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In-Situ Vial Strain Measurements During Freeze/Thaw Processes Using Amorphous Excipients

Ian Flynn, graduate student at Purdue – Webinar Transcription

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In-Situ Vial Strain Measurements During Freeze/Thaw Processes Using Amorphous Excipients

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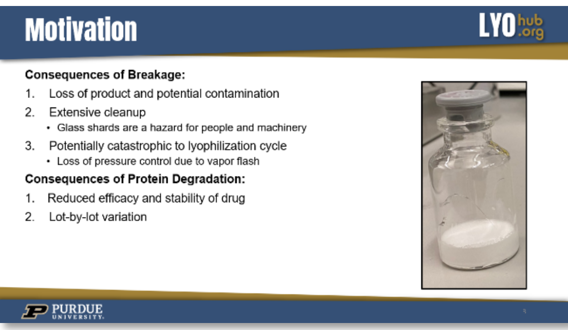
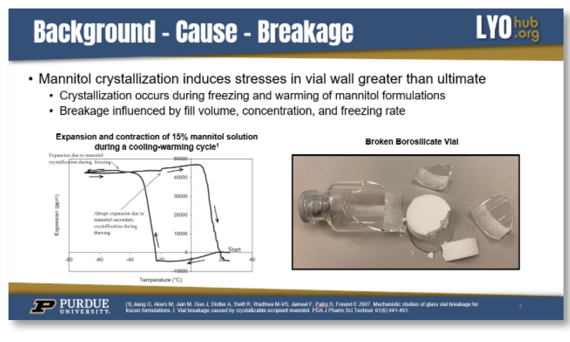
PURDUE UNIVERSITY

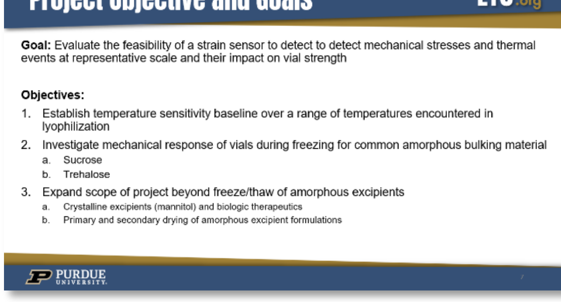
My name's Ian Flynn. I'm a graduate researcher at Purdue University right now working with Professor Alina Alexeenko. And today I'm going to be talking to you guys about the research we've been doing for the past few months on in-situ vial strain measurements during freeze/thaw processes using a amorphous excipients. So there's two main motivations we have for this project right now, one being vial breakage, and another being protein degradation of biologics during the lyophilization process.



So with vial breakage, you have three main consequences that you're going to encounter if you encounter it. You can have loss of product and potential contamination from spilling out of your material, extensive cleanup and hazardous cleanup for people and machinery, depending on what's broken there or how it's broken, and then it may be potentially catastrophic to the lyophilization cycle due to loss of pressure control due to vapor flash. And with protein degradation you could have reduced efficacy and stability of your drug and you can have some lot-by-lot variations, which can be a real pain.

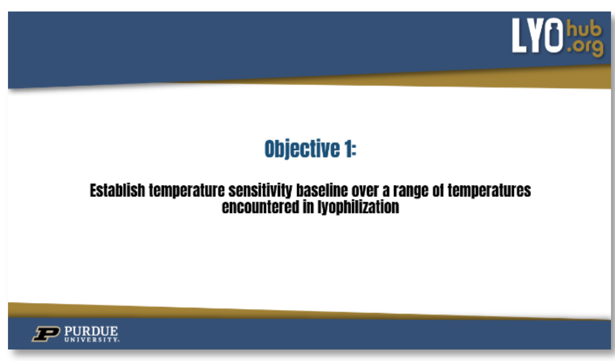


So going into a little bit of background on this. So, mannitol crystallization induces stress in vial walls, which can lead to vial breakage. And this crystallization is going to be occurring during your freezing and thawing. You can see at the bottom right, with 15% mannitol solutions there are large amounts of strain and stress that are being imparted on the vial during mannitol freezing, which can lead to broken vials very easily. And this is going to be influenced by your fill volume, your concentration, the ramp rates are using.

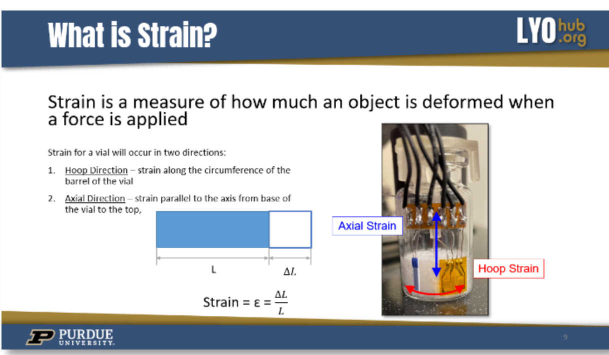


And during freezing, there's changes in the surrounding area which can impart stresses onto proteins, which will lead to denaturation. And this is mostly freeze concentration. The low temperatures can affect the proteins as well as ice formations. The relative contributions of each of these is not super

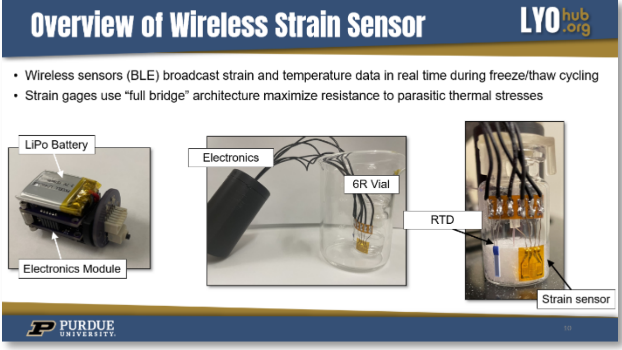
well understood at the moment. So, with this research, we really want to understand better how the ice crystallization in the protein molecules may be denaturing them by shearing on them in the mechanical stresses.



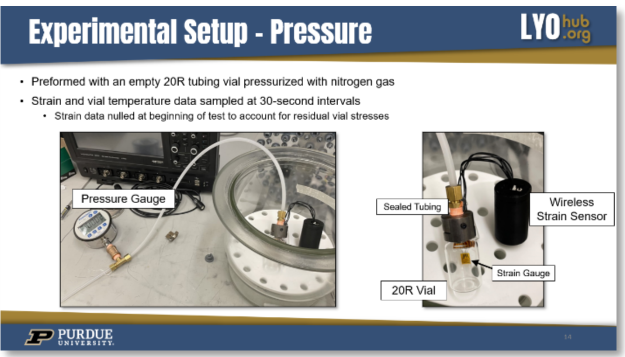
So the goal for this project is really to evaluate the feasibility of a strain sensor to detect mechanical stresses and thermal events at representative scale and their effects on vial strength. We have three main objectives that we're accomplishing this through. First, we really want to establish the temperature sensitivity baseline over a range of temperatures encountered in lyophilization, investigate the mechanical response of vials during freezing for common amorphous bulking materials. So we're really looking at sucrose and trehalose. And then we want to expand the scope of the project beyond just free/thaw with amorphous excipients and start looking at how crystalline recipients such as mannitol and biologic therapeutics, so using like BSA as a model protein, as well as looking at the primary and secondary drying with these amorphous and crystalline formulations.



There we go. So, jumping into objective one, first I want to go over what is strain? Strain is a measure of how much an object is deformed when a force is applied to it. And when we're looking at a vial, there are two directions that we really care about. So axial is going to be up and down along the vial here, as you can see. And then hoop is going to be the circumferential stress that we're going to be seeing in the vial.



And here's an overview of the setup that we're using. So we're using a wireless sensor to broadcast strain and temperature data in real time. So you can see on the bottom right is the electronics that we're going to be using. And this is inside of the lyophilizer, so it's going to be inside of, in this middle picture, this black 3D printed case, which is going to be directly connected to a 6R vial with the strain gauge



directly mounted on it along with an RTD, which you can see in the bottom right here.

Most importantly, the strain gauge we're using is full bridge architecture, which is going to maximize resistance to the parasitic thermal stresses, but it's going to result in kind of a mixture of your hoop and axial strains, which I will get into in just a minute here. And we're getting these strain results from... mechanical stresses produce a change in the filament's electrical resistance which, if you know other given parameters, you can draw or calculate how much strain is being produced.

So first I want to talk about how we're, I guess, decoupling as best we can and figuring out the axial and hoop strain and figuring out what we're actually measuring. So we did this by measuring a 20R vial with one of these strain gauges and then pressurizing it with a well calibrated pressure gauge here and sampling the strain at 30 second intervals while nulling at the beginning of it.

So, we have two different tests we ran with different locations of the sensor. And what we're seeing here is that while you're pressurizing this with inert gas for whichever location, you're going to be seeing positive or negative strain results, meaning that both positive or negative is some type of expansion depending on where the location is.

So, what we found out here is that what we're really measuring is a trade-off between our hoop and axial strain. So, it's the difference between the two rather than a summation or anything else. So, if you see positive or negative strain, they're both going to be expansions outward. I apologize, it's a little confusing, but it's kind of where we are at the moment.

Experimental Setup - Lyophilization

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- Preliminary experiment performed in MicroFD using 3mL fill volume in 6R tubing vial
  - Buffered with vials containing EtOH (200 prf) – goal is to prevent parasitic signals from nearby freezing/thawing vials
- Strain and vial temperature data sampled at 30-second intervals
  - Strain data nulled at beginning of test to account for residual vial stresses

Freeze/Thaw Recipe:

1. Ramp to -6°C @ 0.5°C/min, hold 30 minutes
   - CIN at end of hold step
2. Ramp to -45°C 0.5°C/min, hold 120 minutes
3. Ramp to 20°C @ 0.5°C/min, hold 240 minutes

Electronics Module

EtOH

TC1

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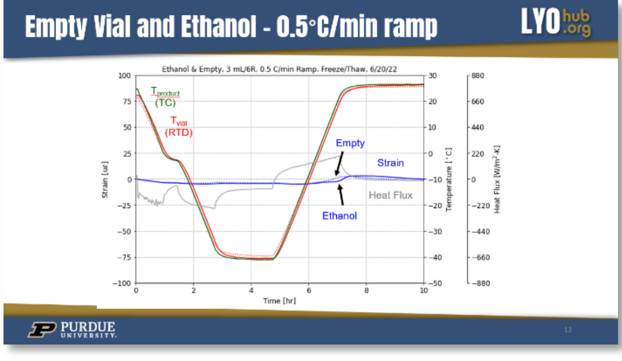
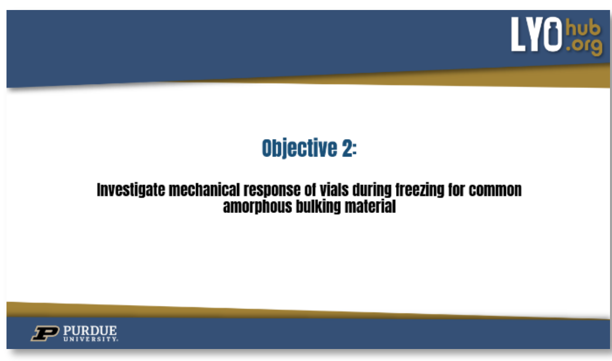
And now getting to the actual experiments we've been running with formulations. So, we've done all these experiments in a Millrock MicroFD using 6R tubing vials with a 50% fill volume. And right here at the bottom you can see our test vial, which is marked TC1 for the thermal couple. And we've surrounded this with anhydrous ethanol vials with the same fill. We're doing this to hopefully prevent any parasitic signals that might be coming about from neighboring crystallization events.

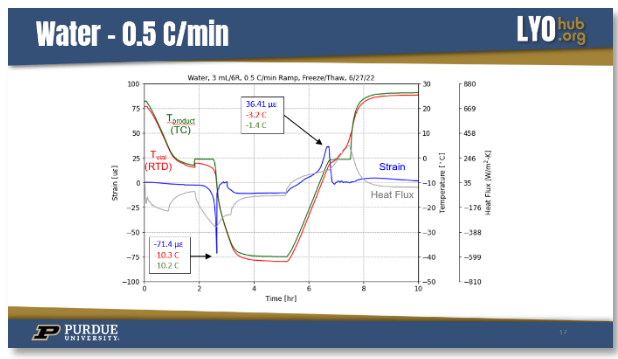


We're also sampling our strain and vial temperature data in 30 second intervals and nulling our data at the beginning to account for any residual stress in the vial. And the recipe that we've used for basically everything I'm going to show you is we're starting at room temperature, cooling down to -63 Celsius at a ramp rate of 0.53 Celsius a minute, keeping it there for 30 minutes before inducing nucleation, then cooling it down to -45 degree Celsius at the same ramp rate, holding it there for two hours, then ramping back to 23 Celsius at the same ramp rate, holding it for four hours.

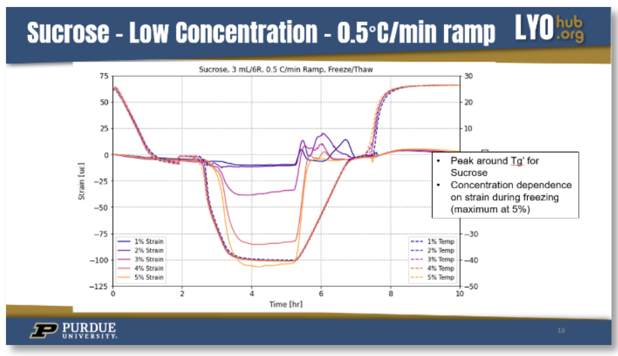
So the first thing we want to do to establish our temperature sensitivity baselines is to get the thermal expansion you'd expect from just a glass as is. So, we did this with an empty vial and an ethanol vial. So the ethanol will not be crystallizing during this, so it should also reflect the strain profile of just the glass's thermal expansion. And as we can see here, the strain is staying between -3 and 3 microstrain throughout the entire process, which reflects a pretty low noise and nice baseline to work with.



And going into some of our actual formulations, first I want to talk about what the solvent we're using will behave like. So water has a large deviation from what we saw for the empty and ethanol vials. So, we see two primary peaks that I want to point out. First peak during here while you're freezing. It's going to be a very sharp peak that's quickly relieved. And then a peak rate here during thawing as you start to expand the ice even more.



The peak rate here, we're not really sure the identity at the moment. It could be from grabbing onto the vial walls and pulling inward until it breaks off, but we really need to investigate that more. And the peak rate here is probably just going to be coming from the thermal expansion of the ice. But I wanted to just mention that because how the profile, once we start adding in our excipients, will start to change.



So right here we have a 1% concentration weight by volume of sucrose, and we can see it already very much so differs from water. And there's three primary characteristics I want you to see at the moment. So let me grab this or... Nope. Sorry, I'm trying to annotate a little bit. Grab a laser pointer. There we go.

So, three things I want to point out is right here we have a large deviation from that strong, sharp peak that we saw before where it's kind of disappearing here. Then we have a peak rate here, which is going to be coinciding right around the temperature of the glass transition for this formulation of sucrose. Then a peak right here, which is around -3, which is where we started to see melting for the pure water. Then once we increase the concentration 2%, so we see a bigger shift. So, we still have our peak right around the glass transition temperature for this formulation of sucrose. Then we have this uneven secondary peak, which will top out at around -22 degrees Celsius. And we lose our peak that was occurring around melting. So, we start to really differ from the profile that we had for water.

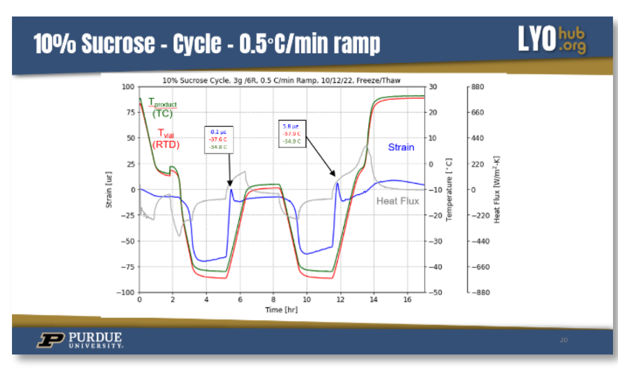
And then further going to 3%, we start to see even more differentiation. So, we start to see a strong strain peak during our freezing period, so we're having a lot of pressure on the outside of the vial compared to lower formulations in water. We have a peak around our glass transition temperature for this formulation. Then an uneven peak follows with a peak around -22 degrees Celsius. And 4% sucrose will follow the same trend, will also increase in our strain during freezing. All the way up to 5% is where we see the largest amount of strain during this freezing period. We lose our peak around our glass transition, and we have nothing really as distinct around these -22 degrees Celsius as we've seen before. There's a little bit of a shoulder occurring in this temperature range, but we've really lost that characteristic.

So the main takeaways we can get from these low concentration studies is we're seeing a peak around the glass transition for sucrose and there's a concentration dependence on strain during freezing. So as our concentration's increasing, our strain is increasing during this whole period. But then as we go into higher concentrations, so going to 6% sucrose, now we start to see a little bit of reversal in the trends that we already saw. So we still see a peak that's occurring right around our glass transition temperature. The strain during our freezing starts to reduce a little bit. So before that, around -110 and up to 6%, it goes to around -70.

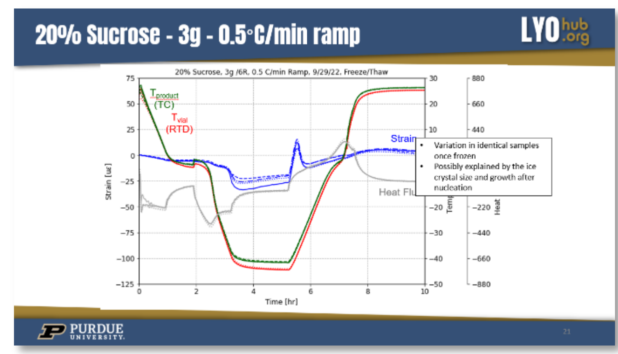
Then as we increase the concentration a little bit more, we see a similar strain profile during this freezing period, but we start to see peak around our glass transition start to rise a little bit more. Then as we continue to increase the concentration to 8%, 9% and 10%, we really start to see this trend kind of reversed from what we saw in the low concentrations. So you have reduced strain during this cold period and our peak around our glass transition starting to rise up to 15%, 20 where we get a very prominent peak right here and the smallest amount of strain that we saw during this freezing period since the 1-2% range. And up to 85%, which really shouldn't be crystallizing, where we see nothing. So that's as expected.

So the main takeaways we get from this is there's a peak around the glass transition for sucrose during heating. Again, for most of these formulations, the only exception being 5% and there's an inverse concentration dependence on strain during freezing with a maximum at 5% once you get above that value. And we see essentially no strain with the 85% since there's no crystallization of ice.

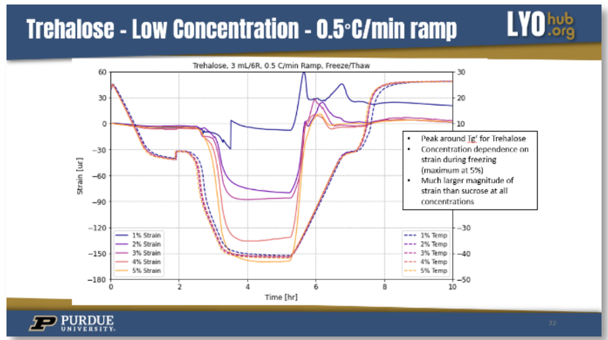
We also wanted to see if an annealing step would affect this at all. So, when we cooled to -45 degrees Celsius, instead of going to room temperature, we took it to -10 degree Celsius, then went back down to -45, then up to room temperature. The idea being after you pass through glass transition, we were wondering if the sucrose was seeping out and moving away from where it was before because it's now rubbery instead of glassy. But with this, it seems that the sucrose, once you pass through glass transition, is almost acting spongier. So, the fact that you could repeat this shows that it's not just seeping away from where it was before because we can see this peak around your glass transition occurring multiple times.



Also wanted to show that this data is very repeatable. So, this is multiple runs with the exact same mask, so same volume of the same solution with as many variables held constant as we could. And we see that for these 20% solutions, there's basically the exact same profile for all of them. There's a little bit of variation during this freezing period, which is probably innate and may come from just different crystal sizes and how the crystals are growing during the nucleation and freezing period. But we're seeing a peak of very similar magnitude around our glass transition the whole time. And then we end up with some small deviations, but relatively the profile stays the same and the magnitudes are comparable at least.



And now moving to another excipient, we have trehalose. So, trehalose is going to behave very similar to sucrose, but with one big caveat being the amount of strain that we're seeing during this freezing period. So, again, we're going to see this little, small peak that's similar what we saw with water, have a peak around our glass transition for this formulation, and a peak around -3 Celsius where we're seeing it for water. Then increasing to 2%, we start to see this strain increasing very rapidly compared to what we saw for sucrose, peak around our glass transition, and a peak around -22 degrees Celsius with the same uneven profile.

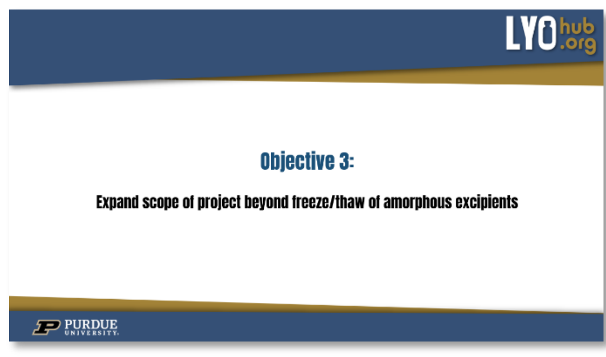


3% we're seeing a very similar trend, although our glass transition is becoming a little bit more of a shoulder and it's less of a well-defined peak. Up to 4%, following this exact same trend, increasing our strain during this freezing, shoulder around our glass transition, peak around -22. Up to 5%, where again, we're losing our peak around glass transition, have a peak around -22, and our strongest amount of strain during this freezing, as we saw before. But most notably, this is almost double the amount of strain that we saw for sucrose, where they're usually considered about similar, but trehalose is imparting much more stress on the vial here.

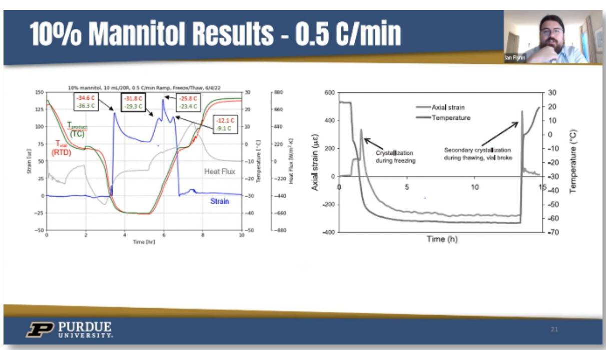
So again, same data that we saw from sucrose. We're seeing our peaks around glass transition for most of these formulations and is concentration dependent on strain. And then once we get to the higher concentration, again, we're going to be seeing something very similar we saw with sucrose, just with increased magnitudes. The biggest difference being at 6%, we're still not seeing this glass transition peak, but as we go to 7%, 8%, 9% and 10%, we're seeing these peaks start to rise up.

There's some kind of odd behavior here where it's not following the trend during this freezing period for 7-10%, but this is still somewhat similar to what we saw for sucrose and it probably needs to be investigated a little bit further. Then up to 15% and 20%, we really start to see this peak start rising back up in the same way that you saw with sucrose. So again, we're seeing this kind of reversal of the trend

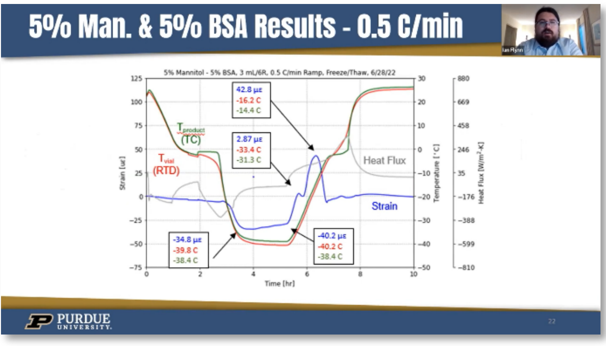
for the concentration dependence on strain, and we're seeing these peaks around your glasses transition for almost all the formulations.



And this is kind of a new topic we're looking at. We're trying to expand the scope of this project a little bit beyond just the amorphous excipients. So, we want to also look at some crystalline excipients. So, we decided to not focus on these originally just because of the much more complex behavior that we noticed right away compared to sucrose and trehalose, which tended to follow that very nice trend.

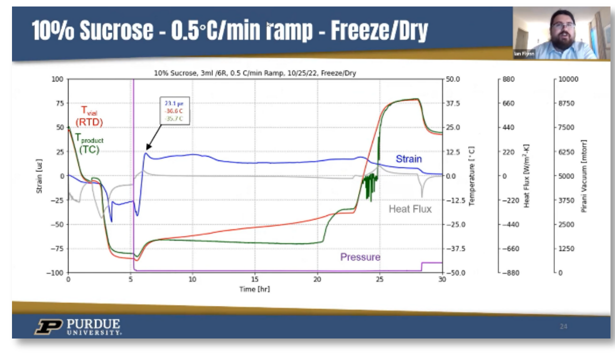


So, mannitol has much stronger crystallization peaks. So, you can see very different profile compared to the very negative strain, a very positive strain here. So, we have a peak during our crystallization as we'd expect for the primary crystallization of mannitol. And then kind of this triple peak behavior during thawing. So other papers, you usually see the secondary crystallization during thawing. So that may be this largest peak grade here. The other two peaks not exactly sure what the identity is. One of them may be a glass transition for mannitol. Another may be the recrystallization of ice as you're freeing it up. It's very unclear right now and it needs to be investigated more.

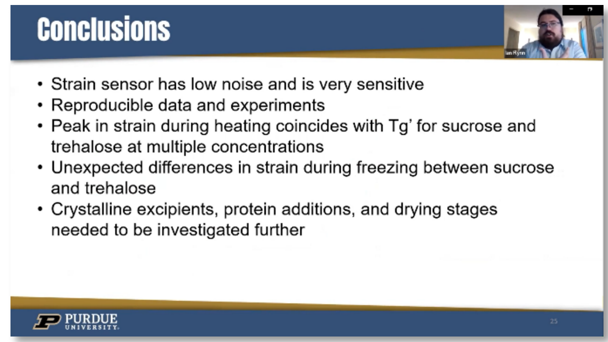


Then once we start adding in some amorphous protein, so with BSA, we start to see a very different profile as you kind of expect once the amorphous BSA starts to inhibit the crystallization of mannitol some. So, we see very different strain during this freezing period. We have a peak occurring around a similar location right here, and then a peak rate here, which coincides very well with the peak that we see for just pure BSA. And one of the more notable things that we're seeing with this is that BSA, similar to the other amorphous excipients we have, has a very strong strain profile right here during freezing, which can impart a lot of stress in the vial and needs to be investigated more.

And also wanted to talk a little bit about full freeze/dry cycles. This is very new. We're still investigating this. This is as of a few days ago really. So, the most important thing we see is we see a very sensitive response to pulling the vacuum and lowering the pressure. So as soon as we lower the pressure, we see a strain response in the vial. And as we start to heat it up, we notice a peak that's occurring right around glass transition. And then after that we see kind of these rolling like slow, broad changes, which may be coming from you're becoming a little bit more rubbery and you're seeing less of this kind of strong strain profiles, which is kind of similar to what we saw with the free/thaw.



In future experiments. We want to try doing some freeze/dry where we're doing primary drying above and below this glass transition temperature to try to see how this strain profile will change when you're not passing this or you're going much above this glass transition.



So, the main conclusions that we can draw from this right now is that we have a strain sensor that is very low noise and is very sensitive to most things going on in terms of crystallization events and changes in pressure. The data is very reproducible. There's going to be some minor variations that are just going to be coming from how the ice is formed and for some other events, but in general, the profiles are going to stay the same. We're seeing peaks in strain during heating, which coincide with glass transition for sucrose and trehalose and almost all the formulations we tested. And there's an unexpected difference between the strain during freezing between sucrose and trehalose where trehalose is imparting much more stress in the vial than the sucrose is. And the crystalline excipients protein additives with BSA and drying stages need to be investigated further. And that's kind in the early stages of the project right now.

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