



Webinar Transcript

Freeze Dry Microscopy

Utilizing FDM as Part of a Complete Thermal Characterization Study for Optimized Lyophilization Cycle Development

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Introduction by Charles Zona

Thanks, everybody, for attending today's webinar on utilizing Freeze Dry Microscopy as Part of a Complete Thermal Characterization Study for Optimizing Lyophilization Development.

Today, we have a couple of presenters. Our first presenter is Dr. Jeff Schwegman. He's the founder and CEO of AB Bio Technologies. Jeff specializes in formulation development, lyophilization cycles, and thermal characterization studies, including freeze dry microscopy and DSC. He's available for speaking engagements and consulting services on these topics that he is going to discuss today.

Our other speaker that we have with us is Ruben Nieblas, of McCrone Microscopes & Accessories. For more than a decade, Ruben has installed microscope systems all over the world, and he also specializes in the installation and use of Linkam thermal equipment, including what he's going to talk about today—the Linkam FDCS196 freeze dry system.

Both Jeff and Ruben will field questions from the audience immediately following today's presentation. They're also developing a hands-on course on

PRESENTERS:

J. Jeff Schwegman, Ph.D.

Jeff is currently the founder and CEO of AB BioTechnologies where he develops formulations, lyophilization cycles, determines residual moisture by Karl Fischer, and provides thermal characterization studies including freeze dry microscopy and DSC.



Ruben Nieblas

Ruben, a technical sales representative for McCrone Microscopes & Accessories, has traveled worldwide installing microscope systems and training customers in microscopy, digital imaging, and the use of Linkam equipment. He also co-instructs the Freeze Dry Microscopy course at Hooke College of Applied Sciences.



lyophilization cycle development and optimization, which will be offered later in the year at Hooke College of Applied Sciences.

If you're interested in freeze dry services or freeze dry microscopy equipment, there will be a contact information slide provided at the end of this presentation, and today's webinar will be available on The McCrone Group's website—it will be recorded so you can reference that at your leisure; at a later date.

Without further ado, I'd like to turn the program over to Dr. Jeff Schwegman.

Dr. J. Jeff Schwegman:

Okay, thank you Chuck, and like Chuck said, I'd like to welcome everybody to the webinar. This is just kind of a general overview of how we incorporate a freeze dry microscopy system as part of complete thermal characterization.

Now for those of you who maybe have been working in lyophilization for a number of years, you know, in the earlier days, the method for optimizing a cycle was just pretty much open the door to the freeze dryer, put your

product in, and push a button, and hope that at the end of the day you get something that at least works, and again, you may burn through 20 to 30 runs, and a lot of product.

Now, you know within the past twenty years—maybe in fifteen to twenty years—there's been a lot of new technologies that have been developed that are putting the science into freeze drying. It's not the trial and error guesswork that we used to do. And one of those pieces of equipment that we use is the freeze dry microscopy system.

So what we're going to do is kind of walk through—over the next fifteen minutes or so for my part—talking to you about why we would do the thermal characterization; why we do the microscopy, and how it can really benefit us not only in how we develop the cycle and optimize the cycle, but also, down the road, if we have problems with a cycle, how this really good tool—set of tools—for diagnosing and correcting failed cycles or failed products.

Now when we talk about characterizing a system, basically, we're doing a couple of things.

We're identifying what our sample is.

Now, obviously the sample forms a solid, but which type of solid it forms is extremely critical in how we design the cycle both in primary and secondary drying because the different phases that can form can behave, and do behave, very differently, so generally what happens is as we freeze the sample down, an aqueous sample—again, sometimes we may be working with co-solvent—but if we're working with an aqueous system, when we start to freeze the sample, we're going to hit a certain temperature where we're going to get a phase change: meaning, ice is going to start crystallizing out of our product. Now this is pure ice, everything else in that formulation, all your additives, excipients, your active ingredients, maybe buffer salts, are going to be pushed around the ice crystals into what we call the interstitial space.

Now eventually, the solids—the other components—in the interstitial space will solidify. And that is what's really key in what we're identifying to the thermal characterization.

So basically, we say there's generally two types of solids that we have formed in that interstitial space: One would be a eutectic (and when I say eutectic, we need to be thinking about a crystalline system).

To contrast that, we may have an amorphous phase, which forms a glassy phase. So then we would be talking about a T_g' (T_g prime), or a glass transition. And it's not uncommon for many of the complex formulations that we work with these days to have a mixture of both. So let's kind of walk through, here, what goes on as we freeze a sample down, and obviously start in solution, and as we're going downward, decreasing the temperature. Again, as I mentioned, finally we have a temperature where ice will nucleate, and we start to grow ice crystals. We do that at the expense of pulling the water out of the interstitial space where all the excipient from the active ingredient is located—super-concentrating that phase, and again, eventually we hit a temperature and a concentration



The McCrone Freeze Dry System features components by Linkam.

where we're going to form either a glassy phase or a crystalline, eutectic again—something that forms a metastable glass or a lyotropic liquid crystal—those are things we would get into more depth about in the hands-on course, but for right now, mostly what we're dealing with are stable glasses and eutectic ones.

Now these are important, because we know where ice is going to melt—ice is going to melt at 0°C —but the interstitial space, whether it's got a eutectic or a glassy phase, those are very different temperatures; and again, this is something that we're trying to identify through the use of these tools, including the freeze dry microscope.

Now, we have to stay below these temperatures. If we exceed the eutectic melting temperature, or the glass transition temperature, T_g' , that interstitial space is going to be a fluid. Now, the problem is, in primary drying the main goal is to remove the ice and the ice channels. Well, if the interstitial space is not a solid, it's not going to support the weight, and it goes through a collapsing event; so, essentially, you end up with a puddle of goo in the bottom of the vial.

This is something we would use, utilize, DSC to do. This is something, again, where the microscope comes into play.

In these two systems, DSC is the gold standard for the thermal analysis, and the microscope is the gold standard for collapse. These two instruments complement each other; we'll discuss that in a little more detail as we go.

But we have to stay below those critical temperatures, again, these are important because these ramps represent, for the most part, the warmest temperature we can go—that's product temperature—during primary drying, without losing...loss of structure of product. We obviously don't want to collapse product.

So I've got a little diagram here showing you, again, phase separation. These are the ice channels; we like nice wide ice channels because they provide a good conduit to get the water vapor out of the product.

And here's the interstitial space. Now, in this case, it's a crystalline solute, and it forms the eutectic phase—it's a glassy phase—which warms the glassy amorphous phase.

Now, these two trap water very differently, so identification is key. In the crystalline phase, you may have, you know, for a completely crystalline system, your product at the end of primary drying is already 99.99 percent dry.

For an amorphous phase, you could still be retaining, you know, 40 percent of your moisture is still locked in this amorphous phase, so identification is key.

Okay, we talked about that determines the maximum temperature that the product can withstand during primary drying without losing structure. How do we do it?

Well, there are a couple of different ways we can do it. These first four bullet points represent what we call thermal analysis. Again, DSC is modulated, DSC is by far the gold standard, and we always couple that with freeze dry microscopy experiments.

So, thermal analysis, for those of you who took physical chemistry, anytime there is a physical or chemical change that occurs in a material, it's going to give off or absorb a little heat from the environment; what we call exothermic or endothermic reaction. And again, that's what the DSC is picking up.

Again, differential scanning calorimetry, they also make a modulated version that we can use mostly for solid materials, or materials that are very complex; maybe having overlapping thermal events.

So basically, we take our sample (this would be just like we were going to take the liquid and put it in the freeze dryer, so everything's got to be the same). If we take the liquid, put it in the sample pan, and we put an empty pan as the reference pan, and cool these down, then warm them up. I'm not going to go too much into heat flow, but it's actually, in the interest of time here, basically looking at heat flow as a function of the heating rate, which we can control. Again, the DSC allows us to control and understand the heat capacity of the different mechanisms that are going on.

(Skip that)...Okay, so here's the system in our lab; a very simple system. Basically, the lid pops off here, there's a sample furnace and a reference furnace. We warm the samples...uh, we cool the samples down, so we start at room temperature, and there's

two curves here: one is the warming curve, and one the cooling curve, so we cool the sample down—boom! We see crystallization occur—that's the ice freezing in the sample.

We get down to a fixed temperature—in this case, it was about -65°C , and then we warm up and go through our melting events. Now the melting—we don't look at the cooling curve, something called supercooling and freezing point depression, which is a little beyond the scope of this. But let's take a look at some of the warming curves that we see. They're very characteristic of what our product is.

So if I see on the warming curve this very sharp peak, that tells me [this is] a classic example of a eutectic melt. I've got a crystalline material in here.

The temperature that we reference is what's called the onset, so it's a tangent drawn to the baseline, and a tangent to the front edge of the peak, and right about there is what we call the eutectic melting temperature.

Glass transition is a little bit different. Tangent drawn here, tangent drawn here, the midpoint of that is what we represent as the glass transition temperature of our product.

This is a good example—I mean, glass transitions are a very low-energy event, so we do have to go looking for them. If we ran a DSC, looked at the warming curve and took this for face value, we'd say, well, there's a glass transition—I'm sorry, a eutectic melt—here, our product is safe all the way up to about -5°C . Well, the problem is buried in the background there's a very low-energy glass transition. If we miss that, you know, we're going to be in trouble. Our sample would collapse. We've got to keep this at much lower temperatures. So, this is where it tends to be a little bit misleading, unless you know what you're looking for.

[Now, okay, some of these got added by mistake...skip ahead a little bit...]

Okay—freeze dry microscopy—so we do the DSC. A sample comes in the lab, we do the DSC, it tells us, number one, what is the thermal event? Is it

glass transition, is it eutectic melt, and what are the critical temperatures that are associated with it? We then go to the microscope, and this, again, what this will tell us is where that sample physically loses structure. Now, just because we see a glass transition doesn't mean that's going to collapse the sample. Again, a little bit beyond the scope of this course, but sometimes we may have a glass transition that occurs, and we see no collapse. So this is the beauty of using both the microscope and the DSC, in that the microscope complements and supports the data that we get from the DSC.

So, this is a...and Ruben will go through some of this in his part...so basically, we take the sample, put it on the microscope, freeze it down to about -45°C , and collect an image. We want to show an image of the frozen layer. Then we kick on the vacuum, and we start drying at a safe temperature. So the DSC is says you got a glass transition—I think in this case it was around -37°C , -38°C —so we're probably drying this at about -40°C , because we want to try and get an intact dry layer. So this is the frozen layer, this was the dried layer, which is intact, and Ruben we will talk about more. There's a magenta filter in here, so if we see a magenta color coming through, we know we're starting to get collapse.

This is the sublimation front. It's a razor-thin line where we're actually converting the ice directly from a solid to a gas. Well then, once we collect this image, we're going to start warming this sample up, because I want to force it into a state of collapse, 'cause it shows me that temperature where we physically start to lose structure.

Now there's two temperatures I'm going to be looking at (and I need to put another slide in here), but basically, as we move across here, this is a temperature gradient. We're slowly warming the sample. And right about here, you start to see these tiny pores open up, and as we get warmer, we finally collapse the whole structure. This is what I would call the onset of collapse—this is where we just start to go through the glass transition, just on the edge of that glass transition we

get what I'll call partial collapse, and this may manifest itself in a product as pores opening up in the product, or even the product shrinking away from the side of the vial...you know, shrinking in on itself, and then finally, we had a temperature, and in this particular case it was -36°C, where we totally lose the structure.

Now, so I look at the DSC data, I look at the microscopy data, and come up with what we call a critical temperature, where we can freeze dry the product safely, or where we actually lose the product, and then we back that temperature down a little bit to make sure we are drying safely.

So, what have we learned from the thermal characterizations? It tells us a couple of things: Is this system crystalline, amorphous, or is it a mixture of both, of the two? It tells us the critical temperatures, or at least tells us glass transition of eutectic melting temperature.

And then the microscopy—the freeze dry microscopy—tells us where that collapsing event occurs.

Now, again, the system will also tell us if we have a metastable system, and if we need an annealing step. We can actually do annealing with the systems again, this is a little bit outside of the scope of this course.

So, thermal analysis studies with the DSC and the microscope allow us to take an empiric, or scientific, approach to lyophilization cycle development. And again, I can't tell you how much time and effort these techniques have saved us. I mean now, instead of taking 15 to 20 runs in the freeze dryer to get something that works, with this information I can go in to develop a fully optimized cycle within about three to four runs. So again, huge time saver, and money saving in regards to development.

So with that, I'm going to turn it back over to Ruben, and he'll tell you a little bit more about the details of the system. Thank you.

Ruben Nieblas:

Thank you, Jeff. My name is Ruben Nieblas, and I'm with McCrone Microscopes & Accessories. In addition to Jeff's presentation, I have a quick introduction into the Linkam FDCS196 freeze drying microscope system.

So just to reiterate what Jeff had mentioned, how we characterize our formulations...we characterize our formulations by using the following instruments: the DSC and the freeze dry microscope, and the information the DSC gives us is eutectic temperature, and glass transition temperature.

The information that the FDM gives us is the actual collapse temperature of our product.

One of the main things is that the glass transition can be anywhere between 5-15° difference, than our actual collapse temperature, and if we are able to freeze dry warmer, we exponentially increase the rate of drying of our product.

So, if we're actually able to freeze dry our product at -25°C, instead of, say, a glass transition of -34°C, we're giving it less energy, so it's costing us less to produce and it will freeze dry a lot quicker.

Again, thermal analysis (studies) tells us if the system is amorphous, crystalline, or partially crystalline, tells us our critical temperatures, and it also tells us if we need to anneal the system and approximately what those conditions are.

So this is an image of the complete system, and I'm going to go over the different components that are on here.

So the first is, just like with any freeze drying system, we need a vacuum pump for the sublimation process. This is a system that is provided—a BOC Edwards 1.5 Pump.

Next, we will actually have a motorized valve on the system. This actually controls the pressure inside of the actual thermal stage itself. Via the software, we can actually regulate

the pressure inside, just like you do on a standard pilot-scale, and/or production-scale freeze dryer.

Next we have a two-liter dewar that comes with the system. The system only uses liquid nitrogen. You cannot use any other type of coolant. Another question that always comes up with this system is: how long would that two-liter dewar last? Depending upon your routine, it can last anywhere between six to eight hours, conservatively. It could last longer; it could last shorter, depending again on how low you're going and how fast, and other conditions.

Next, all of our systems are provided with a polarized light microscope. On a polarized light microscope, we have a polarizing condenser, analyzer, first order red compensator, objectives, and your camera.

So on the microscope, we have our condenser at the bottom, which has the polarizer on it. Next we have our analyzer up on the top. We will have a first order red compensator. We have our objectives.

On the condensers, there is a special lens that needs to be added on to it; this is a long working distance extension lens. This is needed because our sample is higher up inside of this stage, and to therefore properly align the microscope, this extension lens needs to be added on to the condenser, so that we can make sure that our microscope is aligned properly.

Like any other analytical piece of equipment, a microscope cannot be calibrated, however, we need to make sure that it is aligned properly so all the light possible forms through our sample so we can resolve this as much as we can.

Next we have our analyzer, and our analyzer is the same material as a polarizer, except we have to be able to distinguish one from the other, so the one the light passes through first is always known as our polarizer; the one that the light passes through after our sample is always known as our analyzer.

Our first-order red compensator is used in the system because we're using the polarized light accessories as qualitative, not quantitative. We're not trying to measure the birefringence of the material that we have, but as just mentioned, we're using the colors that come up so we can easily see where that onset of collapse and also collapse starts.

Everything is visual with the freeze dry microscope.

Again, we have our different objectives on here. The objectives must have a working distance greater than 4.5 mm. Systems that we provide are a Nikon polarized light system, which includes a 5X, 10X, 20X, and 50X objective.

Linkam has also written their software to only work with the Q-Imaging line of digital cameras, and the one that we recommend is the QICAM Fast.

Going on with the Linkam stage...this is the Linkam FDCS196 stage. Here we have our vacuum port, we have our x-manipulator, we have a y-manipulator, so we can move our sample in any direction, we have our sample door lock. Once I remove the lid, we have a 22 mm silver block, we have a 1.3 mm light aperture diameter. We have a liquid nitrogen inlet, liquid nitrogen outlet, we have our platinum resistor leads, and we have our thermocouple leads, and our sample ramp.

The way that the Linkam system works, you have to think about it like a tug-of-war. The controllers here, it will send an electrical current through the platinum resistor with whatever temperature it is that you told it to. So in our case, we're going down in [tooling?]. So therefore, the controller will also activate the LNP95, which is the liquid nitrogen pump, therefore, the system knows how much the pump needs to regulate, or suck through, the liquid nitrogen, and then the platinum resistor will create the heat going through onto the block. Below that silver block, there is a coil, and then there are channels; so therefore, as the flow of liquid nitrogen comes through, and the electrical current comes in through the platinum resistor, it is accurately controlled at the rate that

you want to heat, or cool. Therefore, if you want to go down 10° per minute, it will regulate that flow, and that current on that platinum resistor to 10° per minute, or 5° per minute. This system can go as slow as .05° per minute, or as fast as 150° per minute in the heating and cooling. And the highest temperature that it will reach is 125°C, and as low as -196°C.

Next we have our Pirani gauge. Our Pirani gauge is what's reading the pressure inside of the stage. This is what's giving the readout to the software, and also that motorized valve that I had previously shown you, so that we can control the pressure inside of the stage.

Next we have our controllers. We have a T95 controller. We have the LNP controller, and we have the LinkPad. Here's a closer look. So again, the T95 is the brains of the system. It will tell the system how much of the electrical current going through the block, the liquid nitrogen is also controlled via the T95 controller, and that will regulate the amount, the flow of liquid nitrogen through the block itself. And, the LinkPad is an actual digital display.

Next, of course, we have...you need a computer to run the entire system, so with that, these are the specifications for the computer. It must be a Windows 32-bit, it can also now be a Windows 64-bit operating system. It must have an available serial port, an available PCIe express card so we can introduce the firewire card for the camera. These are the specifications for the processor—how much RAM, minimum requirement.

These are some of the accessories that are needed...that are used with the freeze drying system. First, we have the G16 sample holder, or what I call the "lollipop," next we have a silicon oil, we have a 16 mm quartz window, and then we have a glass coverslip that we would use.

The way that you would sample load is that you would first introduce the sample holder into the stage. We will then place a single drop of silicon oil onto the block, inside of the ring. Once we place our 16mm quartz

window, which is our substrate, what happens then is that the oil fills in underneath that 16mm quartz window, and therefore, you have even thermal contact from the block onto the substrate. In case there are any imperfections on the quartz coverslip, or if there are any micro-scratches on the block, all of that gets filled in with the oil so that you have that even thermal contact.

We'll place only three to five microliters of sample, that is all that you need on top of the 16mm quartz window, and then we will place this 9mm or 13mm glass coverslip on top of our sample; and then we would actually freeze dry from the outer edge of the coverslip into the center.

This is an accessory that comes with the system. It's the vacuum tweezers. This makes sample loading—especially with those 9mm and 13mm glass coverslips—very easy to do.

So this is the actual view of the software itself; again, very simple to navigate through. On the top, we have our temperature control box, next we have our camera control box. We'll have our vacuum control, we have a live digital window preview, and, just like in any freeze dryer, we have our profile window, which we will be able to tell the rate, limit, time and delay.

The rate, of course, is how fast we need to heat and cool our sample.

The limit is—up to what temperature do you want to reach during that ramp?

The time is—once it reaches that limit temperature, how long do you want to hold it there before it moves on to the next ramp?

And finally, is the delay. The delay allows us to capture an image in whatever interval number you inputted in there. The nice thing about this is at the beginning of the experiment, nothing's really happening; however, we want to document what's going on from the beginning of the experiment through the end, so we can actually regulate the amounts of image capture going through our experiment so that we're not creating so large of

an image format that it's going to be overwhelming for your IT department and your network.

Once the experiment is done, the data chart is... once you finish all of the run, the data chart gives you a graphical interpretation of your temperature profile, it also tells you the pressure that was recorded during the experiment, and each one of those purple dots is an actual image that was captured during that experiment. When you click on, you can actually view all the images in the gallery preview, and their title by the temperature that they were captured at.

Once you've chosen an image, we can now have the image with all of the temperature information placed on the bottom of the image. Onto it, you can also add any type of description on here, such as lot number, permit number, and so forth. And once all of these images are captured, we can also, within the system, grab all of those images, and create an AVI, or time lapse, video of the entire experiment. So something that could have taken us two, three, maybe four hours in that experiment, we can condense it down and view within 30 seconds or less than a minute.

I want to say thank you for coming to our webinar. I'll throw it back to Chuck for any final thoughts.

Questions & Answers

CZ:

Thanks, Ruben. This is the contact information for Jeff and Ruben, for today's webinar. If you have any questions, feel free to contact either one of these guys.

We do have a few minutes here if you'd like to type in a question. We would be happy to answer. We'll wait for a minute here to see if anybody has any questions.

It looks like we've got a question here... "Does vacuum set point impact critical temperature?"

Jeff, would you like to answer that?

JJS:

Yeah, can you read that again? I'm not seeing these pop-ups.

CZ:

Oh, I'm sorry. So the question is: "Does vacuum set point impact the Tc results?"

JJS:

Well, no, it won't. It won't affect the glass transition temperature; it will affect product temperature. So this is why if, I'll say drying at a fixed temperature in the freeze dryer, and we start to fluctuate pressure, we can actually see product temperature change. And in that case, you may exceed your critical temperature, whether that be a glass transition or a eutectic melt. So it won't necessarily affect the Tg'; it will affect product temperature, which could exceed the Tg'.

CZ:

Next question is: "Does it only work with water-based solutions?"

JJS:

No, we can actually... we do quite a few that are sometimes are solvent-based systems as well. I mean you could do... I mean a lot of the diagnostic folks and some of the tissue folks that are freeze drying those products use really odd solvents. So no, it applies to everything.

CZ:

"How do you differentiate Tg and Tg'?"

JJS:

That's a good question. So, basically, there's only one Tg', so a product—you mix it up, it's in its final formulation, and you freeze it down, if there's amorphous components, that amorphous phase has a fixed temperature, where it will go through its glass transition from a liquid to or a solid to a liquid, and it has a fixed water content. Now the water is acting as a plasticizer, so it does affect that temperature, but when you first freeze a sample down, it has a fixed water content and a fixed temperature. That's Tg'. There's only one Tg' for a formulation.

Now as we dry the product, we're starting to pull water out of that interstitial space, and as we do, again, the temperature we can take that before it collapses increases. So there's one Tg', but many Tg values as we pull out the moisture. At the end of the day, when we finish freeze drying, we hope that we've pulled out enough moisture that Tg at the final product is higher than room temperature, obviously.

CZ:

"Does running primary drying at different temperatures affect collapse temperature?"

JJS:

Well, it won't affect the collapse temperature. I mean, the collapse temperature is pretty much going to be consistent regardless of what temperature you're on, or what pressure you're on. Now, that's not to say that you can't start varying these, and not lose your product. There's various combinations of shelf temperature and chamber pressure that we can change, and still keep product temperature the same. Now this is something, too, that was beyond the webinar, but we go into more detail in the course about how we understand those, and determine those. It won't affect where that critical temperature occurs, but product temperature is affected by both chamber pressure and shelf temperature, and understanding controlling those is critical, or you might exceed the glass transition critical temperature of your product.

CZ:

We have time for a couple more questions here. "How can we correlate DSC and SEM data to anneal the system when it's needed?"

JJS:

Well, when you run the DSC, you can actually see an amorphous phase or a metastable phase forming. We can actually conduct annealing studies both on the DSC and the microscope. So if we have a metastable system, the DSC in the microscope will allow us to identify is it a metastable system? Where do we need to anneal it? We can actually do annealing on both of them. Was the annealing effective? And then

use that in cycle development. So it's a great tool for that as well.

CZ:

Okay, and one more here:
"Can this instrument quantify the size of ice crystals under different temperature, and how sensitive is it?"

JJS:

I'll leave that one to you, Ruben.

RN:

Yes, I mean, you can measure the ice crystals that are formed, on here. You can do simple point-to-point measurements of the ice crystals under different temperatures. How sensitive it is going to be dependent upon what magnification you're at, to basically increase the size of those ice crystals for you to measure. How that is actually going to affect the collapse temperature of it—I don't believe that it does. It's just the form of the ice crystals that are being formed. If you want to expand, or make your ice crystals even larger, one of the things that you can do is actually do an annealing step, where you're basically partially melting those ice crystals, and then having them reform as a larger crystal—that would be more for your pilot-scale and your production-scale to...if you can increase that ice size. Again, when you're trying to freeze dry it out, you've got a larger channel where that sublimation process can occur. Do you have anything else to add on that, Jeff?

JJS:

No, I think Ruben's right. It won't change a critical temperature, but it can ... the ice crystal size can change how it freezes. I'm sorry – how it dries. Again, like he said, the smaller the ice crystal, it's going to tend to dry slower, it's going to be a little bit warmer, more resistance trying to get the water out through those tiny pores versus larger ice crystals. But yeah, you can measure that. We've done it at our lab with our microscopy system, but it's pretty much a point-and-click. Measurements are very easy.

CZ:

And I think that we'll end it up with one more: "Can you differentiate the collapse and irregular ice structure?"

JJS:

When you're seeing the, you know, live, as it's being freeze dried, once you've reached that collapsed temperature on an amorphous, you're not going to have any more ice structure. It's going to actually go ahead and collapse. On some of the images that Jeff showed, you'll see that there was basically that black indent—that black line—which is your sublimation front. Once it has started collapsing, there's no more material left behind the sublimation front.

The frozen phase would still be intact because you may have exceeded a glass transition temperature, but we're still below the freezing temperature of ice. So you'll still see that frozen layer remain intact because we still have ice crystals that are helping to support the system. But Ruben's right. So anything behind that we're actually freeze drying, that's where we're going to see full collapse once we see the glass transition temperature.

CZ:

We've got one more here: "What is your explanation for product primary drying at a certain temperature, but they don't create a freeze dry front on the SEM at the same temperature?"

JJS:

Well, I mean, again, this is going to vary a little bit with temperature you run, and again, total solids. We've had to set up our microscopy systems so, for example, we got a sample that had total solids content, a very high total solids content, and the temperature was relatively low. We were running at about -37°C. Well, in that case, maybe it's just going to...there's a huge resistance to get water vapor out, not to mention you're running it cold, so the vapor pressure or sublimation rate, is going to be very low. Now in most cases, we set the system up, turned it on, and walked away from it for several hours, and then come back and then we'll finally see the sublimation front. So that rate, in some

instances, is going to be very low. You may have to set that system up and walk away from it, but then again, that is a good indication that when you try to put this stuff into the vial, it's going to take forever to freeze dry. You can get a little information about how your product is going to behave in a freeze dry by what you see occurring under the microscope.

CZ:

Okay. Well I think we had some great questions. We appreciate everyone's input on that.

Conclusion

CZ:

I'd like to again thank Jeff and Ruben for putting on a wonderful webinar today on freeze drying, and if you have any questions for either Jeff or Ruben, again, their contact information is up.

We'll have this webinar archived on The McCrone Group website shortly, and we will be sending out announcements for the new course that they will be teaching later this year at Hooke College of Applied Sciences.

I'd just like to thank you both again for a great webinar.