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Lyophilization Development: Quality by Design Approach – April, 2020
Dr. Indu Javeri, President & CEO, CuriRx

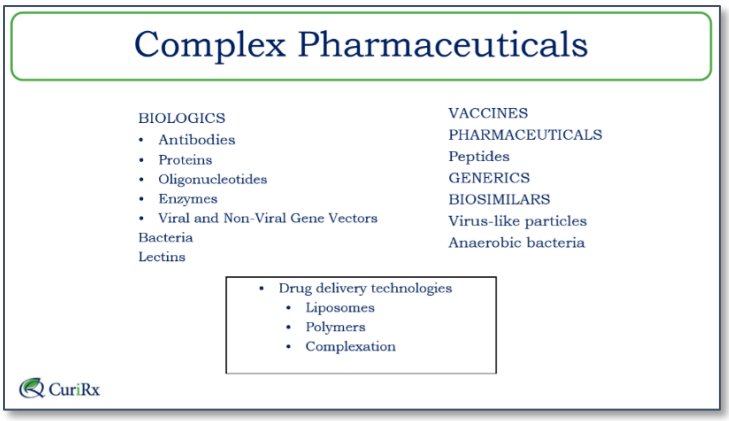
The prevalence of formulation stability challenges for complex APIs, biologics, microbiomes, gene therapy, viral vectors, liposomes, nanoparticles has resulted in the lyophilization of these complex molecules.

Lyophilization is particularly beneficial to parenteral drug developers, as a stable powder for injection can be easily packaged and transferred as a finished drug product. Lyophilization can also be employed to produce stable intermediates in drug product development and manufacturing. Hydrolytically unstable formulation components such as PLGA microparticles or fragile APIs may be lyophilized to create a longer shelf life and accommodate multi-step manufacturing processes. For example, APIs that undergo high energy media milling (AKA nanomilling) may be lyophilized prior to incorporation into an oral solid dosage form.

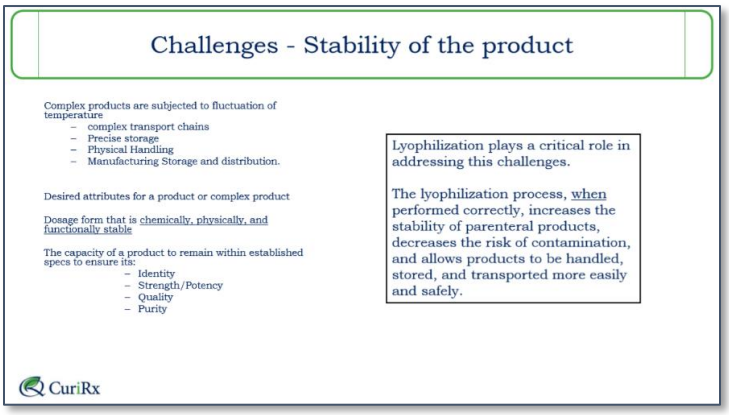
While lyophilization is considered beneficial and is a commercially viable process, it also poses complex formulation and manufacturing challenges. Lyophilization cycles require customized, extensive development for each product, and more so for nanoparticles, microparticles, liposomes, or microbiomes. This may include a series of studies to understand the freezing and drying behavior of formulation components as well as investigations into how formulation strengths or container closure affect the freeze-drying process.

This webinar will discuss these challenges and how to mitigate some of them by implementing a Quality by Design (QbD) approach. The highlights of this webinar are lyophilization of complex pharmaceuticals, challenges, and quality by design approach.

Quality is typically designed from the beginning of a product/process development to the end of the product/process development. When developing injectable lyophilized product, quality is designed into all the different steps of Lyophilization cycle development including technology transfer, scale-up and GMP manufacturing.

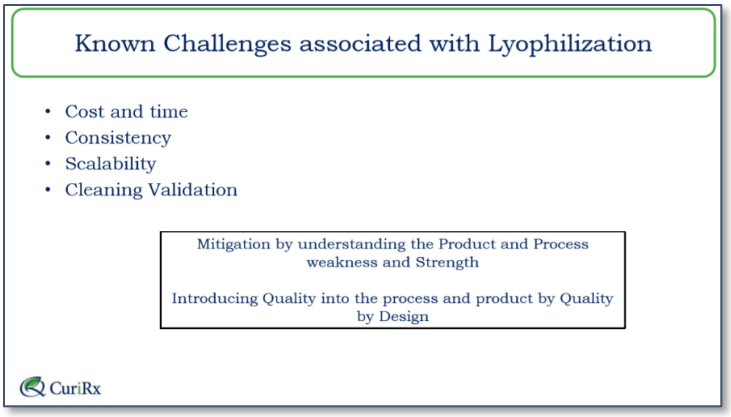


We'll talk about some case studies as well. Some of the pharmaceuticals are complex as listed here. They're delivered either orally or parenterally. For this webinar we are primarily discussing parenterally delivered products; IV, IM and subcutaneous. Many of these products may have stability issues and hence we want to really think about how to make a stable product and lyophilization may be necessary for stability.



Challenges that we see are that the complex products are subjected to fluctuation of temperature in complex transport chains, precise storage, physical handling, manufacturing, storage, and distribution.

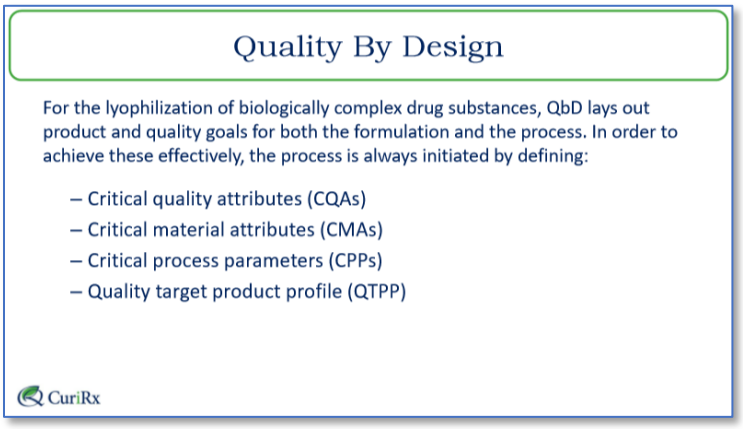
At the end of the day, when we are developing the product, we want to have the desired attributes that produce a dosage form that is chemically, physically, and functionally stable. The capacity of the product to remain within the established spec is to ensure its identity, strength, potency, quality, or purity. This can be addressed by lyophilization and lyophilization therefore plays a critical role in addressing these challenges. The lyophilization process when performed correctly increases the stability of parenteral products, decreases the risk of contamination, and allows products to be handled, stored, and transported more easily and safely.

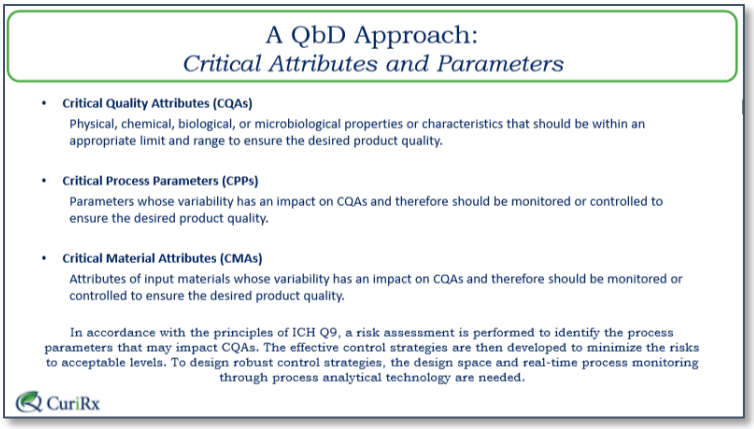


We know the challenges associated with the lyophilization, the cost and time and cleaning validation is something that is associated with the lyophilization development and I'm not going to really touch upon this part in this presentation. What I'm going to talk about is the consistency, scalability and the challenges associated with lyophilization process. And then mitigation is done by understanding the product and the process, weakness and strength and thereby introducing quality into the process and product by quality by design.

The reliability through quality by design is actually well defined in the International Conference of Harmonization Q8/Q9/Q10. These are are developed to provide guidelines to continuously improve quality and produce safer and more efficacious therapeutics. These guidelines, ICH Q8 guidelines define the following concepts; “Quality target product profile”, “Quality critical attributes”, Risk analysis” is provided in Q9. And, to link some of these attributes of the material and parameter to quality critical attributes and design space. The design space is how close or far we are from possible failure situations.

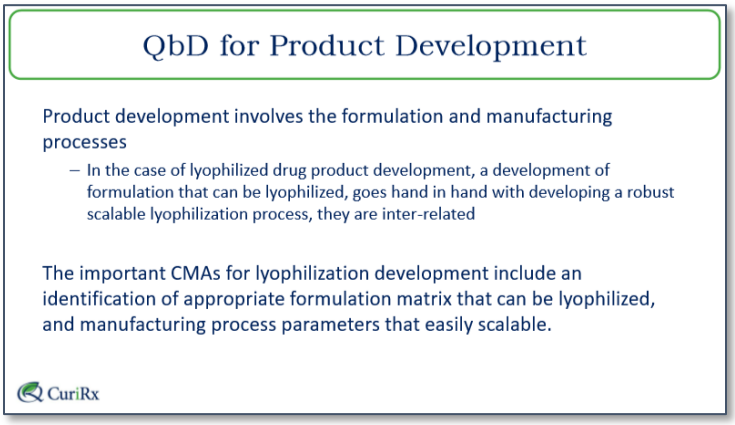
I'm going to take some time in defining some of these attributes. For lyophilization of biological complex drug substance QBD, which is Quality By Design. This lays out product and quality goals for both the formulation and lyophilization process. In order to achieve this effectively, the process is always initiated by defining critical quality attributes that you require. Critical material attributes, critical process parameters and the quality target product profile.





These are the critical attributes and parameters that are defined in the ICH guidelines. Critical quality attributes are defined as physical chemical, biological or microbiological properties or characteristics that should be within an appropriate limit and range to ensure the desired product quality. Critical process parameters are the parameters whose variability has an impact on CQAs and therefore should be monitored or controlled to ensure the desired product quality.

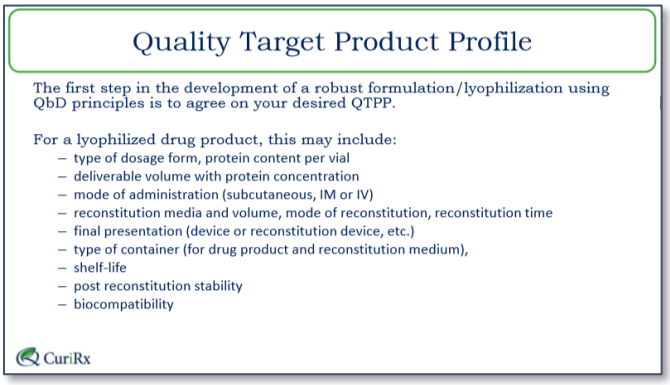
The critical material attributes, the attributes of the input materials whose variability has an impact on the quality attributes, therefore should be monitored and controlled to ensure the desired product quality. They are interlinked because one must really work on each of these aspects to get the desired product quality that one requires. In accordance with the principle of ICH Q9, a risk assessment is performed to identify the process parameter that may impact the CQAs. The effective control strategy is then developed to minimize the risk to acceptable levels. To design a robust control strategy, the design space and time process monitoring through process analytical technologies are needed. There are a lot of things I am hoping that we can walk through as we go forward in terms of how these are implemented within the lyophilization.



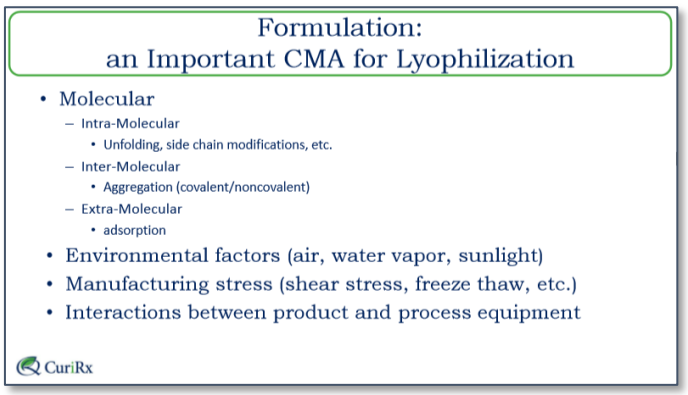
The QbD for product development involves formulation and manufacturing processes. And in the case of lyophilized drug product development, development of formulation is required that can be lyophilized, and that goes hand in hand with developing a robust scalable lyophilization process and they are interrelated. The important critical material attribute, which is what is going into the lyophilization includes appropriate formulation matrix that can be lyophilized and the manufacturing process parameters that are easily scalable.

The CMA, which is a critical manufacturing attribute is really critical when you are doing a lyophilization, what goes into the lyophilization is a matrix for the product. The product matrix becomes important because that is the key that allows us to then have the quality product in the end.

The product development involves the development of the formulation and what is in the formulation dictates the lyophilization development. The objective of formulation development is to identify the right composition and excipients to maintain and support the target product profile, maintaining product quality through various units of operation of manufacturing and appropriate shelf life. This is a very complex statement because “maintaining product quality through various unit operations,” that means that when you're doing a formulation matrix, the formulation matrix has to be done in a way that it withstands all the manufacturing unit operations. That includes compounding the formulation and then going to the manufacturing unit in terms of dispensing into vials and then into the lyophilization. It must withstand that whole process, whole unit operations to then do a lyophilization. So, this becomes possible when interaction between the formulation and the process parameters are fully understood and well controlled. And QBD principles helps us to achieve that.

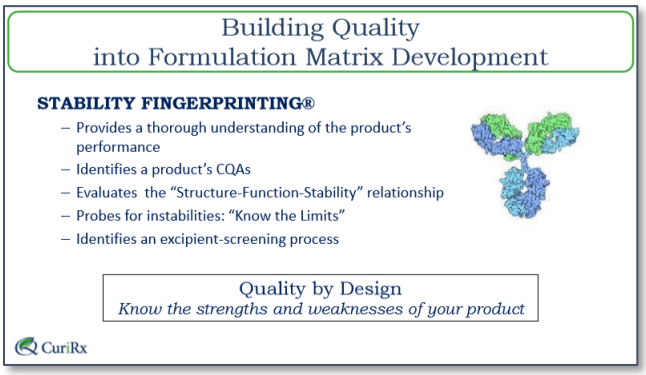


The first step in development of a robust formulation or lyophilization using QBD principle is to agree upon the desired target product profile. And, for the lyophilized drug product, this may include type of dosage form, protein content per vial, deliverable volume with protein concentration, mode of administration, either subcutaneous IM or IV or any other mode of administration. Most importantly reconstitution media and volume, mode of reconstitution and reconstitution time. Final presentation whether it is a prefilled syringe device or reconstitution device, type of container closure, shelf life, post reconstitution stability and biocompatibility. This target profile helps us to determine what is the matrix, that then can be lyophilized, and that lyophilized product then would have the desired of stability and maintain the shelf life.

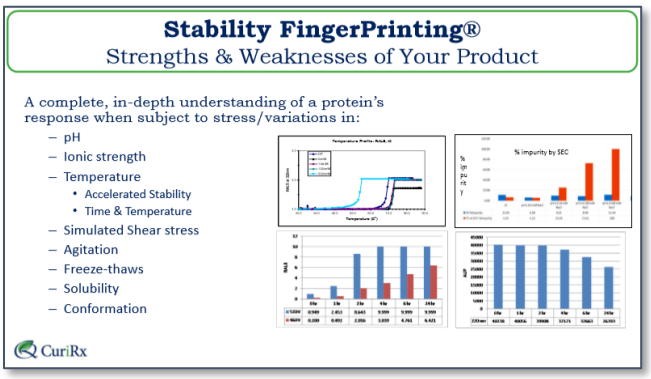


When we look at developing a formulation matrix, which is an important critical material attribute for lyophilization, we need to think about it at a molecular level of the drug substance or the complex drug product, which is inter-molecular. At the molecule level you need to understand intra-molecular relationship, which is unfolding, side chain modification, inter-molecular, which is aggregation covalent or

non-polar. Extra- molecular, which is adsorption. We also need to worry about the environmental factors of air, water vapors, sunlight or light related manufacturing stress, freeze / thaw, shear stress, agitation, then interaction between product and process equipment. This all gets combined to become critical material attributes that then goes into the lyophilization. So how do we build quality into a formulation matrix that is going to go into lyophilization?



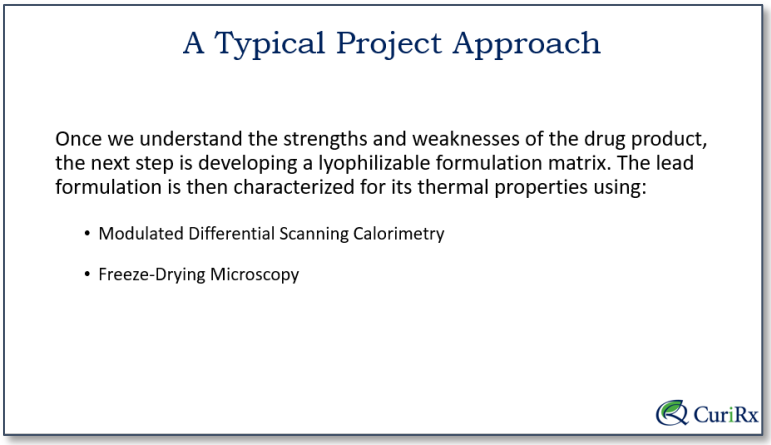
At CuriRx we have developed a stability fingerprinting that allows us to gain a total understanding a product's performance. It identifies the product's critical quality attributes, evaluates structure-function-stability relationships, it probes for instability, knowing the limits. Now we are not discussing design space currently, but we are trying to understand the limits within the product itself and then identify how to have an excipient screening program. In this frame which is "developing a formulation matrix" that goes into lyophilization, the quality by design attributes then help us understand the strength and weakness of the product.



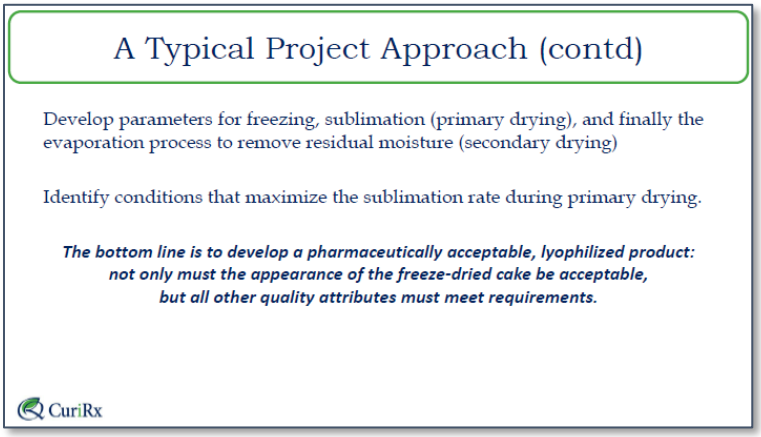
This is just an example of how that is done. It is a complete and in depth understanding of a protein's response subjected to stress and variation in pH and extreme temperatures, simulated shear stress, agitation, freeze-thaws, solubility, and conformation related changes. On the right-hand side of the chart is just an example of ionic strength, as you see the increase of the ionic strength from right to left. And we can see that the Tagg increases as ionic strength of the formulation is decreased. This is done using Right-Angle Light Scattering.

The right-hand box is the impurity by size exclusion of the same formulation at a different ionic strength. And as you see as you increase in ionic strength, we are looking at formation of high molecular based species. The bottom two panels have very similar information that we can derive. The bottom two panels are simulated shear stress of the product. Within 24 hours we can ask, does this molecule have shear sensitivity? And the answer is yes, because within two hours you can see an increase in the light scattering of the formulation. If you were to look at the formulation with size exclusion, you do not see that effect until four hours.

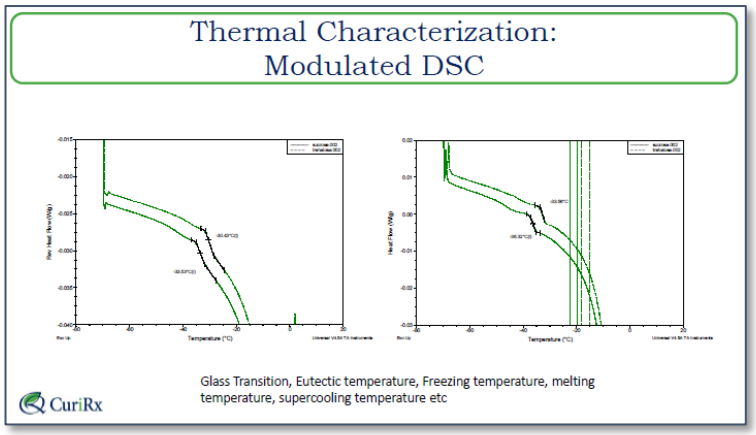
So, we try to include tools, which is the PAT, in order to come up with the formulation matrix that can withstand stand all the manufacturing unit operations in order to develop a formulation matrix that can go into lyophilization.



So, what does a typical project approach look like? Once we understand the strength and weakness of the drug product, the next step in developing is to develop a lyophilization formulation matrix.



The lead formulation is then characterized for its thermal properties using modulator DSC or differential scanning calorimeter, freeze dying microscopy and then to develop parameters for freezing, sublimation and finally evaporation process to remove the residual moisture, which is the lyophilization process. Identify conditions that maximize the sublimation rate during primary drying. The bottom line is to develop a pharmaceutically acceptable lyophilized product, the appearance of the freeze-dried cake must be acceptable, and all the other quality attributes must meet requirements.

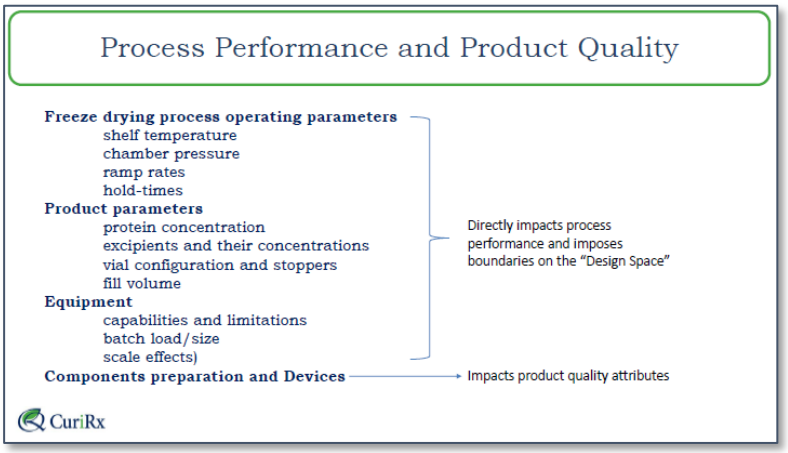


Starting with thermal characterization by modulated DSC. What does that mean, “thermal characterization?” It means that the molecule or a solution can go into three different phases and liquid becomes solid and then solid become gas. That is the principle of lyophilization.

Lyophilization is where we take the liquid and we freeze it and while we freeze it, we are freezing in order to make ice crystalize and then using that freezing, we want to not go back into the liquid stage but, instead, we want to make that into a gas state. So, by doing thermal characterization using modulated DSC, we determine something called glass transition. The glass transition is a transitioning point where the solid can go into the liquid form, but we do not want that to happen. So, determining glass transition of that formulation matrix becomes very critical. Eutectic temperature in many cases when you have a crystalline excipient, you will have an exothermic reaction. Then you want to understand the eutectic temperature. You want to understand the freezing temperature, melting temperatures to supercooling

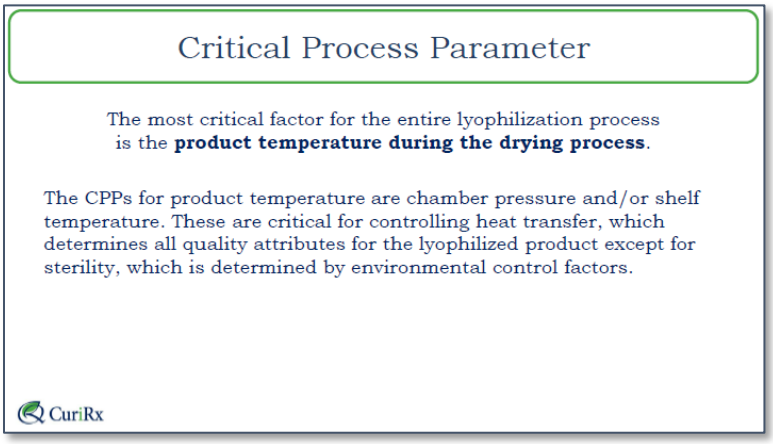
temperature. All these attributes or thermal properties or thermal characterizations can be achieved by a modulated DSC or freeze-drying microscopy.

This is one of example where we have looked at both reverse heat flow and heat flow. On the right-hand panel there's heat flow. On the left-hand panel is reverse heat flow. In one case, the formulation has sucrose as a bulking agent or as a stabilizer and in other cases the formulation contains trehalose, the transitions you note here is called the glass transition, where some part of the solid is transitioning into a liquid form due to the presence of sucrose or trehalose. What that suggests is using this information, we want to maintain that formulation matrix or the product at or below this glass transition of the formulation matrix during primary drying of the lyophilization cycle. That becomes critical for establishing the primary drying temperatures.



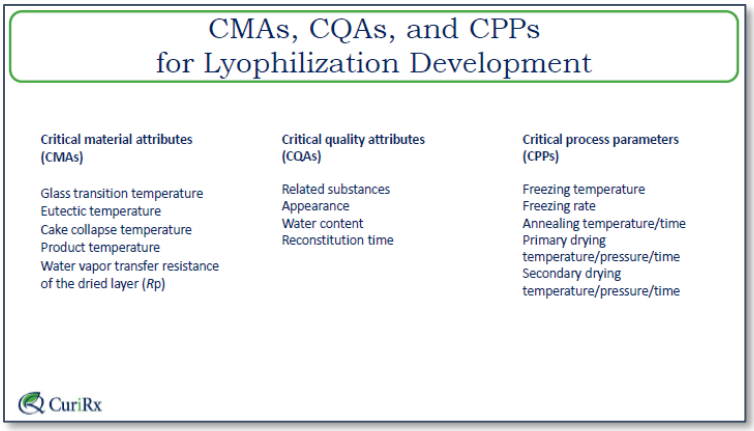
Once we understand the temperature characteristics or thermal characteristics, then we want to ask what is the process performance and the product quality?

The freeze drying process operating parameters are the shelf, the temperature, chamber pressure, ramp rates, hold-times. Product parameters are protein concentration, excipients and the concentration, vial and stopper configuration and fill volume. And the lyophilization equipment. The capability and the limitation, batch load or size of the batch or scale effect. These three really impact process performance and impose boundaries on design space. The component preparation and device impact product quality attributes. And those component preparation like washing the glass while making sure it is particulate free, the devices, all impact the quality attributes.



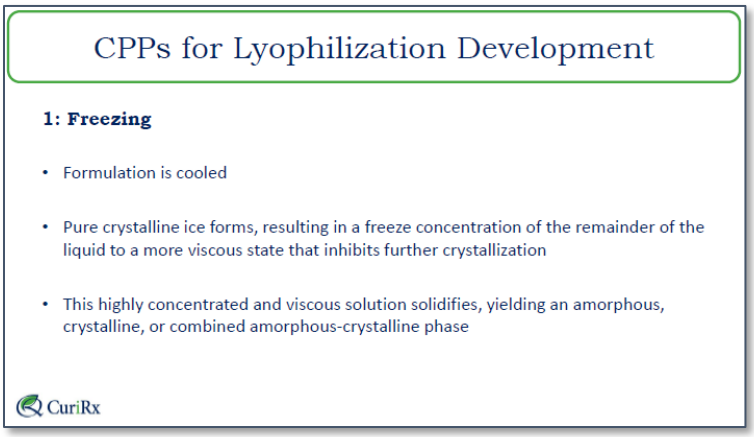
When we look at the critical process parameters for lyophilization, the most critical factor is the product temperature during the drying process. So, the CPP, which is a critical process parameter for product temperature are chamber pressure and shelf temperature.

The critical factor is to maintain the product temperature, but to maintain the product temperature, the critical process parameters are chamber pressure and shelf temperature. These are critical for controlling heat transfer, which determines all the quality attributes for the lyophilized product except for sterility, which is determined by the environmental control factors.

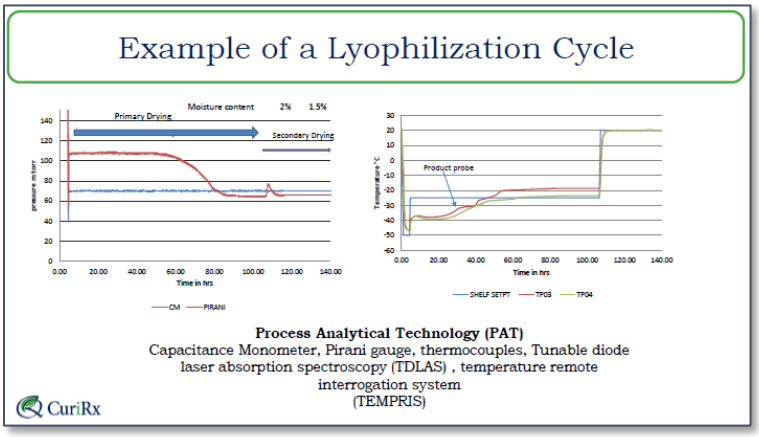


Here are the definitions for lyophilization development that one has to keep in mind, the CMA, which are critical material attributes. CQAs critical quality attributes and CPPs, which are critical process parameters. This is only for lyophilization. So for the CMA, we want to not only have the formulation matrix that can go in or can be lyophilized, but also to understand the glass transition, eutectic temperature, cake collapse temperature, product temperature, water vapor transfer and resistance of the dried layer.

We will talk a little bit about that in next few slides. The critical quality attributes, CQAs, which are related to substance, appearance, water content and reconstitution time. And the critical process parameters are freezing temperature, freezing rate, temperature if annealing is required and time for annealing, primary drying temperature, pressure and time and the secondary drying temperature, pressure and time.



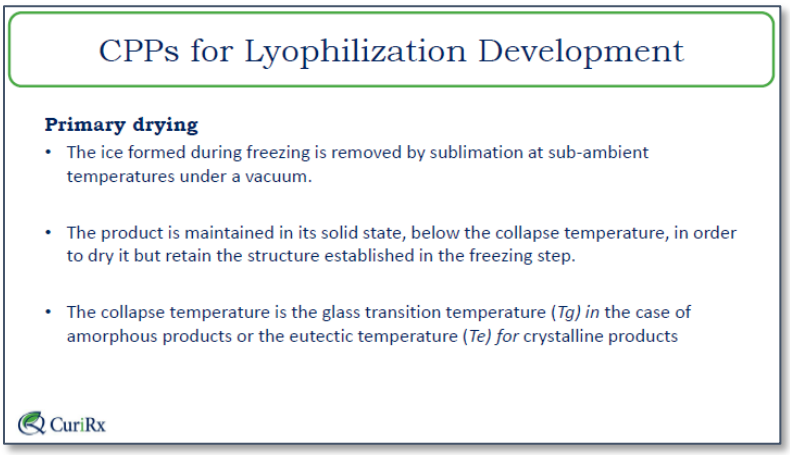
When we talk about lyophilization in general, the first step is the freezing step. The formulation is cooled, pure crystalline ice forms resulting in a freeze concentration of the remainder of the liquid to a more viscous state that inhibits further crystallization. This highly concentrated and viscous solution solidifies yielding an amorphous crystalline or combined amorphous-crystalline phase.



This is an example; the initial freezing time point where we are looking at just the freezing. Within the first few hours, two to three or four hours, we are just freezing the solution. This slide is really busy addressing primary drying, secondary drying in the subsequent slides.

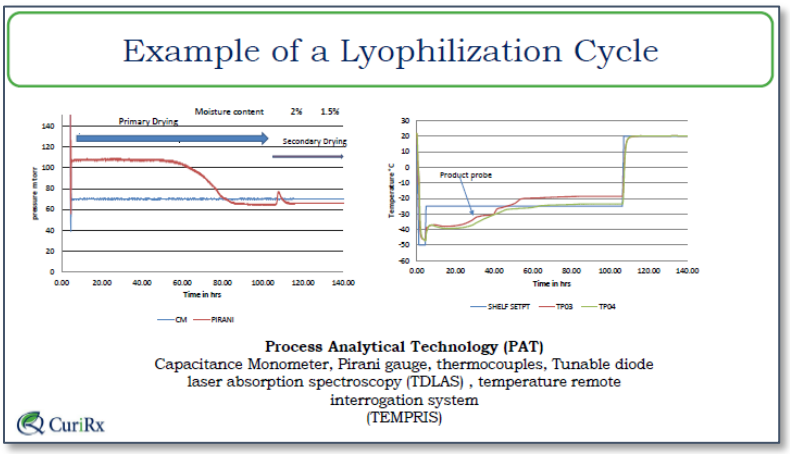
But the key thing I want to point out over here is the process analytical technology, which is a PAT tool. We have a capacitance, monometer, Pirani gauges and thermocouples.

In this slide we're not showing Tunable diode laser absorption spectroscopy but it is one of the tools that is now in commercial usage, temperature remote interrogation system, those are the process analytical technologies that are available to monitor the lyophilization cycle as we're doing the lyophilization cycle development. So here I'm just talking about the freezing time which initially you are freezing and during that freezing you will see a super cooling effect. And you want to bring the entire solution into a very solid state.



Then, we go into the primary drying. The ice that is formed during freezing is now removed. The solid ice is removed by sublimation as a sub-ambient temperature under a vacuum.

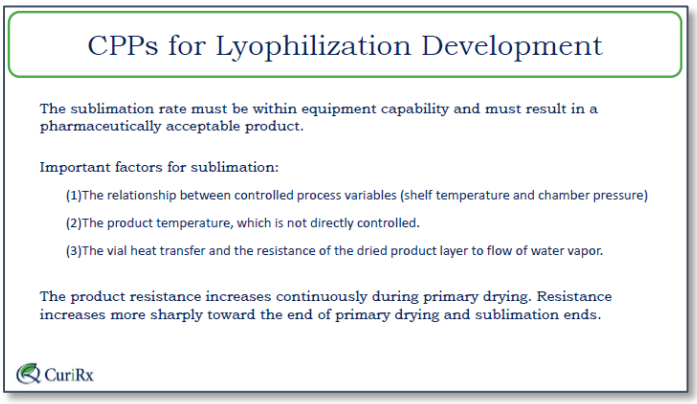
We can just remove the ice, but, we also want to have this product maintained in a solid state. We need to make sure that it does not turn into liquid. We want to make sure that that product is maintained in its solid state by maintaining the temperature of the product in the vial at or below the glass transition. The collapse temperature is the glass transition temperature for the amorphous product and Eutectic temperature for the crystalline product.



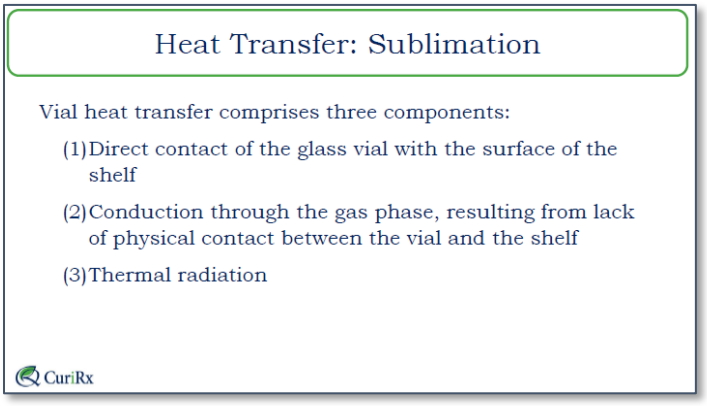
This again is the same slide that was shown earlier where we are looking at primary drying. Primary drying is represented by a thick blue arrow on the left-hand graph. Primary drying is the longest time process that a lyophilizer performs because we are trying to remove the ice or we're trying to make the ice crystals into a gas or vapor form by a sublimation process. The sublimation process happens from the top of the vial to the bottom of the vial. That the heat transfer is from the shelf to the bottom of the vial.

Primary drying becomes a very complex step in the lyophilization. When we are using the Pirani gauge, which is now represented in the left-hand side panel with the red line, as the Pirani gauge goes down, we know there is a reduction of moisture in the chamber and we want to make sure that the Pirani gauge is actually looking at thermal conductivity. The capacitance monometer is basically looking at the pressure in the chamber, the temperature product probe, which is on the right hand panel, which is now represented here as a red and green line, is a probe inserted in the vial.

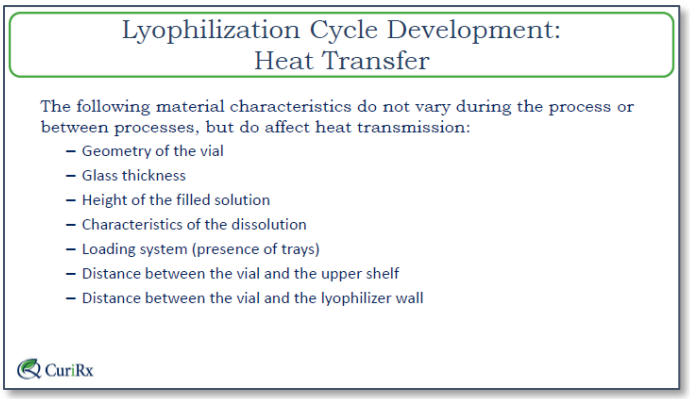
This shows the product temperature that is in the vial and how it is drying. Having this information allows us to develop a primary drying cycle. Again, the variable that we want to really play with this is the temperature and the vacuum, which then allows us to look at the product temperature in the vial that has to remain at or below the glass transition. So, by the end of the primary dryings, we want to make sure that the moisture is 90% removed by just going from solid state to the gas state.



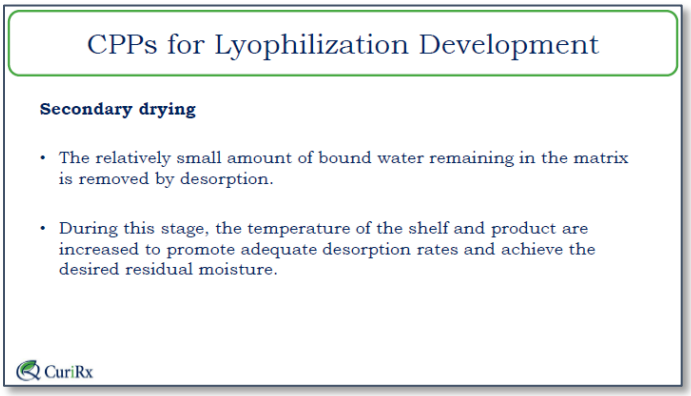
The sublimation rate must be within the equipment capability and must result in the pharmaceutically acceptable product. The important factors for sublimation is the relationship between controlled process variables, which is the shelf temperature and the chamber pressure. The product temperature, which is not directly controlled because you cannot control that directly, but you can indirectly control product temperature by shelf temperature and the chamber pressure. The vial heat transfer and the resistance of the dried product layer to flow off water vapor. Remember sublimation process happens from top to bottom. When we are doing a sublimation, we are creating a resistance at the top and the product resistance increases continuously during the primary drying and the resistance increases sharply towards the end of the primary drying and the sublimation then ends because it starts to form a thicker barrier.



So, what makes sublimation process happens? Vial heat transfer, which is direct contact of the glass vial with the surface on the shelf. Conduction of the gas phase resulting from lack of physical contact between the vial and the shelf and the thermal radiation.

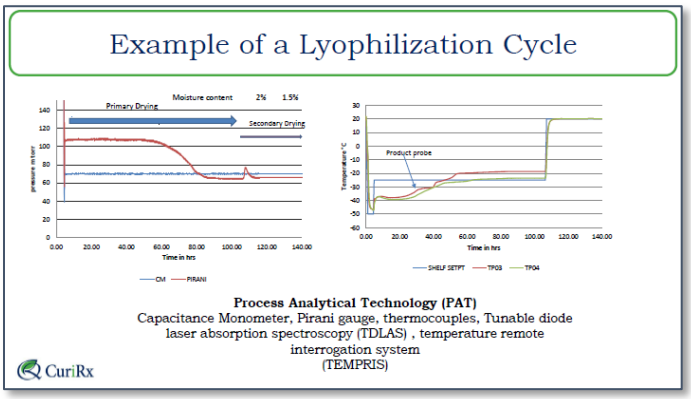


The heat transfer is important to consider and material characteristics have an impact on heat transfer. These important considerations do not vary during the process, but they need to be considered before we start to develop the process.



Material characteristics in need of consideration include the geometry of the vial, the glass thickness, height of the filled solution, characteristics of the dissolution, loading system, distance between the vial and the upper shelf. Distance between the vial and the lyophilizer wall.

Once we have removed 90% of the moisture from the vial or water content from the vial, there is relatively small amount of bound water remaining in the matrix and it is removed by desorption.



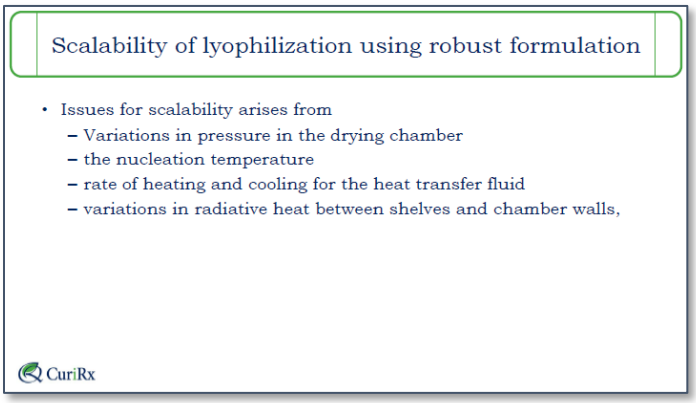
During this stage the temperature of the shelf and the product are increased to promote adequate desorption rate and to achieve the desired residual moisture. I'm going back to the same slide that I had before where we are looking at the secondary drying stage, which is depicted in the left-hand panel, on top of it says moisture content 2% and 1.5%.

What this means is that, as you are start to look at secondary drawing, which is by evaporation or desorption where you have a higher temperature that allows for removing remaining water, there is a point where you need to ask, “what are your specifications for moisture content?”

For each molecule, whether the moisture content is 2% or less is desired, what is the desired moisture content for the particular molecule. And that is determined when you are developing the formulation matrix.

That is when you are trying to determine if this product is susceptible to even small amounts of water? Remember water is very reactive, so, when the water is reactive, it will create the degradation pathway. In some cases, 2% to 5% is pretty good for the product, but in some cases, you may be required to be at less than 0.5% because the water is very active.

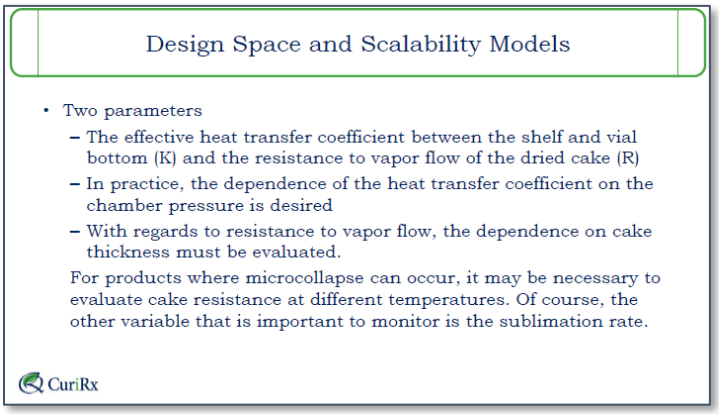
This is very true for some of the coagulation factors that I worked on where you had to really be at a less than 0.5% the moisture content. Secondary drying is where you can remove the vials from the chamber as you are removing the moisture using heat. This will allow you to then determine the kind of percent moisture you require, and you can put the product into the stability program at different time points. So, then you can see the red, does that affect the quality of the product?



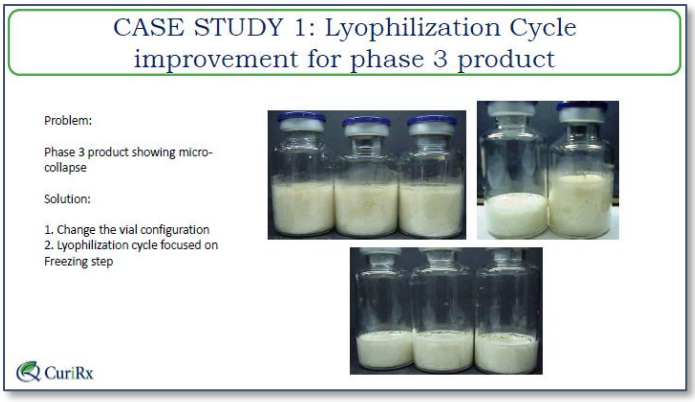
The issue for scalability arises from many different factors. It arises from having a different lyophilizer in the manufacturing, so not only the lab scale but the pilot scale and commercial scale, all maybe different lyophilizers. And the variation in the pressure in the drying chamber, the nucleation temperature. The freezing process, though not much time is spent during freezing, as most of the time is spent in primary drying, freezing is very, very critical because we want to create a nucleation temperature where all the solution is solidified uniformly. And, in a very clean environment nucleation may not be possible. We also need to consider the rate of heating and cooling the heat transfer fluid.

All lyophilizers have heat transfer fluid that is embedded near the shelf and all over the lyophilizer, that is how they are designed. That heat transfer fluid rates may vary from lyophilizer to lyophilizer. There can also be variation in radiative heat between the shelf and the chamber walls. These are all important considerations when you are scaling up.

You generally go from lab scale processing to a pilot scale then to a commercial scale. These considerations become very critical to think about. Some of these things can be taken care of by creating some robustness into the formulation matrix and robustness into the lyophilization process.



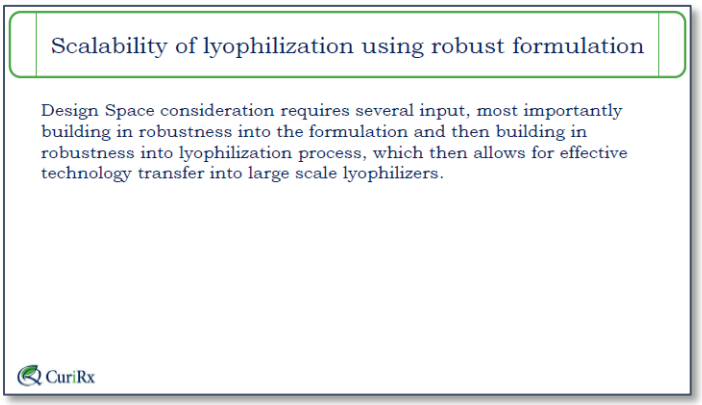
There are two factors or parameters that are critical to understand during lyophilization. They are the heat transfer coefficient that is between the shelf and the vial bottom and the resistance of the vapor flow of the dried cake. In practice, the dependence of the heat transfer coefficient on the chamber pressure is desired. And with the respect of the resistance with the vapor flow, the dependence on the cake thickness must be evaluated. For the products where a micro collapse can occur, it may be necessary to evaluate cake resistance at different temperatures. Of course, the other variable important to monitor is the sublimation rate.



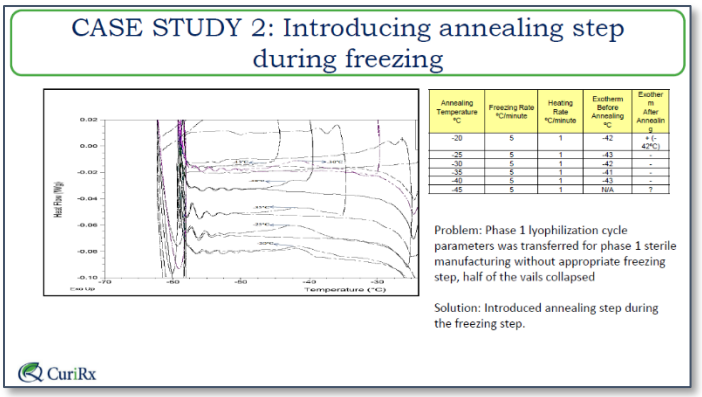
Here is an example, a case study, where we had a project that had come to us that was already in phase three. In this slide, on the left-hand side, the first panel, has the fill height of three vials shown as the same. This is a GMP manufactured at the phase three level using a large scale GMP lyophilizer.

You can actually see in there're micro collapses. So, the solution that we had talked about with the client is to really reduce the fill volume. The design space was done for this product because it's already phase three. However, the design space was not robust enough to understand the risk factor of the micro collapse. The solution was to change the vial configuration because we deemed the vial configuration to be an issue as sublimation happens from top to bottom. There is a resistance that builds up on the top.

We knew that the micro collapse was not due to the excipient nor was it due to the glass transition, but it was a factor of vial configuration. However, as we all know, having a phase three product, you really cannot change much. So, we came up with a solution by just focusing on the freezing step, to create a way that, during freezing, we can create larger pore sizes. By doing so, we were able to give the client a cycle with a modified freezing step, without changing the vial configuration. And it was that step, when incorporated into the phase three lyophilization parameters, that gave them a product without any collapse. A successful case study.

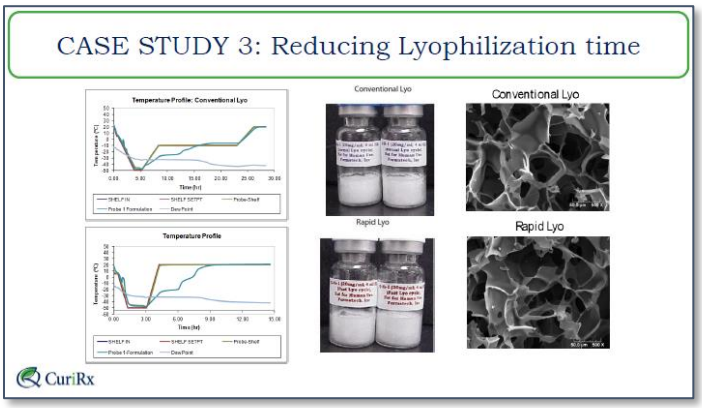


Scalability of lyophilization has several inputs, most importantly, building in robustness into the formulation and then building in robustness into the lyophilization process, which then allows for the effective technology transfer to large scale lyophilizers.



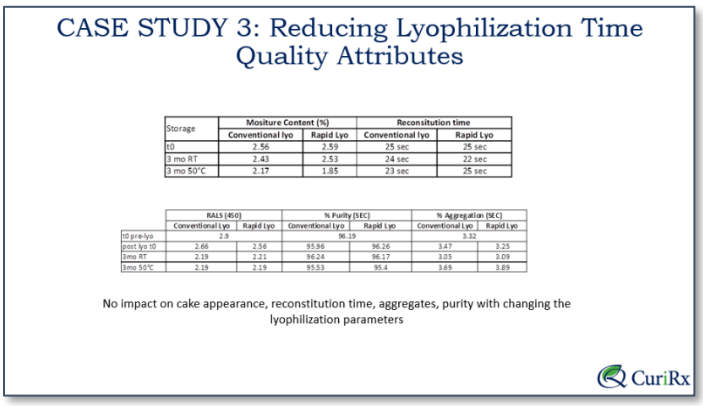
This is another example where we have phase 1 lyophilization parameters transferred for phase 1 sterile manufacturing without an appropriate freezing step. Half of the vials collapsed during the phase one production of the product.

So, upon investigation, we determined that the formulation matrix excipients required annealing step.. We introduced an annealing step during the freezing step and that actually helped in creating a lyophilization cycle to give the product right attributes.



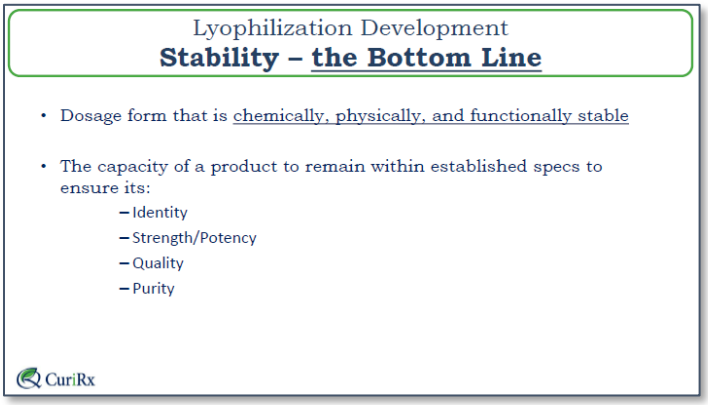
A third case study we are presenting is where we can reduce the lyophilization time if the formulation matrix is correct. Here, we have shown the conventional cycle that took about 30 hours and the second cycle below it took 15 hours.

This where we have taken the same formulation matrix but did two different lyophilization cycles that allowed us to get to what we call, “a Conventional Lyo” and “Rapid Lyo.”



On the very right-hand side you can see an electron microgram of electron microscopy pictures where you see the pore size is very similar, the final cake also is very similar.

By reducing the lyophilization time we did not change the quality attributes. There was no impact on cake appearance, reconstitution time, aggregation, or purity by changing any of the lyophilization parameters.



To recap, lyophilization development is really done to have a stable product. So, the stability is the bottom line, to have a dosage form that is chemically, physically and functionally stable, for the capacity of a product to remain within the established spec to ensure its identity, strength potency, quality and purity.

In conclusion the quality by design approach is introduced from the early stage of understanding the products weakness and strength, creating a lyophilizable formulation matrix then allows for developing lyophilization cycle parameters. Introducing design space studies and then experiments to perform risk analysis builds robustness into the process and quality into the product.

Conclusion

The QBD approach is introduced

- from the early stages of understanding the products strength and weakness
- creating lyophilizable formulation matrix that allows for developing lyophilization cycle parameters
- introducing design space studies
- experiments to perform risk analysis builds robustness into process and quality into the product.

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