Pharmaceutical Development of Antibody Drug Conjugates – Webinar Transcript 2/11/21

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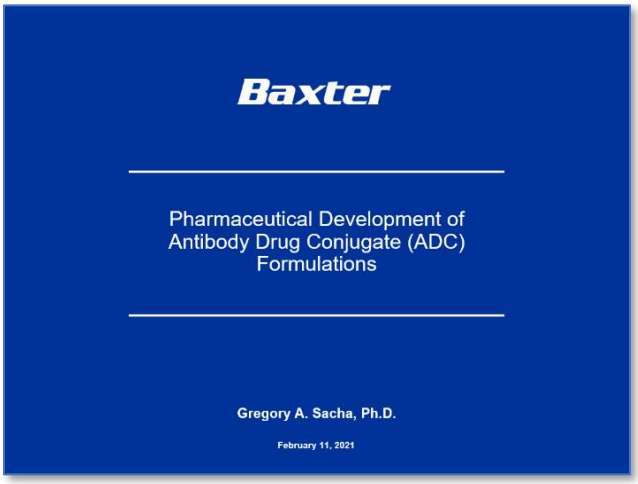

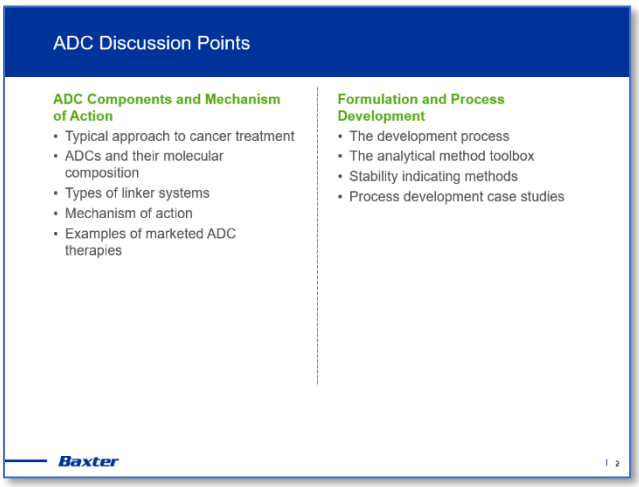

Today we will speak about development of antibody-drug conjugates (ADCs).

We will introduce them, explain a little bit about what they are and then also include our formulation process development.

Let us start by considering current cancer treatment options. They differ depending on the type of cancer.

Solid tumors are typically treated with multiple methods. First surgery, if possible, if you can use it to remove most of the tumor. There are even diagnostic agents out there now that can help highlight the tumors edges around healthy tissue so that the surgeon can follow those highlighted areas and remove all the tumor cell. Other methods include administering chemotherapeutic agents and then also radiation.

Radiation is targeted as much as possible to the tumor area, but by large, radiation can still hit healthy tissue and healthy tissue that is deeply affected and can result in scarring.

Chemotherapeutic agents, the small molecules that we currently have vary widely, but we usually must infuse quite a lot of them on a frequent basis. And we infuse so much so that these small molecules can hit their intended areas where the tumor lies. The big challenge there is when you are giving them in large doses, they also affect healthy tissue with potential severe side effects.

Cancer Treatment Options

Common Treatment Options

- Differs depending on the type of cancer
- Solid tumors treated with multiple method
  - Surgery to remove tumor if possible
  - Administration of chemotherapeutic agent(s)
  - Radiation
- Radiation is targeted as much as possible to the tumor, but healthy tissue can also be affected
- Chemotherapeutic agents are given in large doses to ensure they access the tumor.
  - This also affects healthy tissue
- The results are often severe side effects such as nausea, fatigue, loss of hair

Administration Options

- Oral
- Injection / infusion
- topical

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Patients will lose their hair, they have nausea, fatigue. And this fatigue and nausea often requires treatment with additional medications. So, it becomes a very tough situation for the patients.

These medications are most often given by injection or infusion, but there are oral medications available as well as topical, especially for skin melanomas. Some examples of these chemotherapeutic agents may have heard of, they've been around for a long time, such as antimetabolites.

Examples of Chemotherapeutic Agents

Drug Class	Subgroup	Drug
Antimetabolites	Antifolate	Methotrexate
	Pyrimidine antagonists	Cytarabine, gemcitabine
	Ribonucleotide reductase inhibitors	Hydroxyurea
Alkylating agents	Oxazaphosphorines	Cyclophosphamide
	Nitrogen mustards	Chlorambucil
	Nitrosoureas	Carmustine
	Platinum-based agents	Cisplatin, carboplatin
Topoisomerase inhibitors	Topoisomerase I inhibitors	Irinotecan, topotecan
	Topoisomerase II inhibitor	Etoposide
Mitotic inhibitors	Vinca alkaloids	Vincristine, vinblastine
	Taxanes	Paclitaxel, docetaxil
Antibiotics	NA	Bleomycin, doxorubicin

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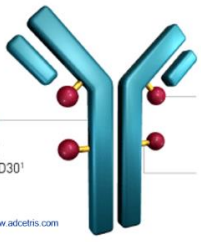
There are ones that are known as cytarabine and gemcitabine. They've been around for a long time. Alkylating agents, such as the platinum-based agents like cisplatin and carboplatin. Those are also well known and have been around a long time, like Nitrosoureas Carmustine and there are the vinca alkaloids, vincristine, vinblastine. If you're a gardener, you may have heard of Vinca vines that we can plant during the summer. And those Vinca vines are exactly where these vinca alkaloids come from.

ADC Composition

- **Three Molecular Entities**
- Monoclonal antibody
- Chemical linker system
- Cytotoxic agent



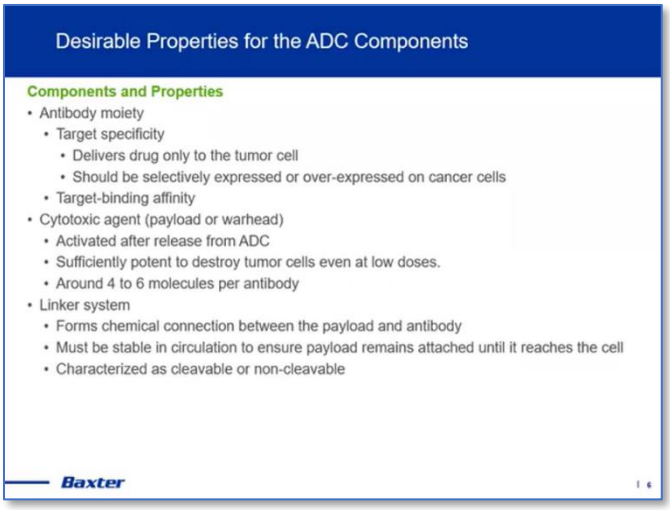
Antibody
The antibody, brentuximab, specific for CD30[1]

Cytotoxic agent
The synthetic microtubule-disrupting agent, monomethyl auristatin E (MMAE), that induces target cell death[1]

Linker
A synthetic protease-cleavable linker that covalently attaches MMAE to the CD30-directed antibody and releases the agent within the target cell[1]

Image from http://www.adcetris.com

So now let us consider an entirely different approach rather than inundating the body with these toxic materials, we can drastically reduce the number of chemotherapeutic molecules that we give to a patient, if we can incorporate them onto an ADC, Antibody-Drug Conjugate. These antibody-drug conjugates are three molecular entities. There is the monoclonal antibody, which in this diagram, that's that blue area of the Y, typically we build antibodies into genes.

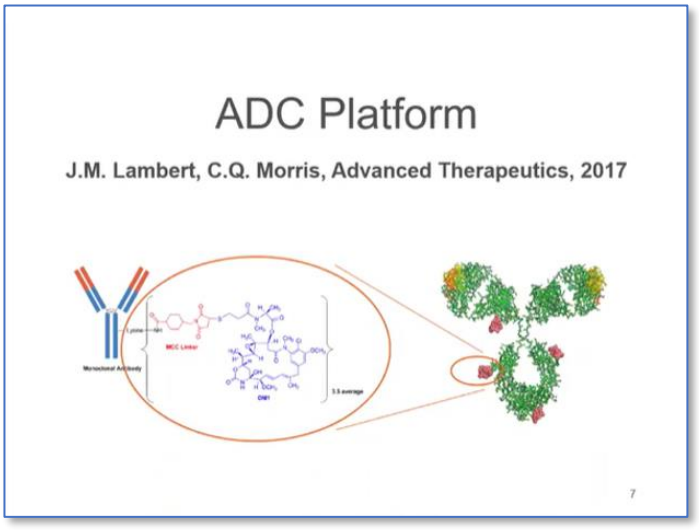


These antibodies can be chosen so that they will preferentially go to cells that are expressing a certain protein or chemical signal, such as a tissue factor. On these antibodies, since our bodies already know how to work with them, we can attach a chemical linker system. That is that yellow line in the diagram, making it very simple looking. They're actually very complex. And the red spheres represent whole individual molecules of Cytotoxic agent, perhaps four to eight individual molecules per monoclonal antibody. That is a drastic reduction in the number of those cytotoxic, the molecules given to a person.

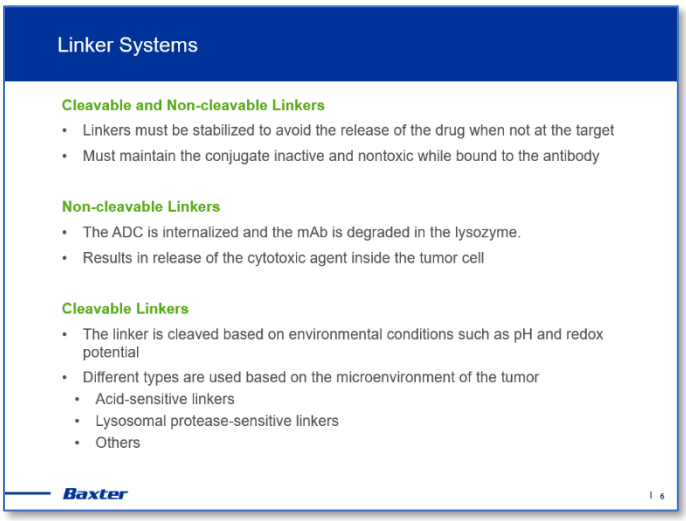
We want the antibody to specifically target a certain cell that is expressing or over expressing a certain molecule. We hope these cancer cells are only expressing that target that the antibodies are going to, or they could be over expressing them. We want this so that the antibody-drug conjugate has a limited effect on healthy tissue.

We would like to have strong target binding affinity. The cytotoxic agent often known as the “payload” or the “warhead” that is attached to that antibody; we would like it to stay firmly attached to that antibody until it reaches its desired point or target. This allows us to provide very low doses but still kill the tumor cells. We want the linker system to remain firmly attached to the antibody until it reaches its desired destination. The older linker systems, when this technology was first developed, tend to not link as well, and you would have free linker and chemotherapeutic agent floating around.

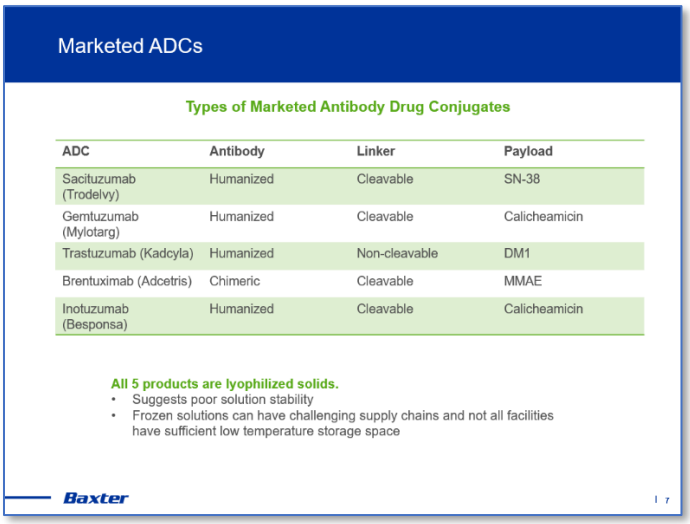
You do not see that as much anymore. These linker systems are firmly attached and they're fairly stable when circulating through the body. And we want that so that they deliver the payload only to the tumor cell preferentially. These linker systems, they are both known as cleavable and non-cleavable.



Here is just another example of ADC platform. The antibody is in blue and red and this large linker system is accompanied by the warhead. We would attach specifically four or six individual molecules to those antibodies.



The linker systems could be cleavable or non-cleavable. We want these to be stably attached so that they don't release this anywhere within the body. They must maintain that conjugate as an inactive substance until it reaches its target. Non cleavable linkers, these ADCs are internalized into the cell where the antibody is then degraded. And that's what releases the warhead. Cleavable linkers will cleave depending on certain environmental conditions, lets say. What is the pH? Perhaps a redox potential.

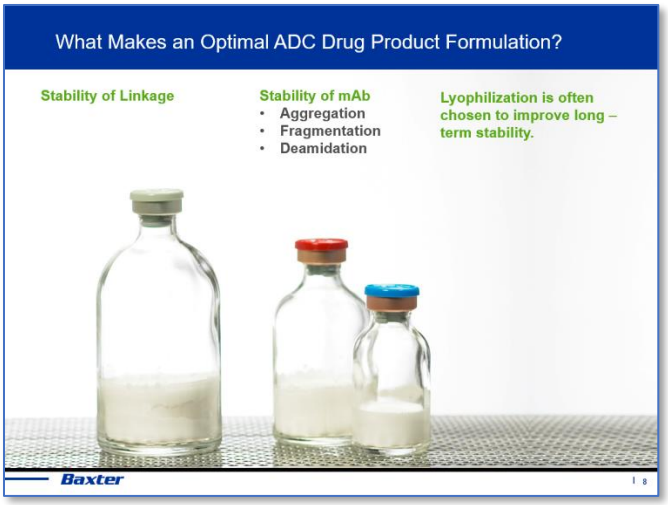


They could be acid sensitive or be sensitive to lysosomal protease, says which will cleave that linker once it reaches its desired location.

Here is just an example of the marketed ADCs. There are five of them that I am aware of, and you'll see on the left side of the table the different ADCs. The next column is the antibody. I placed either humanized or chimeric here. In general, they all use a type of IgG. Typically IgG-1 or IgG-4. Then currently, there's cleavable and non-cleavable linkers that are marketed.

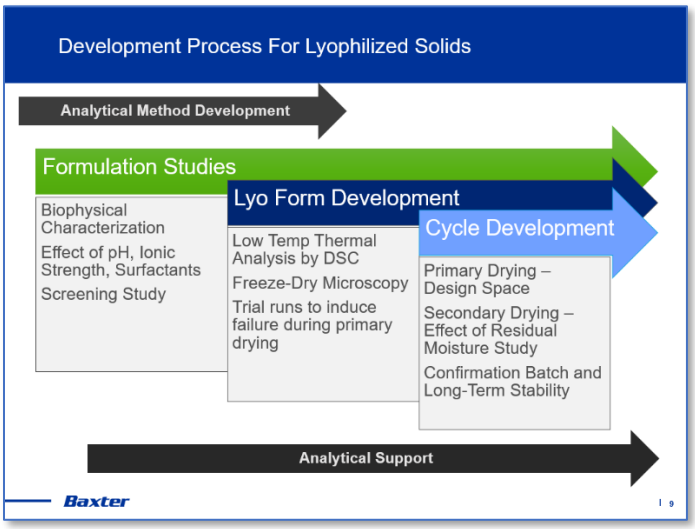
And then each of them is associated with the payload. There are also a couple more that are outside of development. We developed them here at our site. I have patents on the formulations for two of them, both here in the U.S. and in Europe, but we transferred those to our site in Halle, where they're currently making clinical supplies. All five of these products that are currently marketed and the ones that we transferred to Holland are lyophilized. So that tells us something about them.

That means they are not solution stable. So, they may be stable at very low temperatures, but what we have seen, what we have all been aware of with the pandemic, is that cold chain storage can be a huge challenge. Even if you can ship it cold chain and have it remained cold, the facilities that you're shipping to may not be able to have that type of low temperature storage. So in order to get around those incompatibilities with unstable solutions or low temperature storage, we could look at freeze-drying them to extend their stability.



So, what makes an optimal ADC drug product formulation? Well, first, once we formulate it and we process it, that linker system must still remain stable. Again, just to mention, older linker systems which are still available, they tend to not stick as well. And what that means is that, when working with those older linker systems, you would have to work and treat it as a category four compound per safe bridge. And that means working with them in isolators. Otherwise, you risk the health of the people working with them.

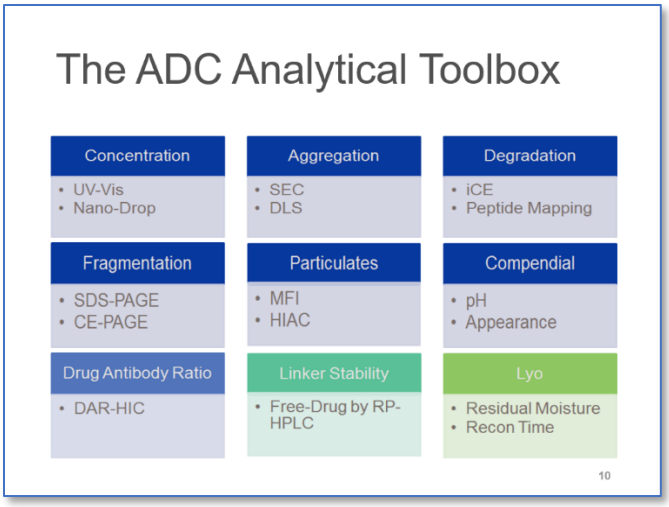
The current linkers that have very good stability, we can work with them as a category three compound and work with them in our laboratory, still taking appropriate precautions just like we do with any other chemical, but we do not have to work with it only in an isolator. We want the monoclonal antibody to be stable. So throughout our studies, we're looking at potential for aggregation or potential for fragmentation or de-emanation. And the way to improve this long-term stability is often through lyophilization.

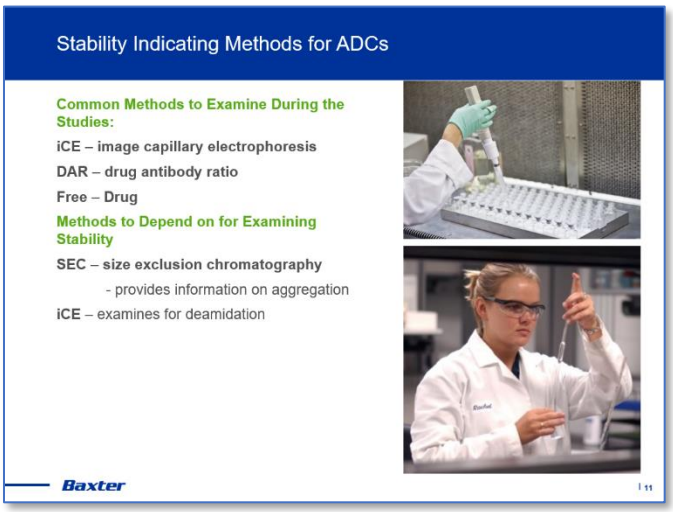


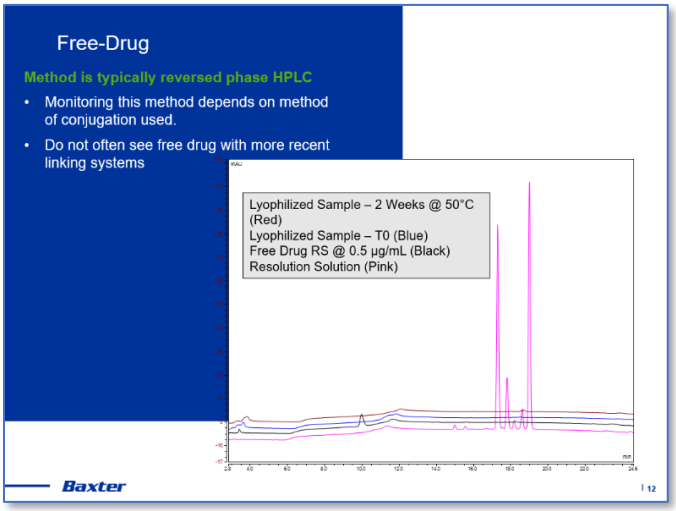
This is just a quick diagram showing you an overview of our process or anybody's process really. We must have good analytical methods in place, in particular stability-indicating analytical methods.

We usually start with our formulation studies, solution stability studies. We want to determine, what's the best buffer? What's the dependence on pH? Is there a dependence on buffer concentration that would make a difference for the stability of this molecule?

That will then provide us the basic data where we can start building our formulation for lyo development processes. And throughout all of these studies we're working in areas that we expect some failure. We want to know where our product will fail so that we can stay away from those areas. If we can design a lot of studies where we never see any failure, we will not know if we are right on the edge of failure or not. So, it is important to be able to test that. And in lyo development, we also test for failure. I'll show you some slides later on. And then we conduct thorough secondary drying studies, where we examine the effect of residual moisture on the stability of the product. And we will provide you some case studies on that as well.





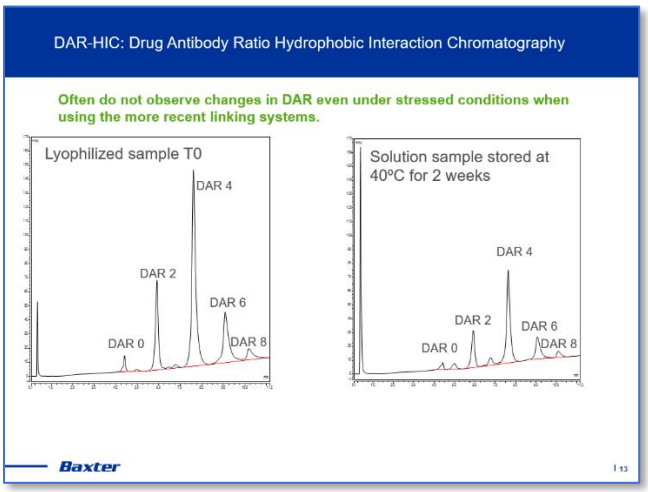


Our analytical toolbox: all these methods are extremely important, but there are a few of them that are more important than others at early stages.

For example, we may look at concentration, but I have never seen concentration change and it does not really represent a great stability indicating method. What we want to know is, do we see fragmentation? Do we see aggregation? Do we see anything that would relate to this not being stable over the long-term? I will show you a slide next that would include that. So part of aggregation would be SEC, DLS, Dynamic Light Scattering. Sometimes those are good to use both at the same time because DLS uses very little solution. It is almost like getting some data for "free." The DAR-HIC is drug antibody ratio with hydrophobic interaction chromatography. We use that to determine if we have our linker systems still in place. And as I mentioned with the newer linker systems, we never see any changes at that. So we might include that particular assay perhaps on long-term stability, just so we have that data.

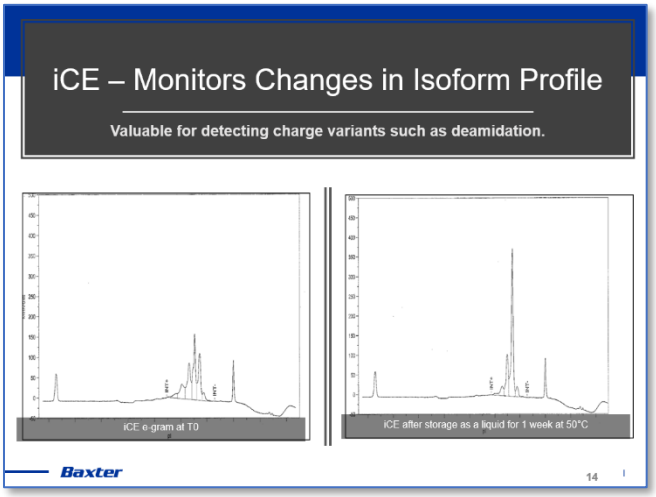
Our stability indicating methods though, the most common ICE, we may look at DAR or a free drug over time, but it depends on the linker system. So really what we focus on is SEC and ICE during the early studies. We find those tell us a lot more information.

Here is an example of free-drug, and this is a solution and lyophilized samples stored at 50 degrees C. We don't often store our solution samples at 50 C but for the next few examples, for some reason we did on these studies and I don't remember the exact reason why when we do, we store at 50 degrees C so that we hopefully can get some data quicker and maybe provide us some idea of what may come when we store it for longer term.

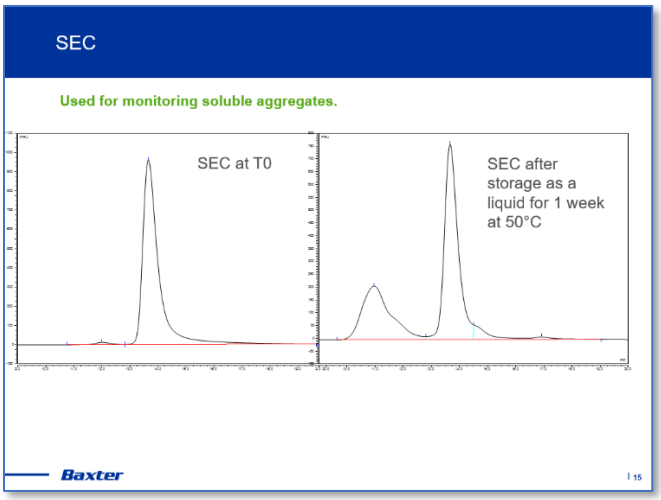


It is frequent in our early studies that we will store freeze dried samples at 50 C for one or two weeks, but also store them at 25 C and 40 C for two and four months. So this is an example of monitoring free drug. Rarely have we ever seen free drug available in the formulations when we are using these newer linker systems.

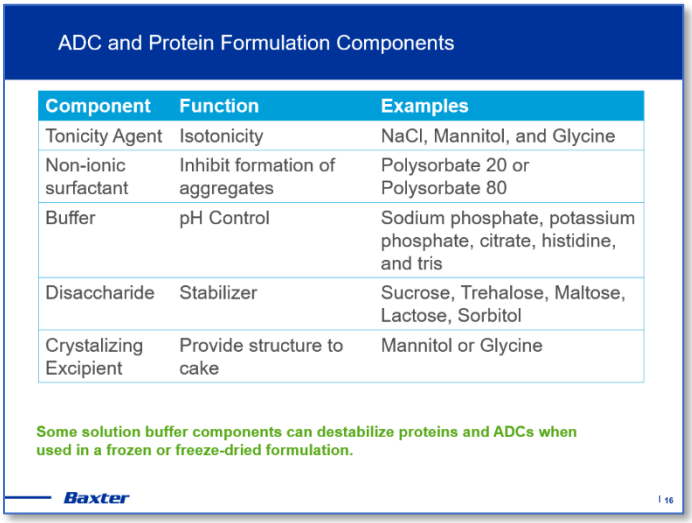
Here is also an example of the drug antibody ratio. Again, even under stressed conditions, I'm showing you here 40 C for two weeks, but we have data for 40 C for up to six or 12 months, maybe two years where you do not see any changes in the drug antibody ratio. So, it's usually just included as a check.



ICE however we do use. We use this because we do see changes over time that may show us different charge isoforms that develop due to the storage conditions, but also due to our formulation conditions. This is just an example. This is a graphic TO and then one after one week at 50 C, we see a large change. And so that tells us this will be an important stability indicating method.

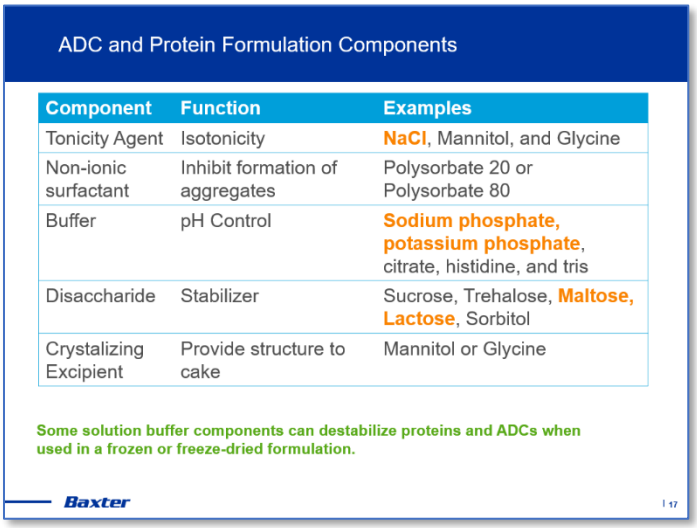


SEC, size-exclusion chromatography, we also use. We monitor soluble aggregates and we do see changes over time. And I will show you some examples, some statistical analysis that we've used where we use SEC and ICE to determine which direction we should go.



So now just let's take a look at the components of a formulation for lyophilized antibody-drug conjugate. These are some common formulation excipients. I wouldn't use all of them but some common ones are listed and why we would use them. For example, pH control or stabilization of the lyophilized solid or providing bulk.

But again, we hope that all our excipients that we are using are inert and they should not interact. However, they do bring some interactions and here are some examples.



One example, this is not really an interaction, but sodium chloride, if we need to adjust the tonicity, we will only adjust tonicity, if our route of administration is direct injection or into the muscle or under the skin, subcutaneous. If it is going to be administered as an infusion, we do not really need to worry about adjusting tonicity for that formulation.

But if we choose to and we are lyophilizing, we try to stay away from sodium chloride. Salts definitely decrease your critical product temperatures. So they lower them to temperatures that make it difficult to freeze or difficult to dry. So, it's better to adjust tonicity with Mannitol and Glycine. We may include an ionic surfactant to inhibit aggregates. I'll explain a little bit more about that later. Buffers. Some buffers that we try to stay away from are phosphates, mainly because they can bring along with them some metals.

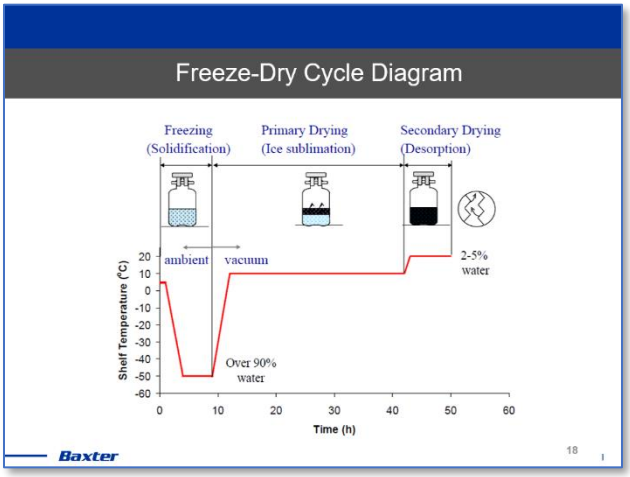
They are known as types of chelators. They are in those lists of antibody-drug conjugates that are marketed. They use phosphate buffers. So they are still used but you just have to be aware of them. The choice of disaccharide. If we have a large molecule, we need to include an amorphous excipient in there. Typically we use Sucrose or Trehalose. Maltose or lactose are available but these are known as reducing sugars.

What that means is that they interact with proteins when they're freeze dried. In particular, they'll result in an interaction called the Maillard reaction that results in browning. That's what leads to meat browning when you cook it. So we stay away from those reducing sugars. We might add a crystallizing

excipient. I often do because it makes it easier to freeze dry and more difficult to lead to failure. So, you could add Mannitol or Glycine.

I have just had a webinar question, “what are the most used cases for Mannitol and Glycine?” Mannitol and Glycine, they do not offer any protection to your protein. And so what means is that you cannot use them alone with a protein. You'll use them to form structure. Mannitol and Glycine, therefore, make a nice scaffold. So, they will hold all the amorphous components in place, but still result in a really nice looking lyophilized solid.

They also allow you to process at much higher temperatures. So it reduces your freeze drying time. So they add a huge advantage. But if you look at those marketed formulations many of them don't include it. I have included them in the formulations I develop only because of that ease for drying.



So, for those of you not too familiar with freeze-drying this is a generalized process diagram. What we have is shelf temperature versus time.

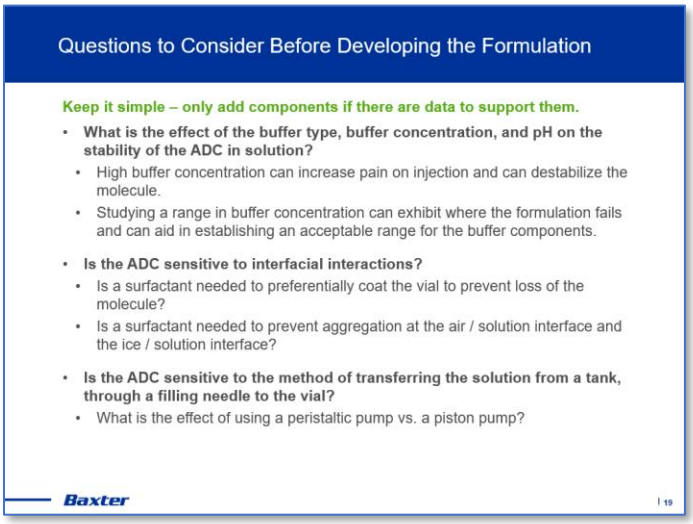
The red line is just shelf temperature. So we first have our freezing step. We cool it to some very low temperature, let's say -40, -50, hold for a certain amount of time and freeze it. And then we could introduce an annealing step if we have a crystallizing component. That's where we would raise the temperature to let's say -15 or so and hold for let's say three hours just to ensure that it crystallizes.

And then we can go into primary drawing. That's where we initiate a vacuum and then we remove that bulk ice. And we remove it based on a difference in vapor pressure of ice in the sublimation front and the vapor pressure of ice in the condenser. That's a whole another lecture, so I'll just leave it at that. But at the end of primary drying, you should probably have around, let's say two to five percent water.

And then we go into secondary drying. That's where we raise the shelf temperature. Pressure is no longer important here because we need energy now to remove the moisture. That is what we call attached water or water that can only be released through diffusion. And so we need energy there to remove it. And for that reason we need to increase our shelf temperature, let's say to 30 or 40 degrees C and hold it for a certain amount of time to reduce moisture.

I will show you later where we conduct the studies to study the effect of residual moisture. That goes into it a little more in depth than this generalized diagram.

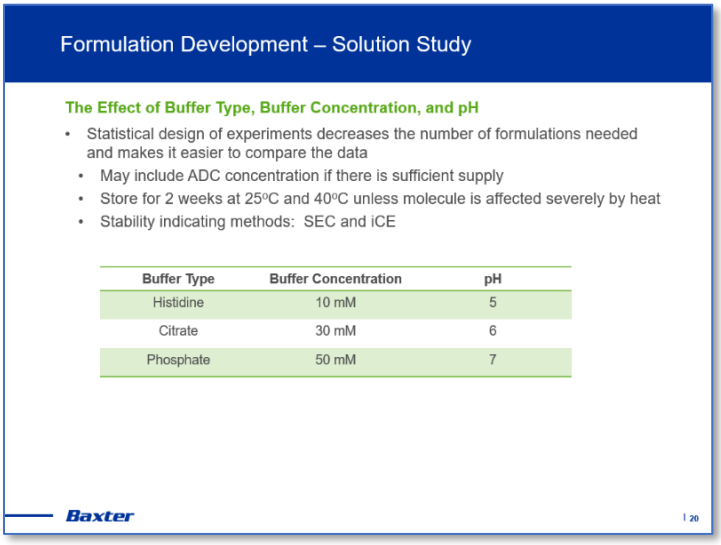
So here are some basic questions to consider before we start formulation. We strive to keep things as simple as possible. So we only add components if we have data to demonstrate that they're needed.



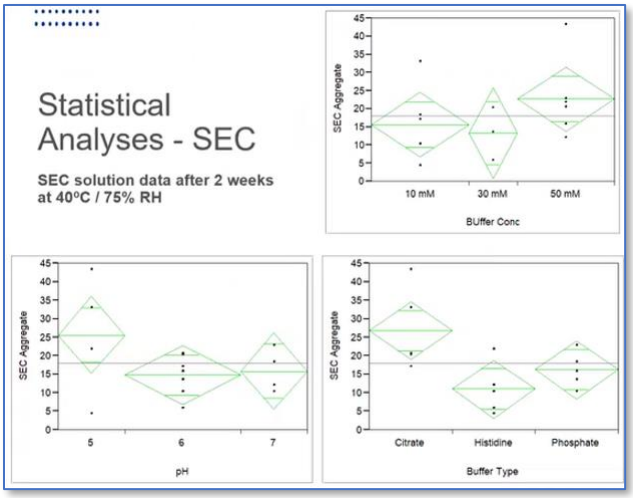
Our first webinar question for proteins and monoclonal antibodies is, **Q:** (from the audience) “what is the effect of buffer type, buffer concentration in pH on the stability of the ADC and solution?” **A:** Well, is there an effect on buffer concentration? We don't want to go too high because high buffer concentration can cause pain upon injection and it can also interact with your molecule. So, we need a steadier range of buffers in our range of pH and a range of concentration.

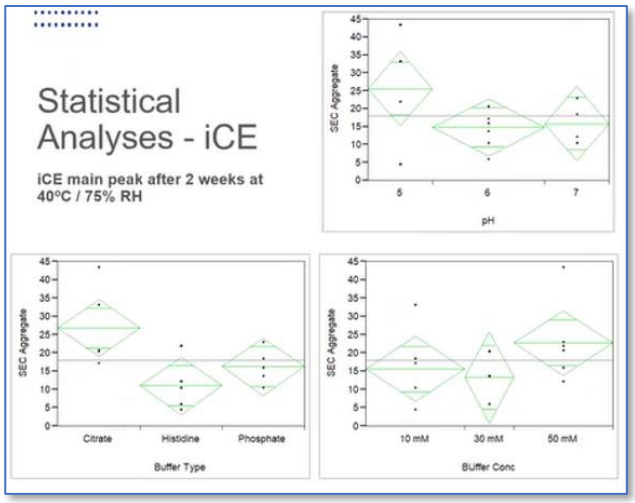
Next Question **Q:** (from the audience) is the ADC or protein sensitive to interfacial interactions? **A:** What we need to know is when we have our formulation added to our vial, do we lose protein or ADC by it preferentially adhering to the glass surface or when we have all these ice crystals form? Now we have a lot of contact of your molecule with the ice surface. It can then unfold an aggregate. Do we have that problem?

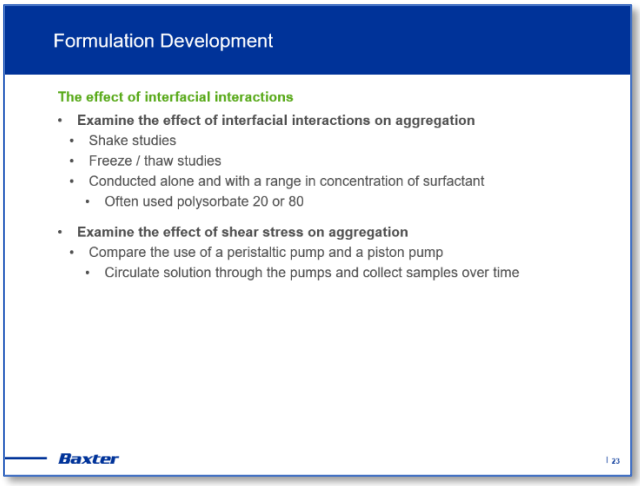
So, there are some studies that we need to do to conduct and determine if we need to add something such as a surfactant to prevent that. Finally, is our ADC sensitive to the method of transferring the solution from the take through the filling needle into our vial? We need to think about how these formulations will be processed at full scale. And that's where these pumping studies come into play.



First, this is the solution study I mentioned. Where we look at the differences between types of buffer, buffer concentration and pH. This sets up for a really nice statistical design of experiments to reduce the number of formulations we preparing, reduce the number of samples we're sending to the lab and reduce the amount of material we're using. So what we can do is formulate these and place them at two weeks, let's say at one to two weeks at 25 and 40 degrees C and test them weekly.







Here is an example of the data that we get from this. This is size-exclusion chromatography and we're looking at the statistical analysis after two weeks of storage at 40 C. First let's look at buffer concentration. There is a slight effect if you increase the 50 millimolar, when compared to 10 and 30, we see more aggregation and that is common. You do not really need to go above, let us say 20 to 30 millimolar in concentration of the buffers.

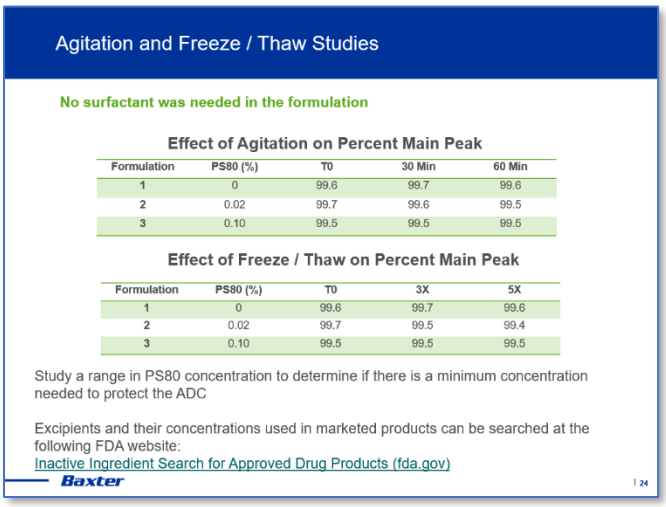
Now let us look at buffer type. Here, histamine and phosphate seemed to be much better with reducing aggregation than when compared to citrate. Next pH. Decreasing the pH to five led to more aggregation. So now that helps us narrow in on our range of acceptable pH.

We will also look at the ICE data. ICE data is telling us the same story. Basically, we want to stay between pH six and seven. We want to avoid the citric buffer. In fact, histamine seems to be a bit better. And in general, with the ADCs that we worked with here in our lab most of them are quite stable and formulated well with a histamine buffer, around pH six. And then again, formulating between 10 and 30 millimolar. What this just helped us do is determine our center point. Now we can formulate at 15 millimolars of buffer and we now have our acceptable formulation range.

Now let us consider formulation development. So effective interfacial interactions. We typically need to design some type of study to look at the effective interfacial phenomenon particularly at the air solution interface, the glass solution interface and the ice solution interface. So what we do is develop or create a shake study and this doesn't have to be violent shaking. This is just shaking so that you constantly turn over the solution to expose the molecules to the air solution interface.

And we'll set up something where we set samples prepared with and without a surfactant and hopefully the range of surfactants so that we know what the minimum amount of surfactants is needed to prevent aggregation. And we'll conduct this shaking for 30 to 60 minutes and then we'll also do freestyle studies where we take the samples and freeze them and then thaw them for, let's say three and five cycles.

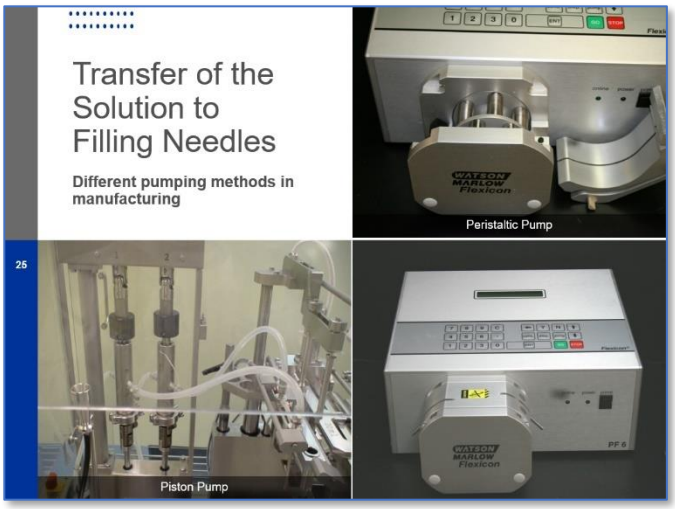
We often use Polysorbate 80 but there is the mixture out there. I haven't seen any literature that suggests one is highly better than the other. Both are used to equal extent. And then we'll also look at a shear stress study because… **Q:** (Question from the audience) a "Do you use poloxamers as surfactants?" **A:** They can be used. We haven't used them widely in our lab. I cannot really tell you a strong reason why. It is just that we have a lot more experience with polysorbate. **Q:** (Question from the audience): "What surfactants other than PS 20 and 80 have you seen commonly used if any?" **A:** The poloxamers like what was mentioned but again we do not often use those. But they are out there. I'll show you a link to a website where you can look at where they're used and what types of formulations are used and what concentrations. Very good questions.



So, let's just jump to the next slide. This is an example, this is an antibody-drug conjugate. These are actually from two different antibiotic-drug conjugates or studied, effect of agitation and effect of freestyle. In here we have polysorbate 80 presented.

In these studies we've prepared samples without polysorbate with a very low concentration. You can even go lower 0.2% but I've seen 0.1% and then I made an artificially high 1.1%. And we look to see if we have any differences in the loss of main peak using SEC and here we do not. And we do not have to include a surfactant in these formulations for the ADCs. This is the link here to the website. There's inactive ingredient search that the FDA has. And it's a wonderful site. They will not tell you this specific product that it's used in but it lists every product out of the market, whether it be topical, injectable or oral that has that particular excipient in it. And at what concentrations.

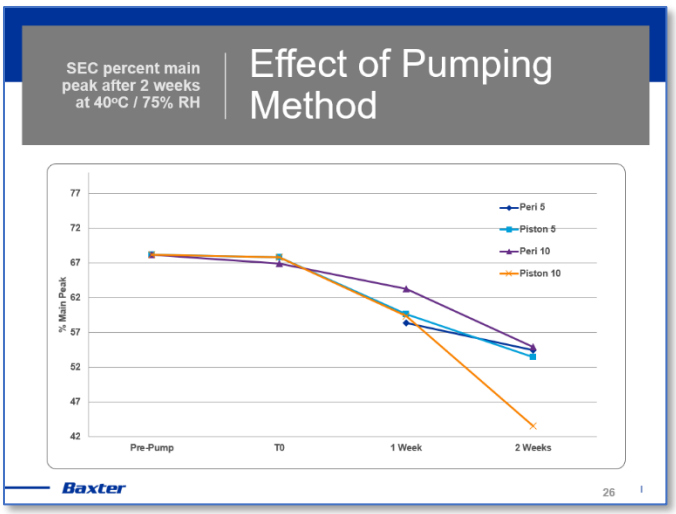
Q: (Question from the audience): "Are ADCs more stable than classic monoclonal antibodies?" **A:** From my experience, I have to say, ADCs we have worked with have been extremely stable or there a certain monoclonal antibodies that show a far less stability so, yeah, it's a good question. And almost makes you feel like it's easier to work with, but it don't let your guard down. You still have to perform these studies because they can still degrade. But just for your reference, check out that website. It really helps to show you what the maximum concentrations have been used. And there are for some small molecule formulations that are delivered by IV infusion, the amount of polysorbate present is far larger than you would expect. So it's a good site to see what's out there.



Several years ago, there was a conference where (I can't remember who presented it) but there was a presentation there about the types of filling methods that were used. And someone had said that you should never use a piston pump and I can't say that. **Q:** (Question from the audience) "Should we use polysorbate after the CMC concentration?"

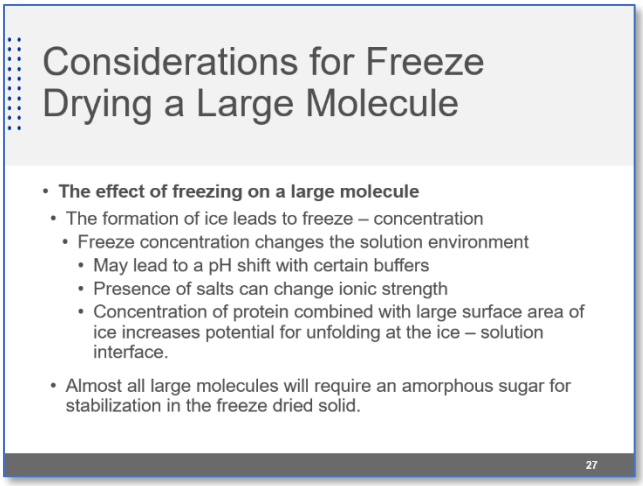
A: By default, you already are. Most of these polysorbates have CMC concentrations that are very, very low. So, you're automatically forming the CMCs.

Back to the filling methods. So there are different filling methods and the manufacturing area. I'm only listing two of them here, but one is your peristaltic pump. That's the two pictures on your left side. That's where we use, let's say most often we're using a silicone type of tubing. That's threaded over those rotating bars and they push let's use it as a positive displacement pump. So they rotate they squeeze and let go of the silicone tubing to transfer the solution. The big challenges with those is it's important to perform studies to determine how long can you use your silicone tubing in that pump before you start to see degradation of your silicone tubing? What we have seen is that there can be times for specific types of tubing since they're being compressed over time, often, they could split at the sides or what's also very common is that the inside of the tubing rubs together and it creates spalling. So you'll end up with small pieces of silicone tubing in your fill vials. So those are studies that are important to conduct before you just use tubing for an extended amount of time. There's also what's known as the piston pump and that's that lower picture on your left. I think I said left two pictures but for peristaltic and not right. But the piston pump, what you're seeing there are two cylinders and inside the cylinder is another cylinder that terms that goes up and down. And it has to be narrow enough to fit into that outside cylinder without scraping. So that means there has to be a gap, that gap has to be filled and it's filled with the solution that you're filling. So, that means it's considered self-lubricating. What it does is that it moves, picks up a volume of solution and then injects it into your vial. The concern here is that over time since that solution is being self-lubricating, that your molecule will build up over time. Some of it remains trapped and is exposed to a lot of shear. For some molecules that's a problem, for others it's not at all. But it's important to conduct a study to determine if it is a challenge.

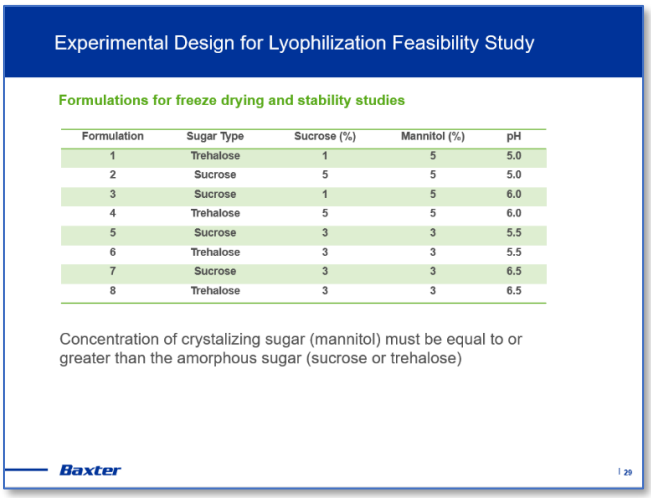


So, let's just look at one challenge. This is where we took solution and we circulated it five times and 10 times through a peristaltic pump and through a piston pump. Why would I want to recycle it? Well, I'm trying to imitate that solution being trapped in between the cylinder for the piston pump over time.

And here we took those formulations and placed them on short term stability one in two weeks. Why would we do that? Well, we learned the hard way. Before we just wanted to know, whether you see particles form over time and just tested them at T's pre pump and then T zero didn't see any changes and then we saw changes later on. What that made us realize is that we need to place these on stability. And what we found is that certain molecules, this is for one particular ADC the piston pump over time wasn't a bad choice. Because we saw an increase in aggregation and large decrease in main peak.



So consideration for freeze-drying a large molecule I'm going to have to fast forward through here I have a lot more slides here than I have the time. We started a little bit late though. We need to realize that there's freeze concentration that occurs. And when freeze concentration occurs, that changes the environment in which your molecule was in so that could lead to other changes. Some reasons we'd want to add excipients well, if you have low solids content, you'll want to add something there for bulk. In general, since we're talking about an ADC, it's a large molecule, we have to have an amorphous excipient there. Amorphous excipient surround those molecules and prevent them from unfolding, maybe due to loss of water or from interacting with each other.



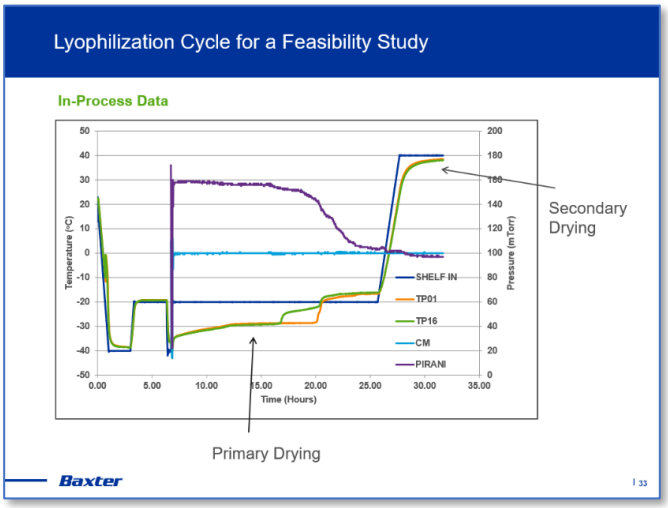
So, we can conduct a study to look at different sugar types we've done this where we look examine the effect of sucrose and trehalose while keeping mannitol constant.

The reason we do this is that if you're going to use a crystallizing component, they have to be at either the same concentration or more than your amorphous component. Otherwise you risk them not crystallizing.

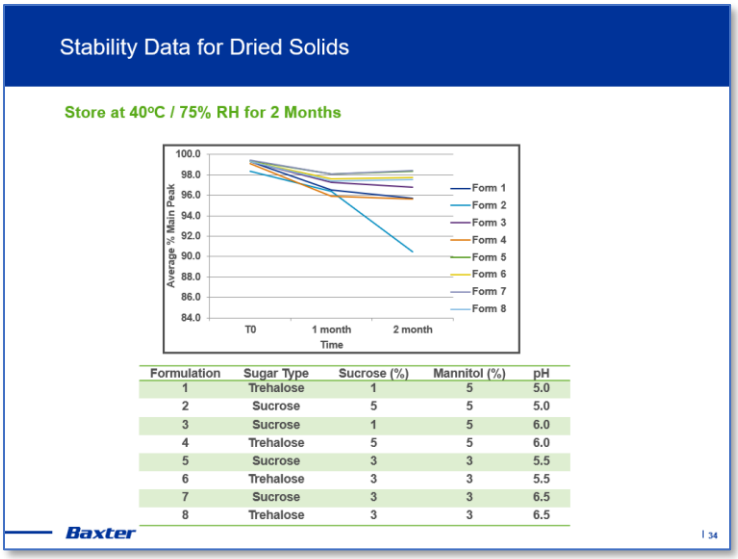
Here is an example of a freeze dryer that has a sample thief on there and on your product chamber and then here's your condenser. So water flows from your product chamber to your condenser.

Just as an overview I've provided that before already but so I won't go into depth here since I don't have much time. But just realize that there are different ways we could freeze things, the slower you freeze it, meaning that if you choose to freeze that let's say 0.1 degrees C per minute, we see that there's a higher degree of super cooling. That means the solution does not freeze until very low temperatures. That creates a very small ice crystals and that could be a problem. We could also include controlled ice nucleation this is an example from Millrock where they use a freeze booster that's where they're injecting a fog of ice into the chamber to act as seeds.

These vials are filled with solution are partially seated with the stoppers that have an escape area. These happened to be single vent, but they can be dual vent.

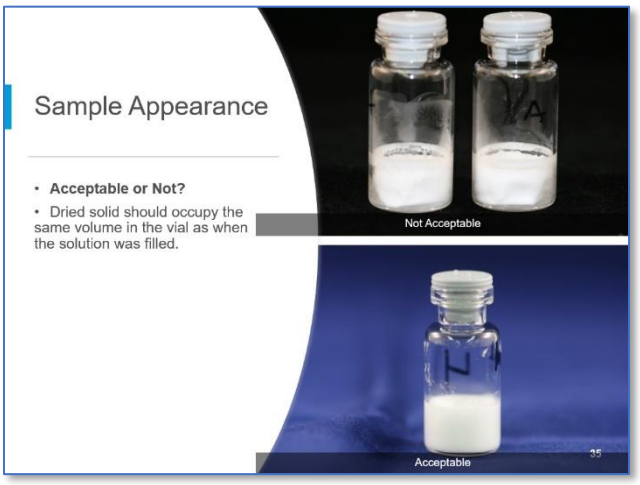


This is an example of in-process data. What I wanted to show you here is the main portion of this is primary drying and we can determine when primary drying is done by a couple of reasons. Again, that's a total different lecture. We can look at product temperature. We could also look at reduction of pressure towards the end of primary drying.



This is an example of stability for these freeze-dried solids. We found that, and this was time and again with ADCs, they're more sensitive to pH than anything else. So formulating at this high pH 6.5 with trehalose is that formulation eight. Over two months, we saw a large decrease in the stability.

Q: (Question from the audience) “In your experience, how comparable is the lyophilization behavior in the ADC to the behavior of the drug-free mAb? **A:** They're pretty much comparable because the monoclonal antibody itself let's say is about 150 kilodaltons. The ADC is very close to that. That linker system and the small molecule that's attached, they don't add much more weight. So they're very close. When we become concerned about... That's a good question, because what we're concerned about when let's say, if we have to use a surrogate to do these studies, lyophlization studies. We might do that because we are concerned about their resistance to mass flow of water vapor from the solid to the condenser and if your surrogate has a far different molecular weight than your antibiotic drug conjugate, it can have less resistance to mass transfer than your antibody drug conjugate and therefore dry, faster and easier. So those are some things to keep in mind.



Let's say our goal here is that our dried solid occupies the same volume as your solution. The top we consider not acceptable. This was a formulation form developed from just sucrose. And when you formulate with a morphous component only, they tend to shrink. In this case, the temperature and also resistance mass transfer of water vapor are high and so you resulted in much higher degrees of shrinking you don't want that.

Sample Appearance

- **Formulation and Process Affect Appearance**
  - Must understand the behavior of the formulation at low temperature.
    - Crystalline or amorphous?
    - How do the components affect each other's behavior?
  - Are the critical product temperatures amenable to freeze drying?
    - Difficult to dry formulations when the product temperature must remain below -37°C.
  - High solids content increases the resistance to mass transfer.
    - May increase drying time or may be difficult to dry.

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In general, we want things to look good because on the market you'll receive a lot of complaints if they have an odd appearance. They could be completely stable in fact, in a lot of these collapsed formulations, we do not really see a large change in stability. It's just that they're difficult to reconstitute and they don't look pretty.

Collapse

Examples of Poor Appearance

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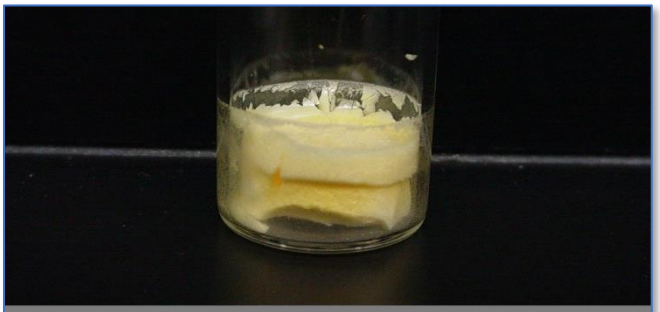
Here are some examples of poor appearance. This is collapse.



Example of Poor Appearance

Partial Collapse

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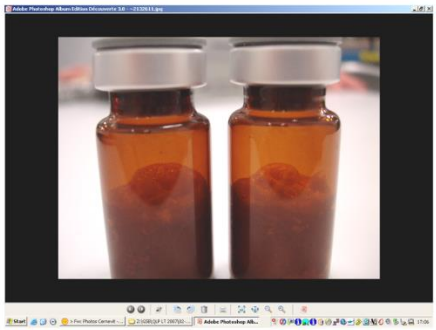
This is partial collapse and again, this was mostly an amorphous compound.

Example of Poor Appearance

Puffing / Bubbling

- Formulation contained glycine that did not crystallize.
- Had to include an annealing step to prevent puffing



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This is an example of puffing and this particular formulation, it contained glycine and the original cycle did not have an annealing cycle. It was completely stable just like that but if you add an annealing step the entire solid looks much more presentable.

Appearance of the Dried Solid

Considerations

- Stability
  - Collapsed solids have more water associated with them.
    - May or may not affect stability of the product.
- Product Complaints
  - May receive complaints from the market that require investigations and responses.
- Suggests that formulation and process are not well understood or controlled.

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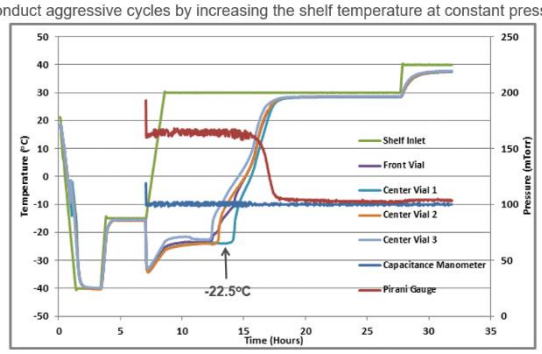
Just some considerations of why you want things to look good.

Identify the Failure Point During Primary Drying

Evaluate Samples for Changes in the Physical Appearance

Conduct aggressive cycles by increasing the shelf temperature at constant pressure.



Primary Drying Conducted at +30°C, 100 mTorr. Maximum Product Temperature = -22.5°C

Baxter

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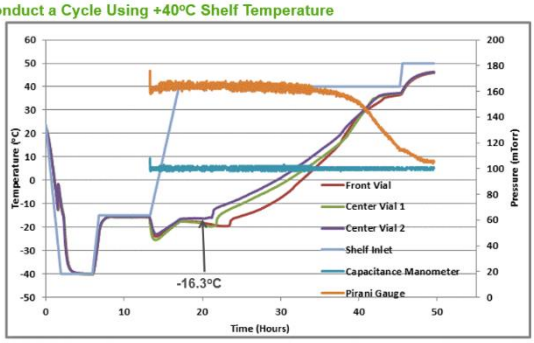
I'm going to jump here; we want to conduct failure identification and failure studies. And that's where we use the same samples over and over again, let's say about 10 of them and we freeze dry it warmer and warmer temperatures. And we want to look at the effect of the shelf temperature on product temperature and appearance.

Identify the Failure Point During Primary Drying

Conduct a Cycle Using +40ºC Shelf Temperature



Primary Drying Conducted at +40ºC, 100 mTorr. Maximum Product Temperature = -16.3ºC.

Baxter

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So I'm going to jump to this one. This formulation is formulated with Mannitol and sucrose. And this is just an example of how stable it is drying. The entire freeze-drying cycle is conducted at four degrees C.

Identify Failure Point During Primary Drying

Appearance of Samples Dried at +30ºC During Primary Drying

All Samples Exhibited Acceptable Appearance as Expected Because Product Temperature was Maintained Below Failure Point

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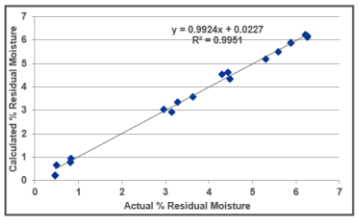
It never came close to the failure point and the samples still look presentable. A slight change but that's just kind of creeping away from the wall. There were still acceptable.

Identification of Secondary Drying Conditions

Determine the Effect of Residual Moisture on the Stability of the Formulation

- Create a calibration curve using NIR and Karl Fischer (KF)
  - Obtain samples during secondary drying at different temperatures to obtain samples with a wide range of residual moisture levels
  - Scan the samples using NIR and confirm residual moisture using KF



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A final point we like to examine the effect of residual moisture and stability. And we do that by taking samples throughout secondary drying conditions and building a calibration curve using near NIR and Karl Fischer.

Collection of Samples During Secondary Drying

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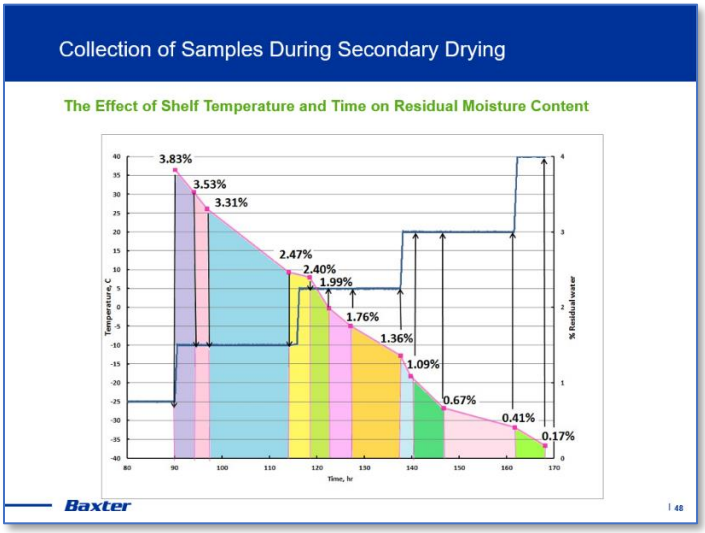
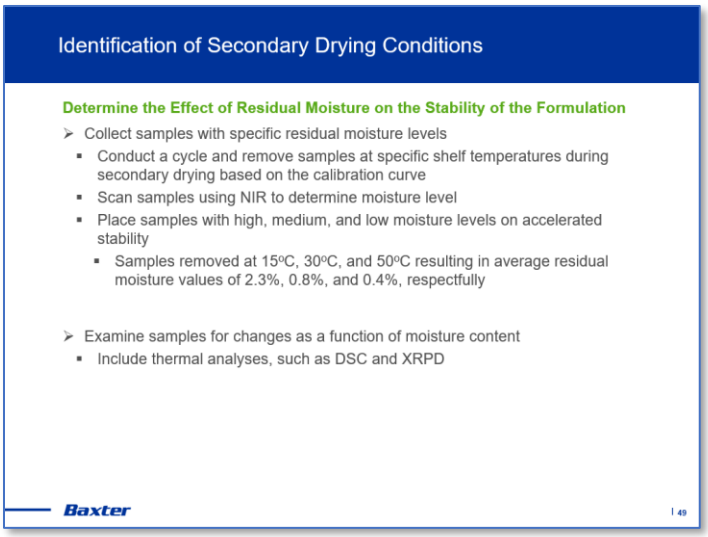
We use a sample thief to start collecting samples throughout the end of primary drying and throughout secondary drying.

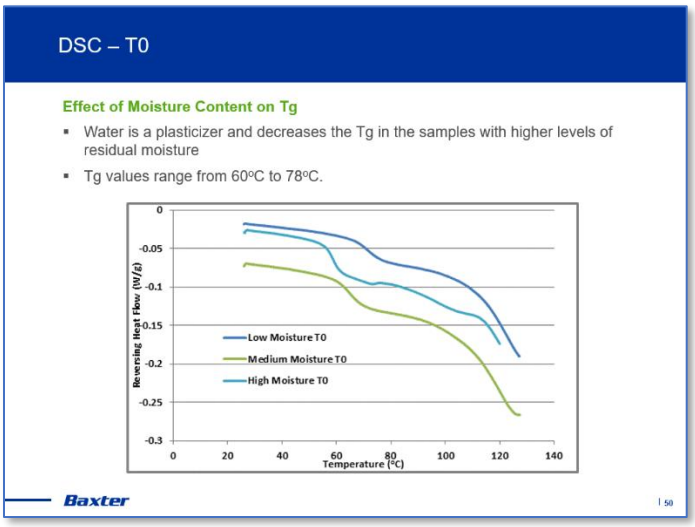


This is an example, here are steps in shelf temperature. And this is how dry the residual moisture is affected by shelf temperature in time at that shelf temperature. Here at the end of primary drying at minus 25 this product had almost four percent moisture.

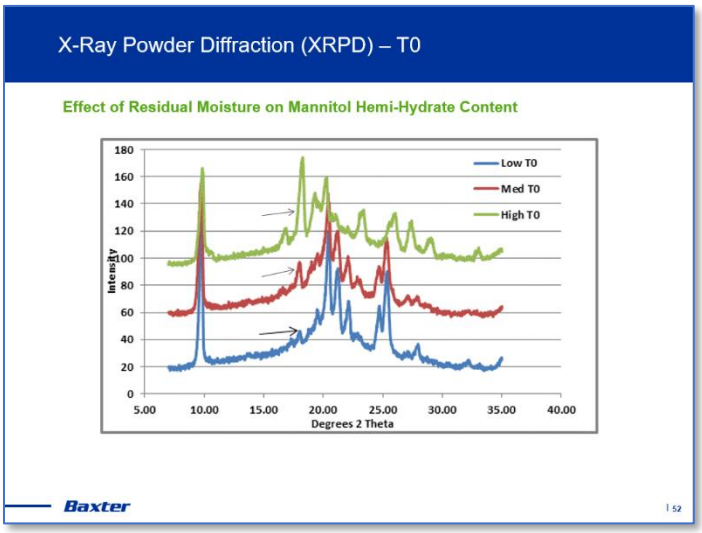
But if you hold it minus 10 for say, a number of hours, moisture drops to around two and a half percent and so forth. And so what we'll do is we'll conduct a study or work removing samples at specific conditions so that we have samples prepared with different levels of residual moisture.



This tends to be a very consistent way of obtaining samples with definite residual moisture contents. We then take these samples, scan them using NIR to attain the exact concentration of residual moisture to zero, and then store them on stability.



Here are examples of how they change this is T zero using DSC. We see with high moisture concentration, the TG of this dried salad shifts to the left. So lower temperatures that means possibly less stability and even shifts after two months of storage, even further.



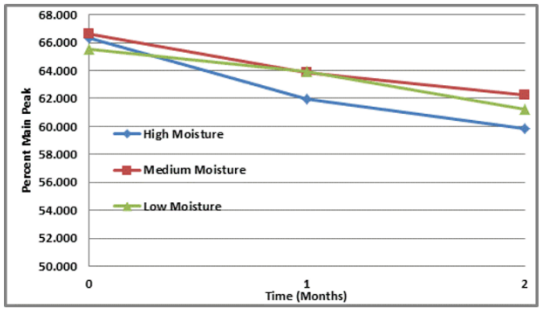
Another thing we look at is extra PD, extra power refraction is where we take these solids, we x-ray them, and they contain mannitol. Here, I'm pointing out with the arrow mannitol hemihydrate.

It tends to form in most conditions when you freeze dry it. And what we're worried about is the loss of that hydrate because as it's lost, it releases moisture. And that could interact with your solids. In the cases where we studied luckily, it hasn't been a problem. Our molecules have still been completely stable, but it's something we still investigate.

Effect of Moisture Content on Stability

Comparison of iCE Data

- Slightly larger decrease in percent main peak at high moisture content



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And then we of course include iCE SCC. In this case, it's showing that with higher moisture you're seeing a little more to degradation.

Summary

ADCs and Product Development

- ADCs are monoclonal antibodies with a linker system that connects molecules of a chemotherapeutic agent to the mAb.
- ADCs are designed to specifically target tumor cells that express or over express an antigen.
  - The ADC will preferably attach only to the tumor cells where they deliver their payload.
- There are 2 different linker systems
  - Cleavable and non-cleavable
  - Most recent linker systems hold the chemotherapeutic agent tightly onto the mAb to prevent release before the mAb attaches to the tumor cell.
- **Keep formulations simple and only add excipients when the data support them**
- Design studies to determine the failure points and establish acceptable ranges for excipients as well as process parameters.

Baxter

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So, I'll quit there because we've already gone well past our time. I tried to answer questions as they went. Are there any additional questions that you can think of before we end? If not, I'd like to thank you for attending. Thank you for your attention and hope it was useful to you.