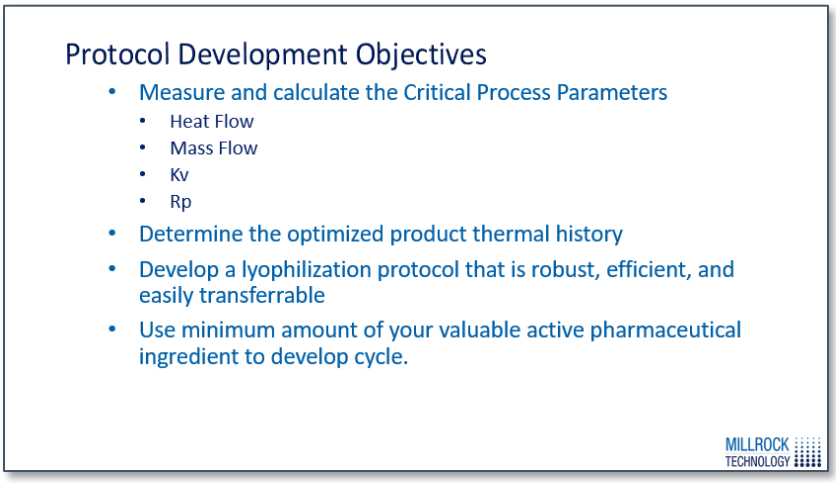
Developing an Optimized Transferrable Lyophilization Cycle using a MicroFD® with LyoPAT®

Spencer Holmes, Applications Engineer, Millrock Technology



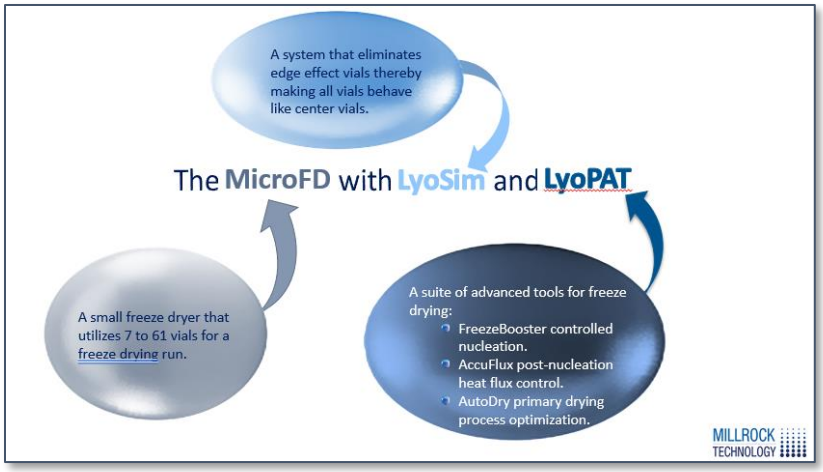
Some of the main protocol development objectives that are critical during the process of developing your cycle are to measure and calculate your critical process parameters, including heat flow, mass flow, the vial heat transfer coefficient (Kv), and the product cake resistance (Rp).

We are going to use these Critical Process Parameters to determine the optimized product thermal history and then use that thermal history to develop a lyophilization protocol that is robust, efficient, and easily transferable for scale up.

Another very important thing is that we want to be able to use a minimum amount of your valuable active pharmaceutical ingredient or API when developing a cycle.

When we are first looking at a new product, a new protein, or a new small molecule that is expensive to develop and in limited quantities, but needs to be freeze dried down the line, we want to be able to develop the protocol using a very small amount of this active pharmaceutical ingredient.

Millrock's solution to this is the MicroFD® with LyoSim® and LyoPAT®. The MicroFD® is a small freeze dryer that uses between seven and 61 vials, depending on the size of the vial, for a full freeze drying run.

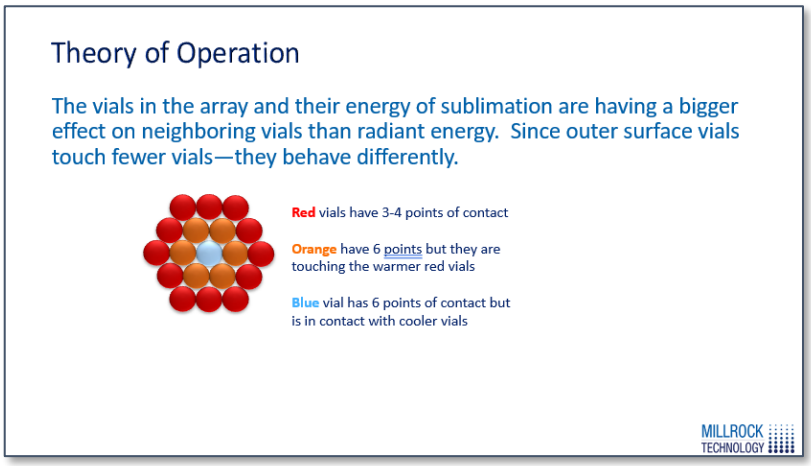


Using a larger tray style unit and simply putting a small array of vials in the center of the tray will not effectively help protocol development, because the vials will not dry or behave representative of a full batch of vials in that system.

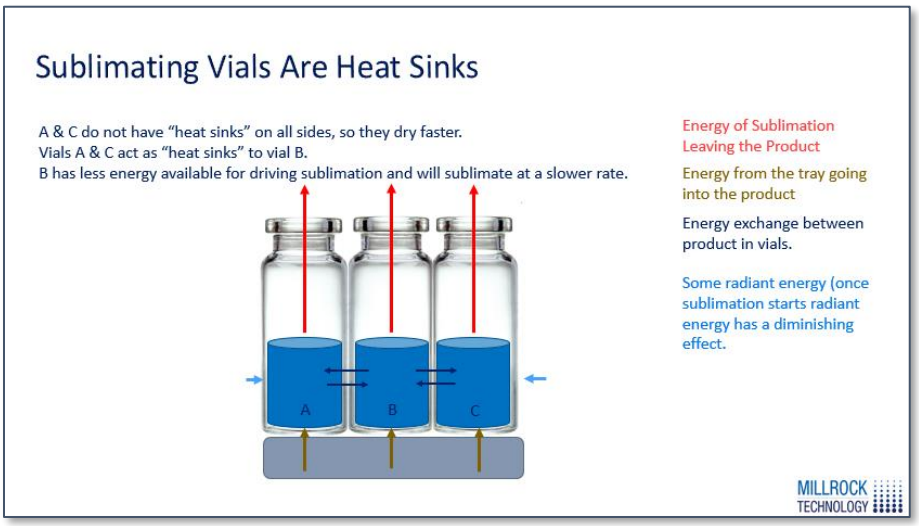
The MicroFD with LyoSim is a system that eliminates the edge effect and makes all the vials behave like center vials. Simply put, the LyoSim is the system within the MicroFD that makes running such a small batch of vials possible.

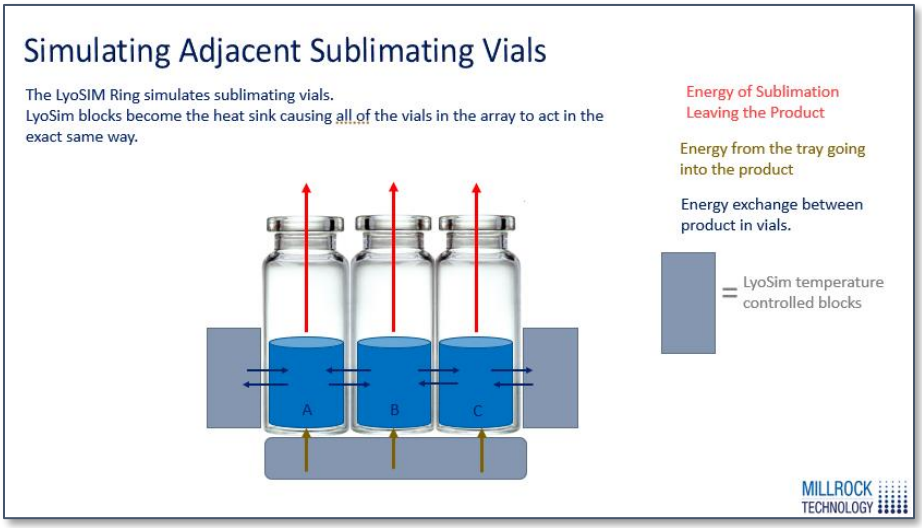
A bit of the theory of operation behind this is based on identifying and eliminating the edge effect which is a well-known phenomenon. For example, in this small batch of vials, most of the vials on the outside only have three to four points of contact with other vials and even within one row of these vials, the orange vials here are in contact with other warmer edge vials, so they still experience the edge effect to a limited extent.

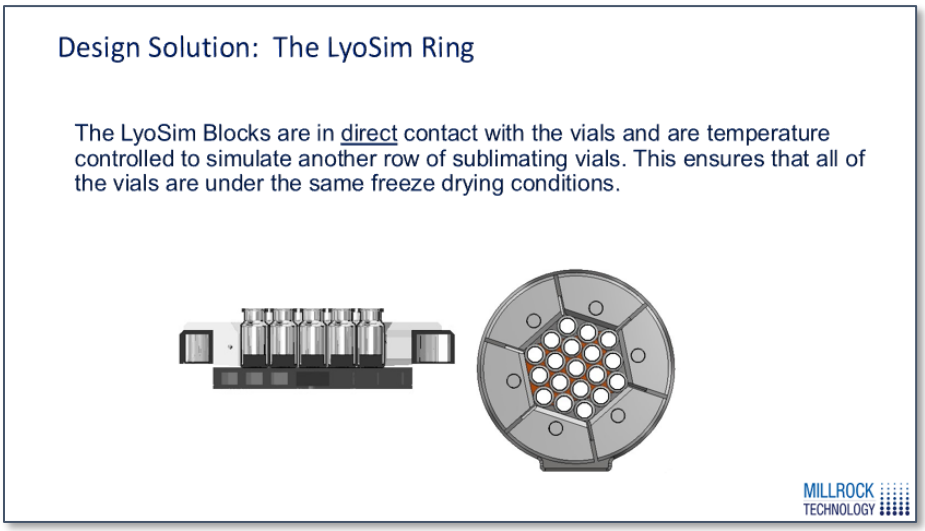
Finally, once we get about three rows back or more, we see vials that are surrounded by six other cooler vials and are considered full center vials. We look at this from a heat transfer perspective. We see that, in the edge, these vials are exposed to extra radiation as well as gas conduction and convection and importantly, they are not in contact with these other very cold frozen vials that are competing with energy through the shelf. This is where that edge vial effect comes from.



The solution to eliminating this edge effect is by bringing in LyoSim blocks. The LyoSim Ring is an independently temperature controlled ring around the array of vials with precisely manufactured blocks are placed on that ring to bring thermal contact with the edge of the vials allowing all





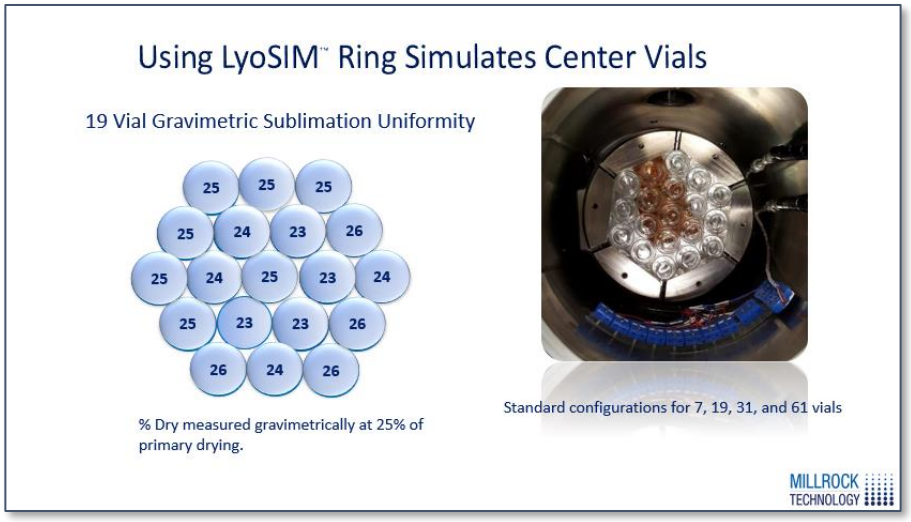


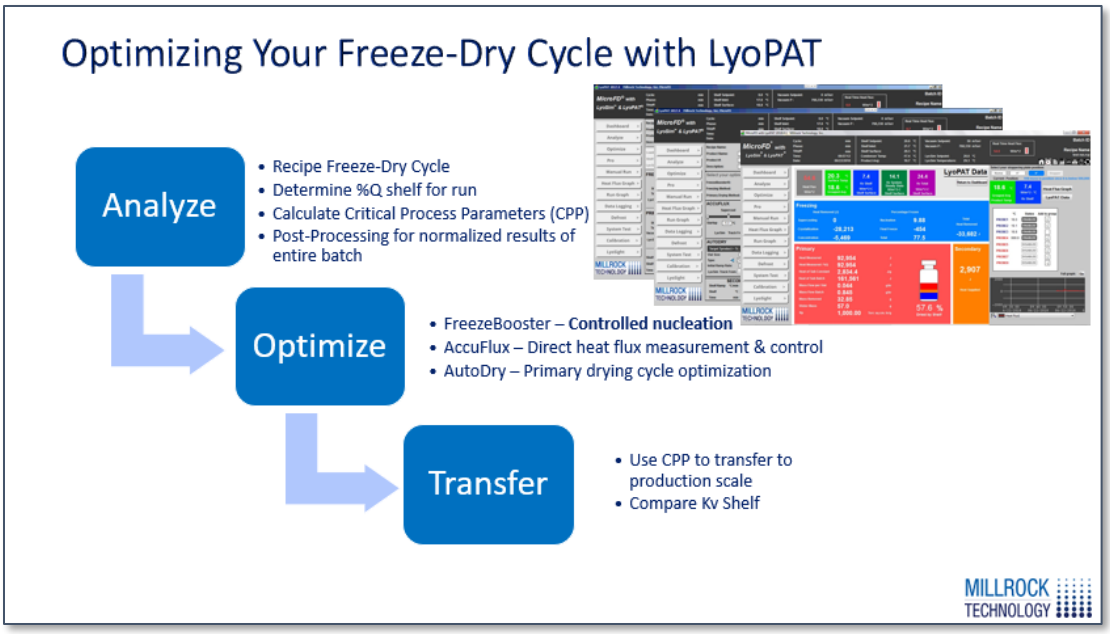
the vials to behave like center vials.

The vials come in direct contact with the LyoSim Ring enabling temperature control around the outside. Then, the temperature of LyoSim Ring is controlled to track the temperature of the vials.

On the outside of this array, the vials are in contact with another cold surface and therefore are not susceptible to the edge effect and behave and dry just like center vials.

Here we can see an actual picture of what this array looks like with the vials and the Ring around the outside and we can see some data that was taken gravimetrically about 25% of the way to primary drying where we record very good uniformity across the batch and do not see any pronounced edge effects on any of the outer vials. We have a range between 23 and 26% dry which is well within the variation you will find across a batch in a larger system. That is a brief description of how the LyoSim Ring is installed into MicroFD to make possible the use such a small array of vials, most commonly 19, to develop a freeze-drying protocol. In addition to the LyoSim Ring, LyoPAT is a suite of advanced tools for freeze drying which include FreezeBooster for controlled nucleation, AccuFlux for post nucleation heat flux control, and AutoDry for primary drying process optimization.



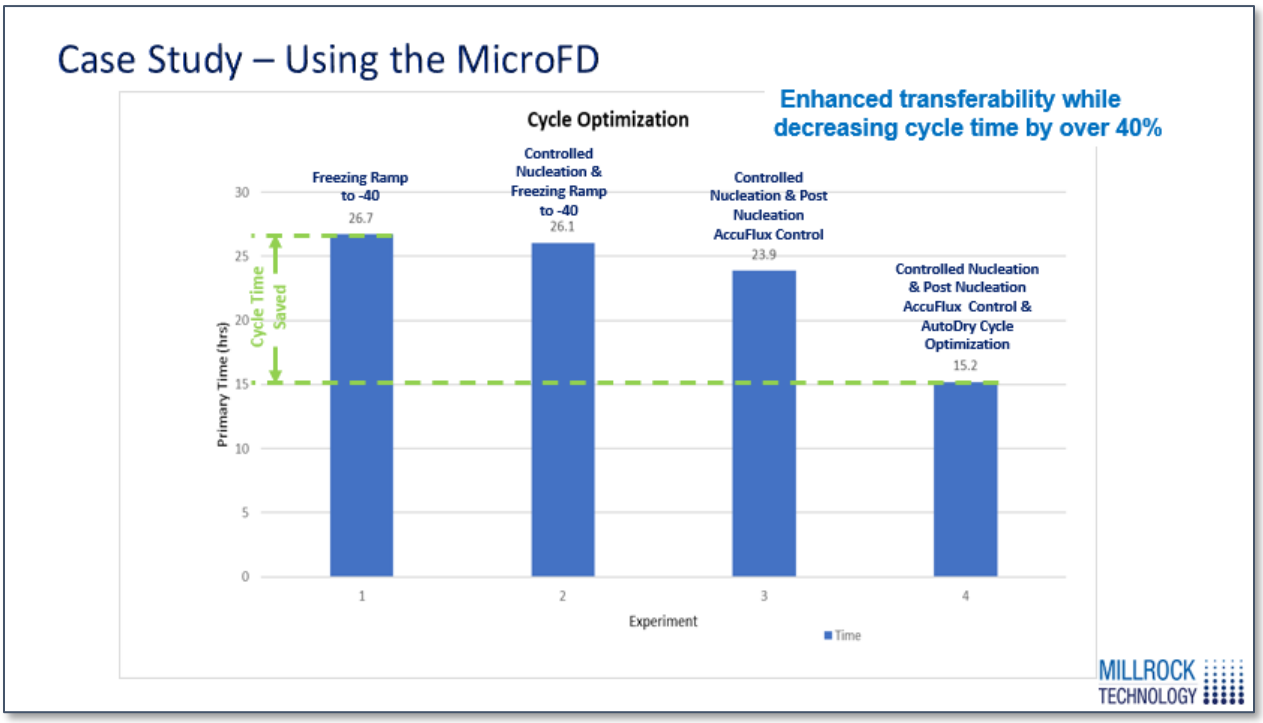


When using LyoPAT, there is a typical sequence that we recommend being used for optimizing your protocol. The first would be to run what we would term an Analyze run which is a recipe based freeze drying cycle that allows you to calculate your critical process parameters and determine your percent Q shelf for the run. That percent Q shelf is a percentage that describes the total percent of energy that is measured coming through the shelf as opposed to the other energy that comes from radiation or convection on the sides. We can also use post processing to normalize our results for this entire batch

and get our full critical process parameters by accounting for this percent Q shelf. In optimize, we then start using the optimization features starting with FreezeBooster for controlled nucleation and AccuFlux for direct heat flux measurement and control as well as AutoDry for primary drying optimization.

AccuFlux is the heat flux sensor that is being used for control and is one optimization feature but in general the heat flux sensor is always present and active for measuring the heat flow into your vials and calculating your process parameters. Once we've used these optimization features to develop an optimized protocol, we can then look at transferring this protocol to a larger system using your critical process parameters to help guide you in the transfer and by comparing the Kvs between different units.

A quick brief case study that we did showed how we could use each step of our optimization. Each one



of these, the first bar here represents an unoptimized simple recipe protocol and then every step along the way represents the addition of one optimization feature. First, it involves adding controlled nucleation then controlled nucleation with the post nucleation heat flux control and then finally, controlled nucleation with post nucleation heat flux control and in primary drying the AutoDry for cycle optimization. Here we can see that through these three optimization features, we have been able to cut down our primary drying time by over 40% for this example cycle.

Analyze

Program the Run and Start Freeze Drying

Freezing Options
- Step program – 10 steps

Primary Drying
- Step Program – 16 steps
- End of primary drying. Pirani – Capacitance Manometer convergence.

Secondary Drying
- Single step – ramp and final hold conditions

LyoPAT automatically provides all critical process parameters including Kv, mass flow and resistance.

MILLROCK TECHNOLOGY

Analyze

Example of a "Conventional" Cycle

Step 1
Use an existing recipe in the MicroFD.

- 5% Sucrose, 2mL in 6R vial, 19 vials
- Freezing
  - Ramp 1/C min to -40C and hold
- Primary Drying
  - -25C and 60mT
  - EOPD determined with Pirani and CPM convergence
- Secondary Drying
  - 20C and 60mT for 1 hour

MILLROCK TECHNOLOGY

Analyze

Critical Process Parameters Determined in MicroFD

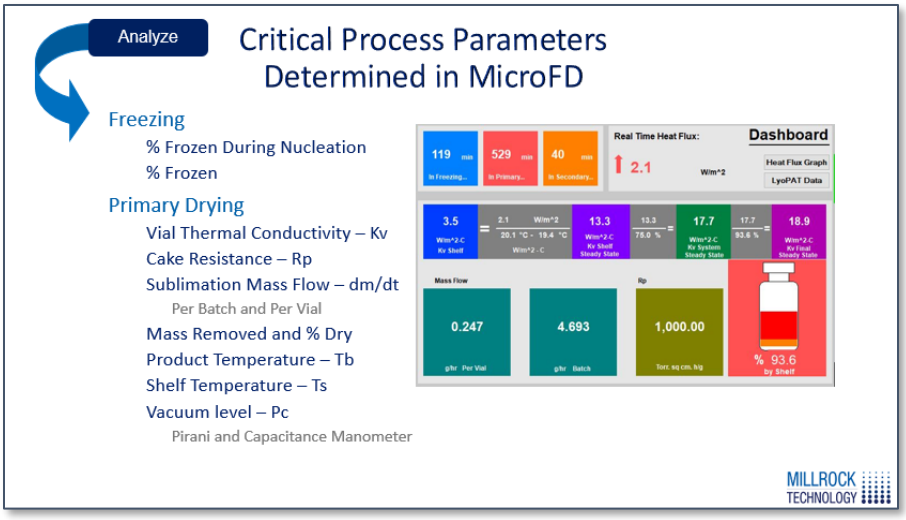
Freezing
- % Frozen During Nucleation
- % Frozen

Primary Drying
- Vial Thermal Conductivity – Kv
- Cake Resistance – Rp
- Sublimation Mass Flow – dm/dt
  - Per Batch and Per Vial
- Mass Removed and % Dry
- Product Temperature – Tb
- Shelf Temperature – Ts
- Vacuum level – Pc
  - Pirani and Capacitance Manometer

MILLROCK TECHNOLOGY

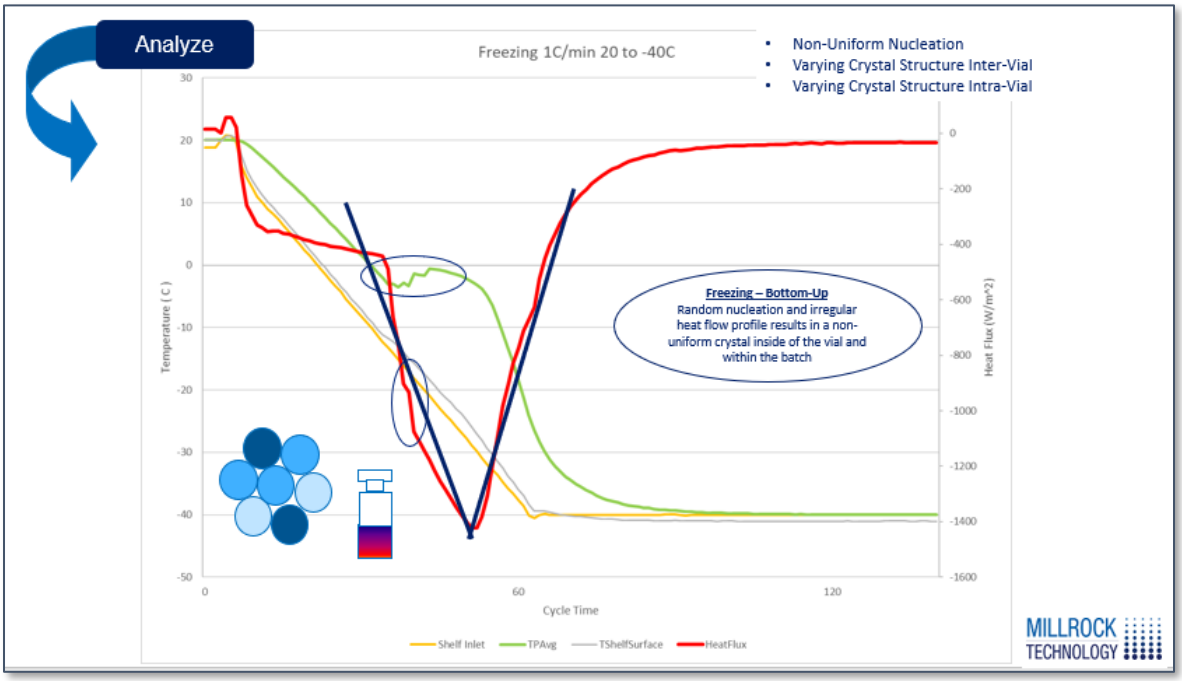
Again, the first step of this process would be to run a standard recipe based-freeze drying cycle. This will mostly be based on a conservative recipe based on products that have run before or other published knowledge out there.

In this initial analyze cycle LyoPAT automatically calculates and provides all of the critical process parameters including Kv, mass flow, and product cake resistance. It makes these calculations and measurements from the heat flux sensor on the bottom of the shelf and with the additional data of product thermocouples in the vials, it can be used to calculate a Kv as well as the mass flow and cake resistance. Our conventional cycle in this case was a 5% sucrose solution with two milliliters in a 6R vial and 19 vials in that little array. This was frozen at one degree C per minute to -40C and in primary drying it was a simple recipe of -25C and 60 millitorr with the end of primary drying determined by the convergence of the Pirani and the CPM.



During the cycle is the first time we measure our critical process parameters. Again, the heat flux can be used to measure all of the process parameters we see here, most importantly the vial thermal conductivity, the cake resistance as well as the mass flow.

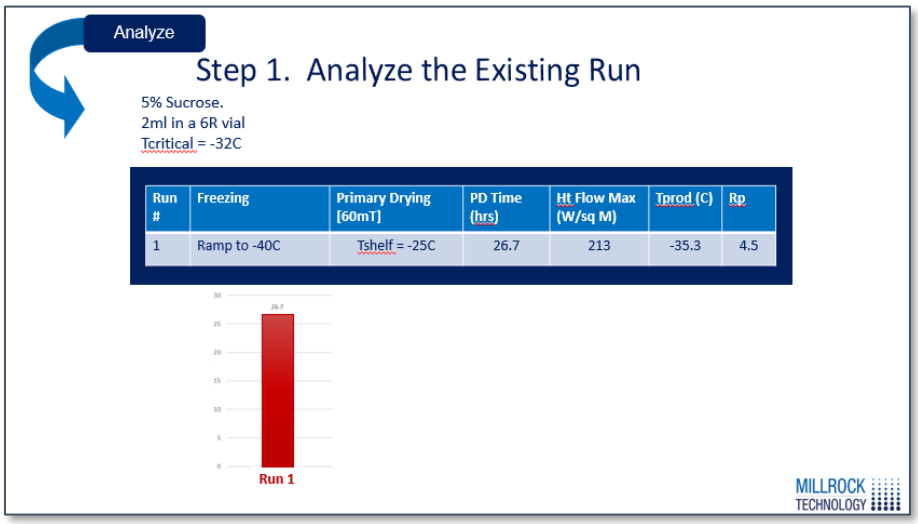
When we are looking at the freezing side of this recipe cycle, the initial freezing recipe we see here, we see a few distinct things. First, we see that for each of these little ticks in this TP average graph represents one vial nucleating at a random temperature. We see several different random nucleation events as the vials are freezing and then followed by a deep 'V' of heat flow.



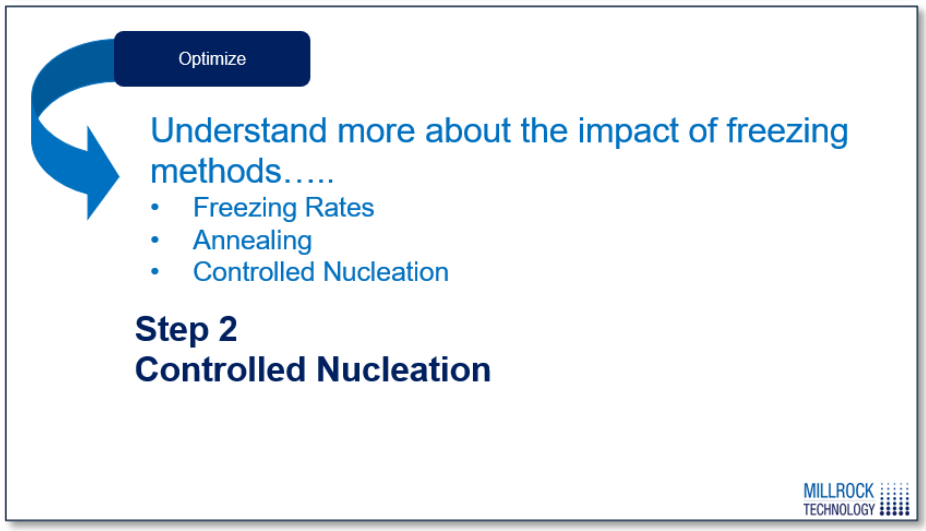
What this really means is that as these vials are nucleating randomly at different temperatures, they have a non-uniform starting point for crystallization across the batch as well as after they have nucleated. As the product temperature stays near its freezing temp and the shelf continues to drop, the magnitude of the heat flux continues to increase drastically. The rate of freezing early on is much less than the rate of freezing towards the end which leads to a varying crystal structure inside each vial. Both across the batch and within the vial, we have non uniformity in our crystal structure.

One thing to note here is that, looking at this data here, we can see that most of the vials have nucleated around -%C or even warmer which is actually a very warm temperature for nucleation for random nucleation to occur. Typically, in production systems, you'll see a much lower degree of super cooling before nucleation occurs. That will come into play later when we look at the results from this.

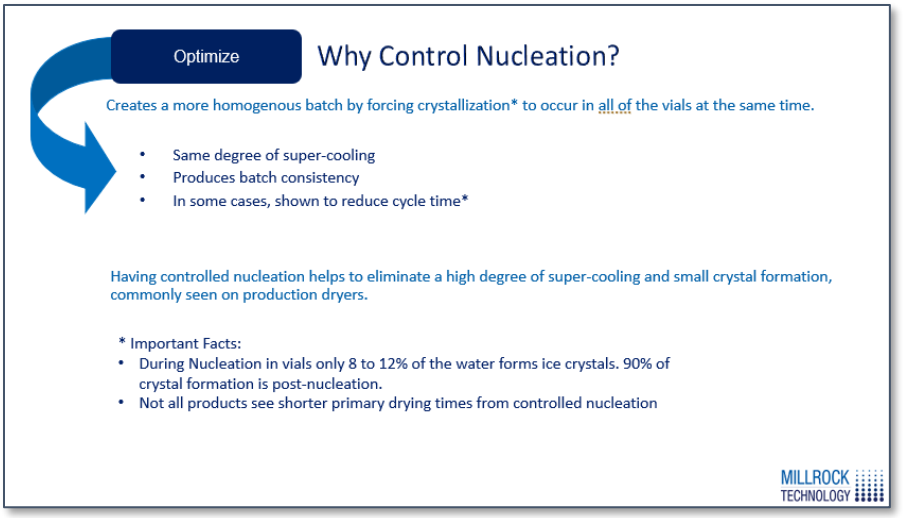
Again, here we see not much to compare it to now, but the initial run here took 26.7 hours of primary drying at minus 25C. We've reached a max heat flow of about 213 W/m² and resulted in a steady state product temperature of -35C and a max Rp of 4.5.



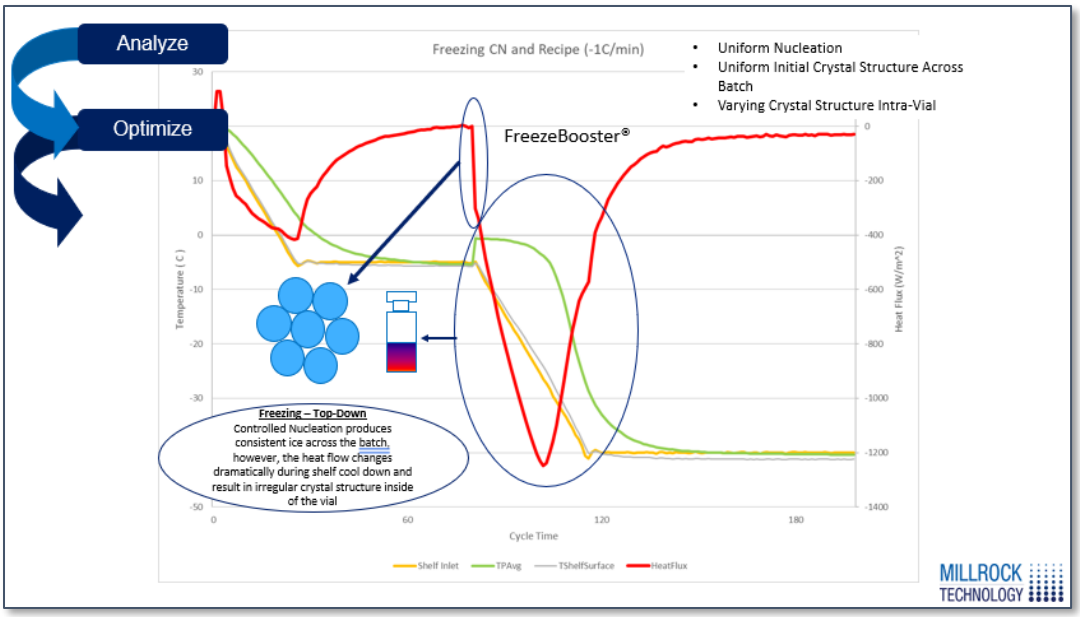
The first step in optimizing your protocol is to look at freezing which is really the foundation of your freeze-drying cycle. A couple things to look at when freezing are varying different freezing rates, adding annealing steps, or adding controlled nucleation, each of which can have different impacts on your product.



Annealing is commonly used to help increase the frozen crystal structure of the vial and controlled nucleation is used to ensure that all the vials have the same degree of super cooling which produces batch consistency.



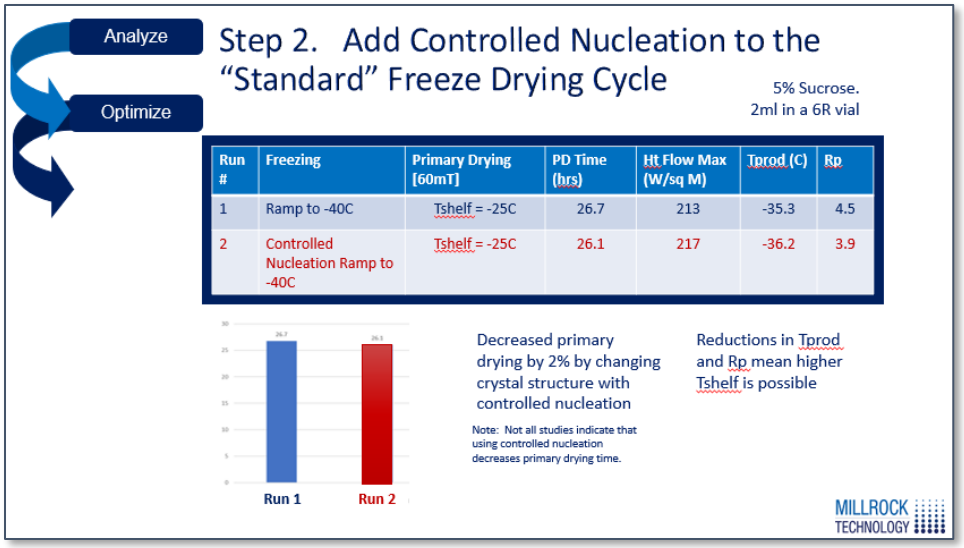
In some cases, using controlled nucleation can be shown to reduce cycle time but it's important to note that that is not the primary benefit of controlled nucleation. The primary benefit of controlled nucleation is to create uniformity across the batch and consistency between batches, so you always have the same degree of super cooling. That is what we're going to focus on while varying freezing rates and adding annealing steps are also ways of improving your frozen crystal structure, the primary thing we're going to focus on here is controlled nucleation. Going back quick, one thing to note is that during nucleation only approximately 10% of the water in the vial freezes and forms ice crystals. The rest of that water remains in liquid form in an ice slurry and fully crystallizes after the nucleation event.

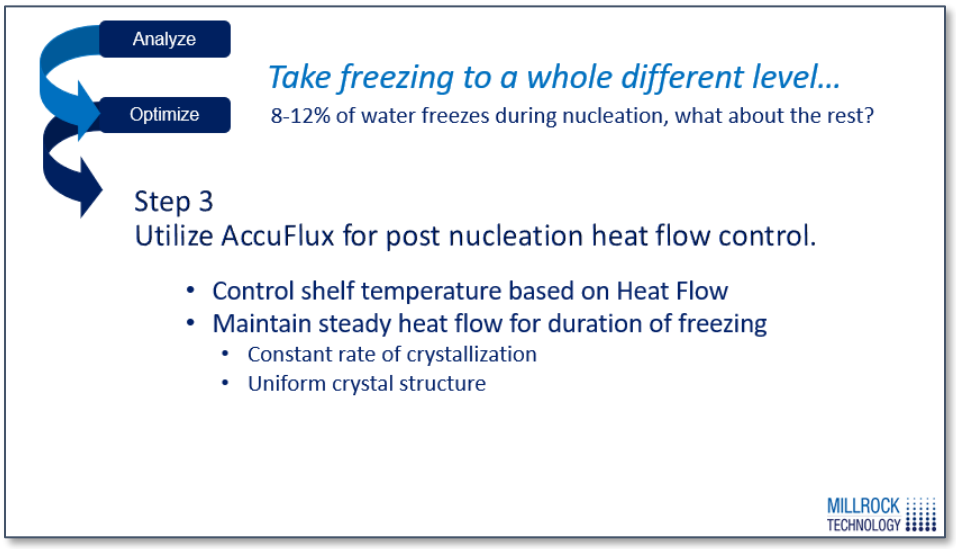


Going forward, when we look at freezing data for this, we see that using controlled nucleation we were able to cause all of the vials to nucleate at the same time which creates consistency across the batch at the start of freezing. But this is still followed by this deep V of heat flow where we've had our nucleation event, we can see all of the thermocouples jump up at the same time and then we started right after nucleation ramping our shelf temperature down to our final freezing set point which caused this deep V of heat flow. What this means, again, is that early on in freezing, when our product is near its freezing temperature of maybe minus one degree and our shelf is at minus 10, we've got approximately a 10 degree differential between the product and the shelf. We have a heat flux of -600W/m². Later on, as

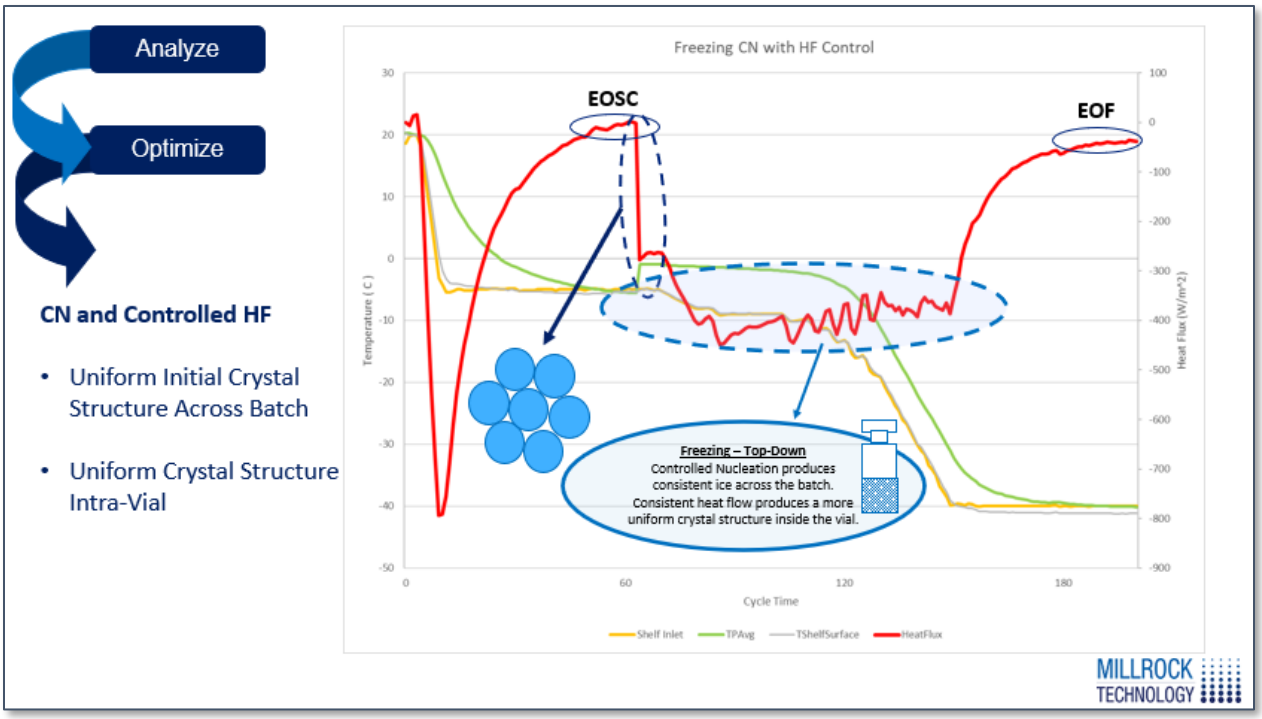
the shelf continues to drop and that other 90% of the ice is still forming within the vial, we've increased to an almost 20 degree temperature differential between the product and the shelf and our heat flux has doubled to -1200W/m². This means that while we still had a uniform start of freezing across the batch, we still have inconsistency and non-uniformity within the vial because that rate of freezing is not consistent for the entire time that the ice crystals are forming. But it's still an improvement on random nucleation.

When we look at the data we have afterward and just going back again, quick, one, again, the main point of controlled nucleation is that we're forcing all of these vials to nucleate at the exact same temperature of minus five degrees in this case. Here we can see that a very minimal decrease in primary drying time, only about 2%, which could be just statistically random. One reason for this is because as we looked at in our initial run, our vials were nucleating at around minus five degrees C anyway where in a production system they could have super cool much colder than that and had a much higher cake resistance. In our initial run, they ended up nucleating at a relatively high super cooling temperature. Between these two runs, controlled nucleation did not change the temperature of nucleation too drastically but it did cause all of them to nucleate uniformly which resulted in a slight decrease in primary drying time and a slight decrease in the product cake resistance. The next step for optimizing freezing after controlled nucleation is to worry about the other 90% of the water. We looked at the fact that about 10% of the water freezes during nucleation but what about the rest? The rest we see if you immediately ramp your shelf temperature down after nucleation does not freeze at a constant rate.

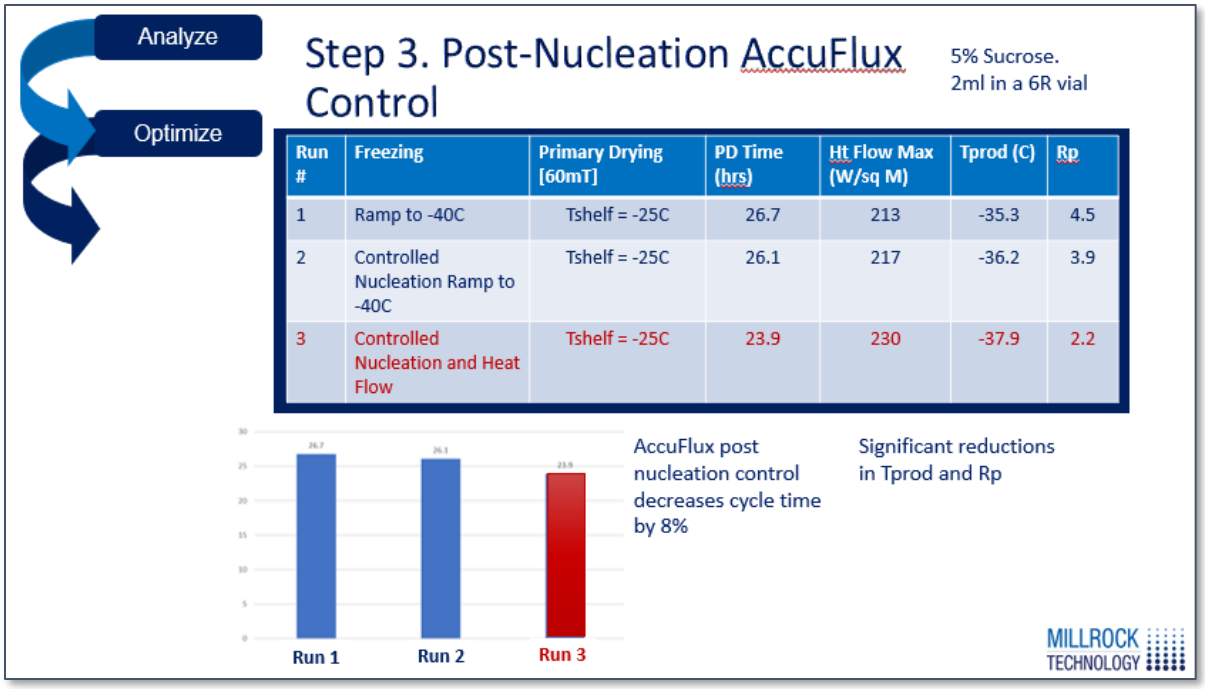




For the next step of optimization during freezing, we use our AccuFlux for post nucleation heat flow control. What this does is it controls the shelf temperature based on the heat flow and it controls the shelf temperature to maintain a steady heat flow for the duration of freezing leading to a constant rate of crystallization and a uniform crystal structure within each vial.



Again, we have that controlled nucleation event where the temperature of all the vials increases at once and we have one sharp decrease in heat flow at the point of nucleation, but then instead of immediately ramping the shelf temperature down at a certain rate of half a degree a minute or one degree per minute, we instead use the AccuFlux feature to control the shelf temperature, to maintain a set heat flux. For this run, that setting was about minus 400 watts per meter squared and we can see that the heat flux is not maintained perfectly at minus 400, but it is a vast improvement on that deep V of heat flow we saw earlier. Instead of ramping that shelf temperature down right away leading to a non-uniform structure within the vial, we've now through controlled nucleation created a uniform starting point across the batch and with AccuFlux heat flow control have created a uniform crystal structure within each vial.

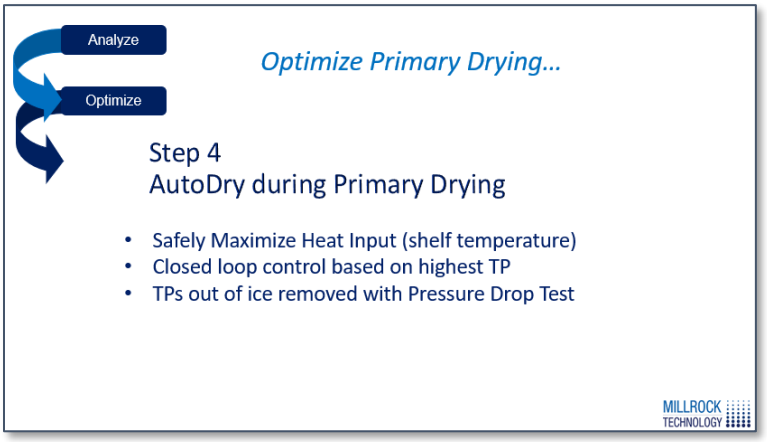


Run #	Freezing	Primary Drying [60mT]	PD Time (hrs)	Ht Flow Max (W/sq M)	Tprod (C)	Rp
1	Ramp to -40C	Tshelf = -25C	26.7	213	-35.3	4.5
2	Controlled Nucleation Ramp to -40C	Tshelf = -25C	26.1	217	-36.2	3.9
3	Controlled Nucleation and Heat Flow	Tshelf = -25C	23.9	230	-37.9	2.2

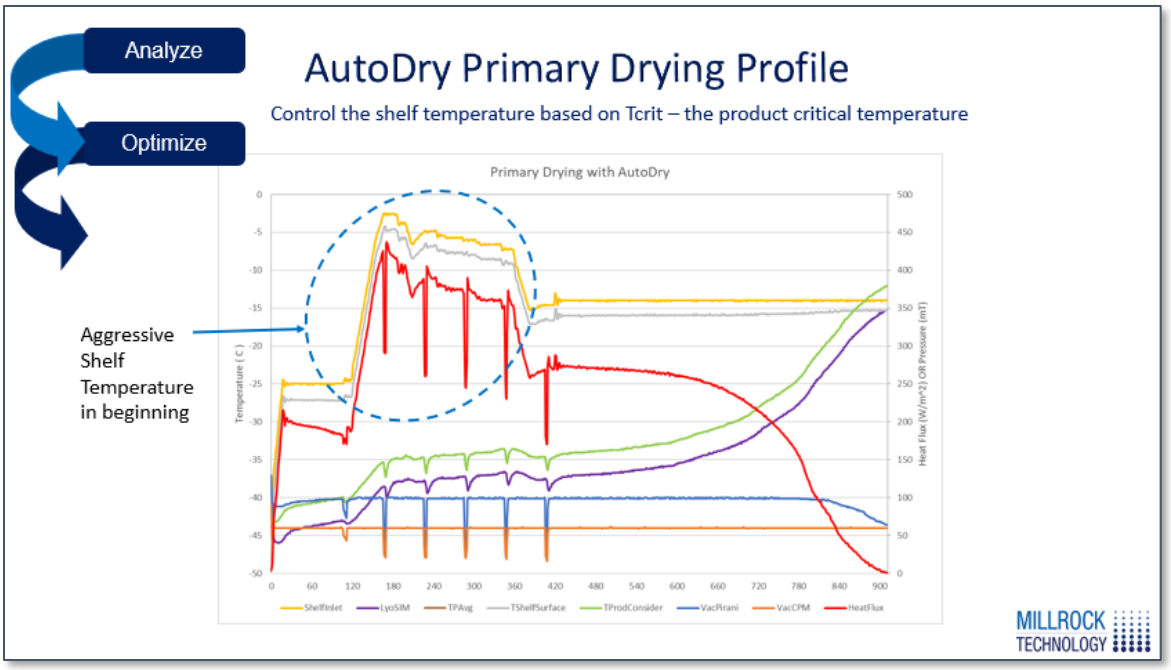
The results for this speak strongly. Here again, we don't see a drastic reduction in cycle time, about 8% here, so it's definitely something that is statistically significant reduction but not an incredible time savings there, what you notice instead though when we look at a product temperature and the cake resistance is a drastic reduction in both. Although we kept the same drying recipe which is a relatively conservative drying recipe at -25C so we weren't able to shorten that drying time drastically by keeping that same drying recipe, we drastically reduced the product temperature and the cake resistance.

By having a uniform crystal structure within that vial, it really reduced the resistance and allowed the product to dry at a much lower temperature. This tells us that while up here, in our initial run, our product temperature was at -35C, our product temperature here was over two and a half degrees lower, which means we could have, with this optimized freezing protocol, increased our shelf temperature quite a bit and shorten that drying time quite a bit. But for the sake of progressive optimization, we left drying the same.

Now that we've, like I said, completely optimized freezing, we look at optimizing primary drying using AutoDry. We just saw earlier that once we have optimized freezing completely and our product temperature is much lower than it was before, we can increase that shelf temperature in primary drying. But the question can be raised, how far can we raise that shelf temperature safely?



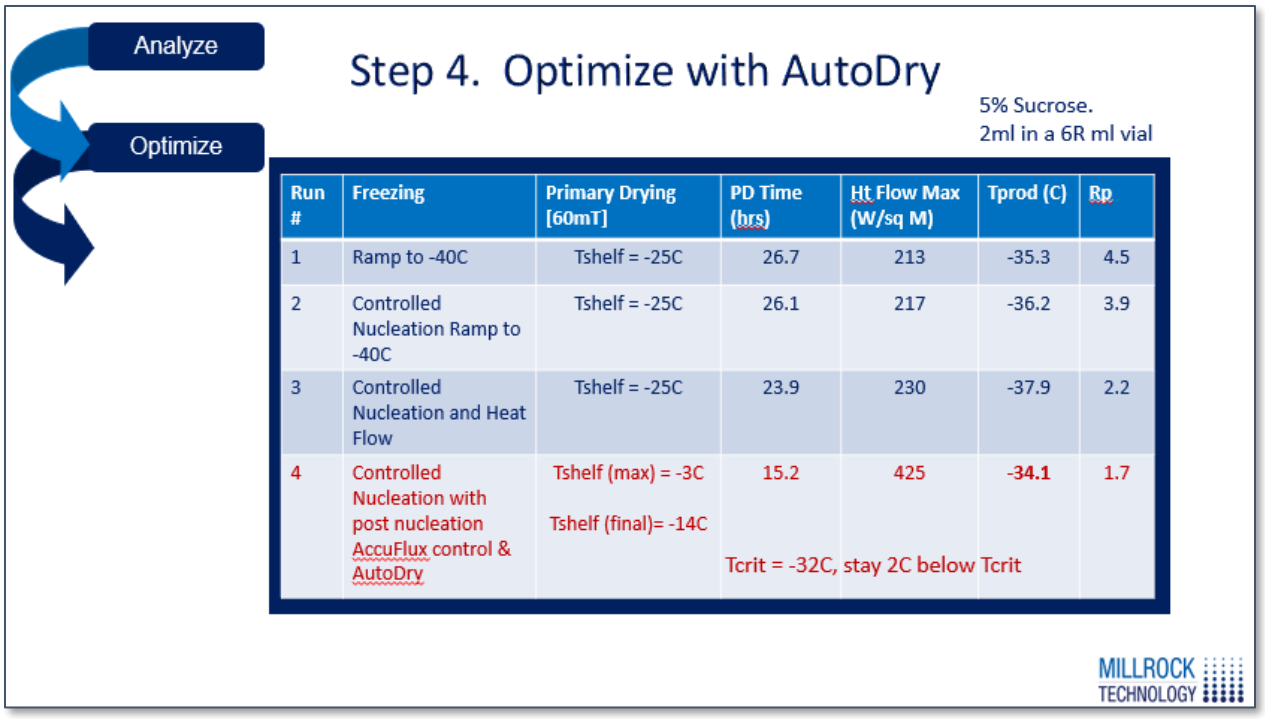
This is where AutoDry comes into play. AutoDry safely maximizes your shelf temperature and your heat input while keeping your product safely below its critical temperature based on closed loop control looking at the highest thermocouple in the batch. It also monitors the thermocouples as it is drying to determine when they are pulled out of ice with a pressure drop test.



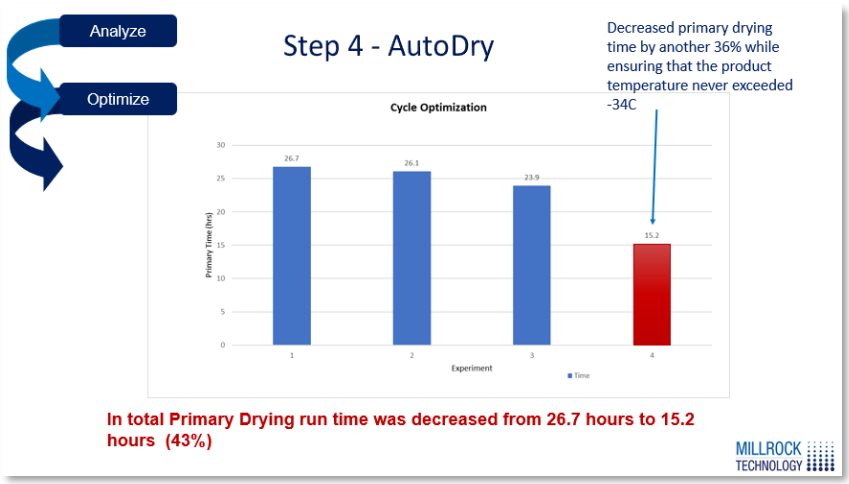
What we see with AutoDry is that early on in primary drying when you have no dried layer and no cake resistance, that's when you can safely increase the shelf temperature much higher than you normally would because you have no cake resistance and all of that energy is going to go straight to sublimation.

Later, once you start building up a dried layer, that shelf temperature will need to be brought back down to keep your product at a safe level at the bottom of the vial. What AutoDry does is you tell it here to go to first initial safe baseline temperature. Here we used our -25C which was what our standard cycle was in previous runs and then we allow it to adjust to reach steady state for a 90-minute period at the start, this is an adjustable setting, and then the AutoDry program automatically increases the shelf temperature to the point to maintain that product temperature below its critical temperature with a safety offset. For this cycle, with sucrose which has a critical temperature of about -31C to -32C, we programmed it to have a critical temperature of minus 32 and use a two degree safety offset. It would keep increasing the shelf temperature until the product temperature reached about minus 34 degrees C and then as that product temperature slowly starts to rise a little bit, it starts bringing the shelf

temperature back down to a safe level. Throughout this process, it's also conducting pressure drop tests which we see here, which it uses to determine when thermocouples are removed from ice. Any thermocouple that's still in ice when the pressure is dropped from its normal set point here at 60mT down to about 30mT, the temperature of that thermocouple as that vapor solid equilibrium is shifted will also drop and any thermocouples that do not experience that temperature drop can then be considered out of ice so we know not to use them for control. Simply put, AutoDry maximizes the shelf temperature early in primary drying and then lowers the shelf temperature later to keep your product safe.

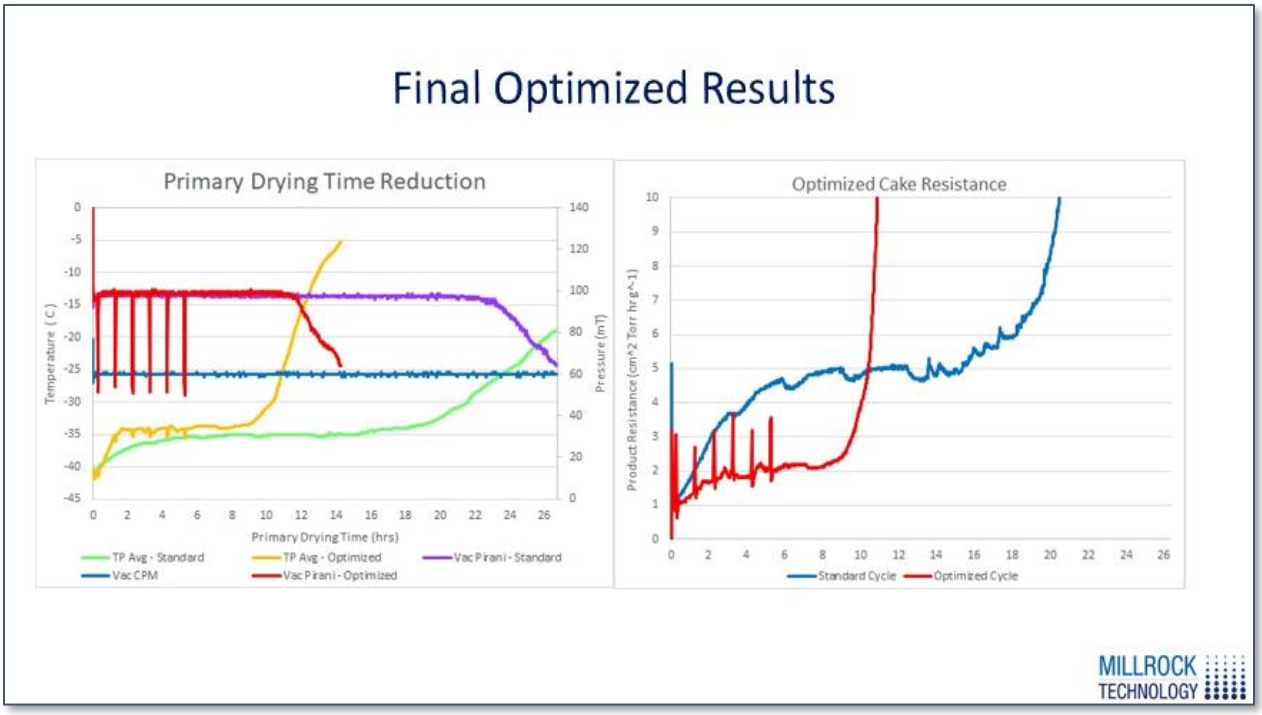


When we look at the results from this, this is where we see the drastic reduction in drying time. Previously, we looked at the other three slides or three graphs were on freezing, we are optimizing freezing and just use a very basic recipe in drying. This was also based on using those same three optimized freezing steps. Once we had an optimized freezing profile, we can then use AutoDry to optimize our primary drying. What we see here is that AutoDry was able to increase the shelf temperature much higher than we normally thought was safe and conservative in our previous cycle. It reached a max temperature early on in primary drying of about -3C and then as our cake resistance started increasing and our product dried, it reached the maximum -3Cand then it leveled off to about -14C. This cut our primary drying time down significantly over 11 hours less than what it originally was and we saw that we were able to double the maximum heat flow early on in drying all while keeping our product temperature safely below its critical temperature. Again, our critical temperature here was minus 32 degrees C since we were using sucrose and we also put in a two degree safety offset. We kept our product below minus 34 degrees C.

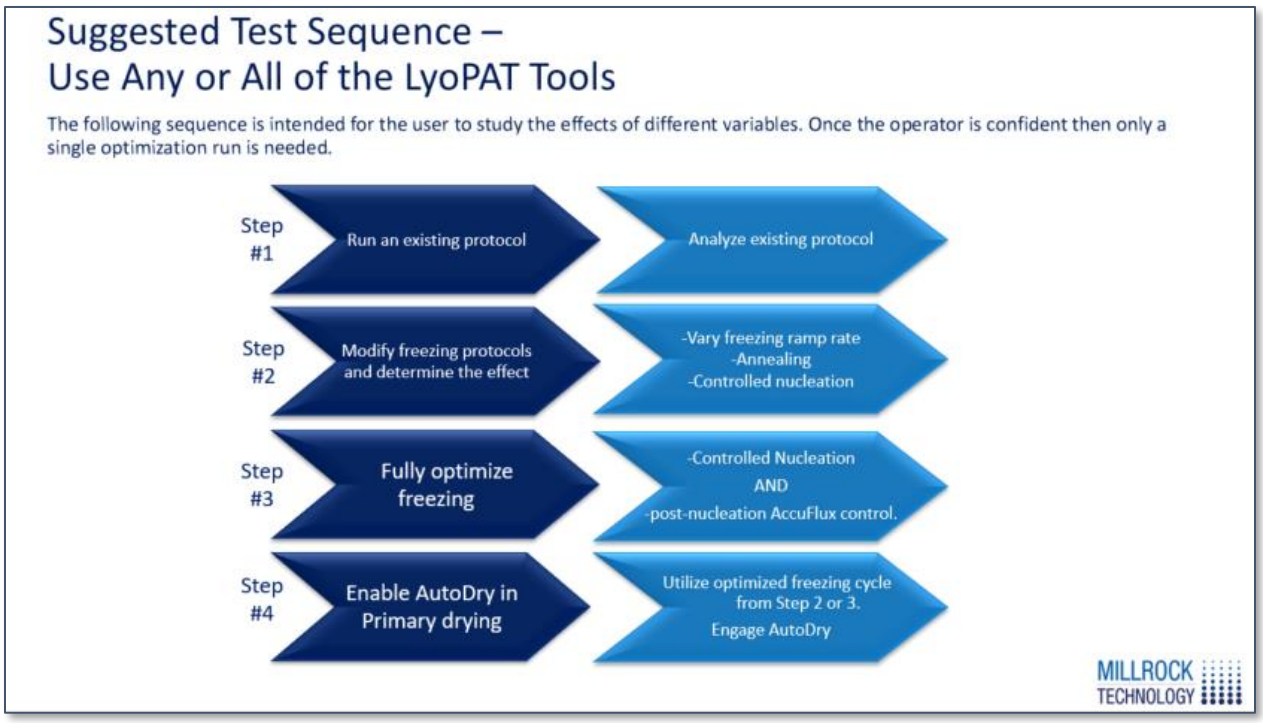


And then the overall results for that show that we were able to overall reduce our primary drying time by 43%. But again, I'll reiterate that much of this optimization did not occur in primary drying, but occurred in freezing to start. We had to lay optimized foundation within our batch in our frozen state before we can fully optimize in primary drying.

If we just went straight from run one to run four and used just AutoDry without controlled nucleation or heat flow control, we would have seen some time savings, time reduction in primary drying but not nearly what we were able to see after optimizing freezing.

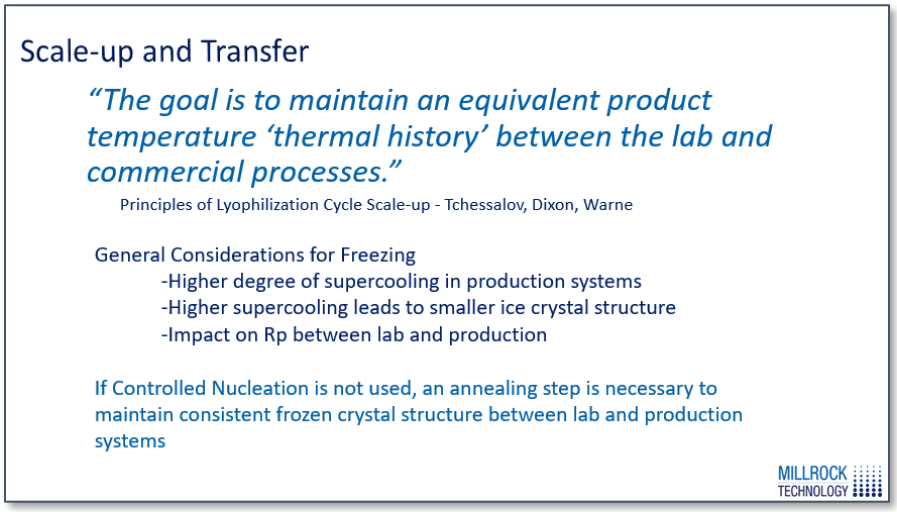


Here we can look at our final optimized results and we see our product temperature and pressure convergence for the initial run in the optimized run. We can see that our product temperature while higher than the initial run was still safely below our critical temperature and we can see we have also significantly reduced our cake resistance through this optimization process.



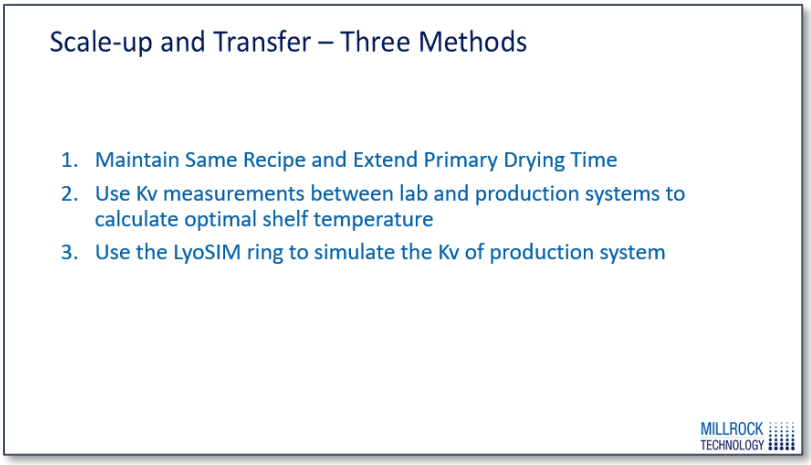
In summary, for the process of analyzing this or optimizing this protocol, we started with a basic recipe protocol based on what a conservative recipe would be for our product and then we began optimizing freezing through controlled nucleation is mainly what we used but varying freezing ramp rates and annealing are also other methods. And then we finally fully optimized freezing by using controlled nucleation and post nucleation heat flow control before finally once we had an optimized frozen product using AutoDry for a fully optimized primary drying cycle as well.

That's the section of this presentation that deals with optimizing protocol. Now, we are looking at how the MicroFD with LyoPAT can be used for transferring a protocol.

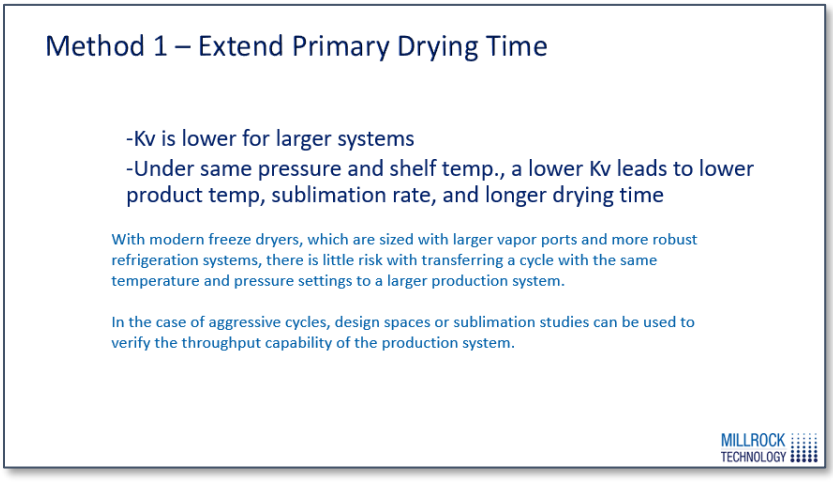


Broadly stated, as this quote shows is the goal of transferring a protocol is to maintain an equivalent product thermal history between the lab and commercial processes. This thermal history includes both the product temperature as well as the heat flux that the product's undergoing.

Most of what we are going to take a look at for transfer involves transferring considerations in primary drying. There are also a couple considerations during freezing as well. The main consideration being that in production systems which are typically in a much cleaner environment, the product will experience a much higher degree of super cooling, which leads to a smaller ice crystal structure when those vials finally do nucleate. If random nucleation is used in both the lab and in production systems, we'll typically see a higher Rp, a higher cake resistance, in the production systems than in the lab. For this reason, if controlled nucleation is not being used and just random nucleation is present, an annealing step may be necessary to maintain a consistent frozen crystal structure between the lab and production systems. Even for some products that do not gain a benefit from annealing in the lab systems, there's benefit towards adding an annealing step to allow for consistency once you start transferring to a production system.

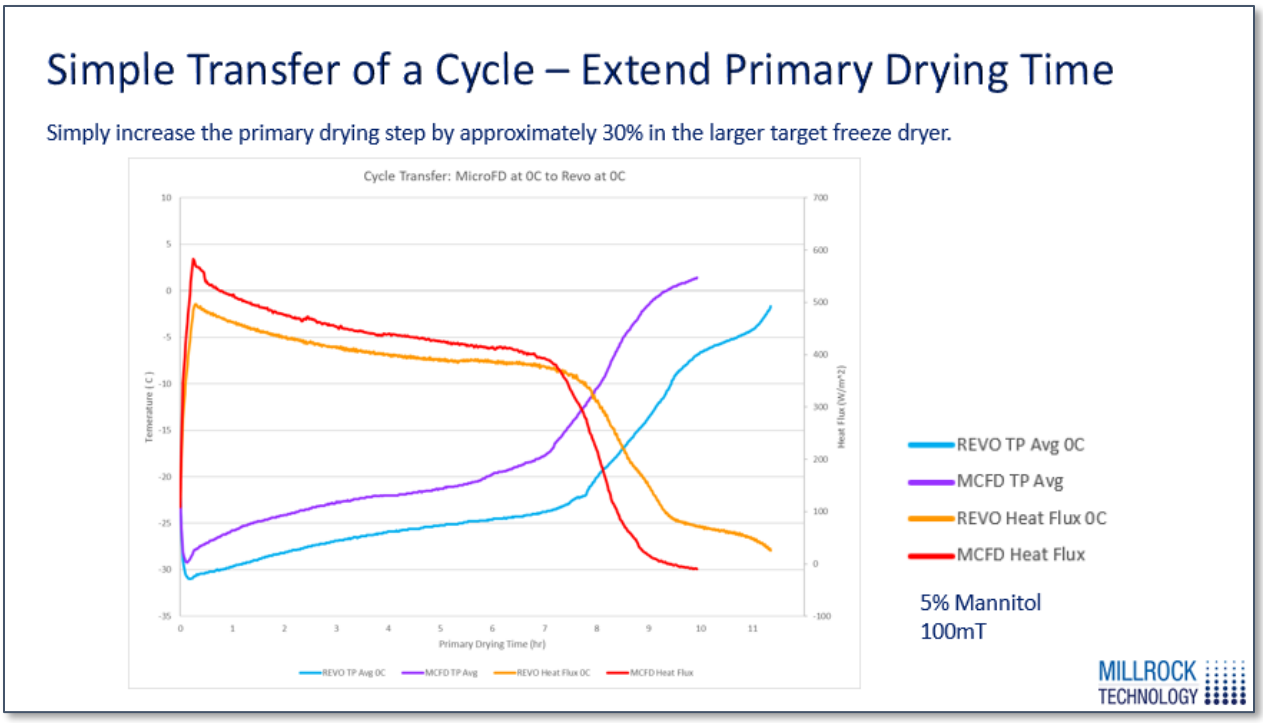


Past freezing, when we are looking at primary drying, there are three main methods that we look at for transferring the cycle. The first and simple method is to maintain the same recipe and extend the primary drying time. You're going to keep the same shelf temperature and pressure and extend primary drying time in your production system. The second would be to use Kv measurements between the lab and production systems to then calculate the optimal shelf temperature for your production system. And then the third, a newer method actually came from a customer of ours, is to use the LyoSim Ring to simulate the Kv of a production system allowing you to develop your cycle from the start on the smaller MicroFD.

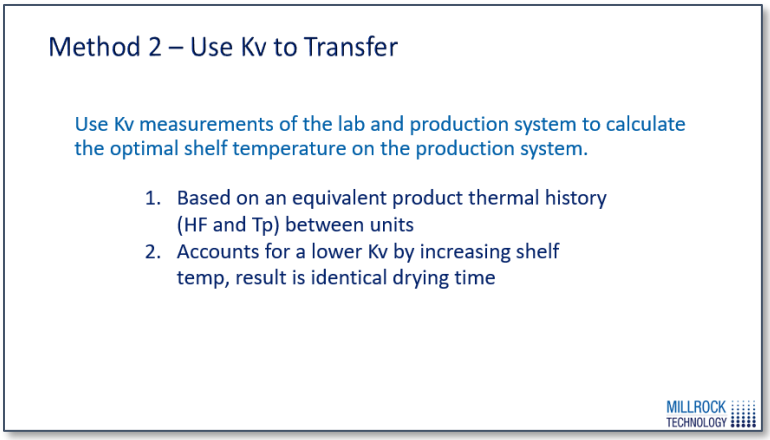


Method one, extending primary drying time, is based on the observation that Kv is generally across the board lower for larger systems. So with the same pressure and shelf temperature, the lower Kv in a larger system leads to a lower product temp, sublimation rate, and longer drying time. With modern freeze dryers which are sized with larger vapor ports and robust refrigeration systems, there's little risk with transferring a cycle with the same temperature and pressure to a larger production system. In the past with systems that had restricted vapor ports or

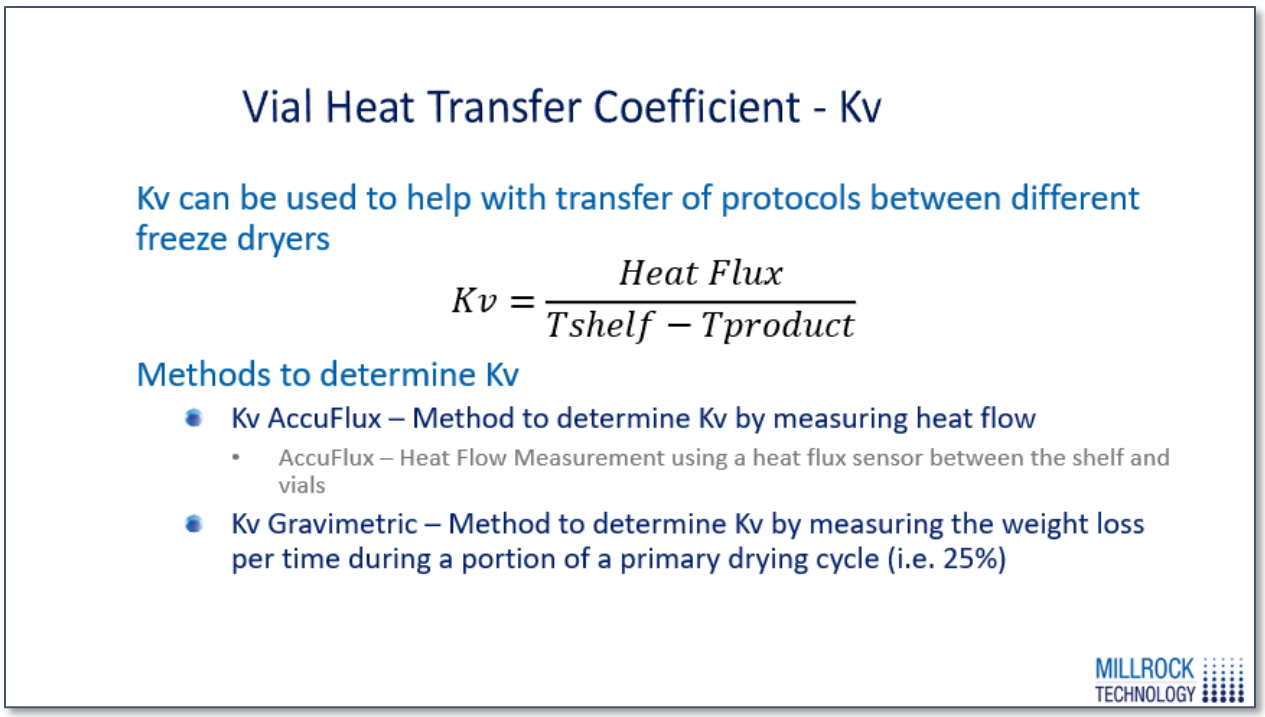
undersized refrigeration systems, there were a lot of concerns that the larger production systems would not be capable of handling the protocol that was developed on a smaller system. But with modern systems that have these considerations taken in mind, this concern is greatly reduced. In the case where you are using a relatively aggressive cycle and that is still a concern, a simple design space or sublimation study can be used to verify the throughput capability of your production system and that's pretty much as intense as you need to go when you're transferring using this simple method because it's across the board and generally going to have a lower Kv for the production systems as the shelf size increases the percentage of edge of vials decreases so the percentage of impact of that that edge effect has on the overall batch decreases and we see a lower Kv.



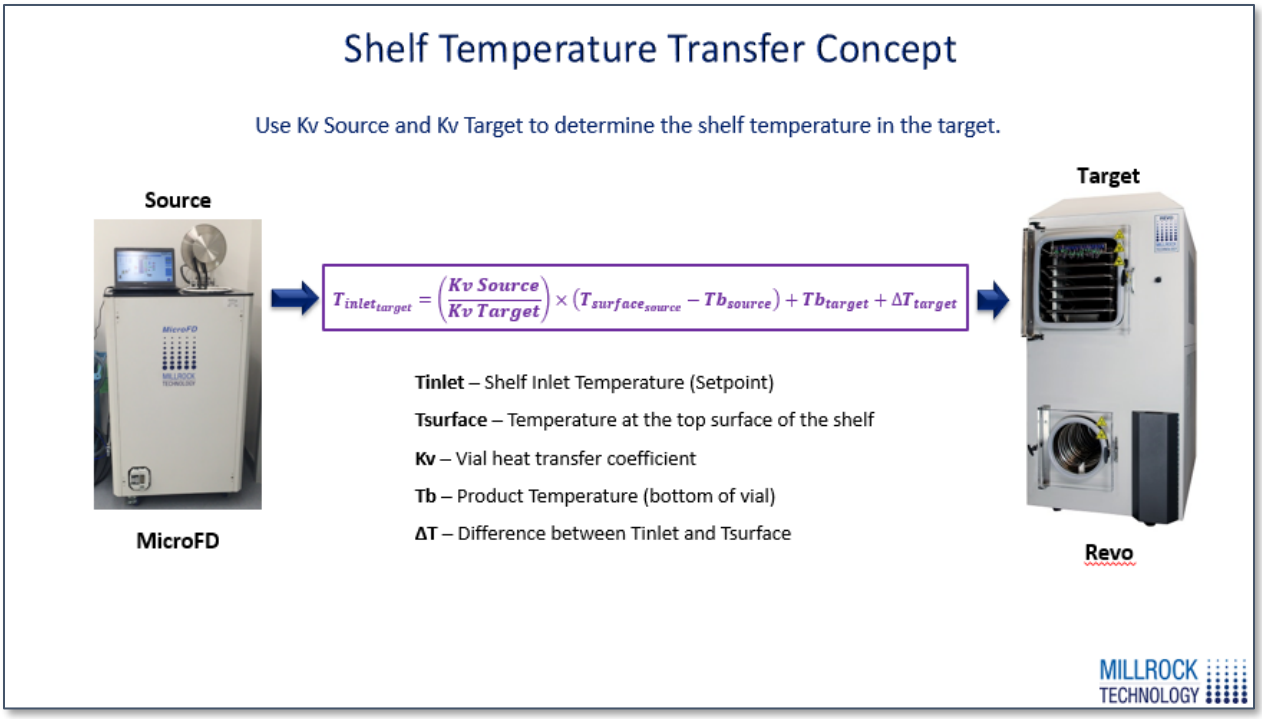
And then as we can see here, that lower Kv leads to a lower product temperature in this light blue line. You see the product temperature in a larger REVO system that we migrated. We started with a MicroFD in red and purple here at zero degrees C and then move that same product and recipe to a larger REVO system which is still a lab scale but much larger than the MicroFD. With that lower Kv, we had a lower heat flux which is essentially a lower sublimation rate, lower product temperature, and we saw a longer primary drying time. That is a very simple method and typically a lot safer than most people seem to consider. We hear a lot of concerns with transfer but from customers' evidence what they've stated, they've had very little difficulty scaling up to a larger system by simply keeping the same temperature and pressure. All they must do is increase that primary drying time and your product is pretty much guaranteed to run safer and at a lower temperature than it did in your lab system. This is a simple method, but it is not as optimized or as efficient as other methods for transfer.



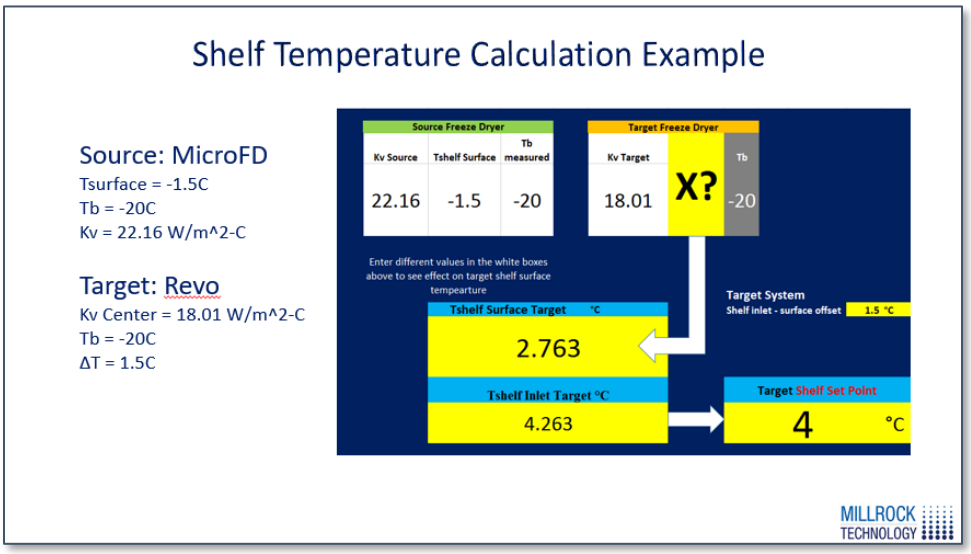
Method two for cycle transfer would be to use the Kv measurements between the lab and production system to then calculate the optimal shelf temperature on your production system. This is based on having an equivalent product thermal history, both heat flux and product temperature between the units and it accounts for a lower Kv by increasing the shelf temperature resulting in an identical drying time.



A quick refresher on Kv here it is a coefficient that describes the heat flux that is seen proportional to the difference in temperature between the shelf and the product. We measure Kv in two main ways using AccuFlux which is using that heat flux sensor to directly measure this heat flux here and then with the product temperature and the shelf temperature or gravimetrically which are more intensive measurements by weighing the mass loss of the vial through primary drying. Whichever method of Kv measurement is used, it does not matter. One note is that this Tshelf can either use the shelf inlet temperature or the shelf surface temperature and while there are benefits to either item measurement when you're comparing Kvs, the most important thing is to make sure that that point of shelf temperature measurement is consistent. If you're using the shelf surface temperature on one system, you need to use the shelf surface temperature on the target production system and the same goes if you're using shelf inlet temperature.



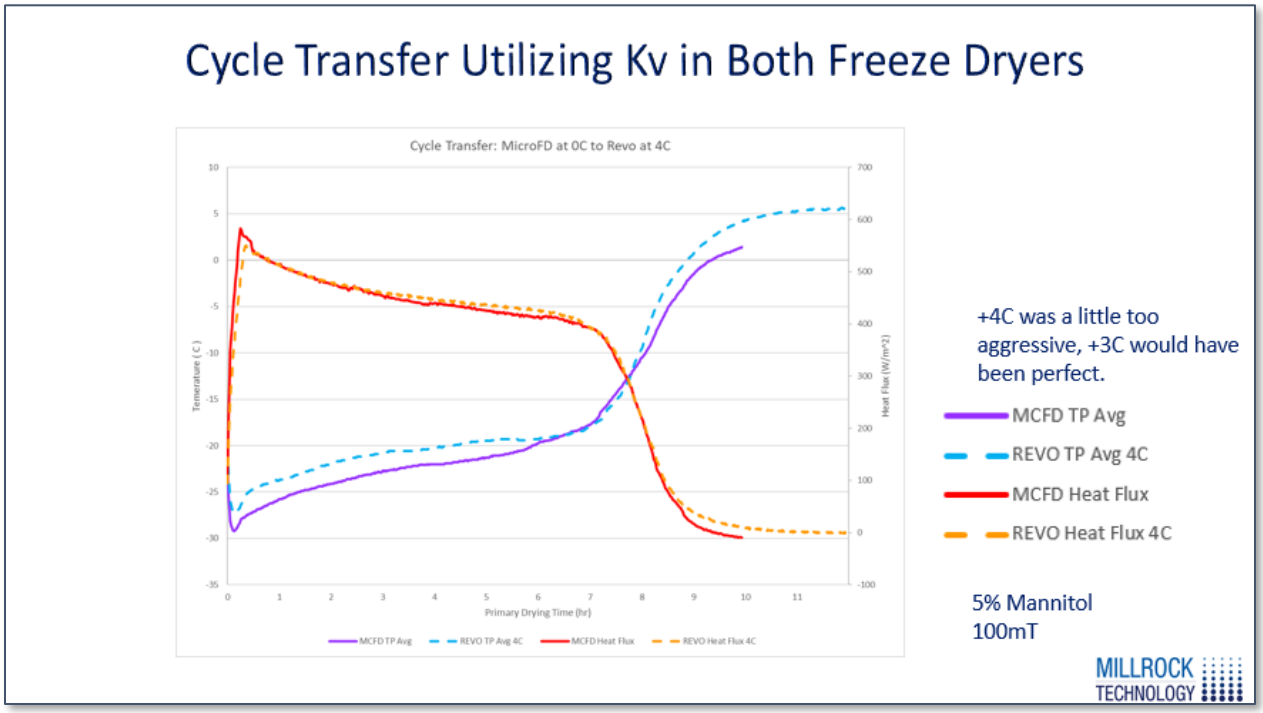
The shelf temperature Kv transfer concept is again based on maintaining that same heat flux and product temperature between the systems. This equation can be found by rearranging this formula for Kv and then setting the heat flux between the two systems equal to each other and essentially what it is, is it's a way of determining the inlet temperature by looking at the ratio of the Kvs between the source and the target unit. In general, if we have a higher Kv on our source unit which is generally the case, it's going to tell us that in order to maintain the same product temperature, the same heat flux on a system with a lower Kv, we're going to need to increase that temperature differential between the product and the shelf which means increasing the inlet temperature. For this specific example, the Kv was calculated using the shelf surface temperature. Since we don't control based on the shelf surface temperature, we have to use a factor of delta T which is the difference between the T inlet and the T surface.



When we plug these numbers in, and we're looking at this specific example, on our source unit which was our MicroFD which would be our smaller lab unit, we had a Kv of about 22 W/m²-C, a product temperature of -20C, and a surface

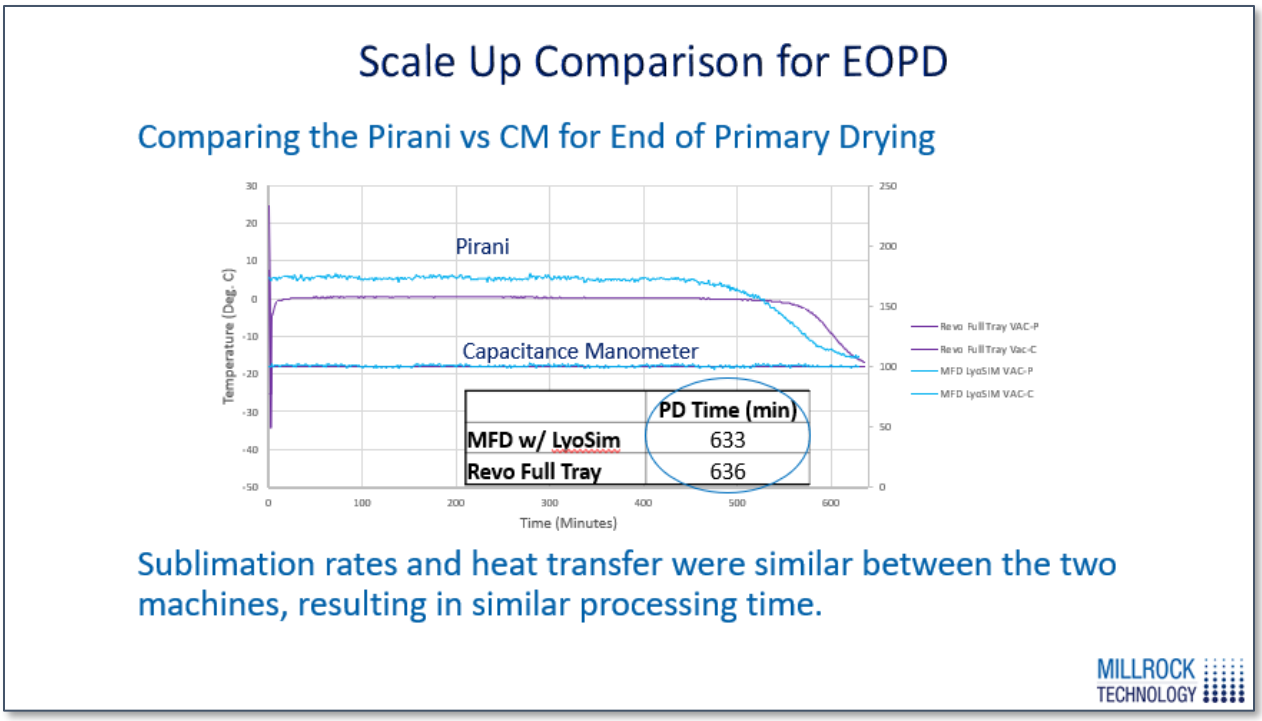
temperature of -1.5C, and we want to transfer this cycle to our REVO which has a Kv in the center of the batch of about 18W/m²-C with a similar product temperature and about 1.5C difference between the shelf inlet and shelf surface temperatures. By comparing the Kvs between these two units, we were able to calculate that in order to maintain the same heat flux between the MicroFD and the center of the REVO, we needed to have a shelf surface temperature of 2.7C and because there's a 1.5 degree difference between the inlet and the surface, this meant that we needed to control the inlet at about 4.2-4.3C which we rounded to 4C. One thing that's important to note is when you're looking at this Kv transfer concept, it does allow for more informed decisions when you're transferring. By which I mean, when we're looking at transferring to a larger system, there are two main considerations that can be used. Because the KV on the edge is going to be higher than the KV in the center of the batch, we can either transfer to maintain identical thermal history for the center vials or for the edge vials. In this case here, we transferred to maintain an identical thermal history for the center vials. While the center had a Kv of 18, the edge vials in this system may have had a much higher Kv that may have even been higher than the MicroFD so we decided to transfer for the center vials which may have resulted in a cycle that would be too warm or too aggressive for the edge vials. If we wanted to transfer with all of the entire batch in mind and make it conservative for the edge vials, we would instead use the Kv of the edge here which would give us a different target temperature. But again for this example, we transferred focusing on the majority of the batch which was the center vials.

What we see here is again the red and the purple of the MicroFD run at zero degrees C and then in the



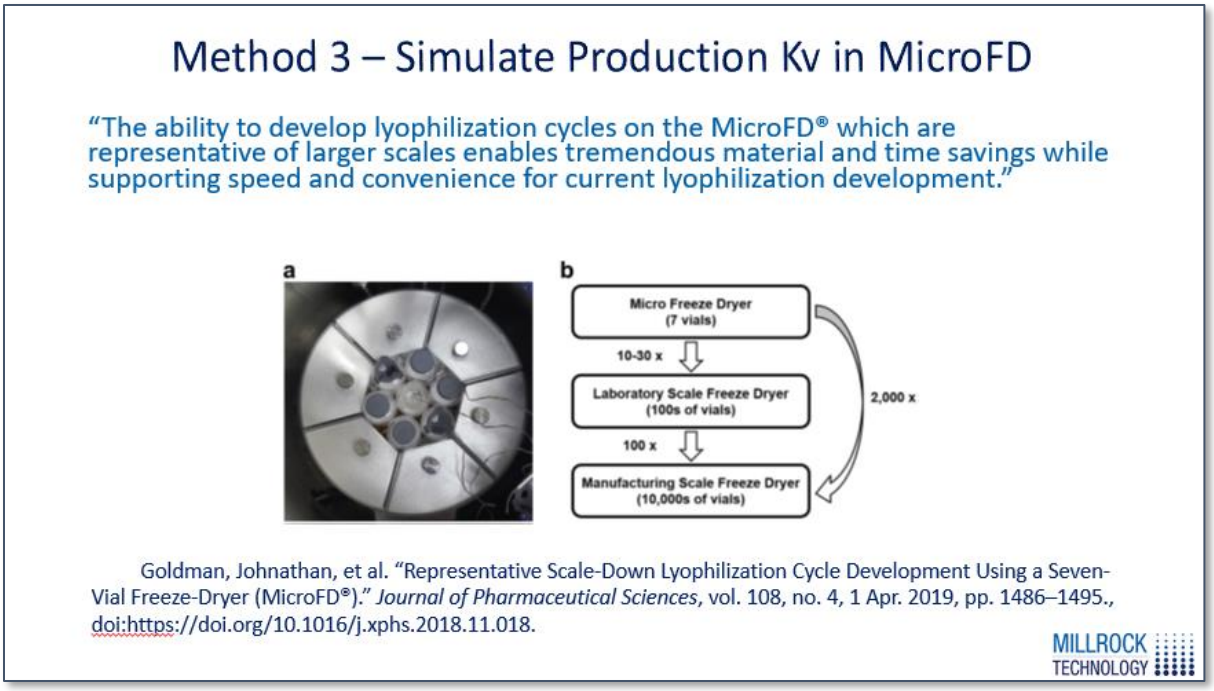
dotted orange and dotted light blue lines, we see the center vials of the REVO run at 4 C. The result of this was a virtually identical heat flux profile between the vials of the MicroFD and the center vials of the REVO. Again, the edge vials in the REVO which we did not consider for this transfer may have had a much higher heat flux and a much higher product temperature. It may have been too aggressive for the edge vials but for this case of transfer, we assumed the product that we just wanted to focus on the

center vials. Again, we increased our product temperature as well. It was a little bit higher in the REVO early on in the cycle but to the end of the cycle, before they all start popping out of ice and completely drying, we see that those product temperatures overlap very closely as well. In general, we may have considered this a little bit too aggressive and perhaps 3C would have been perfect but what it broadly shows is that in order to maintain the same thermal history between systems when transferring rather than simply extending your primary drying time, this can be done relatively easily by simply comparing the Kvs between these units.



An extra piece of data here shows the primary drying time between these two cycles, the MicroFD at 0C and the REVO at 4C, was within about three minutes of each other. The sublimation rates and heat transfer were very similar between the two units resulting in similar drying times.

Method three which is a newer method that was proposed to us by a customer is to use the LyoSim of the MicroFD to simulate the Kv of a larger production system. What they did, and this has only been demonstrated on arrays with seven vials, is instead of controlling this LyoSim temperature to be equal or very close to the product temperature within the vials to eliminate the edge effect, they actually cooled this LyoSim temperature several degrees below the product temperature of the vials here effectively slowing down the sublimation in the vials causing them to have an effective Kv that was equivalent to a larger production system.



They were able to use the LyoSim to slow down the vials in here and simulate the Kv of a larger system and then they were able to fully develop a cycle inside this small system using just seven vials. They were able to test it from bringing it from the micro freeze dryer to a lab to manufacturing scale and then eventually go straight from the micro freeze dryer to a large manufacturing scale provided that they can characterize the Kv in each of these systems. They can simulate it in the MicroFD, developed their protocol this way, and then transfer it directly to a larger system.

In summary, those three methods of transfer were one, to keep the same product temperature or keep the same shelf temperature and pressure and extend the primary drying time on your production unit, two, to compare the Kvs between your lab and production unit and use those to calculate an adjusted shelf temperature on your production unit, or three, to use the MicroFD LyoSim to simulate the Kv of a larger system and then use that to develop your cycle directly in the MicroFD. Overall, in summary, we were able to do this testing and the optimization and the transfer of the cycle could now be done with as little as seven to 61 vials depending on the size of the vials that fit within that LyoSim Ring and they still provide the same certainty and the same results that you'd find in

a production unit for the added benefit of significant time and money savings. It's such a small scale it makes freeze drying development enjoyable.