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Introduction

Commercial freeze-drying has improved significantly over the last 20 years both in terms of the engineering of the equipment, and in terms of the understanding of the science behind the process. Even though great strides have been made in lyophilization, occasionally there are problems that arise during the process that not only may leave scientists scratching their heads, but in a worst case scenario, may also bring manufacturing to a screeching halt until the problem is found and resolved. In the case where manufacturing is halted, the scientists and engineers within the company are under extreme pressure to quickly diagnose the problem, resolve the issues, and get production up and running. In the case of freeze-dried products, it may be very difficult to identify the problem as there are many issues that may cause physical and or chemical damage to the product. Additionally, unless there is a scientist or engineer on staff who truly understands the science behind freeze-drying, diagnosing and fixing problems may be very problematic.

While some problems will require the expertise of a lyophilization expert to solve, other smaller problems, with some basic detective work, may be diagnosed and eventually corrected by a novice. Either way, following a precise line of questioning will help in understanding what is truly wrong with the product, why it is occurring (whether formulation or process based), and will give clues on how to solve the problem.

Correctly Identifying the Failure

When a product failure is detected, there are several key questions that must be asked and several key pieces of data that should be acquired. Listed below are questions that should be asked when a product failure occurs:

- Did the cycle run correctly according to the program?
- Was the solution formulated and filled correctly according to the batch record?
- Were the correct vials and stoppers used, and were they prepared (cleaned and sterilized) correctly?
- Were there any changes in the excipients or active ingredient (different supplier, different lot, etc)?
- What are the finished product testing results, and what was the reason for failure (physical appearance, purity, residual moisture, etc)?
- Did the entire batch fail or were there only specific vials or trays that failed?
- If specific vials or trays failed, where were they located within the freeze-dryer (shelf number and location on shelf)?

Listed below are several items that should be requested for inspection:

- The printout from the cycle
- Several samples of product including product that failed testing and product that passed testing (control)
- The thermal characterization data for the formulation including the amorphous/crystalline character, the potential for metastable glass formation, the critical temperatures associated with the different phases (Tg', Te, Tc), and the freeze-dry microscopy data

Identification of a physical failure is made by conducting a visual inspection of the failed product and comparing it to product that meets specifications. Correct identification of the failure will greatly help in resolving the problem.

The different physical failures commonly observed in freeze-dried products are melt back and full or partial collapse. Melt back occurs when samples are warmed to secondary drying temperatures prior to completing primary drying. If primary drying is not allowed to go to completion, there will still be a slug of ice present at the bottom of the vial or tray. When the product is raised to the secondary drying temperature, the ice will melt leaving a dissolved layer of material at the bottom of the vial or tray. Figure 1 shows a vial of product that failed due to meltback.

Figure 1

Full or partial collapse occurs when the product temperature exceeds or partially exceeds the glass transition temperature of the product. It must be understood that collapse can occur either during primary drying or during secondary drying if the ramp rate is too aggressive to secondary drying conditions. Figures 2 and 3 show full and partial collapse, respectively.

Figure 2

Figure 3

Once the failure has been identified, the next task is to work through the maze of the various reasons why the failure occurred, and to identify a plan on how to fix the problem. By taking an empirical approach, it is possible, in many cases, to correct problematic formulations and cycles.

Case Studies in Failed Products

Parenteral contract manufacturing companies see a wide, diverse range of products. While every attempt is made to produce these products flawlessly, occasionally batches will fail when first transferred into the facility or scaled up from

development. When these failures occur, the development scientist is usually the first person called upon to diagnose and correct the problem. The following case studies come from a reputable contract manufacturing company, and represent several product failures and the steps taken to identify and correct the problems.

Case Study 1

The development group was approached because samples of a lyophilized product were collapsing on stability both at 25°C and 40°C. The samples at 40°C were collapsing after 4 weeks, and the samples at 25°C were collapsing after 8 weeks. Figure 4 is an image showing a fully collapsed sample that was taken after 2 months at 25°C, and a control that had been kept at -20°C.

Figure 4

The formulation and analytical data were as follows:

API: Peptide, Amorphous – 200µg/mL
Excipients:
40 mM Citrate buffer
pH 4.5
10 mg/mL mannitol
1 mg/mL sucrose
Tg': -32°C
Potency/Purity Post Lyo:
202 µg-mL/100%
Moisture: 3.5%
Fill Volume: 1mL
Vial Size: 3 mL

The cycle used to dry the samples was as follows:

Freezing:
5°C to –50°C at 1°C/min
Anneal at –10°C for 2 hours
Cool to –45°C

Primary Drying:
Shelf Temp: -28°C (product = -37°C)
Vacuum: 100 mTorr
Condenser: -80°C
Hold Time: 24 hours

Secondary Drying:
Shelf Temp: 25°C
Vacuum: 100 mTorr
Condenser: -80°C
Ramp Rate: 0.4°C/min.
Hold Time: 3 hours

As discussed above, the first step is to identify the failure. In this particular case, the failure was collapse, which means that the product exceeded it's Tg value on stability. The next step was to think about what influences the Tg in a product. It is understood that water acts as a plasticizer in amorphous solids. As the residual moisture increases, the Tg of the product goes down. Over time the Tg went below the storage temperature of the product and resulted in collapse. The next question to answer was how water was getting into the product. Upon further investigation, it was found that the moisture was coming out of the stopper over time. The stoppers used for this product were sterilized by running them through an autoclave. Under the high temperature, high steam conditions, the stoppers were absorbing moisture which they would release to the dry product (trying to reach equilibrium) over time. The Tg for this particular product dropped sharply in the presence of water vapor, so additional steps in the stopper preparation were needed. By adding a 4 hour dry heat step under vacuum, enough moisture

was pulled from the stoppers so that leaching into the product was no longer an issue.

Case Study 2

For this particular product, all of the samples that were being freeze-dried were completely collapsed after the cycle. Figure 5 shows an image of one of the samples that had fully collapsed. In addition to obtaining samples, the printout from the cycle, the analytical data from the testing, and the data from the thermal characterization study were all requested.

Figure 5

The formulation and analytical data were as follows:

API: Peptide, Amorphous – 1mg/mL
Excipients:
15 mM HEPES buffer
pH 7.5
7 mg/mL sodium chloride
15 mg/mL glycine
5 mg/mL trehalose
Tg': -45°C
Potency/Purity Post Lyo:
0.73 mg-mL/72.8%
Moisture: 20%
Fill Volume: 4mL
Vial Size: 10 mL

The cycle used to dry the samples was as follows:

Freezing:
5°C to –45°C at 1°C/min
Hold at –45°C for 4 hours

Primary Drying:
Shelf Temp: -40°C
Vacuum: 250mTorr
Condenser: -77°C
Hold Time: 17 hours

Secondary Drying:
Shelf Temp: 25°C
Vacuum: 150mTorr
Condenser: -77°C
Ramp Rate: 0.1°C/min.
Hold Time: 10 hours

The failure was identified as collapse, and attributed to exceeding the products collapse temperature (either Tg' during primary drying or Tg during secondary drying). The printout from the cycle was inspected, and is shown in below in Figure 6 (note this does not show the secondary drying step).

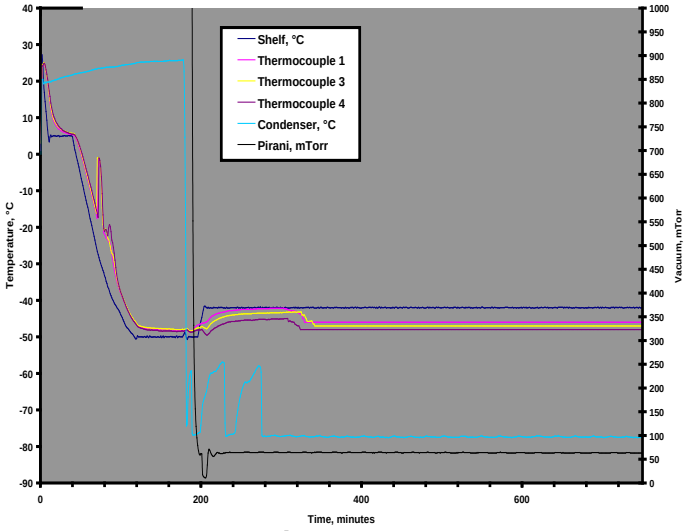

Figure 6

Upon inspection of this cycle printout and the thermal characterization data, it is clear why this product is collapsing. As shown, shelf temperature during primary drying is set to -40°C. As the product proceeds through primary drying, it's temperature will rise due to increased resistance from the dried layer that is being created as the sublimation front passes down through the product. According to the thermal

characterization data, this particular product has a Tg' of -45°C, which is exceeded before the completion of primary drying. By looking at the sample temperature probes, it is clear that this is occurring during primary drying.

The first thought to fix this problem might be to simply lower shelf temperature to keep the product below the Tg' until primary drying is complete. However, this product had some inherent problems that needed to be addressed prior to attempting another production run. The problem with this product is that it needs to be reformulated to improve the thermal properties. The Tg' for this product is excessively low (-45°C), and while a development scale freeze-dryer was able to successfully produce intact product, the less efficient, production dryer could not keep the product below it's Tg' and collapse resulted. Even if a production freeze-dryer could keep this product cold enough, it should be reformulated for the simple fact that keeping a product this cold during primary drying significantly suppresses the sublimation rate. While intact product may be obtained, the cycle would be so long, it would be prohibitive as part of a manufacturing process. For this particular example, by removing some of the problematic excipients (sodium chloride), and substituting excipients with better thermal properties, it was possible to raise the Tg' for this product from -45°C to -28°C, and the product could now successfully be dried in a reasonable amount of time.

Case Study 3

This particular product was being manufactured on an extremely large scale with many batches (millions of doses) being produced. The cycle was designed in development based on it's thermal characteristics, prior to being scaled up to large scale manufacturing. Batches of this product were then mass produced without issue until a problem occurred where 5% of the vials in one batch failed. As such, production shut down until the failure could be identified and corrected. This drug was very expensive, and the manufacturer did not want to risk throwing out fifty thousand doses per batch for additional failures.

In addition to obtaining samples, the printout form the cycle, the analytical data from the testing, and the data from the thermal characterization study were all reviewed. The sample obtained is shown in Figure 7. The control was a solid white cake (not shown).

Figure 7

The failed samples had a purple brown discoloration on the top of the cakes, and there was a ring of meltback on the bottom.

The formulation and analytical data were as follows:

- API: Small Molecule, 173 g/mol
- Solution Concentration: 450 mg/mL
- Eutectic Melting Temperature: -12.3°C
- Vial Size: 20 mL x 20 mm
- Lyophilization Cycle Ran Flawlessly
- Significant Degradation Products Detected by HPLC
- HPLC Potency: 150%

Additionally, the operators were questioned about the location of the failed samples within the dryer, and according to their statements, the

vials were randomly located throughout the chamber.

The first step taken was to identify the failure. While it was initially unclear about the reason for the discoloration on the top of the cakes (it was suggested that oil or residual cleaning solution was dripping into some of the vials during filling), it was clear that the ring on the bottom of the vials was caused my meltback. Based on this, it was clear that these particular vials, for whatever reason, were not completing primary drying. It was known that this was not a dryer issue (edge effect, etc), because other vials next to the failed vials were acceptable.

The next step was to look at the printout from the cycle. It is shown in Figure 8.

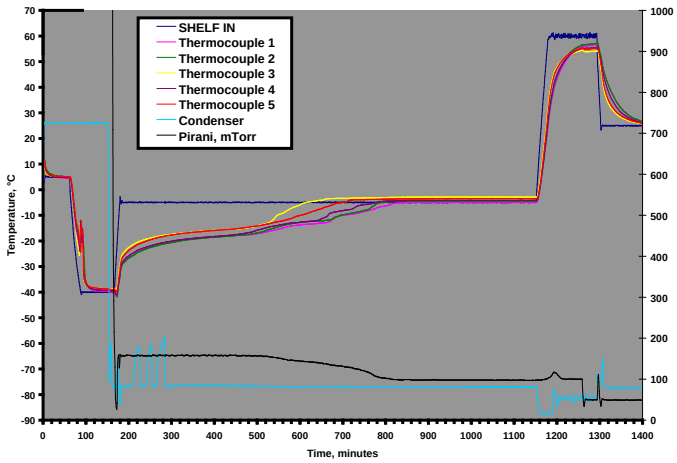

Figure 8

This cycle was compared to printouts from other cycles, and no deviations were found. Based on this, it was known that the freeze-dryer was not the problem.

The next step was to look at the analytical data. The HPLC results showed that a significant amount of degradation was occurring in these samples. Additionally, the potency of these samples was near 150% of target. Knowing that the time it takes a sample to complete primary drying is based on the temperature (which was perfect in this cases), and the total volume of ice to sublime, the development group suggested to engineering that some of vials were being overfilled prior to being loaded into the freeze-dryer. This would account for the samples melting back since there was now more ice to sublime, and would explain the increased potency in these samples. Upon further inspection of the filling line, a computer glitch was found which caused an additional half dose to be filled into the vials. Occasionally during a filling operation, the operators must stop the line to correct a problem (tipped over vials, improperly seated stoppers, etc). When the filling process was restarted, the computer because confused and would fill half of a dose in several vials that had already received a full dose. The discoloration was later determined to be due to the aggressiveness of the secondary drying phase. This particular molecule was very robust in regards to temperature, so secondary drying took place with a shelf temperature of 60°C. For the vials that melted back, the water would boil under the vacuum, percolate up through the product, and quickly degrade. One of the degradation products that formed happened to be colored (purple brown).

Conclusions

Understanding why batches of freeze-dried products fail, and the ability to resolve these issues can be a major challenge for the development scientist. By understanding the thermal characteristics for a particular product , asking the right questions, and being able to correctly identify the physical failure, allow the development scientist to methodically work through the process of correcting problematic cycles and or formulations.

About the Author: J. Jeff Schwegman, Ph.D. is a formulation and process development scientist in the pharmaceutical industry, where he has over 17 years of experience in developing freeze-dried products consisting of both small and large molecules. He is currently the founder and CEO of AB BioTechnologies which offers teaching and consulting services in pre-formulation, formulation, and lyophilization cycle

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