Introduction To Freeze-Drying – General Overview
Presentation Transcript 11/15/2023
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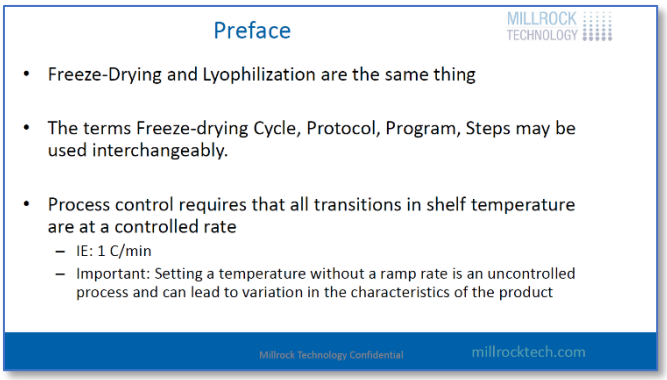
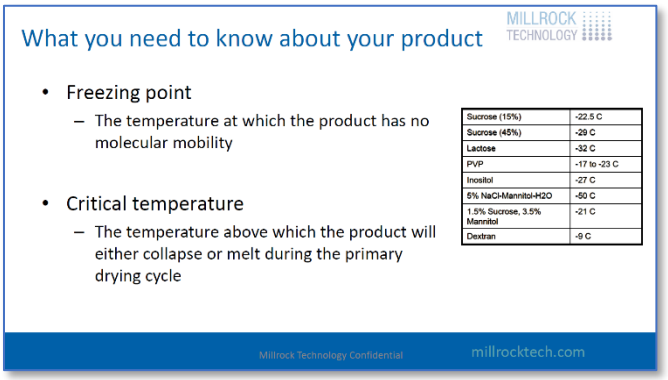
Hello, my name is TN Thompson, Millrock Technology. I'm going to be talking about an intro to freeze-drying today, and this is just a general guide on how to approach freeze-drying, give you an idea of what some of the considerations are to help you develop your protocol and process in a more efficient manner.



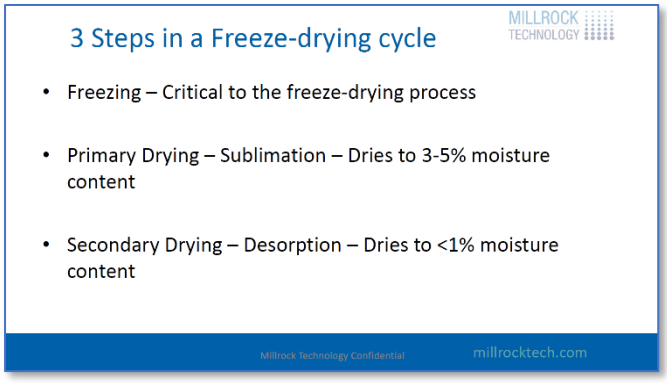
Freeze-drying and lyophilization are the same thing. When we talk about that in the industry, you could do search terms on lyophilization or on freeze-drying. Essentially, you're going to get the same type of result. The terms freeze-drying cycle, protocol, program, and steps are used interchangeably throughout this conversation. This is going to refer to that recipe that's actually being used to process your product. It's important to understand that process control, which is what is required for a freeze-drying process, needs control in all transitions and shelf temperature, and they have to be done at a controlled rate. I say this upfront because a lot of times people say, "Well, I'm going to be going to -40 on my shelf."



You can do that, but if you don't have a controlled ramp to do that, sometimes it might take half an hour, sometimes it might take an hour, and that difference can have a large impact on your process.

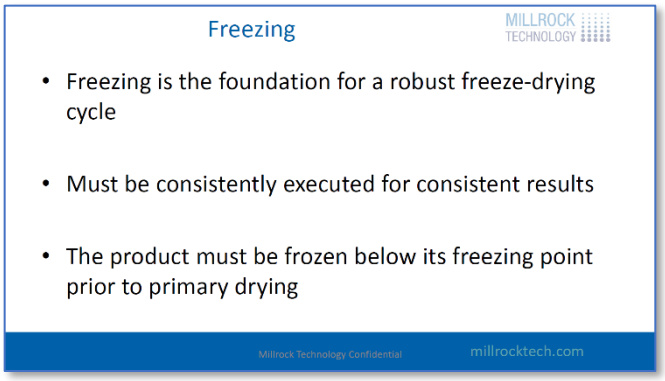
To start this process, you need to know something about your product, and it's critical that you know this information. The first thing is the freezing point, and that's going to be the temperature at which the product has no molecular mobility. Typically, things with solvents or salts are going to have a much lower freezing point than those that don't. There's also another parameter that's called the critical temperature, and this is the temperature above which the product will either collapse or melt during the primary drying cycle, so that's during the sublimation event. This is very important, because the process has to maintain the product temperature below the critical temperature in order for you to have a very robust cycle and to have the efficacy that you're looking for in the finished product.

On the very basic side of things, there are three steps in a freeze-drying cycle. There's freezing, primary drying, and secondary drying.

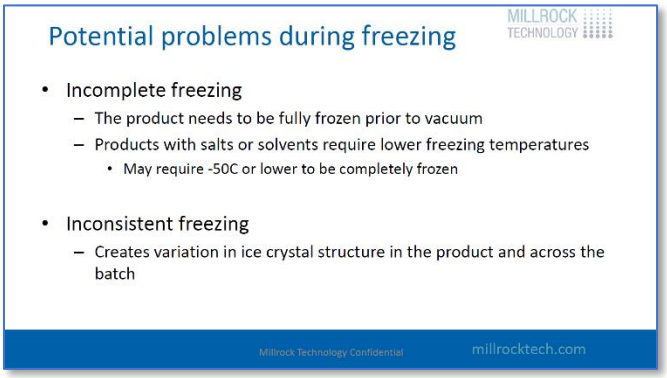


Freezing, which is actually the most critical step in the entire freeze-drying process, is the foundation for freeze-drying. If it's not done properly, then you can't have a robust cycle. Primary drying, which is what people refer to as freeze-drying, is sublimation, and this is going to allow us to remove moisture down to the three to 5% moisture content range. This may or may not be sufficient dryness for your product. In some cases, you need a secondary drying cycle, it's called desorption. And the temperature of the shelf has increased. And this can be used to dry the product to less than 1% moisture. Be aware that there are some products that can be too dry, and it affects their efficacy once the freeze-drying process is done.

Freezing is the foundation for a robust freeze-drying cycle. It must be performed consistently to get consistent results in the freeze-drying process.



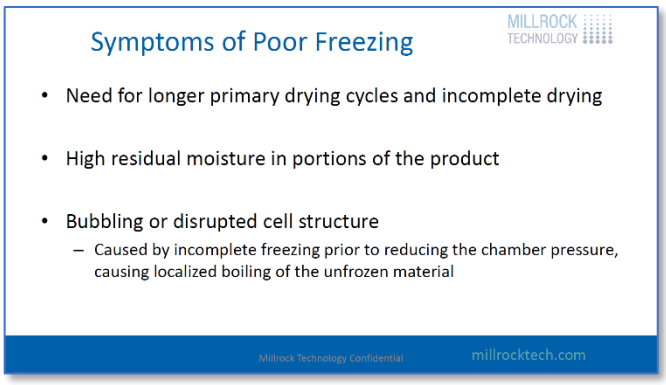
The product must be frozen below its freezing point prior to primary drying. If it's not, it's going to cause problems with the process. So, you have to fully understand not only the freezing point, but how long it takes your product to freeze. The thicker the product, for example, the longer it's going to take.

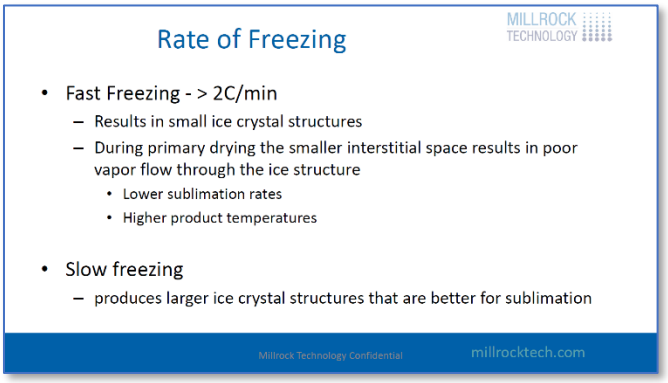


What are the potential problems during freezing? The first is incomplete freezing. Since the product needs to be fully frozen prior to pulling a vacuum on it, it's very important that you understand that your product, number one, is frozen, and it's been frozen long enough that all of the product is frozen. For example, if you have a thick material, that can take a very long time to freeze, and it's important to understand that all of your product is frozen. Inconsistent freezing is another problem. Inconsistent freezing can create variations in ice crystal structure in the product and across the batch, which results in variation to the process.

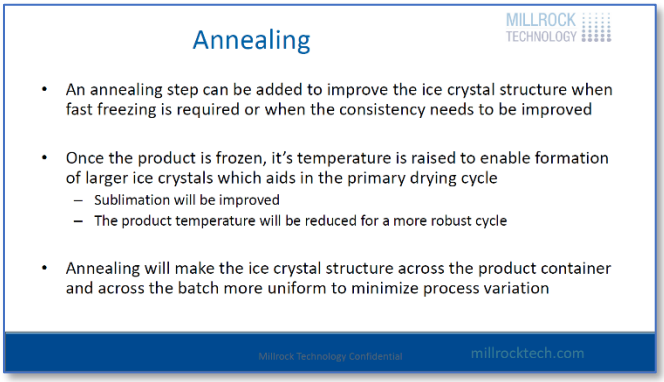
So, when I say inconsistent freezing, what does that mean? That means that sometimes it's frozen at one degree C per minute, sometimes it's frozen at five degrees C per minute, sometimes it's frozen to -15 instead of -30. That's going to lead to process variation, so it's highly recommended that you freeze in the freeze dryer when you can, and it is done at a controlled rate, so you get consistent ice crystal structures across your batch.

Symptoms of poor freezing. Poor freezing can lead to longer primary drying cycles and incomplete drying. Can result in higher residual moisture content at the end of the freeze-drying run, and if you have a batch product or a bulk product, that can result in just portions of your product having high residual moisture. Can also lead to bubbling or disrupted cell structures, which is caused by the incomplete freezing process, and once you start to reduce the chamber pressure, you can get localized boiling of the unfroze material causing problems. So, how does the rate of freezing affect the process? It actually has a very big impact on it.



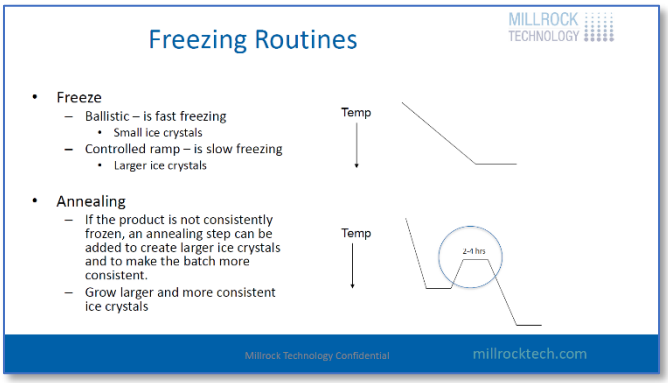


First, we'll discuss fast freezing. Now, fast freezing for this discussion is going to be anything greater than two degrees C per minute. Typically, a ballistic freeze would be something like if you put your product into a freezer and just let it get cold as fast as possible. Number one, that's uncontrolled, and number two, is probably a rate faster than two degrees C per minute. Fast freezing results in small ice crystal structures. What does that mean? Well, it means that during the primary drying cycle when sublimation is going to occur, there's a smaller interstitial space between the molecules between the ice crystals, and that results in poor vapor flow through the ice structure. So, it actually inhibits or hampers the sublimation process. So, it's going to lead to lower sublimation rates, and ultimately higher product temperatures because the vapor can't escape as you're adding heat, then the sublimation rates lower and it leads to an increased product temperature. Slow freezing, on the other hand, we'll consider that anything two degrees C per minute or less, produces larger ice crystal structures that are better for sublimation. So, it's important to understand and control that freezing process.

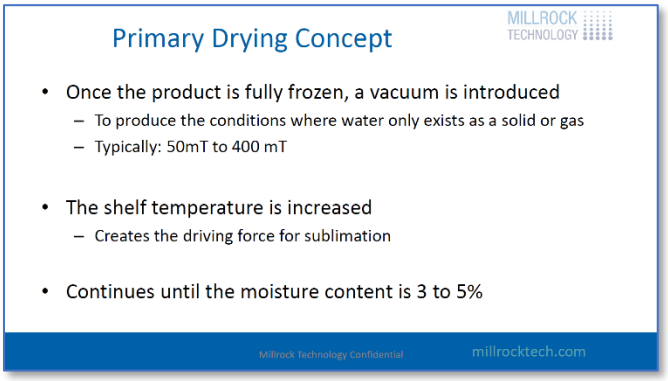


There's an ability in your freezing process to actually overcome any process variation, and that's called annealing. Annealing is the process where we increase the product temperature after it's been frozen to a higher temperature, let's say, -20 or -15 degrees, hold it there for a period of time, depending on your product, might be one to three hours, for example, and that allows the ice crystals to grow in size and to make it actually more consistent across the batch. So, if you've got variation inside your product, annealing will actually make it more consistent. And then if you have multiple trays or multiple vials, it'll actually make it more consistent across the batch. So once the product's frozen, the temperature's raised, larger ice crystals are created, and then it's going to enable an improved sublimation rate.

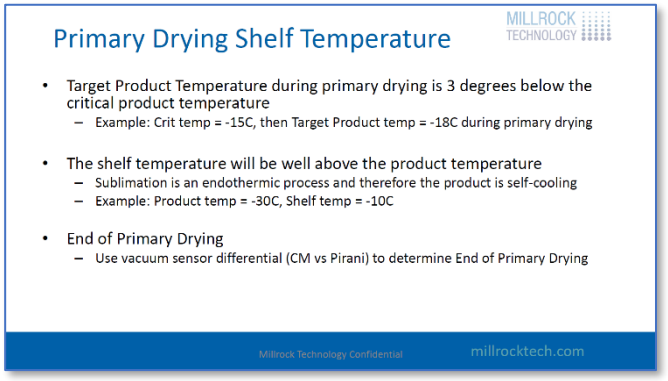
And it's also going to protect the product better, because the product temperature's going to be reduced during the primary drying cycle. So, the annealing will make the ice crystal structure across the product container more consistent, and across the batch more consistent.



So, let's take a look at freezing routines. The first is just what we'll call freezing. If it's ballistic, which means it's at a fast freezing rate, you're going to create small ice crystals. If it's controlled and ramped, then we're going to get larger ice crystals. So, we've got a time down to temperature, and then we get down to temperature and we hold. If our freezing process is inconsistent or needs improvement, we can always add the annealing step where we have a ramp rate down to a temperature hold for a period of time, raise the temperature of the product to let's say -20, -15, hold it for a period of time, and then freeze it again before we go into primary drying. This is going to grow larger ice crystals, and it's going to make them more consistent.



Primary drying. The concept of primary drying is to reduce the vacuum in the system to a point where the water can no longer exist as a liquid. It can only exist as a solid or a gas. Typically for freeze-drying, we reduce the pressure to 50 to 400 millitorr approximately for the freeze-drying cycle or the primary drying cycle. At that point, the shelf temperature is increased, and that's going to create the driving force for sublimation. So, the shelf temperature is going to be always above the product temperature. This is going to continue until the moisture content is in the three to 5% range.

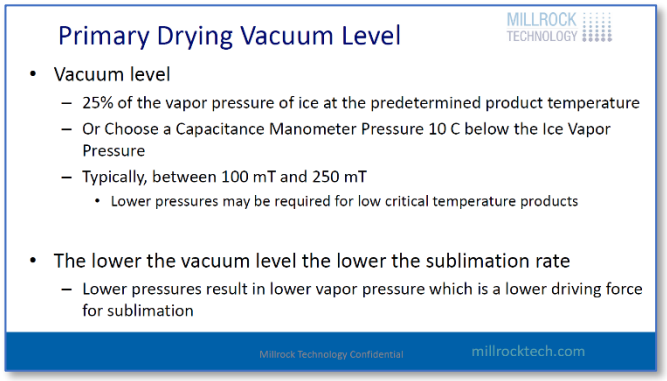


So, what is the primary drying shelf temperature? How do we set that? You take your target product temperature during the primary drying cycle, and you're going to try to keep that three degrees below the critical product temperature. For example, if our critical temperature's -15, then the product temperature is going to be about -18 degrees.

The shelf temperature, since it's going to be above the product temperature because we're driving energy into the process... Sublimation is an endothermic reaction, so the product is actually going to be cooler than the shelf during sublimation.

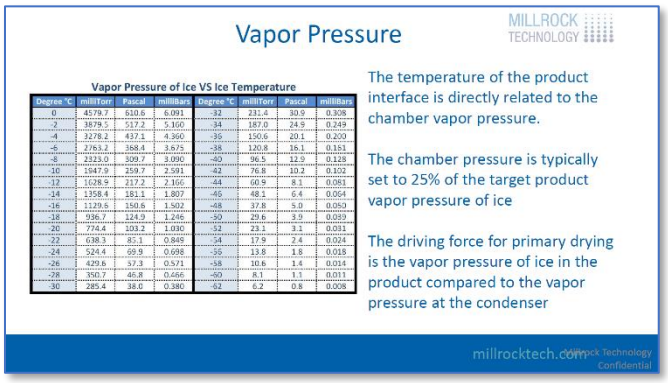
An example of product temperature. If we're shooting for, let's say, -30 degrees, we could actually have a temperature somewhere around -10 degrees. So, it is significantly higher than the product temperature, and that's going to be the driving force, as I've mentioned. We can use the end of primary drying determination using a vacuum sensor, a Capacitance Manometer versus the Pirani, to determine when we've actually reached the end of the cycle. We'll get into that in more detail as we move forward.

So, let's talk about vacuum during the primary drying process. The vacuum level is typically set to 25% of the vapor pressure of ice at the predetermined product temperature. And that's typically done when using a Capacitance Manometer for control. So, we choose a Capacitance Manometer pressure 10 degrees below the ice vapor pressure. Typically, that pressure is going to be between 100 and 250 millitorr for almost every application.



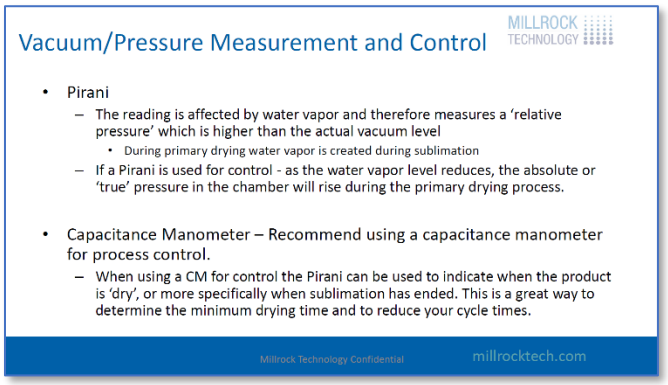
And it's important to understand that the lower the pressure in the chamber, that means that the product's going to be running at a lower temperature and that your sublimation rate's also going to be reduced. The lower the vacuum level, the lower the sublimation rate.

This is a vapor pressure chart; it gives us an idea of the type of vapor pressure at different ice temperatures in the process. So, if we're trying to run somewhere around -18 degrees on our product, we said that we're going to set the chamber pressure to about 25% of that. So here, let's just say it's a thousand. We try to be running at about 250 millitorr, and that's going to be about -32 ice vapor pressure in the product itself. That's going to keep our product actually closer to -18 because of the driving force of the sublimation event.



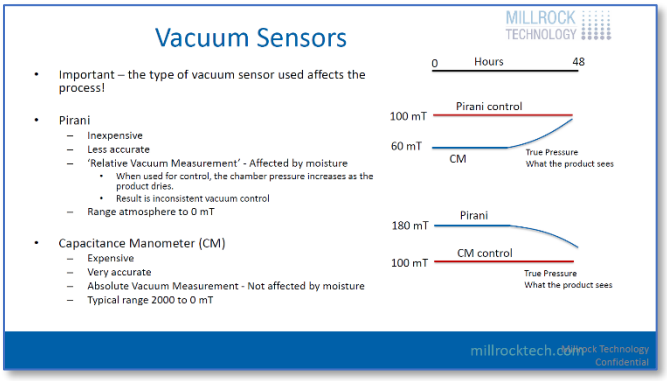
Degree °C	milliTorr	Pascal	milliBars	Degree °C	milliTorr	Pascal	milliBars
0	4579.7	610.6	6.091	-32	231.4	30.9	0.308
-2	3879.5	517.2	5.160	-34	187.0	24.9	0.249
-4	3278.2	437.1	4.360	-36	150.6	20.1	0.200
-6	2763.2	368.4	3.675	-38	120.8	16.1	0.161
-8	2323.0	309.7	3.090	-40	96.5	12.9	0.128
-10	1947.9	259.7	2.591	-42	76.8	10.2	0.102
-12	1628.9	217.2	2.166	-44	60.9	8.1	0.081
-14	1358.4	181.1	1.807	-46	48.1	6.4	0.064
-16	1129.6	150.6	1.502	-48	37.8	5.0	0.050
-18	936.7	124.9	1.246	-50	29.6	3.9	0.039
-20	774.4	103.2	1.030	-52	23.1	3.1	0.031
-22	638.3	85.1	0.849	-54	17.9	2.4	0.024
-24	524.4	69.9	0.698	-56	13.8	1.8	0.018
-26	429.6	57.3	0.571	-58	10.6	1.4	0.014
-28	350.7	46.8	0.466	-60	8.1	1.1	0.011
-30	285.4	38.0	0.380	-62	6.2	0.8	0.008

How do we measure vacuum in a freeze-dryer? There are two types of sensors that are typically used. The first is a Pirani. The Pirani is a less expensive offering for vacuum measurement. However, the nice part about a Pirani is we read from atmosphere all the way down to zero millitorr. The problem is that the Pirani reading is affected by water vapor, and the primary drying cycle creates water vapor. So, if you're using these for control, the true vacuum in the system is going to change throughout the process as the water vapor content changes, and water vapor content reduces during



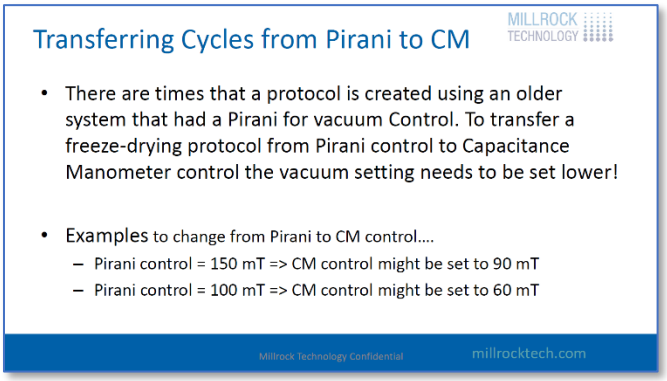
the cycle. So, we're going to talk a little bit more about what that looks like. The other type of vacuum sensor is a Capacitance Manometer, and we recommend using this for process control because it's the most consistent. And as you move into production, almost every production system uses a Capacitance Manometer. It's important to know that the Capacitance Manometer is not affected by water vapor, so you're going to get the true vacuum reading at all times.

Well, that's kind of confusing, isn't it? We have two vacuum sensors, but one is affected by water vapor, the other one isn't. And often we have both of these in a system.



So, let's take a look at the differences between the two, when they operate. Again, the Pirani's an inexpensive, less accurate measurement system, and it does what I call the relative vacuum measurement where it's affected by moisture. Older freeze dryers, I'm going to say older, 30 years old plus, will control with the Pirani, but if you put a Capacitance Manometer inside the system, as that water vapor goes away, the true vacuum would actually be coming up, although you're not going to see it when you're looking at Pirani control because it's not changing. The true vacuum in the system actually increases through the process. This could be a problem, because if you're going to transfer this cycle from your older freeze dryer to either a new freeze dryer or a production freeze dryer, it will not transfer properly because the new system's going to be using a Capacitance Manometer.

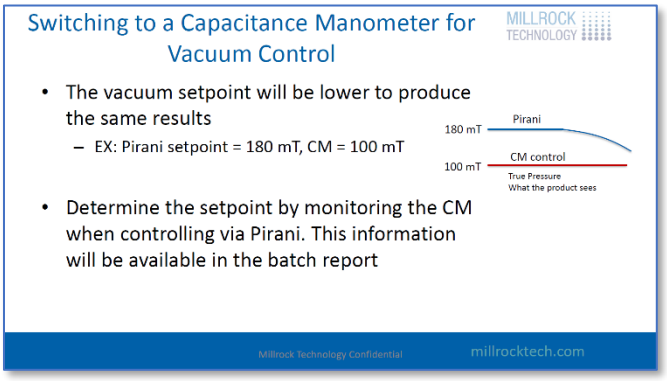
Let's take a look and see what that looks like. If you're using a Capacitance Manometer, the Capacitance Manometer will control the process at the true pressure, and that's going to be consistent across the process. And what had happened is your Pirani will actually run at a higher vacuum level, because the vapor is making it read higher, and as the vapor content in the system drops, or the water vapor presence drops, then this is going to come down until it almost matches the Capacitance Manometer. In the dry state, they're going to be pretty much the same. So, if we're controlling from the Capacitance Manometer, you're always going to get a true cycle.



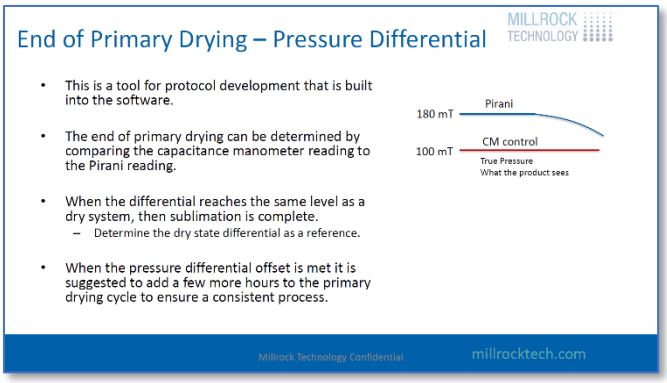
Again, just a little more detail on if you were going to transfer a cycle from a Pirani to Capacitance Manometer, you're going to need to understand where to set the Capacitance Manometer.

Let me give you a couple instances. If we were controlling our process in an old system at 150 millitorr using a Pirani, we might set the Capacitance Manometer to 90 to get the same process results, okay? This is critical. I've seen people who have actually transferred from a Pirani system to a steam sterilizable freeze dryer, and the process would not work because the new pressure was higher than it should have been because it was using a Capacitance Manometer. I'm going to show you that in a second as well. Another one, if we were controlling at 100 millitorr with the Pirani, we might set the Capacitance Manometer to 60

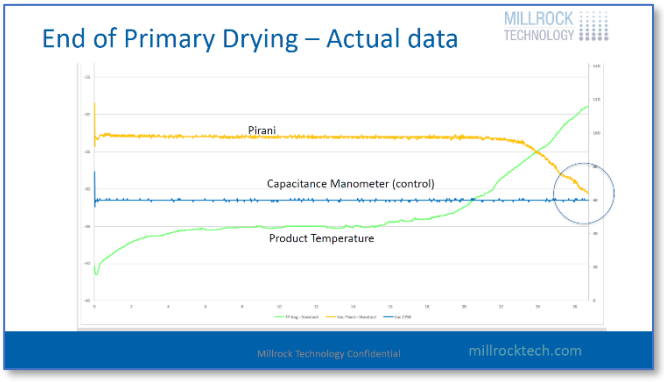
millitorr. Very critical that that's understood for transfer. So, when you're switching between vacuum measurement systems, again if we were up at 180 with the Pirani system, you're going to have to set your Capacitance Manometer at 100, or somewhere in that. So, you're going to have to determine the setpoint by monitoring the Capacitance Manometer while you're controlling with the Pirani, and this is going to give you that offset so you can actually do the transfer.



The beauty of having the two in a system is that you can determine the end of primary drying. So, during the primary drying process, as that Pirani comes down in pressure, we can actually determine what the end of primary drying is, because when there's no water vapor present, then we can actually know that we're at the end of primary drying. Now, you determine that differential for a dry system by measuring the difference between the Pirani and the Capacitance Manometer in a dry state, and once that reaches the differential, let's say it's five millitorr just as an example, then what we suggest is that's the end of primary drying. You just add a few more hours of hold at the existing pressure and temperature to verify that everything in the system has reached its dry state, and then you can move on to secondary drying.

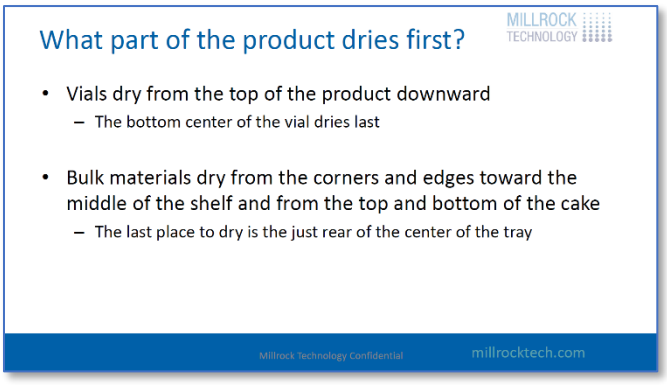


Here's a real life example of a Pirani. We have set the Capacitance Manometer at 60 millitorr in this case. The Pirani's reading is up around 100 millitorr. At the end of the cycle, the water vapor starts to decrease until it reaches the end of primary drying.

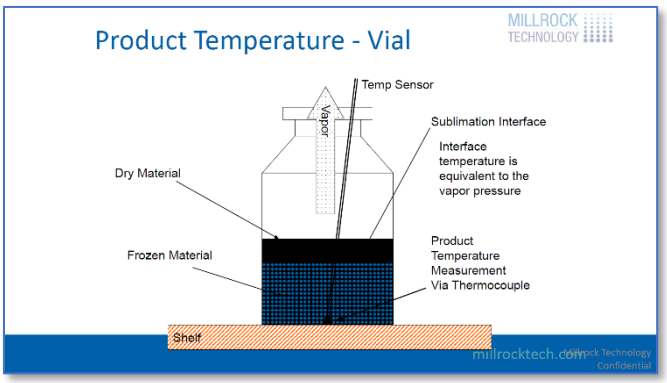


Now, also look at the product temperature. What happens is the product temperature here is maintained at about -35, and we're running... Unfortunately, we don't have the shelf temperature on this chart. I think we're running the shelf temperature at about -20 degrees or something like that. So, it does run much cooler than the shelf temperature. And then you can see the product temperature coming up, which means that there's no water present or no ice present, and that's the end of primary drying.

What part of the product dries first? Well, it depends on your container. If it's in a vial, it dries from the top of the product downward, and the last place to dry is the bottom center of the vial. In bulk materials, when they're in trays, it dries from the corners and edges toward the middle of the shelf, and from the top and the bottom of the cake toward the middle of the cake, like a sandwich. So, the last place to dry in a bulk system is just behind the rear center of the tray.

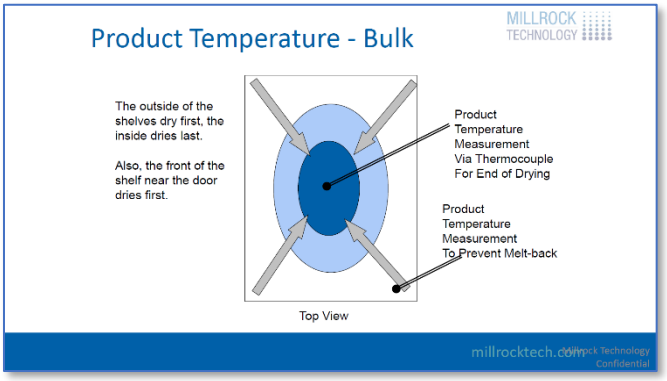


Let's see what that looks like. Here's a vial, and the dryer layer builds up from the top. And this is ice down here. This has been dried here, and the water vapor leaves the system.

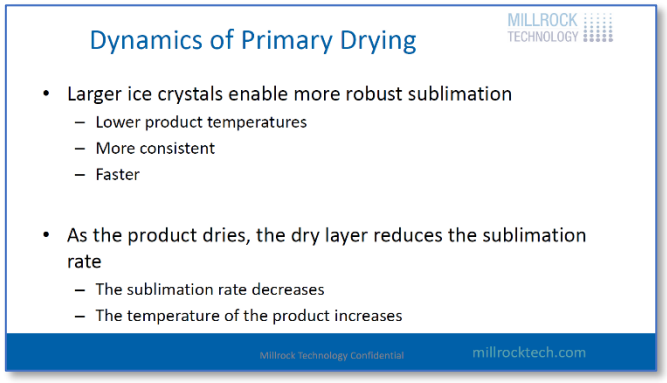


In a bulk system, looking at the top of the tray looking from the top, the edges dry first because there's more heat conduction coming on from here, and ends up that it dries last in the middle of the shelf. This is kind of important, because that's where you want to put your thermocouple if you're trying to measure the product temperature.

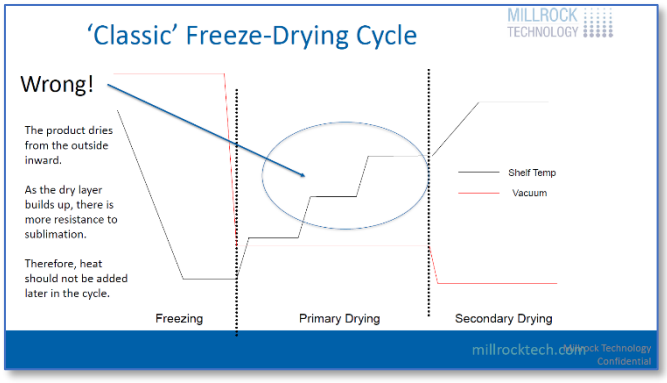
One more comment on the last slide was we also were building up resistance, and that resistance is critical to how you design your cycle. So, we'll talk about that in a second.



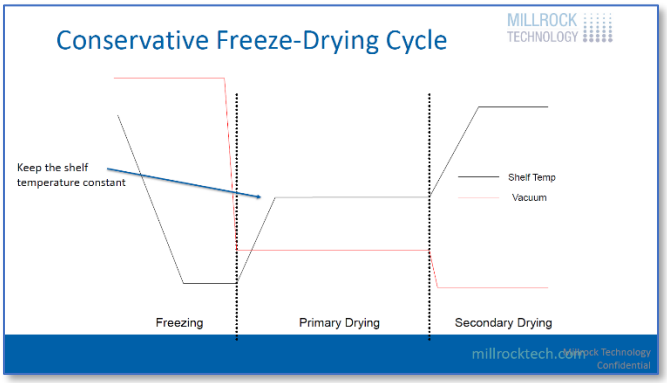
So, the dynamics of primary drying. We've discussed some of this stuff. Larger crystals enable more robust sublimation that will result in lower product temperatures, a more consistent cycle, and a faster drying cycle. So, it's not only going to be faster, but it's going to be more robust at the same time. That's why it's so important to take the time to freeze properly. As the product dries, the dry layer reduces the sublimation rate. The sublimation rate decreases over time because of the dry layer, and that means that the temperature of the product's going to increase.



This is what you will see in a lot of papers that were written 30 or 40 years ago as a classic freeze-drying cycle. Here we freeze the product, we pull a vacuum on it, and we start to add shelf temperature, and that shelf temperature is increased during the cycle.



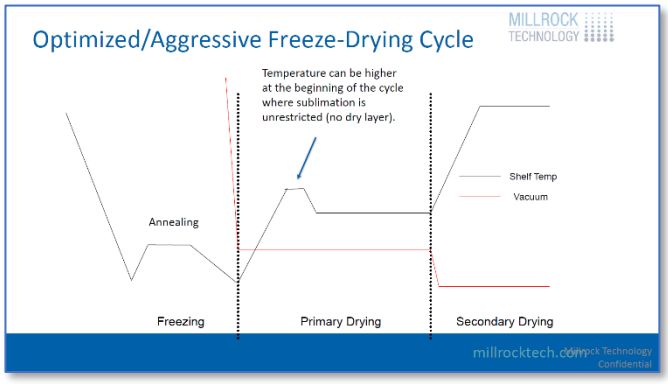
Unfortunately, we just learned that a dry layer builds up during the cycle, and if that dry layer is building up and you're adding heat, there's a good possibility that you're going to melt back or collapse your product and you're going to get a bad process. This type of cycle is a bad cycle. If you have something like this, chances are that you're having a higher failure rate, or a much, much longer cycle than you actually need.



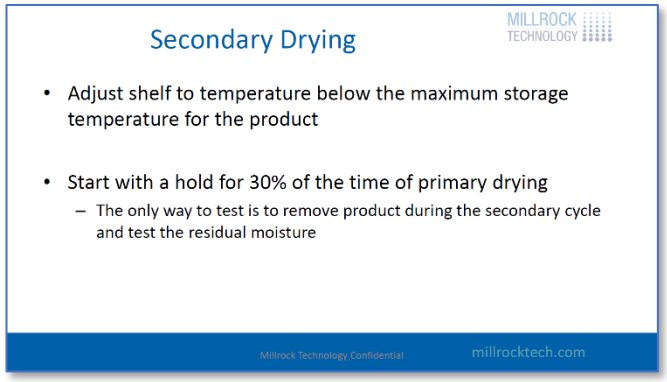
This is a conservative cycle. You freeze the product, bring the shelf temperature up to a level that it can maintain during the entire process until secondary drying is required. This is going to be very consistent every time you run it, and it's going to give you a process that's repeatable and robust and very friendly to the product.

As we discussed, we were talking about the dry layer building up.

Well, when you don't have a dry layer, literally you can take the shelf temperature and drive it up pretty aggressively at the beginning of the cycle, because there's no resistance to sublimation. And as that dry layer starts to build, we can then bring down the shelf temperature and control it at some predetermined point for the entire cycle. This is what I call an optimized or aggressive cycle.



A lot of people don't use this, but it's basically demonstrating the point that there's more sublimation at the beginning in the run than there is at the end of the run, and this is almost inverse of what that first slide was of what I call the classic or wrong cycle.



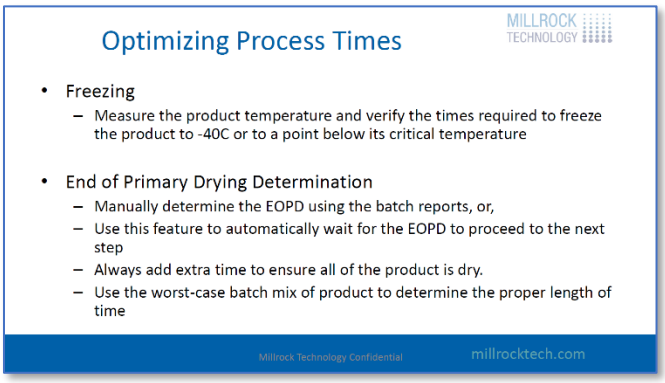
Once primary drying is done, we can go into secondary drying. And secondary drying, you simply adjust the shelf temperature to below the maximum storage temperature for the

product. So, if your product denatures at 30 degrees, you do not want your shelf above 30 degrees.

In a lot of collagen or tissue applications, you might be able to go to 40 or plus even more degrees higher in temperature.

Typically, the first question people ask is how long is secondary drying? A rough rule of thumb is 30% of the time for primary drying. So, if you have an eight hour primary drying cycle, it's two hours or so. If you've got a 15 hour cycle, it's five hours. That's going to give you a pretty robust and conservative cycle. The only way to test that is by actually removing the product at the end of that cycle and measuring the moisture content. Now, be careful, because a lot of people remove their product after secondary drying and they don't put it into a sealed system. And unfortunately, what happens is any moisture in the air will start to be reabsorbed into the product, and it can make it look like the product wasn't dried properly. So, it's very critical at the end of your cycle, if you're in a tray or something like that, you seal that in an inert atmosphere and keep it from reabsorbing moisture. In a vial, obviously we're sealing the vial with a stopper under a slight vacuum. By the way, it's under slight vacuum, not heavy vacuum because if there's too much vacuum in there, it will also tend to draw any moisture in from the residual air around it.

So how do we optimize our process times? Well, it's fairly straightforward. First, you want to measure the product temperature and verify the times that are required to freeze the product to -40, or to the point below its critical temperature. For example, anything with sodium chloride in it or salt in it should probably be done to -50 rather than -40. -40 is a pretty classic temperature where most things are frozen by then. But again, if you have any organic solvents in there, they're not going to be frozen there. You want to go down as far as possible.



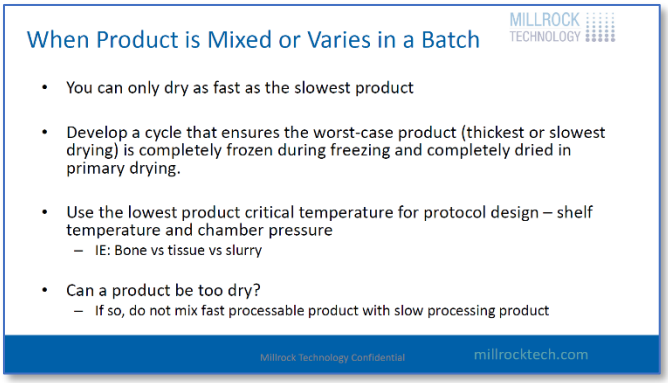
By the way, anything that has an organic solvent in it, that organic solvent's going to come out first when you pull the vacuum on the system during primary drying. So, you've got to be aware that that's going to happen immediately. And for the first two to three hours, that's what you're going to be getting out is the actual solvent. To further optimize your primary drying cycle, we recommend using End of Primary Drying Determination, which is the Capacitance Manometer versus the Pirani, and that will give you a really good idea of how long your drying cycle is. And then just add some period of time just to make sure that you dry longer than is required for your product. So those two things will really help you out.

Not everybody freeze-dries in vials. Now, almost everything you read about in publications is vials, vials, vials. Believe it or not, probably the bulk of freeze-drying is done in bulk. No pun intended.

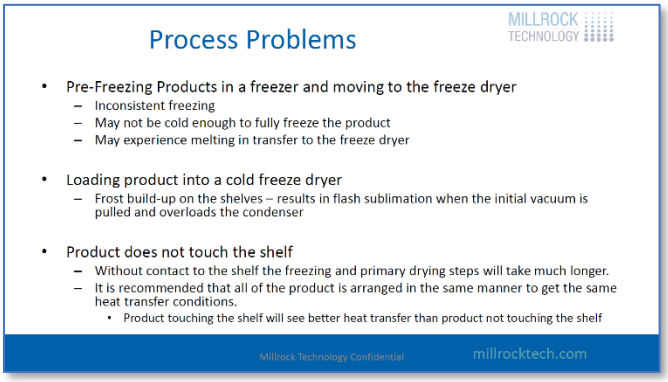
Freeze-drying is actually more familiar or more frequent in non-vial applications. People put their product in trays. They might have their product in bags. They might have their medical devices or some other types of

containers. So, what I'm trying to talk about here is how do you freeze dry if you're not freeze-drying in vials, because these bulk materials present unique challenges.

There's variation in form. There's variation in content and material. There are varying containers: bags, trays, Petri dishes, tubes. And if you're freeze-drying in a tray, it's important to understand that the thicker the product, the longer the freezing and primary drying cycles. We really don't recommend anything greater than one inch. Optimal is about a half inch thick, because the thicker you go, the harder it is to freeze the product all the way through. And when you're in the primary drying cycle, there's more resistance to the sublimation process as the product dries. So, try to minimize the thickness of the material to maximize the robustness of your process.



Some people put mixed batches in their freeze dryer. Be aware that you can only dry as fast as the slowest product. So, whatever the thickest is, whatever the biggest is, whatever the longest drying process is, everything needs to dry at that rate. So, you have to always develop your cycles for the worst case conditions, not the best case conditions, and use the lowest product critical temperatures if you're doing this. A lot of times there'll be bone or tissue or slurry in a system, or collagen or some other materials. I mentioned it before, but I'll mention again, can a product be too dry? There is the possibility of product being too dry. Some proteins have that problem. It's important to understand what the proper residual moisture content should be.



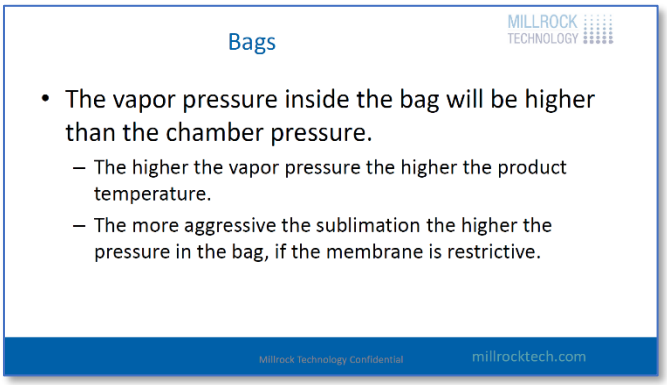
Common process problems. Pre-freezing products in a freezer and moving to a freeze dryer. If you throw your product in a freezer, in a bag, in a tray, in whatever, and sometimes the tray touches the bottom of the freezer, sometimes it touches the shelf of the freezer, sometimes it's stacked on something else, you're going to get different freezing rates for the product, and that's going to lead to an inconsistently frozen product, and therefore the ice crystal structures is not consistent. We've already discussed that's a problem. Another problem might be that the freezer might not be cold enough. You might be putting it into a -15 degree C freezer instead of a -80 degree freezer. That may not be freezing the product, especially if you have a solvent in there or sodium chloride in there, or a lot of other sugars by the way. It just doesn't have to be those materials. So, if you're doing that, this can lead to melting in the freeze-drying process, or inconsistencies in your freeze-drying process. You could also experience melting when you're transferring it to the freeze dryer.

If you're freezing outside of the freeze dryer, we recommend annealing in the freezing step. Now, it's also a problem if you're trying to load that product into a cold freeze dryer. If you have a cold shelf in the freeze dryer and you open up the door to put your product onto a cold shelf, let's say a -40 degrees shelf, you are going to get moisture on the shelf. That's going to be frost. When you first start the

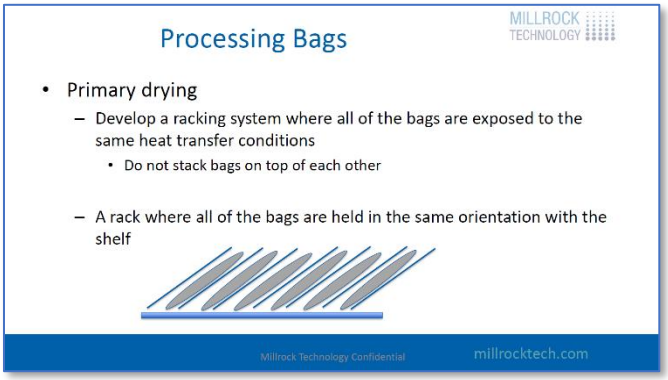
system up and you pull a vacuum, that frost is going to flash sublimate. Instead of having what the system's designed for, maybe one liter per hour of condensate, you might be pushing off five liters per hour, and it could overload the condenser. It's critical to understand that could be a problem, so we've tried to stay away from loading on a very cold shelf. Maximum low temperature might be zero degrees on a shelf, just to minimize the amount of frost that is created.

There's also the case where a product does not touch the shelf. If you're freeze-drying in bags or you're freeze-drying in medical devices, you might have some product touching the shelf, some product not touching the shelf, or none of the product touches the shelf. What we recommend in those cases is if some of the product never touches the shelf, then none of the product should touch the shelf so you have consistent heat transfer across your batch. It's very important to understand that everything should freeze at the same rate, and everything should dry at the same rate.

Also, if you're using bags, it's important to understand that the vapor pressure inside the bag during sublimation is going to be higher than the chamber pressure. So, you have a barrier to sublimation going on there, and if you have a very aggressive sublimation rate, the pressure could be high enough inside the bag to actually cause a collapse condition or a melt back condition of the product that's in the bag. So, you need to do your tests to show that you're not melting that bag.



Here's an example of what's recommended if you are processing in bags. A simple rack like this would do. It basically would put all of the bags in the same condition so they're getting the same heat transfer.



Some final thoughts. Freeze-drying is a robust process, and when the steps are performed properly, meaning they're controlled in every aspect, the results are consistent and repeatable. That's critical. If you're having variation in the quality of the product that's coming out of the freeze dryer, something in the process is not consistent. Make sure that each step has process control to eliminate variation. If you're doing tissues or collagens, you can be a little more aggressive, because you actually have a structure or what's called a matrix that supports the material. So, collapse isn't really the problem. It's the melt back portion of things that's the problem. All right. That's all I have to talk about today. I'm hoping that this was useful for you. And certainly, if you have any questions, please give us a call.