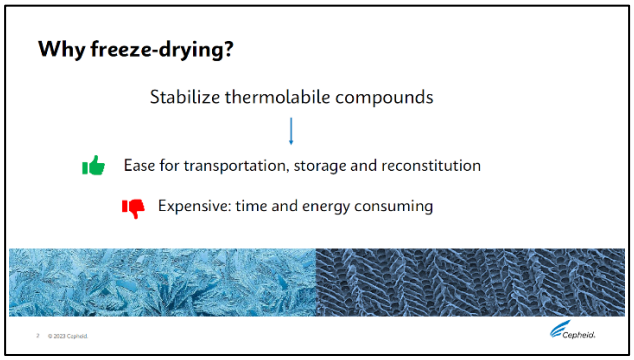
Freeze-drying cycle parameters considerations in relation to formulation characteristics for LyoBeads – Edited Webinar Transcript 6/29/2023

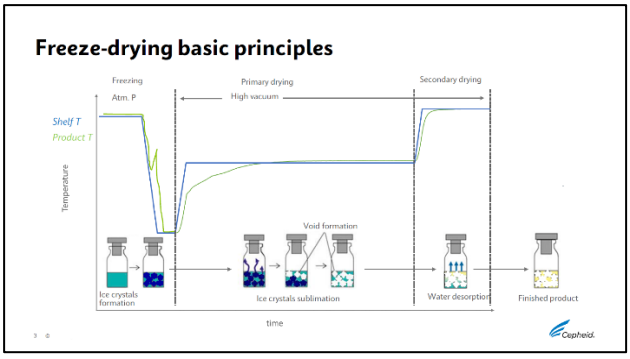
Presented by, Maria Badal Tejedor – Associate Principal Scientist, Freeze Drying - Cepheid

Okay, so good morning. Good afternoon, everyone. My name is Maria Badal and I'm currently working as an associate principal scientist with lyophilization in Cepheid. Cepheid is a diagnostic company that develops and manufactures individualized diagnostic kits for detection of infectious diseases among other things.

My background is in material science applied to surface chemistry within pharmaceuticals. And then for the last three years I've been working with Cepheid with lyophilization also diagnostics. I'll go through my presentation so you can type your questions in the chat box and then I will read them after I have finished the talk. Today's talk is about freeze drying cycle parameters, considerations in relation to the formulation characteristics for the specific system of LyoBeads.



Why do we freeze dry? That's an important question. What we want to achieve with freeze drying is to stabilize thermolabile compounds that cannot be dried by applying heat because they could actually lose their structure and they will no longer fulfill their function. There are also certain advantages associated with freeze drying. It's that once the solution is dried, it's easy to transport, it's easy to store, and then it provides long-term stability. And then we also have a porous structure in the form of a cake that is easy to reconstitute. But there are also some disadvantages associated with freeze drying. It is expensive. It's a process that is time and energy consuming and, if we want to make it efficient, we need to really devote time to develop the proper formulation and then set the proper drying parameters to actually not extend the drying time very long. And for that, it's also necessary to have the right knowledge of the technique.

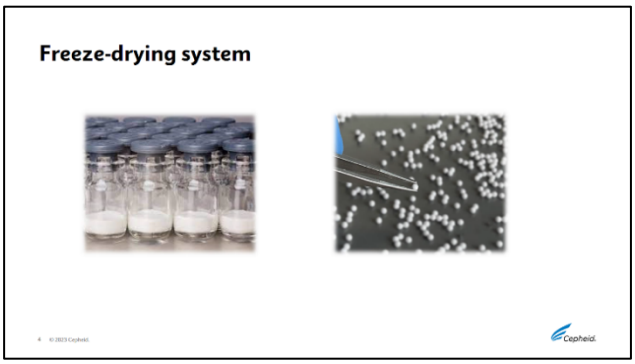


What I'm showing right now is a typical drying cycle. So, what I am showing is the evolution of the temperature during the drying cycle and then in the blue line, we see the settings for the shelf temperature inside the chamber of the freeze dryer. And then in green, we see the evolution of the product temperature during that time and how this follows the set temperature. But the freeze drying cycle is divided in three main steps. The first is freezing, the shelf temperature, is

decreased from room temperature to minus 55, 60 degrees atmospheric pressure. And usually, the standard settings that we have, is that the solution is placed into a vial and the vial is cooled down and frozen.

And there we can actually differentiate two main phases. One is the ice crystal phase and the other one is the so-called “freeze concentrate,” where all the other components that were in solution before, they get in close proximity to each other. And, in some cases, we can even differentiate a third phase if we have a component that actually crystallizes. So, then we have the crystalline phase of that component. And then after freezing, we move into primary drying, which is the second state in freeze drying. And then for that, we reduce the vacuum. We apply high vacuum and then we need to provide energy into the system for sublimation to happen. So, we increase the shelf temperature and then what happens, is the ice crystals that formed during freezing, they basically transform from solid phase to a vapor phase during primary drying and then they leave behind all the voids into the cake.

So, we get a very porous and fragile structure. After primary drying, we move to secondary drying, also having the same high vacuum in there. What we want to do is to desorb the remaining water that was trapped in the freeze-concentrate because when we froze, not all the water has the ability to actually grow into the crystal phase, into the ice crystal form. And then some of the water molecules will remain trapped in the freeze-concentrate. We want to make the product as dry as possible. So, we want to desorb that water. We do it by even increasing further the shelf temperature to 25 degrees approximately. And then after that we have our finished product.

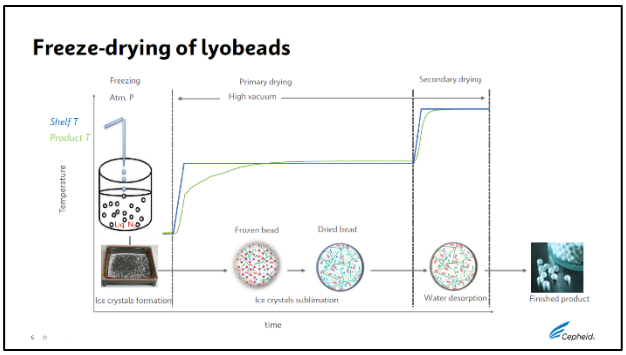


So, the common setup or the most common setup, is to actually lyophilize in the form of vials. But what we're going to do right now is to lyophilize in the form of beads.

That means that the process is going to change. The freezing step is going to be different. And we will also have other requirements for the LyoBeads because we want to handle these beads. These beads will be transported later on. So, we need them to be stiff enough to not break on the way.

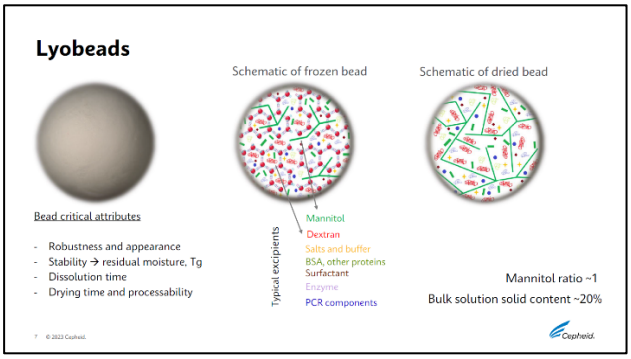
Let’s look at the freeze drying process when we are handling beads. What we do to freeze our solution in the form of beads, is to drop that solution into a tank. And that tank is filled with liquid nitrogen.

So basically, what happens to the droplet when it meets the liquid nitrogen is that it freezes immediately. So, it has a quench cooling and then the frozen bead precipitates inside the tank filled with nitrogen. So manually, liquid nitrogen together with the beads, it's transferred into a tray. The trays are loaded into the freeze dryer. We run our process, our freeze drying cycle. And then after also manually collect these trays and then we put all the beads together into bottles because these bottles will probably be transported and stored before they go into the manufacturing line to be filled into the final packaging.



If we look again at our drying cycle, we see how now the freezing has changed. What we have is a quench cooling freezing of our beads. When we drop the beads in liquid nitrogen, these are transferred again into the chambers. So, the shelves have been pre-cooled. The beads are loaded in shelves that have a temperature of minus 50, 60 degrees. So, we have our frozen bead that will also go for primary drying. Primary drying is also performed at a high vacuum and then we start providing energy into the system by increasing the shelf temperature so that all ice crystals will sublimate. But in this case, since we had quench cooling, small crystals have formed. They didn't have the opportunity to grow into very large crystals and perhaps it will have a component that has the tendency to crystallize. It did not have the time to crystallize either.

In this case, when we are performing the drying, several things will be happening at the same time. We will increase the shelf temperature, we will give energy into the system, until sublimation starts to happen. The energy will not only be used for sublimating the ice crystals, but also to increase the product temperature. And at the same time, we'll have other events going on in the case of having a crystalline component. So, after our bead has dried, we also move into secondary drying to absorb the water that was trapped into the freeze concentrate as in the case before. And then after increasing the temperature and secondary drying and giving time to desorb the water, we will have our finished product.



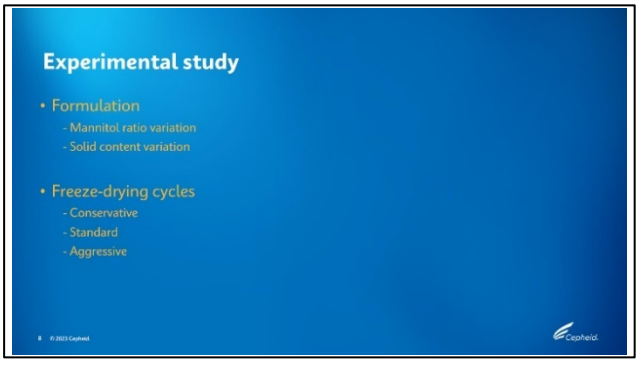
But LyoBeads need to meet certain requirements to be able to be used for its purpose. And then some of the critical attributes that we want from our LyoBeads is that they are robust enough. They have a good appearance and especially they do not look very much collapsed.

Collapse is detrimental especially for the drying process as water might be trapped if the structure has collapsed. So, we want to have good stability for our beads, so the residual moisture has to be low and also the glass transition has to be as high as possible. So, to be able later on to store these beads at

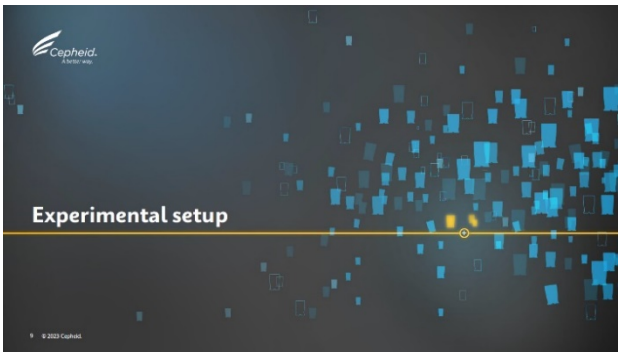
higher temperatures. We also want the bead to have a good porous structure so that it has fast and quick dissolution time for reconstitution later on. And then of course we want to have a system that makes it possible to dry in a short time as possible. So, for that we need to be careful about which components that we choose to formulate our beads. One of the most commonly used excipients are mannitol and dextran. These two excipients provide a structure to the beads.

And in the case of mannitol, it's an excipient that crystallizes so that it forms like a scaffold into the structure that holds all the other components on it. Dextran also gives hardness and a structure to the bead and will also give robustness, but these components will not be alone in the formulation. We need other components that are necessary to fulfill the functions of the bead. And in the case of our beads, they're going to be used to perform a PCR reaction. We have other components that are important for the reaction. We have our biological molecule that will be stabilized into the matrix. And then of course we need to add other components that will provide stability to the enzyme. Those can be proteins or those can be small molecules in the form of non-resistant sugars. And then the protein also comes with a buffer with some salts.

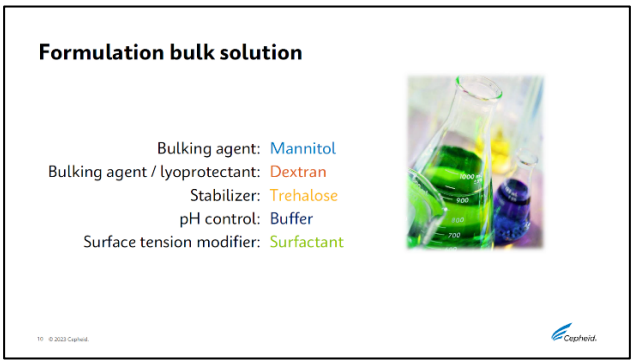
And then in some cases, it's also necessary to add a surfactant to process the liquid formulation through the tubing system. But all these components need to be added into a certain amount to be able to fulfill the critical attributes of the beads of robustness. And for example, in the case of mannitol, it's important to consider that it needs to crystallize during drying. And if it doesn't, it does not fulfill a scaffold function, but it's actually detrimental to the system acting as a plasticizer. So, it's important to keep in mind that the mannitol ratio should be around one. And the same happens with the solid content of our bulk solution. We need to have a certain amount of solids to actually have a round bead that holds its structure. All of these parameters are going to be studied in the following study that we have performed.



And then we are also going to investigate which are the best drying parameters to be applied depending on the characteristics of each formulation. So, what we have done in our following experimental study, is to design different formulations with different mannitol ratios, also with different solid contents.

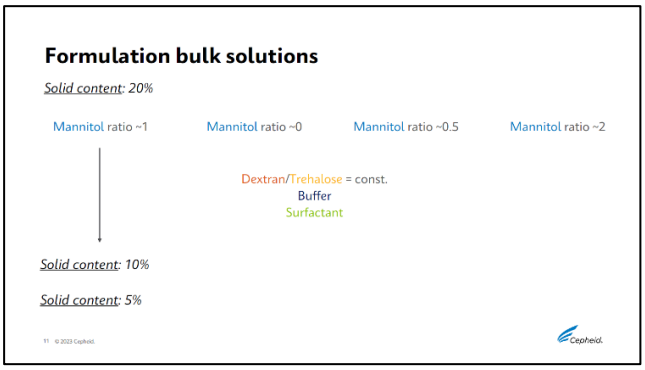


And we have dried those with different drying cycles and experimental setup is as follows: The materials that we have used in our formulation are mannitol that acts as a bulking agent, and it has crystalline properties. We also have the dextran that acts as a bulking agent, and it has lyo-protectant properties. We use Trehalose that is commonly used as a stabilizer for biologics. And then of course we have a buffer

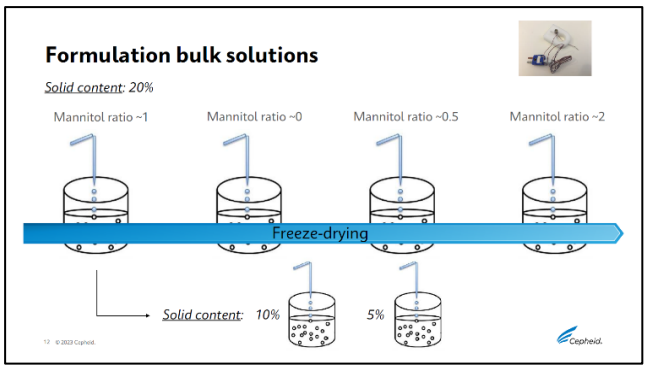


and a surfactant. The buffer will control the pH to keep our active molecule stable when the active molecule gets into the formulation. Right now, we are just testing a blank formulation and then we have surfactant that is needed for the processability of the solution.

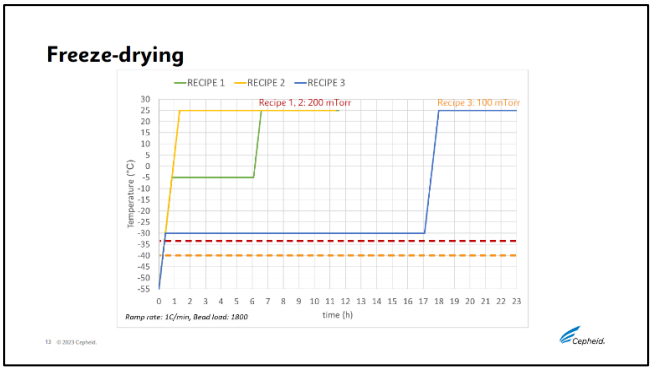
So, these are the four different systems that we are going to investigate. When the solid content, is of 20%. So, we have changed the mannitol ratios, so we have a mantle ratio of one.



We have no mantle in the formulation, and then we have a very low mannitol ratio of 0.5 and then a high amount in content of mannitol when the ratio is of two. All the other components were kept constant, so the amount of buffer and surfactant was constant for all the formulations. And then we were barring the amount of the trehalose in the formulation to meet actually the percentage with the mannitol ratio. But the correlation between dextran and trehalose was kept constant. And then also lower solid content was the behavior of lower solid content formulation was also investigated, but it was investigated in the case of having a mannitol ratio of one.



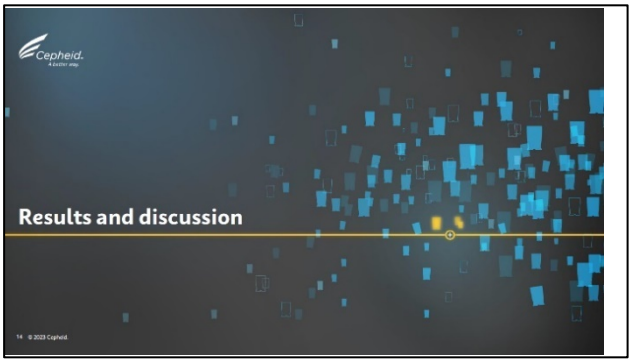
All these solutions were prepared and then were dropped in liquid nitrogen, and then thermocouples were connected to some beads in each cycle. And then they were also connected to each of the systems. And then three different batches were loaded and freeze-dried with the three different programs that we are testing.



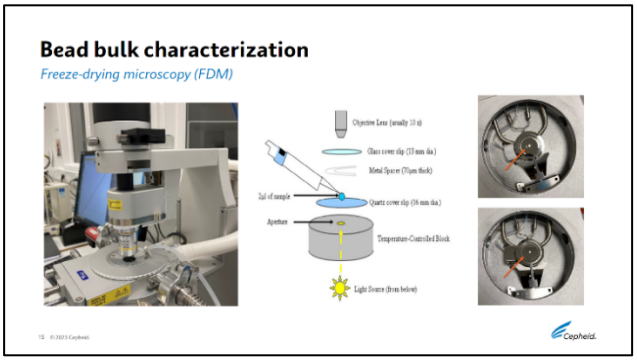
So, these cycles are the cycles that we evaluated. Cycle one has more standard lyo conditions. So, the energy during primary drying is given not in such large amounts since we actually set the shelf temperature to minus five degrees. And then secondary drying, it's performed at 25 degrees. And then it's also important to consider what's going to be the pressure inside the chamber because the pressure is going to set in the case of the

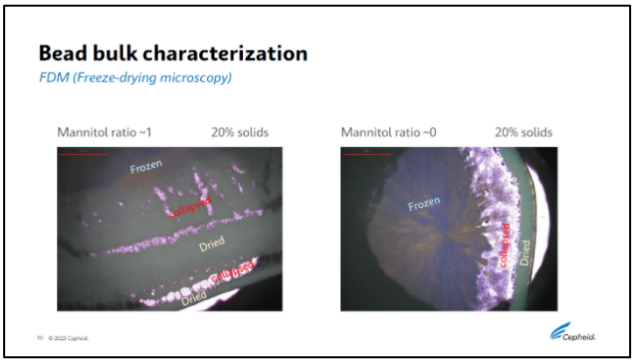
LyoBeads when sublimation will start. And it correlates very well when a sublimation actually happens.

And when the theoretical sublimation for the given pressure is set in the table. So, in this case, the vapor pressure over ice for 200 millitorr, is minus 34 degrees. And then if we move to cycle two. Cycle two, it's a more aggressive drying cycle so that we heat up all the way to 25 degrees. We just apply one ramp and then the set pressure is also 200 millitorr, which means that sublimation will start at minus 34 degrees. And then the third experimental cycle that we are going to test, is a more conservative cycle. So, we needed to actually prolong the drying time to be able to finish drying, to complete drying. And then in this case, the shelf temperature for primary drying is minus 30 degrees and then the set pressure is also lower, which means that sublimation of this cycle will start at minus 40 degrees.

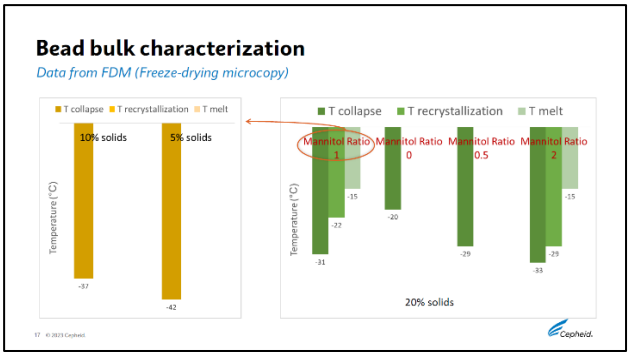


So, when those cycles were run, what we did was to analyze and characterize our formulations, to see which were the critical temperatures of each of our formations. And then we also evaluate how the temperature in the beads is increased depending on the cycle that is run. And then later on we look at the appearance of the bead. So how the beads look and how the inner structure and outside structure of the bead was. But first of all, let's start by characterizing our formulation. For that, we have used freeze drying microscopy. For those that are not very familiar with the technique, what freeze drying microscopy is about, basically what it does is to mimic the conditions that we have in our lyo cycle, but it does it in a little small chamber. And then we just need a drop of our solution to run the experiments.





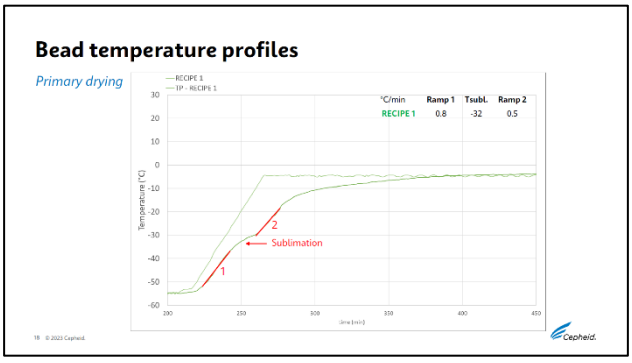
A droplet is placed between two glass slides. Those glass slides are placed on a stage that will actually freeze and cool down the solution and will heat it up later. And then vacuum is applied inside the chamber. We follow the behavior of this droplet with the camera and what we can observe are the different features in a case of having mannitol present in the formulation when the solid content is 20% and then the mannitol ratio is one. We face two different drying fronts. We see first in opaque color how the solution is frozen. And then as we start heating up the solution, we see also a kind of gray opaque how it dries first. It collapses after it becomes transparent. And then we see how the solution becomes opaque again with a gray color, which means that we face a kind of restructuring in recrystallization of the solution, having a second drying front that will collapse later on.



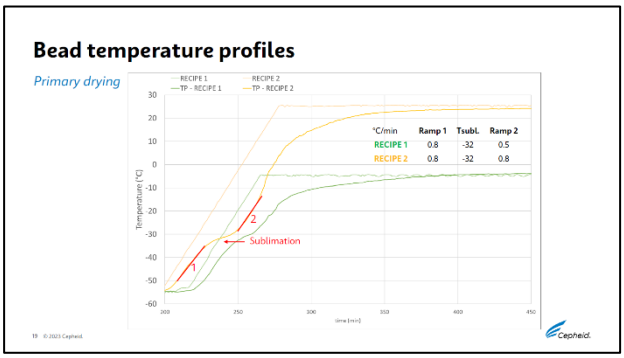
But then of course what we see with mannitol, is not present in the formulation is a completely different behavior. The solution freezes and then the drying front starts and then the solution just collapses. So, there is no restructuring. Of course, when we don't have mannitol present in the formulation, we still have 20% of solids. But if we compare these two analyses, with and without mannitol, we see how in a case of having mannitol present at this ratio, we find a drying front, then the solution is collapsed, then we find a drying front again, and then we collapse the solution. While this doesn't happen when mannitol is not present in the formulation. But, if we translate this into numbers, what we can see for the different solutions, is that direct crystallization behavior of mannitol usually occurs when mannitol is present in a ratio of one or larger than one. And in a case of having mannitol ratio of two, so we have a larger amount of mannitol in our solution, we need this crystallization front even earlier after collapse has happened.

So, we have a lower temperature gap between these two events, which will be beneficial later on for our drying. The gap is larger when we have a mannitol ratio of one, and we do not see this crystallization when there is either of course no mannitol, but with mannitol, it's in a very low amount. The recrystallization is probably hindered by the other excipients, and then we have a more depressed collapsed temperature, and no crystallization is happening.

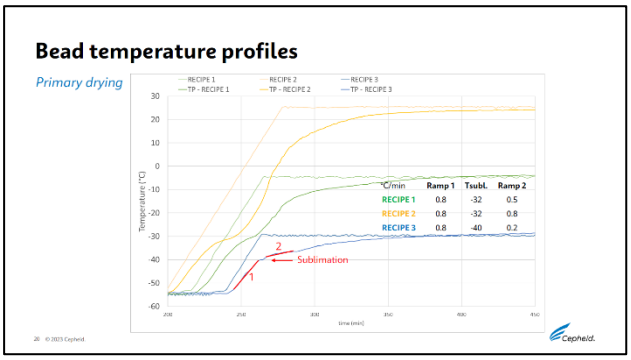
But when we evaluate the solution having a mandatory ratio of one, but in this case with a lower percentage of solids, what we see is that the collapsed temperature of our solution, it's depressed and there is no event of crystallization for mannitol. So, it's important to keep in mind where we land with our critical temperatures because what is important during the drying cycle is to not overpass the critical temperatures before the beads have been actually dried. But we need to follow what's going to be the drying process by our thermocouples and we will look at where we need to be and how the cycle is actually going to input the energy to the system and at what speed that cycle is going to put the energy into the bead.



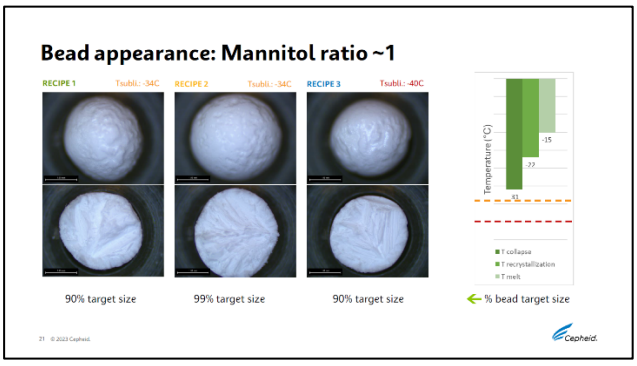
The faster we give energy, of course, the easier it's that we reach these collapsed temperatures. But let's have a look first at each of the cycles and what the bead temperature profile looks like in each case. So, we look at primary drying, for cycle one, we increase the shelf temperature to minus five degrees, and then here we can see what the temperature profile of the bead looks like. So, the bead follows the set ramping rate until sublimation starts. It's important to keep into account that here we had a set pressure of 200 millitorr, which means that sublimation will start around minus 34 degrees. So in this case, if it starts at minus 32, 33 degrees it aligns very well with what the theory says and after sublimation, then part of the energy is used to sublimate water and part of the energy is used to increase the temperature of the bead.



So, it does it at a slower pace than it did before sublimation started. But in a case of having an input of energy much larger, as is the case for cycle two where we increase the shelf temperature to 25 degrees directly, we see since we are using the same pressure that sublimation starts at the same point and then the rate to increase the temperature after sublimation, it's much faster. So, we input energy much faster if we use a higher shelf temperature for primary drying. And then in case of having a more conservative drying cycle, which is cycle three, we see the same shape for our curve. And then sublimation starts at minus 40 degrees here. It starts early since we are using a lower vacuum in our system, we will start sublimating at the same speed and then after sublimation, the temperature in the beads will increase slower.

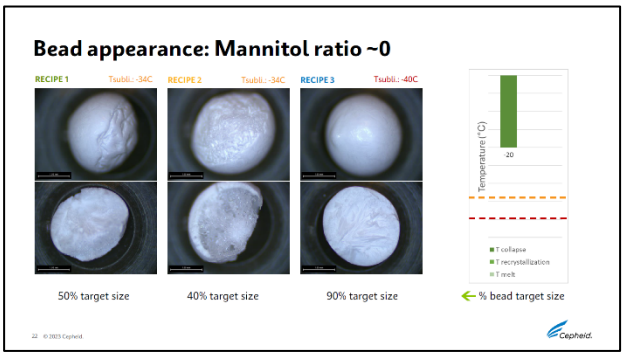


So, depending on where we actually set our shelf temperature, we will be providing energy into the system faster or slower, which will allocate more or less time for our bead to really complete drying before it has actually met any of the critical temperatures that will collapse the system. But what do the beads look like in each case when we are using and applying any of these drying cycles?

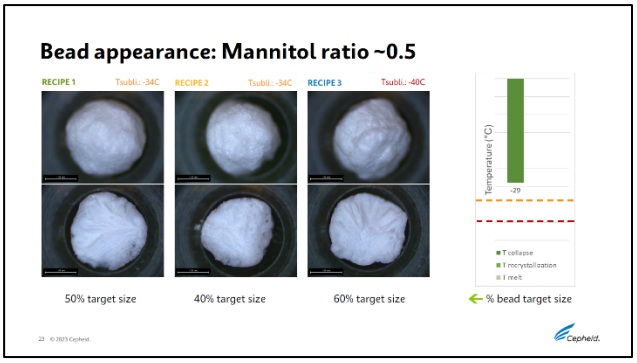


So, for example, in the case of having a mannitol ratio of one, we have a little bit of shrinkage, but still, it meets its target size. So, it has not collapsed in the inner structure in the cross section as we can see here. And then the overall size, most of the beads keep their initial size.

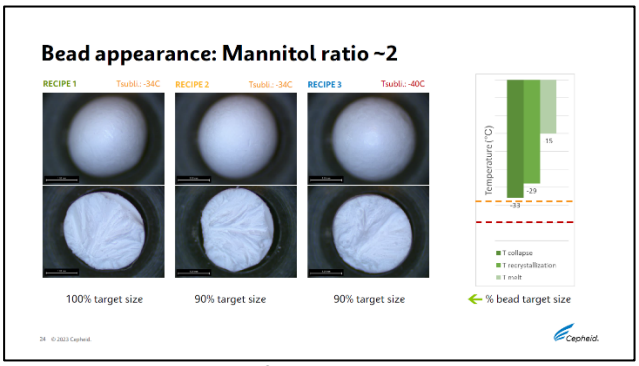
So, any of the cycles work well with the system. And then of course from the point that we start sublimation, we are closer to the collapsed temperature. But since we actually get a change of properties due during crystallization later on as we increase the shelf temperature the properties of the beads will change. The mannitol will actually come out of the structure. So, it will prevent the whole structure of the beads from collapsing.



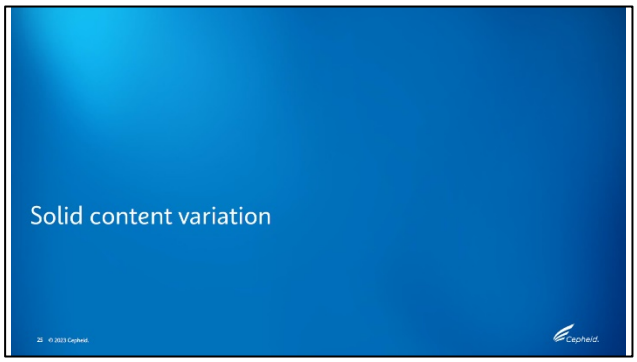
But if for example, we look at a system with no mannitol, with no crystallization happening, we have a system that has a higher collapse temperature, which allocates more time for drying from the point that we start sublimating. But then very fast cycles or very aggressive cycles where the ramping rate after sublimation is fast, they will not allow enough time to actually dry the system before it reaches the collapse temperature. This doesn't happen when we are using a more conservative drying cycle where we have a perfect bead that did not shrink, it had the perfect size, and it did not show any collapse signs in the inside. So, in that case, we started sublimation earlier. We did it slower. We raised the product temperature slower so that we allocated time to fully dry the system before it meets its collapse temperature.



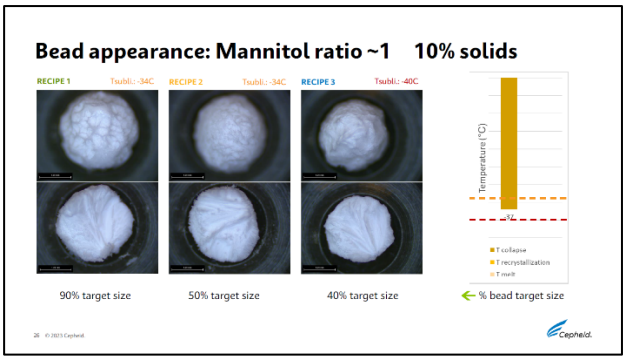
When we look at the system that had a mannitol ratio of 0.5 and we see the appearance of the beads in each of the cases, we see how the structure of the bead has shrunk. So, we do not meet the target size. The bead has collapsed in the inside as well, and then the surface of the bead looks quite shrunk as well. So, for this system, mannitol did not fulfill its function, as it needed the time to crystallize to do that. Perhaps more time was needed since we actually started to sublimate the ice crystals until the temperature of collapse is reached in the product. And then at the same time, probably all the other excipients already hinder the crystallization of mannitol, so it could not crystallize. And in that case, it acts mostly as a plasticizer rather than a bulking agent.



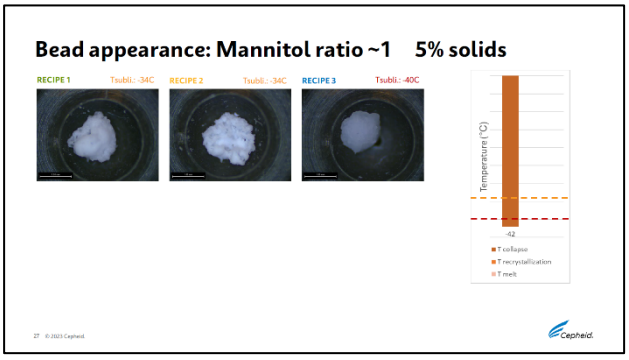
And then when the mannitol ratio is of two, all the beads had a very good appearance. They were smooth and round. They did not collapse in the inner structure. Even though as we can see here in the graph, when we start sublimation for the more aggressive drying cycles, we are very close to collapse, but crystallization happens right after. So, it's very probable that the solution gets another confirmation that transforms already the properties and then it increases the collapse temperature farther. And then probably it takes it up to the so-called melt temperature.

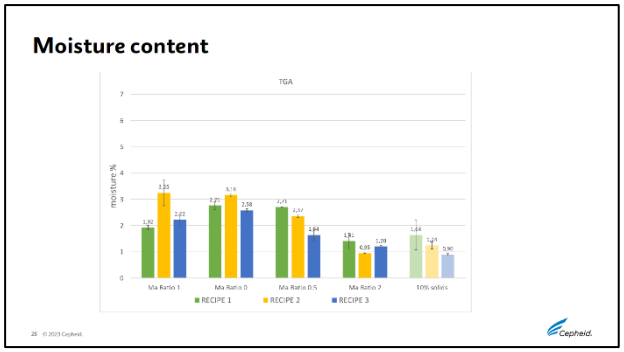


But what would happen if instead of having a 20% of solid content, we have less solids in the solution, even though we still have a mannitol ratio of one. So, as we saw in the characterization, the collapse temperature of this solution is quite low. And then for our cycles, this collapse temperature, it's met before sublimation has started for the more aggressive cycles and very close for the more conservative cycle. And as we can see in the appearance of the beads, they all collapsed. They were all very brittle. They cracked easily. And then both the surface collapsed and also the inner structure.

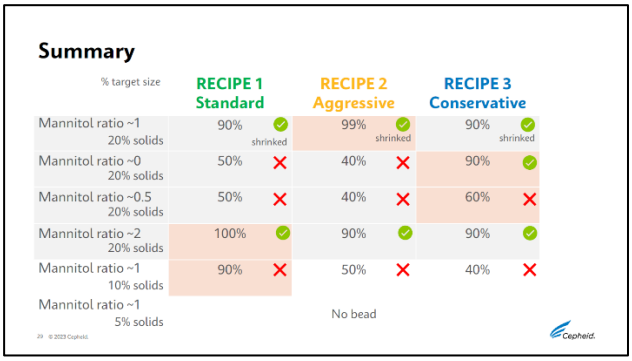


None of the beads of course fulfilled the target sites except for one. That most of the beads were there in the size of dropping the bead. And that happened for the standard drying cycle. But still the beads would not fulfill all the desired criteria. And then of course, if we reduce the solid content even further, we cannot hold the structure with any of the cycles. And still probably the amount of solids is not enough to be able to handle and process those beads. And as we can see also in the characterization, the collapse event for this system, it's very low. And then we have the settings over cycles to start sublimation at higher temperatures than the collapsed temperature of this solution.





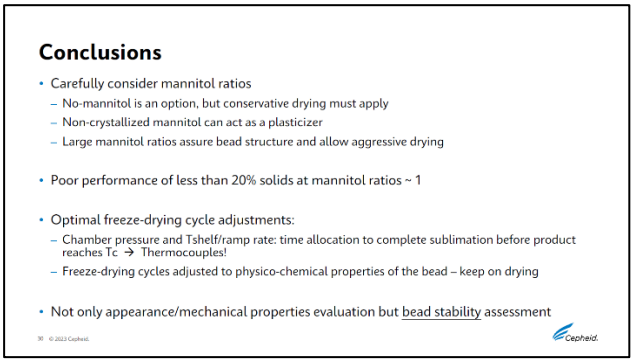
It's important also to look at the moisture content we want for stability issues. We want the bead to be as dry as possible. And in this case, for some of the cases, the moisture was relatively low. For some others, it was a little bit high. There was not a clear correlation that could tell us that some formulations actually trapped less moisture than others and that the cycle is better than the other to actually remove the moisture and what perhaps needs to be adjusted? It's the secondary drying to make sure that the moisture content after drying is reduced to the minimum as possible.



So, to summarize the results of the study, we can say that when the mannitol ratio is of one and the solid content is of 20%, all the three cycles worked, even though the bead shrank a little bit, but perhaps this is because of the amount of mannitol. So, mannitol should have been into the higher amount so that none was prevented for crystallization. But all the beads or most of the beads in each cycle will actually keep their size. So, it's possible when mannitol is present to the formulation, it's possible to run a more aggressive cycle. So, when we do not have mannitol into the formulation, we need to be careful with the settings. And then we need to run a more conservative cycle that applies energy into at a bit smaller and slower pace so that we make sure that we fully dry the bead before we reach any of the collapse temperatures.

When the mannitol ratio is of 0.5, that means that we'll have little mannitol into the formulation. Mannitol acts mostly as a plasticizer, so it does not fulfill its function. And then either it probably acts detrimentally towards the properties of the bead, or we really need to run very conservative cycles, which will turn out in very long cycles that we do not want in manufacturing. And then of course when we have a high amount of mannitol, it easily crystallizes and then it's possible to run short cycles, more aggressive cycles. And the bead looks perfectly fine and nice. And also, it is the right size. When we decrease the solid content, even though we still keep the mannitol ratio at one, the beads shrink and then it doesn't have a proper structure. It provides a very fragile structure that it's not possible to handle later on. And then when the solids are even lower than 10%, then there is no bead remaining.

And then of course, in each of the cases there is an optimal cycle that works best. And of course, we want to be able to run as much of an aggressive cycle as possible. For that case, it's good to have a component that crystallizes mannitol into the formulation, but it's still, it's possible to dry without mannitol. But in that case, we need to be more careful to the rate that the energy is given to the system.



And yes, to conclude, I would like to highlight that we need to carefully consider the mannitol ratios. So not having mannitol in the formulation, it's an option, but then we need to think about more conservative drying cycles. Mannitol when it does not crystallize, it's very detrimental to the formulation and especially to the structure that is formed after freeze drying. And then instead of acting as a bulking aid, it acts as a plasticizer.

And then of course a large mannitol ratio will assure the structure of the bead. And then it will allow us to dry aggressively and fast. So, we will be able to run short dry cycles. But in the case of LyoBeads, if we reduce the solid content, there is a poor performance of the bead, at least when the mannitol ratio is of one. I mean there is still room for testing less amount of solids and then having either no mannitol into the formulation at all or having a high amount of mannitol. In that case, perhaps we can still have a bead that is round, nice, and it holds the structure, but that has to be tested.

And then of course the freeze drying parameters should be adjusted, and then it's important to consider what's going to be the chamber pressure because that will define at the point that we start sublimation. And then depending on which is going to be our shelf temperature, then we will give energy faster or slower.

And then that will allocate time for sublimation to be completed before we actually reach any of our critical temperatures. But that should be controlled with the thermocouples. And then depending on the physical chemical properties of the bead, we will be able to run more or less aggressive cycles. But for example, in a case of having just one ramp cycle, that's possible to do as well with formulations that need more conservative settings. But then in that case, we would need to adjust the ramping rate. So, we can set a ramping rate slower so that we actually allocate enough time since sublimation has started to complete the dry. But then after we can still keep on drying at that rate and keep on increasing the temperatures. So, we do not necessarily need to stop in low temperature, but we can actually go to our final temperature, but then adjust the rate that we are heating up our system. And then of course in this case, we evaluated the appearance and then the mechanical properties of the bead. But it's important also to evaluate what's going to be our bead stability when we have an active component in the bead.