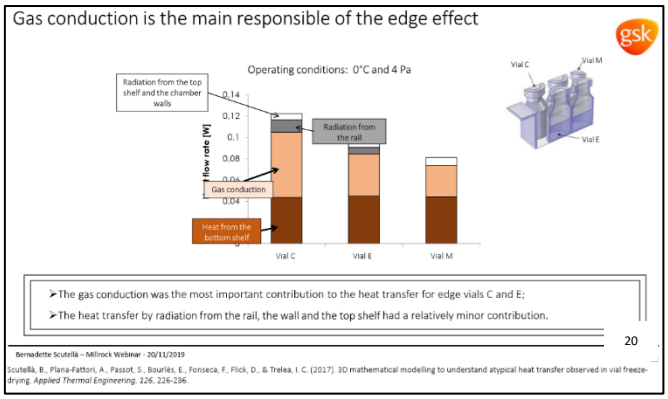
Focusing on edge vials, even within the edge vials, we can see that the heat transfer is not the same for each one. Some vials, which are in contact with the loading rail, will present a higher heat transfer than the vials that are not in contact with the rail. Usually, the edge vial effect is considered to be due to the radiation from the wall, and to the conduction from the rail and, it is expressed in terms of Kv. This way of expressing the edge factor maybe inaccurate as by definition Kv is calculated proportional to the shelf temperature, product temperature and the bottom area of vial. But the heat transfer received at the edge vial is received on the walls, on the sides of the vial and is due to the radiation of the wall or towards the mechanism taking place in the drying chamber, which are not actually proportional to the center of the shelf.



Slide 19 We have developed 3D mathematical modeling in order to predict the edge vial effect in different configurations. For the standard case, we have first developed the geometry of the container. By

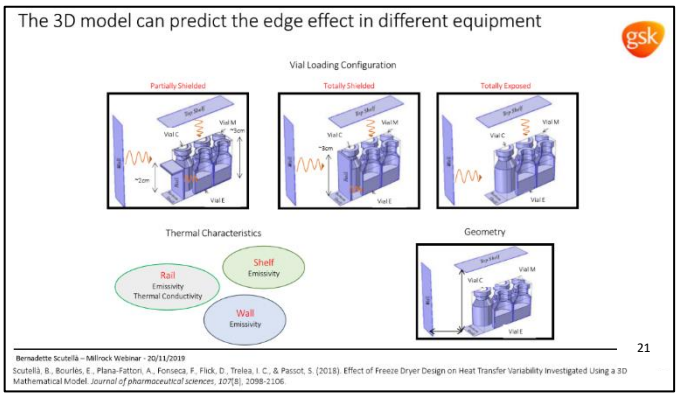
dimensional analysis we have performed before we were able to reproduce, by using COMSOL Multiphysics software, the sublimation of the vial. We reproduced a portion of the chamber, replicating the vial in order to have five vials. Two edge vials, and one real central vial. And then, we have at the bottom and the top shelf, the rail and the wall. Once the geometry was defined, we included the three main heat transfer mechanisms to the model, which were the contact conduction with the bottom shelf, the radiation from the shelves, the wall, the rail, and the conduction through the gas to the drying chamber and to the vial box, which is actually often neglected when considering the edge vial effect.

After validating the model, the model is in a steady state and the product in this case was ice, we were able to understand the different contributions of the heat transfer mechanism on the total heat transfer and on the edge vial effect.



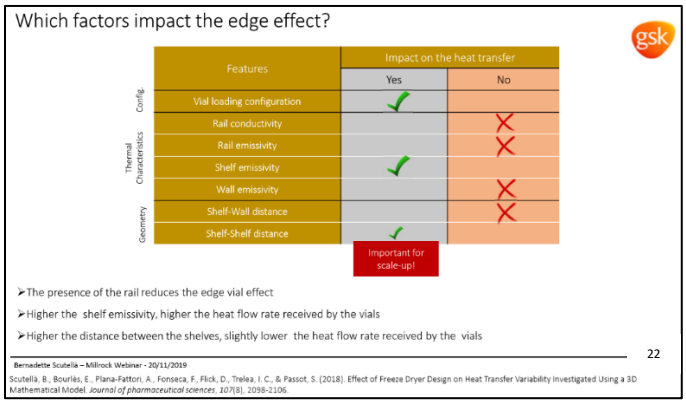
Slide 20 In the graph, you can see the heat flow rate for the vial C in contact with the rail. The vial E far up from the rail and the vial M, which is the real central vial. We have calculated the heat from the bottom shelf (as expected was the same of course for all the vials), the gas conduction and then the radiation from the rail, the top shelf and the chamber walls. By doing this exercise we have assessed that the gas conduction is mainly responsible when it comes to the edge vial effect and to the difference in heat transfer between edge vials and edge vials with the central vial. For example for the vial C, gas conduction impact was more than 50% on the total of the heat flow rate, whereas we have a much smaller than expected contribution of radiation from the rail, the shelf and the chamber walls, which goes between 5 and 10% for all the vials considered.

Slide 21 The model that we have developed is quite flexible and allowed us to evaluate the edge vial effect under different conditions, which has proven to be very useful when we need to perform scale-up.

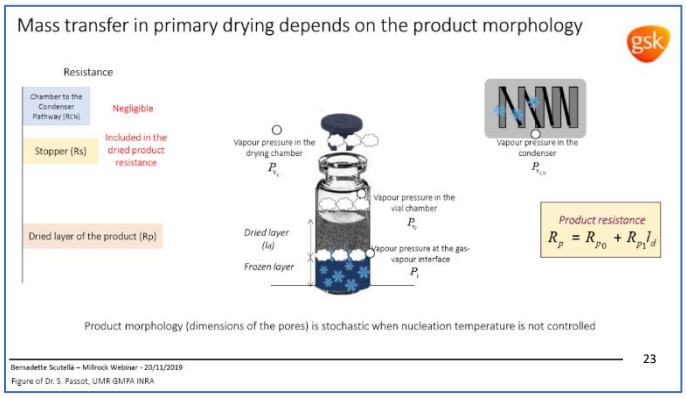


We were able to reproduce different vial loading configurations. For example, edge vial factor if you're totally shielded from the rail, which is our common practice when it comes to pilot scale of freeze dryer, because it's easy to load the vial into the equipment as compared with a commercial freeze dryer where there is often auto loading.

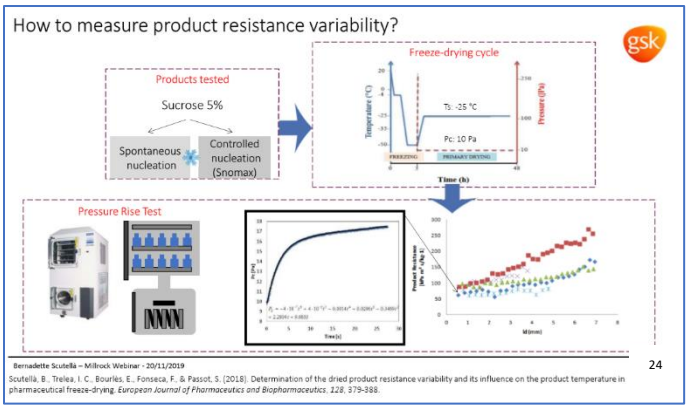
This can help in assessing how heat transfer variability will change between pilot scale configuration and commercial scale configuration and process if our operating variable are adopted to be scaled-up. Also, we can assess the impact of different thermal characteristics especially shelf emissivity, wall and rail emissivity, and finally we can modify the adopted geometry to the scale of the freeze-dryer that we are using.



Slide 22 By doing different simulations we arrived at the conclusion that among different factors we have, the higher ones that impacted the scale-up are the vial loading configurations, the shelf emissivity and the shelves distances. It was important to gain this kind of understanding, because we can then quantify this information before we do the scale-up to commercial equipment.



Slide 23 As many of you may know, the mass transfer takes normally from the interface between the frozen layer and dry layer during sublimation. The water vapor goes through resistance imposed by the dried layer, which is commonly known as the "product resistance." Then the water vapor goes from the vial chamber, through the stopper resistance to the drying chamber and finally, the water vapor from the drying chamber through the condenser pathway, to the condenser. Among the three resistance areas, due to the mass transfer, the chamber to the condenser pathway is usually negligible with an importance of less than 3%. The stopper resistance accounts for an impact of about 10% and is often included into the dried product resistance.

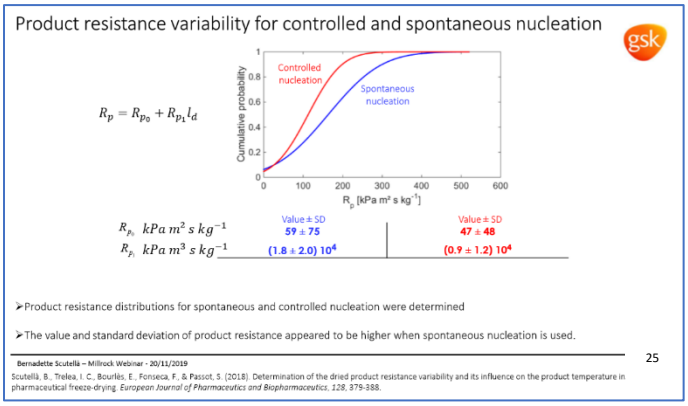


Slide 24 Now we will focus on the dried product resistance and experiments performed without any stopper. In this work the product resistance was related to the dried layer thickness by linear relationship. Depending on the product you may have different kinds of equations. The scope here was to evaluate the variability of the product resistance, which is linked to the dimension of the pores. The dimension of the pores also depends on the value of nucleation temperature, which is stochastic among the vials. Also, if the nucleation is not controlled the vials may have a different nucleation temperature as well as a different product structure.

So, what was our strategy to measure the product resistance variability? Here we have considered a 5% sucrose solution and we have performed freezing via spontaneous nucleation by adding a nucleation agent, Snomax. Then we have run sublimation tests, which include the top freezing and primary drying

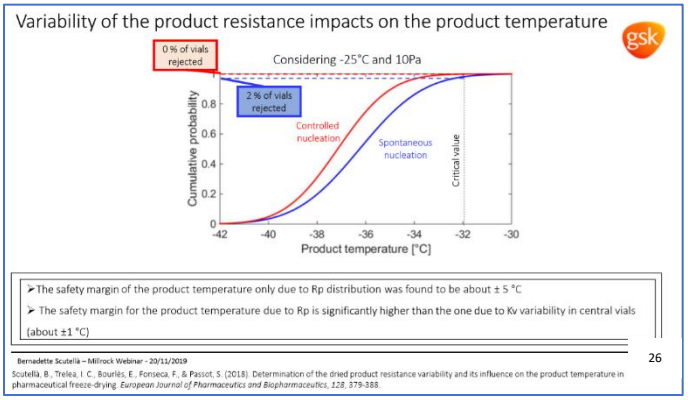
by using a shelf temperature of -25°C and chamber pressure of 10 Pascal, and finally, we have performed the pressure rise test by using a Millrock Technology freeze dryer.

The pressure rise test consists of a test in which we close the valve between the condenser and the chamber, while the vials continue to sublime. So, at the end we have the drying chamber is full of vapor but the pressure still continue to increase and from the fitting of this curve by binomial equation by doing the derivation of the time equal to zero, we were able to determine the mass flow rate via the EDL gas law equation, and so, from the mass flow rate we determine the product resistance value in function of our Ld, dry layer thickness. These experiments was the performed at different times during primary times in order to obtain the complete evolution of product resistance with the dry layer thickness and it was repeated also five times within the same freeze dryer with freeze dryer and by using the same freezing method and the same solutions of 5% sucrose solution. Then the experimental data were fitted by using the linear equation and it was possible then to determine the coefficient of the fittings.



Slide 25 In this case of Rp zero and Rp one and the standard deviation of this coefficient in case of controlled nucleation and spontaneous nucleation. From the standard coefficient and considering the operating variable that we want to explore, we were able to determine Rp distribution. So, this case expressed as a cumulative probability.

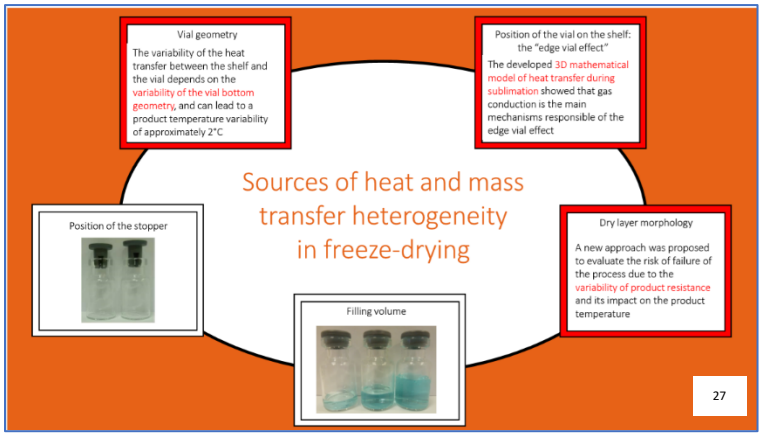
So, you can see that the spontaneous nucleation had the product resistance higher than the controlled nucleation because usually spontaneous nucleation creates much lower product temperature of nucleation whereas controlled nucleation in this case was set to nucleate at -4. From the distribution by knowing the distribution of the product resistance and by using the classical equation of design of Professor Pikal, we were able to determine the distribution of the cumulative probability of the product temperature.



Slide 26 This is the variability of the product temperature only linked to the product resistance, and it was found that we were around plus minus five degrees, which is actually a lot. But we need also to evidence that these experiments were not performed in a clean room. The environment was not controlled. We also did not filter the solution, so this larger variability of the product temperature maybe due also to these factors. Once that we have the cumulative probability of the product temperature is possible to understand, to evaluate, which is the risk of failure of our process. So, for example, if we consider from the variability of Rp, and we calculate the cumulative probability of the product temperature for a specific operating parameter. And we

consider the critical value, the glass transition temperature for our solution, we can calculate from the cover, which is the percentage of vial that will have a product temperature higher than the critical value.

So, for this spontaneous nucleation was 2% and for the controlled nucleation was 0%. After determining the parameters via the design space, this can be a check that can be done and that can reassure us regarding assessment of the product resistance, mass transfer variability.



Slide 27 To conclude this webinar, we hope we have given some insight on how to develop a freeze-drying cycle, especially in considering the vial geometry, which we saw can lead to a product temperature variability of about two degrees. Also, to present to you more information that we have developed for the edge vial effect, showing that the gas conduction is the main mechanism responsible of this heat transfer variability, and also how to take into account the variability of the mass transfer and of the product resistance.