CASE STUDY

CONSIDERATIONS FOR DEVELOPMENT OF A LYOPHILIZED BIOSIMILAR

The patents for a number of parental biologic formulations are destined to expire in the United States between 2016 and 2027. Some of the well-known biologics include adalimumab, nivolumab, bevacizumab, trastuzumab, eculizumab, rituximab, infliximab, and pembrolizumab. All of the biologics listed here are monoclonal antibodies and all will be available for investigation as a biosimilar. This provides development opportunities for many companies, but also provides significant challenges. The biosimilar must closely match the analytical testing requirements for the biologic developed by the innovator company, as well as many other possible requirements (1-2).

There are several common concerns for direct comparison of biosimilars with an innovator product. The production of the large molecule may utilize a different cell expression mechanism. This may lead to changes to the physical structure of the molecule. These changes may lead to exposure of functional groups that are not exposed in the innovator product, which may result in a faster degradation pathway. There may be issues with variances in analytical data that may lead to questions about the similarity of the molecules. The expectations for analytical testing and comparison of the data are often the primary consideration when developing a biosimilar. However, there are also quality targets for the drug product that may affect the outcome.

Lindsay Wegiel, PhD
Research Scientist
Baxter BioPharma Solutions

Gregory Sacha, PhD
Senior Research Scientist
Baxter BioPharma Solutions

The innovator for the product establishes quality targets that are based on the original process development studies. The opposite can be true for the biosimilar product--the process must be designed to deliver the targeted product.

Initial planning for development of the biosimilar drug product includes identifying the main excipients for the formulation. This is relatively straightforward. However, the formulation may have included an organic solvent, and organic solvents are removed during lyophilization. This can make identifying components of a co-solvent system quite challenging.

This co-solvent system can directly affect the formulation and process development, so it is important to get it right.

The formulation for the biosimilar will be highly scrutinized by regulatory agencies and must closely match the formulation for the innovator product. For example, a biosimilar may exhibit sensitivity to interfacial interactions that are the same as those exhibited by the innovator product and a surfactant may offer protection from the interactions. The molecule may be sensitive to shear stresses imparted by the filling method (i.e. peristaltic vs. piston pump). Possible solutions are to avoid one mechanism of filling or perhaps offer protection to the molecule by adding a surfactant and conducting studies to demonstrate that it is acceptable. However, it may be difficult to justify the addition of the surfactant if one was not used for the innovator product. Another area of concern is if the product must have the exact same appearance as the innovator product. The reason for this concern is that a difference in appearance may suggest a difference in product quality. A general rule of thumb is that the biosimilar

should appear the same or better than the innovator product. Quality targets that affect the drug product are cake appearance and level of residual moisture in the dried solid. Both can be affected by the cycle used to lyophilized the formulation.

ACCEPTANCE CRITERIA FOR CAKE APPEARANCE

There is often an expectation that all lyophilized cakes must look perfect. However, a perfect appearance is not always possible due to the excipients used. When an amorphous sugar is used, it is expected that there will be some amount of shrinkage from the vial walls (Figure 1). Additionally, cracking of the solid may occur as a result of stresses during the drying process (Figure 2). Shrinkage and cracking are typical and are not often a sign of poor quality or stability. The main concerns are residual moisture, reconstitution time, and results from a stability-indicating analytical method. If the cake has poor appearance but meets the mentioned specifications, then there are no concerns about quality of the product. One should be concerned if the amorphous component crystalizes as this can cause major changes in stability.

Figure 1: Example of Cake Shrinkage

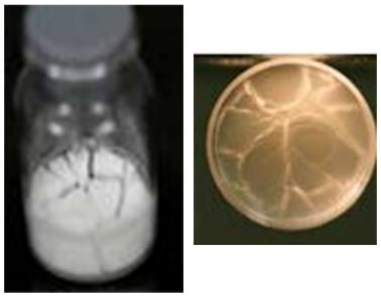

Figure 2: Example of Cake Cracking

IMPORTANCE OF THE VIAL

Does the biosimilar vial need to match the vial for the innovator product? Marketing may prefer that the vials are a perfect match; however, this may not be feasible. Branded products may utilize a custom vial. Sometimes these custom vials are also a custom size. Customization can increase the cost of the vial and this can increase the overall cost of manufacturing. Custom vials can have a long lead time for

ordering and this may risk a delay in the project timing. Most drug product manufacturers qualify certain readily available vial types and sizes. It is cost effective and more efficient if one of the available vial types can be chosen. This helps to ensure that the vial works well on the manufacturing line, and that equipment parts are already present at the manufacturing facility.

There are many sizes of vials available, and there are two common types. One type is a tubing vial and the other is molded (Figure 3). The size and type of the vial can affect the drying time for a lyophilized product. A narrower vial will have a higher fill height, and this will require more time for drying. It is recommended that the fill volume be no more than one-third of the overfill volume. The vial type also plays an important role. Molded vials typically have thicker walls, and may be less uniform. This can affect the heat transfer through the vial and increase the variability in the batch. Vial manufacturers are aware of these differences, and there are now molded vials that appear more similar to a tubing vial. Tubing vials are created from a cane of glass that is heated and cut to form the specific vial.

Figure 3: Example of Tubing Vial (two vials on left) vs. Molded Vials (two vials on right)

RESIDUAL MOISTURE IN THE DRIED SOLID

Residual moisture in the dried solid is affected by the components of the formulation, secondary drying shelf temperature, and secondary drying time. The level of residual moisture is important because it can directly affect the stability of the product. The effect of residual moisture can be different based on the type of molecule. Small molecules often exhibit poor stability when exposed to moisture. However, large molecules often require some

residual moisture for stabilization. The innovator may have a specification for the moisture level, but these specifications typically state that the moisture needs to be less than a certain amount. There is a balance, as too much moisture can affect the excipients in the formulation. Higher levels of residual moisture decrease the Tg of the formulation and result in poor stability when stored at temperatures above or at the Tg. Therefore, it is important to evaluate not only the moisture level of the innovator product, but have data on a range of moisture levels for the biosimilar such that an educated target for moisture level can be made.

QUALITY BY DESIGN APPROACH FOR EXAMINING THE EFFECT OF RESIDUAL MOISTURE

Studying the effect of residual moisture on the stability of the molecule is needed to make a data-driven decision on the moisture specification for the product. This requires preparing samples that exhibit a range in residual moisture levels, and storing them on accelerated stability for 2 to 4 months. A couple of methods for preparing samples have been used by researchers. One method exposes lyophilized samples to different relative humidity conditions using saturated salt solutions to obtain solids with different levels of residual moisture. This is an inaccurate method of preparing samples because the minimum relative humidity levels can range between 6% and 11% when using saturated salt solutions. Another method is to remove samples from the start of secondary drying and at defined intervals at a specific shelf temperature for up to about 10 hours. One sample from each time point is tested for residual moisture to obtain the approximate residual

moisture level for the samples removed at the specific time point. This method assumes that the residual moisture levels are equal for all vials at each time point, and does not provide actual residual moisture values for all vials.

Another approach to preparing samples that exhibit a range of residual moisture levels is to remove samples at the end of primary drying and after equilibration at specific shelf temperatures during secondary drying. Approximately 2-3 lyophilized samples are removed from the product chamber using a sample thief (Figure 4). The shelf temperature is then slowly increased in a stepwise fashion, removing approximately 2-3 vials at each shelf temperature after there has been enough time for the product temperature and Pirani gauge to equilibrate. All of the vials that were removed during the cycle are scanned nondestructively using NIR, followed by confirmation of the residual moisture destructively by Karl Fischer. The data are used to create a calibration curve for testing residual moisture using NIR. Data from the calibration curve are used to identify the shelf temperatures that will result in the desired moisture levels. A second batch of samples are prepared and freeze-dried. Samples are removed at the secondary drying shelf temperatures that will result in the targeted high, medium, and low levels of residual moisture. These vials are scanned using NIR to confirm the residual moisture level nondestructively using the calibration curve. The samples are then placed on accelerated stability.

Figure 4: Example of a LyoStar with a Sample Thief Door

CASE STUDY

A biosimilar was recently developed based on an innovator product that was filled into a custom 15 mL vial and utilized a reconstitution volume that was less than the original fill volume. The concentration for administration was part of the specifications for the product. It was desired that the biosimilar appear and behave the same as the innovator product. This resulted in a few challenges. First, using a custom vial would add 8 months to the time line due to the lead time in receiving the vial and acquiring the change parts for the filling equipment. Next, the concentration of the solution filled into the vials was unknown.

Figure 5: Image of the Innovator Vial (left) and Biosimilar Vial (right)

Ultimately, the risk to the time line was not acceptable. A 20 mL vial was chosen that was already qualified on the filling line (Figure 5). This not only saved time, but substantially reduced the overall cost of the project by not having to purchase new change parts for the equipment. This created a minor challenge of having a different fill height for the product.

Both the fill volume and concentration for the innovator product were unknown. Concentration can affect thermal behavior as well as the stability of the product. Therefore, it was desired that the biosimilar had the same solution concentration as was used for the innovator product. While the reconstitution volume was known, it was evident that the fill volume was less than the reconstitution volume as the reconstitution volume resulted in a solution that was over half the total vial volume (10 mL). The innovator product exhibited shrinkage of the solid away from the vial. Shrinkage is common for amorphous formulations, but can

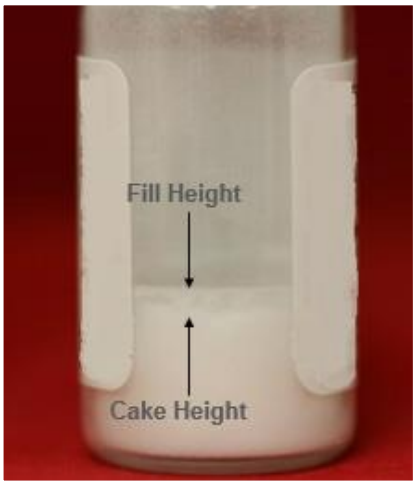

Figure 6: Image showing the fill and cake height differences

make it difficult to determine the original fill height. There is often a mark left on the vial from the original fill height even if the cake shrinks or crumbles (Figure 6).

The original concentration was determined by slowly adding water to the vial until the solution reached the level of the mark on the vial. The concentration was then measured and the fill volume was calculated. The fill volume was determined to be 6.7 mL. This volume was filled into the biosimilar vial and the height was slightly lower than for the innovator product (Figure 7). One of the quality targets for the biosimilar was for the dried solid to have the exact same appearance as the innovator product. The innovator product exhibited a cake with cracking and dusting of the solid on the interior surface of the vial (Figure 8). It was possible to design the lyophilization cycle and adjust the conditions for the end product to reproduce the cracking (Figure 8, next page). The concern was if the dusting of the solid on the glass surface could be reproduced. The behavior of the solid during shipping produced the desired results.

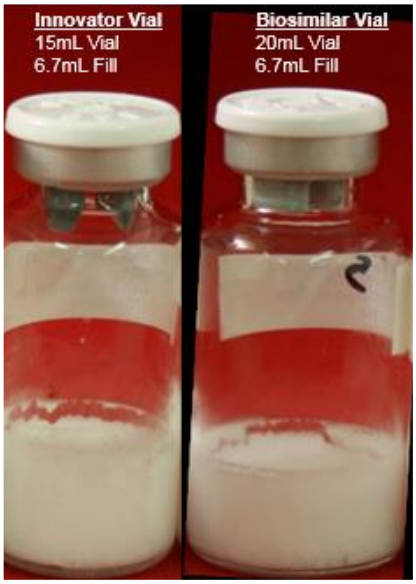

Figure 7: Image Comparing the Innovator Configuration to the Final Product

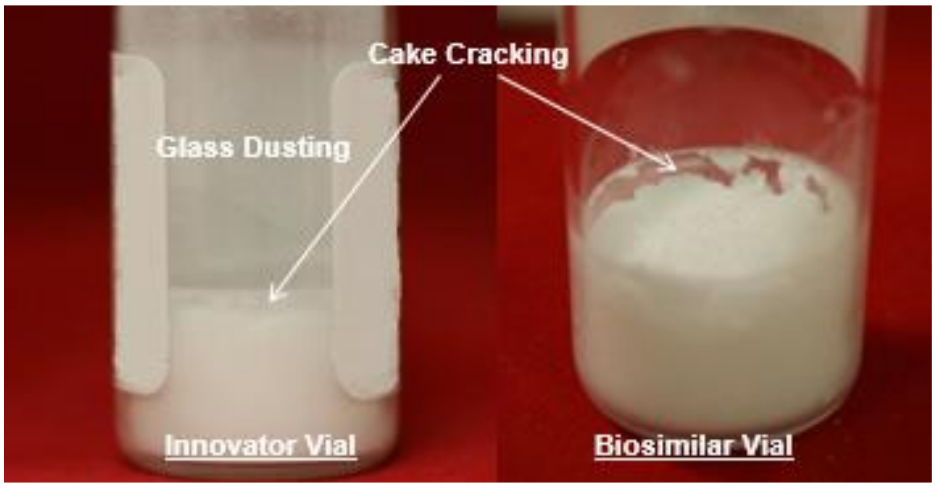

Figure 8: Image Comparing the Cake Appearance of the Innovator Vial (left) to the Biosimilar Vial (right)

The moisture level was the next development concern. Although the specification for residual moisture was known for the innovator product, there was no knowledge of the minimum acceptable residual moisture. This was a concern because the stability of large molecules can be negatively affected if the level of residual moisture is too low. The first step was to create a NIR calibration curve so that the vials could be tested for moisture nondestructively, as well as obtaining information about the moisture level at different secondary drying temperatures. A freeze-dry cycle was conducted whereby the shelf temperature during secondary drying was increased in a stepwise manner starting at 0°C, and increased in increments of 10°C until 40°C (Figure 9).

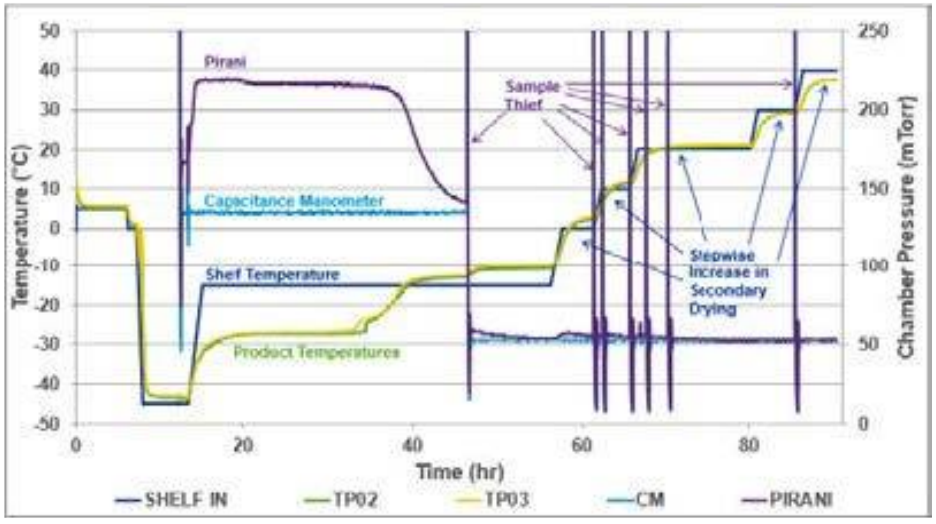

Figure 9: Lyophilization Process Parameters for the NIR Calibration Curve Freeze-Dry Run

The samples were scanned using NIR and the moisture level was determined by Karl Fischer. A calibration curve for the NIR was created whereby the moisture level was predicted within ±0.3% (Figure 10, next page).

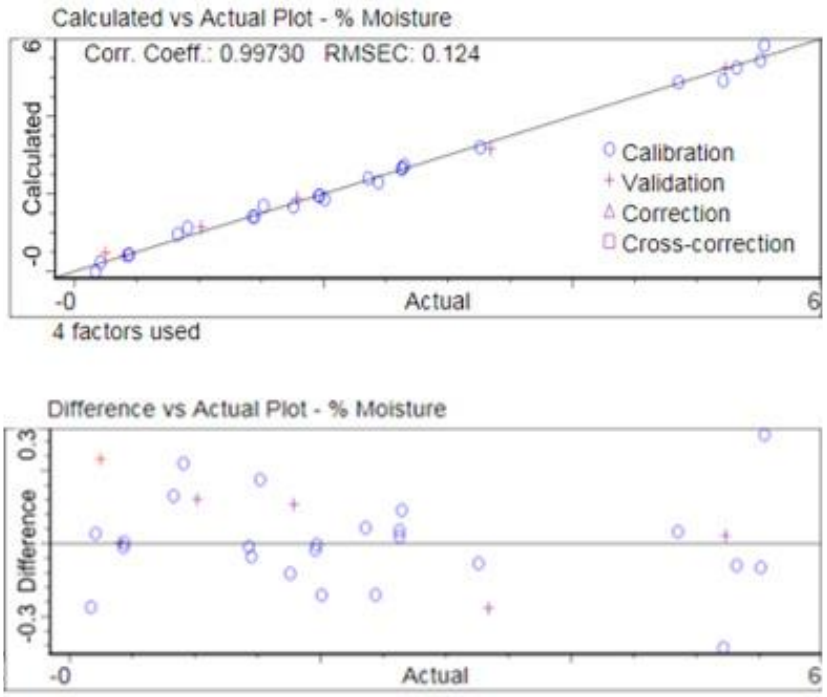

Figure 10: NIR Calibration Curve Plots

The innovator moisture specification was less than 2% but most of the vials had moisture levels around 1%. Data was needed to determine how the moisture level affected the stability of the samples. Samples were prepared using a freeze-dry cycle that targeted moisture levels of 5%, 3.5%, 2.5%, 0.4%, and 0.2% using the data from the secondary drying of the NIR calibration curve cycle (Figure 11). During this run the secondary drying temperature was increased in 4 steps at -5°C, 2°C, 30°C, and 40°C with thieving of samples at the end of primary drying and at each secondary drying temperature (Figure 12, page 8). The vials were close to targets with moisture levels of 5.4%, 3.6%, 2.5%, 0.6%, and 0.4% as determined by NIR (Table 1, Page 8). These vials were placed at 40°C/75% RH for 2 Months.

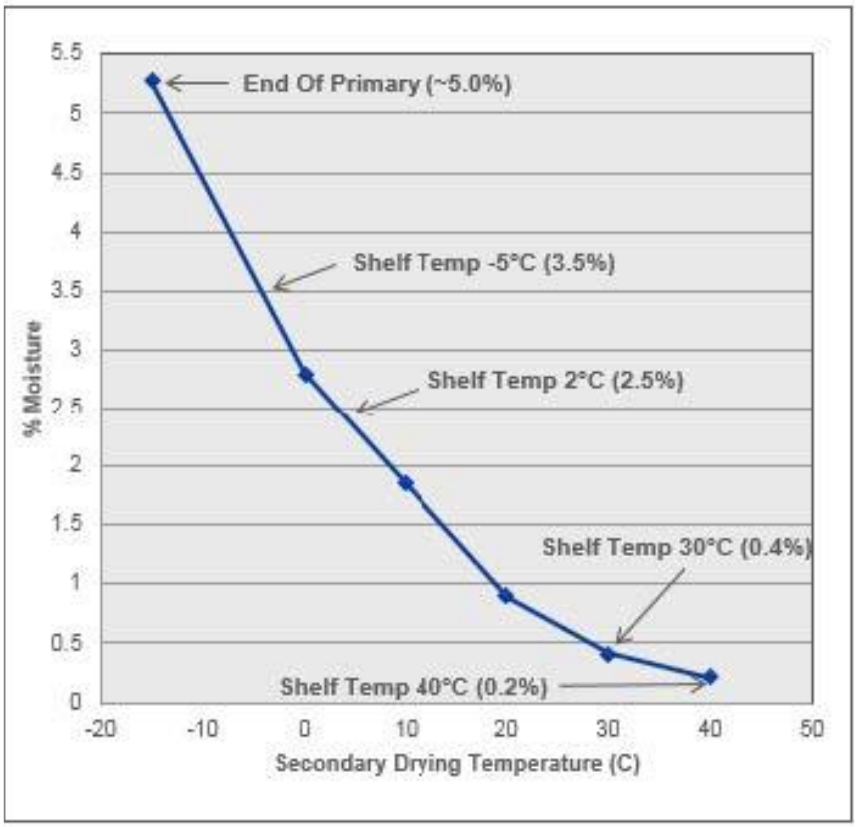

Figure 11: Secondary Drying Temperature vs. Percent Moisture Graph

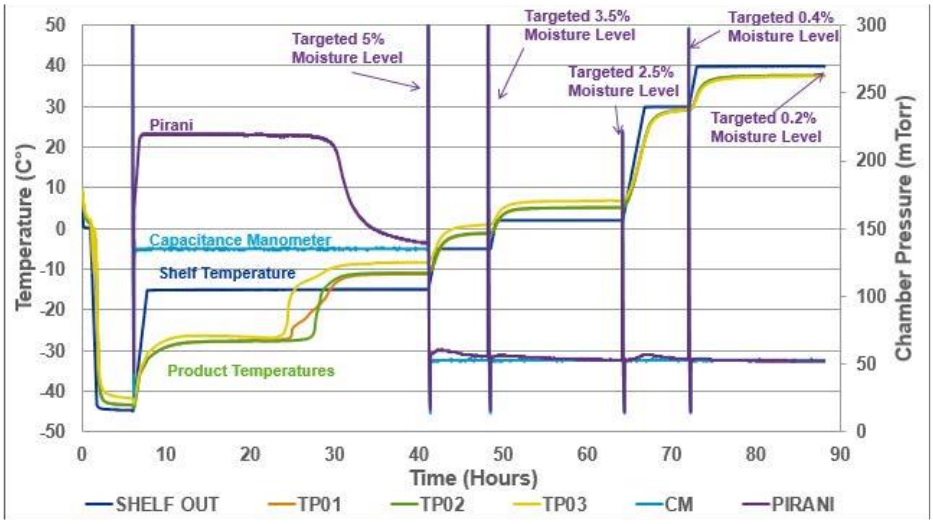

Figure 12: Lyophilization Process Parameters for the Moisture Study Freeze-Dry Run

Sample	Moisture	Average	Std.
EOP 1	5.42	5.44	0.06
EOP 3	5.49		
EOP 5	5.47		
EOP 6	5.36		
-5°C 1	3.57	3.61	0.05
-5°C 2	3.66		
-5°C 7	3.56		
-5°C 11	3.65		
2°C 2	2.35	2.48	0.10
2°C 8	2.55		
2°C 9	2.58		
2°C 10	2.45		
30°C 3	0.66	0.63	0.05
30°C 4	0.61		
30°C 5	0.67		
30°C 6	0.57		
40°C 2	0.20	0.18	0.02
40°C 3	0.19		
40°C 4	0.16		
40°C 7	0.18		

Table 1: Table of Moisture Values as Determined by the NIR Calibration Curve for the Moisture Study Freeze-Dry Run

The iCE data indicated an increase in the percent basic species for the low moisture samples. This suggested that there may be a lower boundary limit for the moisture level for this product. The data suggested that there was improved stability when the moisture level was above 0.6%. The data exhibited a small decrease in stability at 2 months once the moisture level reached 5.4%. Most residual moisture specifications only provide the acceptable upper limit.

Therefore, the effect of residual moisture on the stability of the product would have been unknown if the stability studies were not conducted. The residual moisture for the innovator product was around 1% and data from the stability study for the biosimilar demonstrated that a decrease in stability could be expected when the residual moisture level was at or less than 0.6%. The data aided in establishing the desired residual moisture for release of the product which was set at just above 1%.

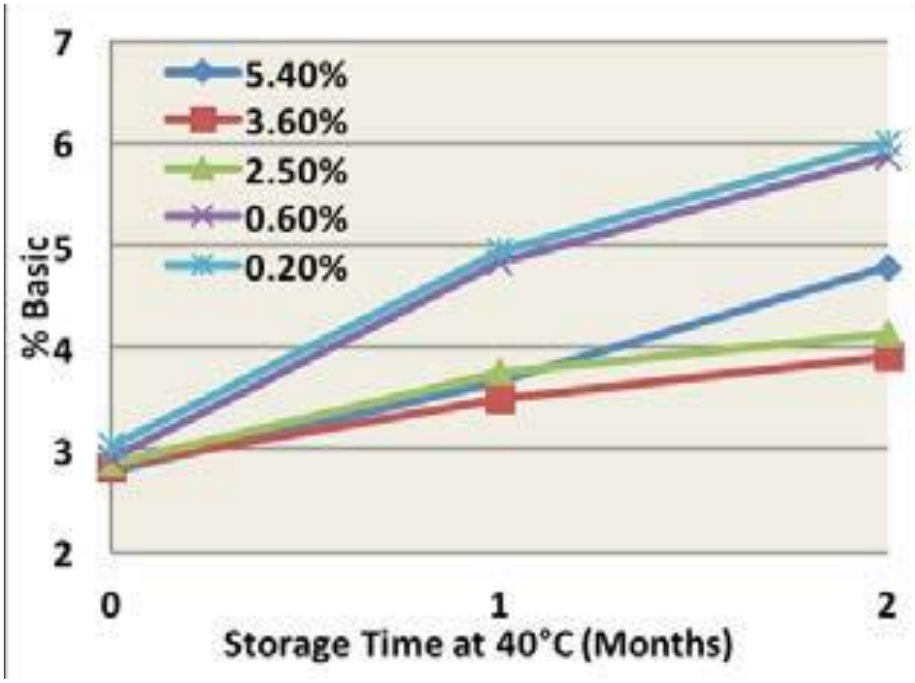

Figure 13: Percent Basic iCE Results from the Accelerated Moisture Study

OUTCOME/RESULTS

The product and process were successfully transferred to full-scale manufacturing. The learning points from the project provided valuable information about product appearance and process development. For example, it may not be necessary

to utilize the exact same vial to obtain the desired appearance for the dried solid, and it was determined that the change in vial size and fill height would have little to no impact on the results after conducting process development. Additionally, the study exemplified the importance of thoroughly understanding the effect of residual moisture on the stability of the product. This aided in adjusting the secondary drying cycle to ensure that the product was not too dry.

REFERENCES

1. US Food and Drug Administration. Guidance for Industry: Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product. Rockville: US Food and Drug Administration; 2015.

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Baxter Healthcare Corporation
One Baxter Paay Deerfield, Illinois, USA 60015
Email: biopharmasolutions@baxter.com
Website: biopharmasolutions.baxter.com

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