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Container Closure Integrity and Deep Cold Storage

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Effects of Deep Cold Storage at -80°C on the Container Closure Integrity of Sterile Product Vials

Derek Duncan

CERTAIN STERILE PHARMACEUTICAL PRODUCTS REQUIRE DEEP COLD STORAGE, EITHER AT -80°C OR EVEN CRYOGENIC TEMPERATURES (DOWN TO -196°C). LIVE VIRAL VACCINES, GENE THERAPIES, OR PRODUCTS THAT CONTAIN ACTIVE CELLS (CELL THERAPIES) OFTEN NEED DEEP COLD STORAGE TO MAINTAIN STABILITY AND/OR ACTIVITY. STUDIES HAVE SHOWN THAT DEEP COLD STORAGE TEMPERATURES CAN INTRODUCE RISK TO THE CONTAINER CLOSURE INTEGRITY (CCI) OF VIAL-RUBBER STOPPER COMBINATIONS TRADITIONALLY USED TO FILL STERILE PHARMACEUTICAL PRODUCTS (1-3).

Observations of overpressure have been reported in stoppered vials after storage at -80°C. When syringes were inserted into these vials, the plungers moved upwards and once the syringe was removed, product sprayed from the punctured hole indicating a substantial overpressure inside the vial. This situation raises concerns not only about the stability and efficacy of the formulation, but also about the safety of the administering healthcare professional and the patient, especially if the product vial contains a live viral vaccine.

EFFECTS OF DEEP COLD STORAGE AT -80°C ON THE CCI OF STERILE PRODUCT VIALS

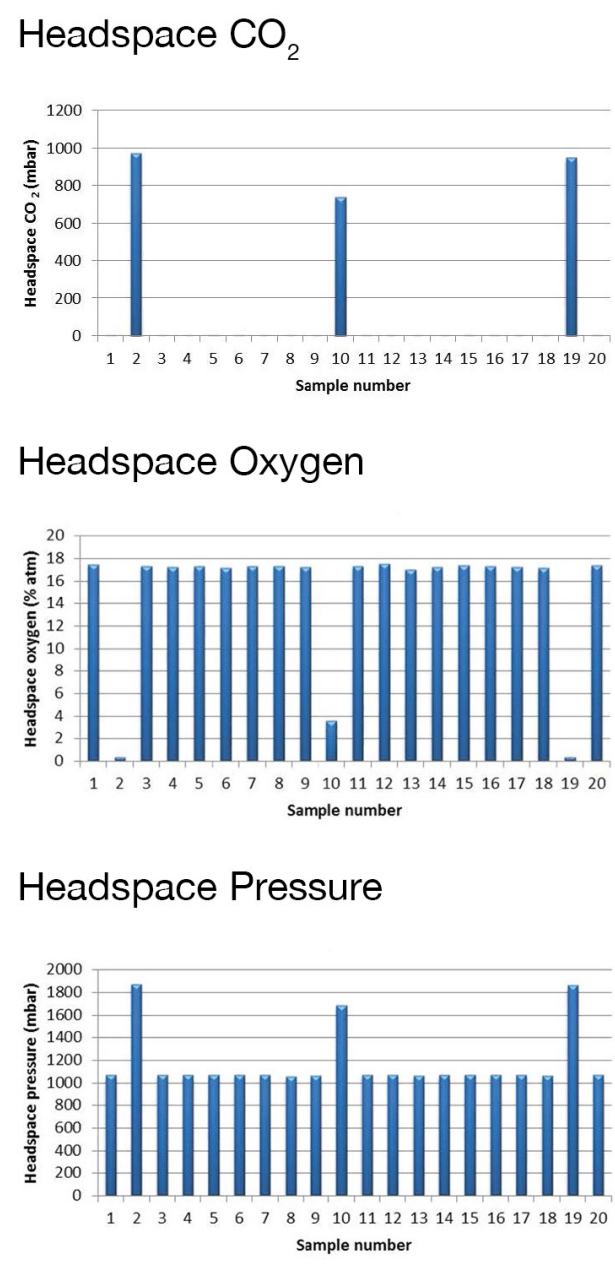
Although this vial overpressure was occasionally observed and reported, it was not until recently that analytical data was generated that clearly demonstrated the root cause (1). When vials suffering from overpressure were tested using laser-based headspace gas analysis, the results showed headspace conditions drastically different from the headspace conditions initially achieved by the manufacturing and vial sealing process.

For example, 20 vials were filled with media and sealed under atmospheric air conditions. After one week of storage at dry ice temperatures (-80°C), the vials were analyzed using non-destructive laser-based headspace gas analysis. The results displayed in FIGURE 1 identify three vials with overpressure as well as elevated levels of carbon dioxide and depleted levels of oxygen.

CCI Failure During -80˚C Storage as Root Cause of Vial Overpressure

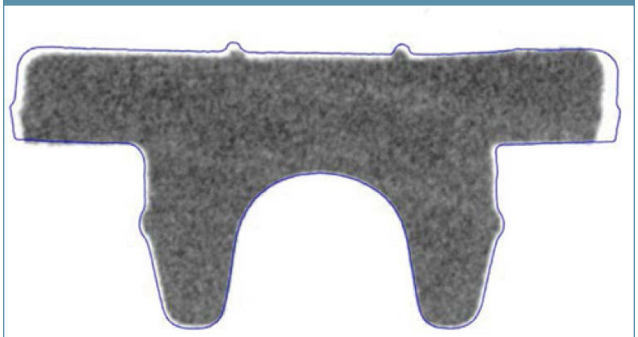
The resulting vial overpressure in FIGURE 1 after storage on dry ice (-80°C) is the consequence of a temporary loss of CCI during the deep cold storage period. Cold, dense carbon dioxide gas ingresses into the leaking vials during this storage period, displacing the initial air headspace. The cold, dense carbon dioxide from the storage environment is trapped after the vials reseal once removed from cold storage and allowed to come up to room temperature. This results in depleted oxygen, elevated carbon dioxide content, and elevated total pressure levels in the headspaces of the vials that leaked.

FIGURE 1: Headspace carbon dioxide, oxygen, and pressure measurements on 20 vials filled with media and sealed under atmospheric air conditions. The three different headspace conditions of three vials indicate these vials had temporarily leaked while stored on dry ice.



EFFECTS OF DEEP COLD STORAGE AT -80°C ON THE CCI OF STERILE PRODUCT VIALS

FIGURE 2: An x-ray tomography image showing shrinkage of a rubber stopper at cryogenic conditions. The blue line shows the form of the stopper at room temperature. Photo courtesy of Cedric Gysel, Janssen.



There are several material phenomena that lead to this temporary loss of CCI. Commonly used rubber butyl stoppers lose their elastic properties at these low temperatures because the glass transition temperatures (T_g) of the rubber formulations lie between -55°C and -70°C. In a range of temperatures around the T_g, the rubber stopper becomes brittle. In addition, the packaging components shrink at varying rates due to the different rates of thermal expansion of the materials (glass vial, rubber stopper, metal crimp) leading to possible gaps at the material interfaces. FIGURE 2 shows an x-ray tomography image showing shrinkage of a rubber stopper of roughly 8.5% by volume at cryogenic conditions. Most of this shrinkage occurs at temperatures warmer than the T_g. By contrast, the shrinkage by volume of a borosilicate glass vial will be an order of magnitude less. When the rubber stopper loses its elastic properties and if gaps appear between the sealing surfaces due to material shrinkage, there is a risk that seal integrity could be lost.

In addition, as the temperature drops from room temperature (RT) to -80°C, the headspace gas pressure in the vial drops from 1 atm to 0.66 atm resulting in a pressure differential with the storage environment. If CCI is lost during -80°C storage, the non-sterile cold, dense gas from the storage environment (i.e., air from a -80°C freezer or carbon dioxide from dry ice) rapidly leaks into the stored vial. When the leaking vial is taken out of cold storage and warms up to a temperature above the T_g, the stopper regains its elastic properties, the packaging components expand to their original forms, and the sample can reseal, trapping the cold, dense gas from the storage environment inside. As the vial continues to warm up to RT, an overpressure builds up in the vial. This temporary leak could risk the stability as well as sterility of the pharmaceutical product and would not be detected by traditional CCI test methods.

Mitigating the Risk of CCI Failure During -80°C Storage

There are several approaches for mitigating the risk of CCI failure during -80°C storage.

1. Robust packaging development studies implementing appropriate CCI test methods are needed to choose appropriate vial/stopper combinations and to demonstrate sealing performance during deep cold storage.
2. In addition, studies have shown that robust capping and crimping that consistently achieves appropriate

stopper compression minimizes the risk of CCI failure. Loosely capped vials are more prone to suffer from CCI issues during deep cold storage than tightly capped vials—the use of residual seal force (RSF) testing can help qualify capping and crimping lines.

3. Finally, polymer vials are increasingly being considered for deep cold storage applications. Polymer vials will shrink at similar rates to the rubber stopper and there is some evidence that the polymer-to-polymer interface provides an advantage to maintaining a good seal during deep cold storage.

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Holistic Approach for Ensuring Container Closure Integrity of Product Requiring Deep Cold Storage

Derek Duncan and Roger Asselta

Introduction

Live viral vaccines and gene and cell-based therapies sometimes require deep cold storage temperatures (-80°C down to cryogenic, -196°C) to maintain the activity and efficacy of the formulations. Studies have shown that these deep cold storage temperatures can introduce risk to the sealing performance of the packaging components (1). It is therefore critical that robust development work is done in a holistic framework to choose the appropriate packaging components and to qualify the sealing process such that the risk of container closure integrity (CCI) issues during deep cold storage and transport is minimized.

The work done so far investigating CCI of stoppered vials at -80°C has identified the following critical areas for ensuring CCI at deep cold storage temperatures:

- The choice of appropriate packaging component combinations taking careful consideration of worst-case fits and stack tolerance.

"To seal a stoppered vial, an applied stress (sealing force) deforms or compresses the elastomeric stopper against the container sealing surface."

- Qualification of the capping & crimping process to produce robust consistent stopper compression for sealing.

CCIT Methodologies to Perform Method Development Studies

In order to quantify the risk of -80°C storage to the CCI of stoppered vials, CCI test methods are required that can generate robust science-based data during development studies. Over the past few years, a significant amount of data has been generated for deep cold storage product using CCI test methods based on headspace gas analysis. Most of this work has been performed in development to support the choice of appropriate packaging components and to qualify the sealing process. There are also examples of 100% CCI testing of GMP product (2).

Laser-based headspace analysis as a technique for CCI testing has several advantages including:

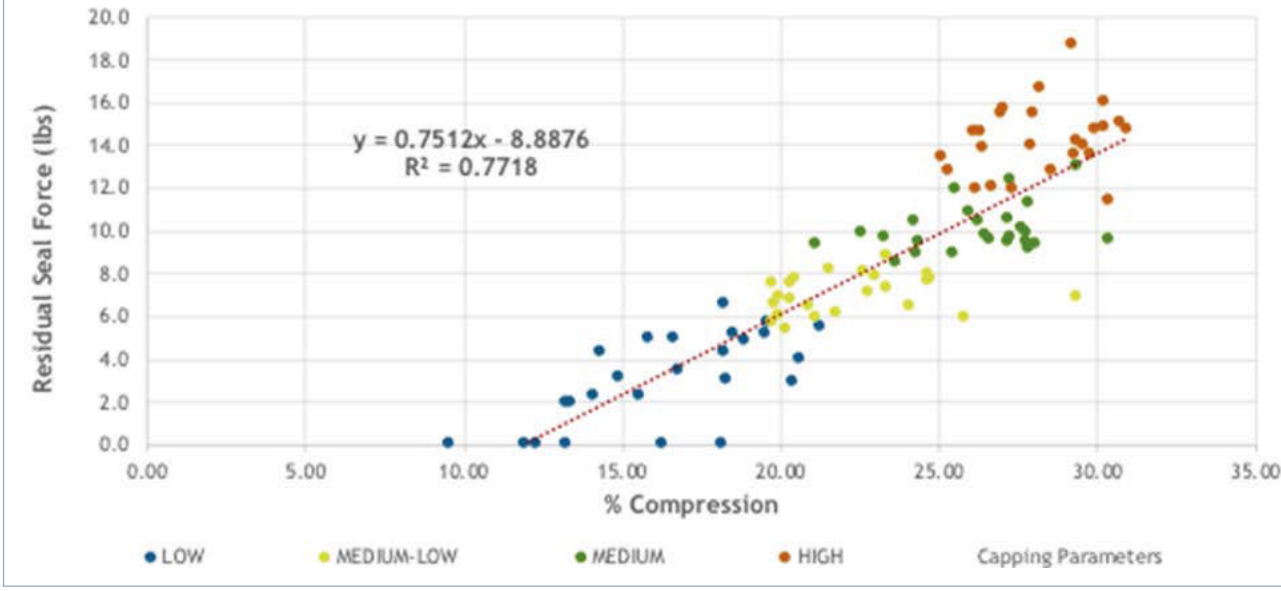
- The measurement is non-destructive meaning samples that have been analyzed for CCI can be kept for other purposes.
- The measurement is rapid; a measurement time of a few seconds allows for data to be generated in a straightforward manner on hundreds (or thousands) of vials to gain statistical insight into the sealing performance as a function of various parameters.
- Product vials can immediately be measured upon removal from the cold storage environment; no thawing is required.

Using headspace analysis for CCI testing leverages the fact that a leaking vial will exchange gas with the surrounding storage environment. When the vials are initially stored at -80°C from room temperature, a partial vacuum within the headspace develops due to the decrease in temperature. If a leak is present, the atmosphere of the storage environment will flow into the vial headspace. When the leaking vial is brought out of cold storage, the vial can reseal trapping cold dense gas inside. As the vial continues to warm up, the trapped gas expands creating an overpressure in the vial. This type of leakage during deep cold storage can be identified by measuring an increased headspace pressure or, in case of storage on dry ice, by elevated carbon dioxide levels.

Residual Seal Force for Qualifying Capping and Crimping to Produce Good Sealing at -80°C

To seal a stoppered vial, an applied stress (sealing force) deforms or compresses the elastomeric stopper against the container sealing surface. This induces a corresponding strain in the stopper, creating a contact stress at the vial/stopper interface. The strain is "locked-in" by applying and crimping an aluminum ferrule over the stopper. This locked-in

FIGURE 1: Residual seal force vs. rubber stopper compression for various capping and crimping settings.



compression, or stored internal energy, is known as the Residual Seal Force (RSF). RSF is the stress a compressed rubber stopper flange continues to exert on the vial's sealing surface after crimping the aluminum seal and is a measure of vial seal tightness. By correlating RSF measurements to stopper compression and CCI testing during packaging development, they may be used as a predictor of the risk to container closure seal integrity. In particular, studies have shown RSF measurements to be a suitable qualitative test method to evaluate seal quality in deep cold storage applications (3). FIGURE 1 summarizes the results of a packaging development study correlating RSF to the rubber stopper compression achieved by varying the capping and crimping settings.

As shown in FIGURE 1, RSF testing can be used to characterize the seal quality of capped vials through a correlation with stopper compression. These values can be used to establish the optimal cap sealing process parameters for various types of capping equipment, which can then facilitate the comparison and consistency of seal quality of sealed vials manufactured on different equipment in different facilities (4).

The data in FIGURE 2 demonstrates how RSF testing enabled the characterization, optimization, and eventual qualification of a capping and crimping line. Initial RSF testing showed the capping and crimping line produced vials with relatively low RSF values (~35 N) and a large vial-to-vial variability. Optimizing the capping and crimping parameters improved the line performance such that vials were being produced with higher RSF values in a more consistent range of values.

As mentioned earlier, robust capping and

FIGURE 2: Results showing how RSF testing can enable the optimization of capping and crimping parameters to consistently produce tightly sealed vials.

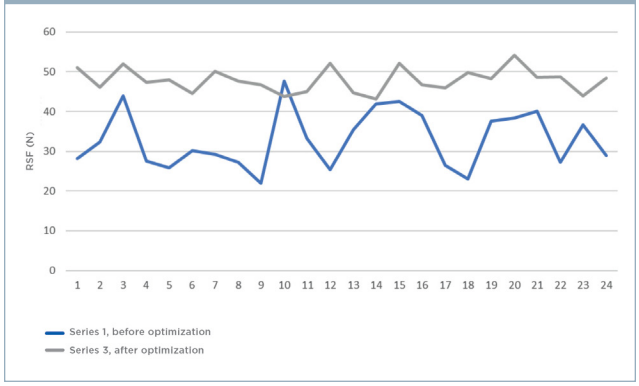
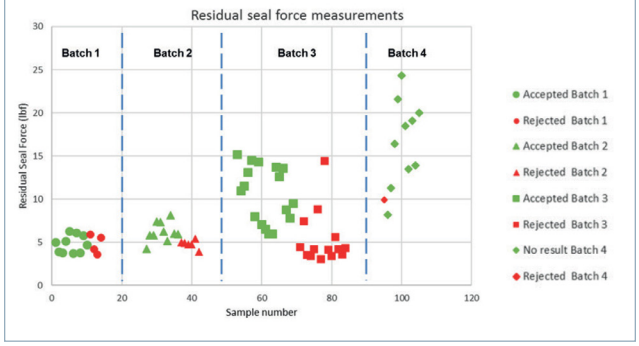


FIGURE 3: RSF vs CCI correlation data showing that low RSF values correlate to increased risk of CCI issues.



crimping can be critical for product vials to maintain CCI during deep cold storage. In addition to conducting RSF correlation studies with stopper compression, studies can be done to investigate the correlation between RSF and CCI. FIGURE 3 shows the results of a study performed on vaccine product vials that were stored at -80°C. The results of non-destructive CCI testing using headspace analysis were correlated to RSF measurements made on the same product vials. The red data points represent product vials that were rejected as having lost CCI during cold storage according to a leak limit defined as part of a headspace CCI test method, while green data points represent product vials accepted as having maintained good CCI. The results in FIGURE 3 show that low RSF values correlated to an increased risk for losing CCI. As part of a holistic approach for lowering the risk to CCI during deep cold storage, the manufacturer initiated a program in which the capping and crimping lines where optimized and qualified using RSF measurements, and finished product was CCI tested using headspace analysis as part of in-process control activities.

Summary

Deep cold storage at temperatures around -80°C can introduce risk of temporary loss of CCI of stoppered vials. Identification of appropriate primary packaging components and the qualification of a robust capping and crimping process can lower the risk to CCI. A holistic science-based approach involves executing packaging and process development studies to generate robust data demonstrating the consistent production of vials that maintain good CCI during deep cold storage and transport.

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Seal Quality Testing: An Introduction to Residual Seal Force

Roger Asselta

Why measuring the residual seal force of a compressed rubber stopper in a vial is an important seal quality test

Since the early part of the last century, crimped, stoppered vials have been a primary packaging system for parenteral medicines. The system has proved effective and reliable in containing and protecting the quality, safety, and efficacy of many injectable drug products. Still widely used today the storage requirements of some vaccines, biopharmaceuticals and gene and cell therapies such as deep cold storage present new challenges to the robustness of the traditional vial systems. Traditionally, drug products may have be stored and transported at or near room temperature (20°), chilled (4 to 8°) or frozen (down to -20°). Today, some vaccines and biopharmaceuticals require temperatures down to -80° and some cell therapies need cryopreservation (-150 to -195°).

For a vial system to be suitable for its intended use of containing and protecting the quality, safety and efficacy of the parenteral drug product it must be “well sealed” and maintain integrity through administration to the patient. This life-cycle approach mandates a thorough understanding of Container-Closure Integrity (CCI),

SEAL QUALITY TESTING: AN INTRODUCTION TO RESIDUAL SEAL FORCE

Maximum Allowable Leakage Limits (MALL), and Inherent Package Integrity (IPI).

Container–Closure integrity is defined in *USP* <1207> as "the ability of a package to prevent product loss, to block micro-organism ingress, and to limit entry of detrimental gases or other substances, thus ensuring that the product meets all necessary safety and quality standards".

USP <1207> introduces two important concepts in considering parenteral packaging integrity. First, the Maximum Allowable Leakage Limit (MALL), which is the greatest leakage rate (or leak size) tolerable for a given product–package that poses no risk to product safety and no or inconsequential impact on product quality. The second concept is Inherent Package Integrity (IPI), defined as the leakage rate of a well-assembled (sealed) container/closure system (CCS) using defect-free components. Defect free does not mean perfect or even nominal, but that they conform to specification. Packaging component and system variation must be well understood to determine IPI.

MALL establishes the requirements for CCI that are necessary to protect the critical quality characteristics of the drug product. Inherent Package Integrity is the ability of the container closure system to meet those needs. Understanding a drug product's MALL and the intended container's IPI should be integrated into the risk assessment process and part of a holistic approach in product life cycle management.

An important seal quality test to assess how well a vial is sealed is the measurement of the Residual Seal Force (RSF) of the compressed rubber stopper (*USP* <1207.3). Correlating RSF to a Container Closure Integrity Test (CCIT) method and evaluating changes over time and conditions will determine the system's Inherent Package Integrity.

Since the 1970s and long before its inclusion as a seal quality test in *USP* <1207.3>, RSF has been used in assessing parenteral vial sealing. To seal a stoppered vial, an applied stress (sealing force) deforms or compresses the elastomeric stopper against the container sealing surface and induces a corresponding strain in the stopper, creating a contact stress at the vial/stopper interface. The strain is "locked-in" by applying and crimping an aluminum ferrule over the stopper. This locked-in compression or stored internal energy is the Residual Seal Force. RSF is the stress a compressed rubber stopper flange

FIGURE 1: The compression curve (green) is a combination of the viscous and elastic responses to the stress from the tester load. "The knee" (yellow) is where additional deformation occurs. An algorithm is applied, using the 1st (purple) and 2nd (blue) derivatives to accurately identify that knee.

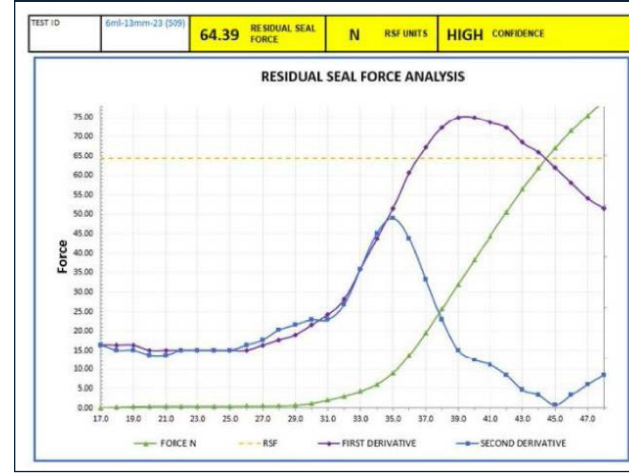
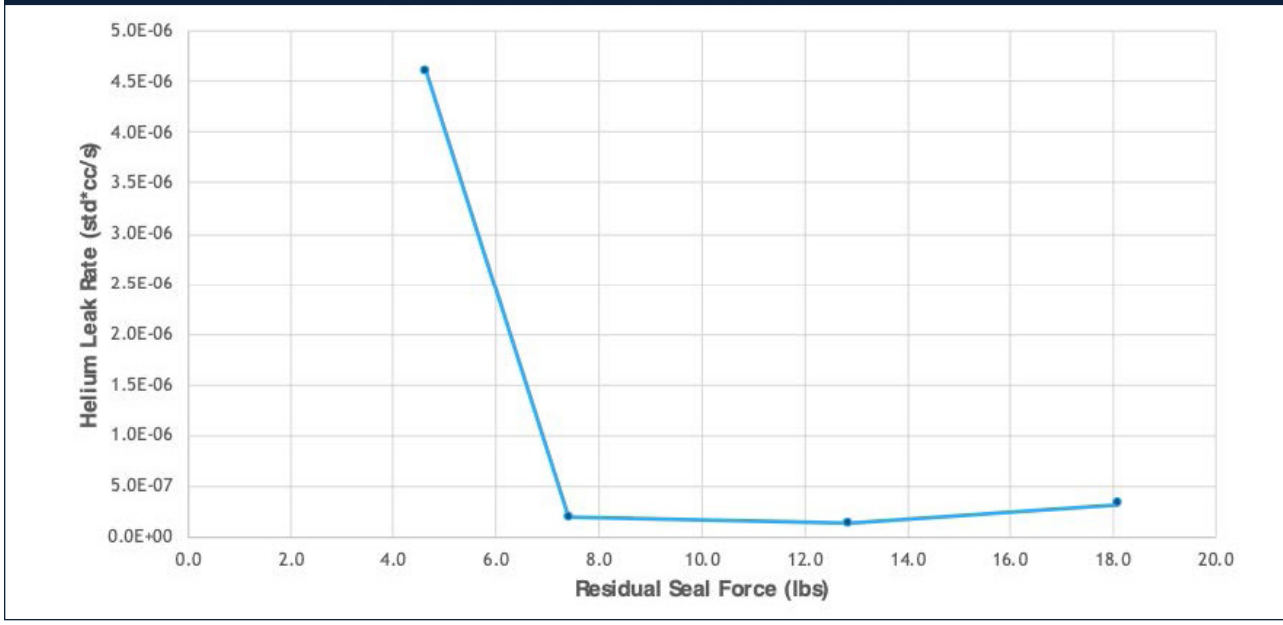


FIGURE 2: Residual seal force versus helium leak rate.



continues to exert on the vial's sealing surface after crimping the aluminum seal. RSF is a measure of vial seal tightness.

RSF is determined by identifying the point at which an applied force to the sealed vial further deforms the elastomeric stopper. Initially this was performed visually using a stereoscope, to observe when the bottom of the crimp first moves down as the force is applied. Techniques using universal testers were developed to evaluate the further deflection of the deformed rubber and plotting a stress/strain (distance v. force) curve. The tester exerts force on the Cap/Stopper. When the tester force exceeds the closure compression force, graphically the stress-strain slope (rate of change) drops. The bend or "knee" in that curve indicates the point of further deflection and is identified as the Residual Seal Force.

In 1993, John Ludwig and colleagues applied sophisticated algorithms to evaluating the stress/strain curve providing more objective analysis. Meanwhile, Dana Morton (Guazzo) demonstrated the correlation of RSF to stopper compression and leak rate cut-off, showing the higher the compression of the stopper flange, the higher the RSF value, the lower the leak rate.

These early works led to the application of RSF testing in the package development and validation of parenteral vials systems, including: the choice of materials and components, understanding the effects of material and component variation, confirmation of seal tightness, characterizing a well-sealed vial during assembly and crimping, establishing optimum crimping process parameters and control, and aid in appropriate crimping process validation.

In 2010, Orosz and Guazzo demonstrated a correlation of RSF to leaking marketed vials and they were able to differentiate between

well sealed vials and those presenting a "sterility" risk. "RSF values may be used in effectively setting up vial cappers and for monitoring the crimping process. With an understanding of compression and leak rate cut-off, RSF can be further used as a predictor of leakage risk". In 2012, Zuleger established qualitatively that an "appropriately tight capping and crimping process" is important for storage at -80°. Duncan and Asselta (2015) demonstrated RSF as a suitable qualitative test method to evaluate seal tightness in deep cold storage applications: "RSF measurements can be a useful tool in quantifying seal tightness and be predictive of CCI failure at low temperatures."

Mathaes and his colleagues showed how: "The RSF tester can be used to characterize the resulting residual seal force of a capped vial independent of the capping equipment used, which can facilitate the comparison of seal quality of drug product units manufactured in different facilities." Additionally, they demonstrated that a suitable RSF range that would still show full CCI, is recommended specific for each CCS combination and can be established using different capping equipment. Ovadia et al. (2019) did extensive work evaluating the applicability of RSF testing in evaluating capping processes and understanding variation, writing: "The ultimate goal of capping is to achieve long-lasting CCI of the container closure system. Thus, the relationship between RSF and CCI should be understood to allow the use of the RSF tester during routine commercial manufacturing". As critical as CCI is to a parenteral product, aesthetic quality or "pharmaceutical elegance" should be considered. Oni et al. (2019) demonstrated how RSF can be used to balance CCI with aesthetic quality and can influence the selection of components.

Combining RSF with CCI test methods and other tools such as x-ray tomography and predictive modeling provide a robust and holistic approach to assuring a parenteral vial system's container closure integrity through its life cycle.

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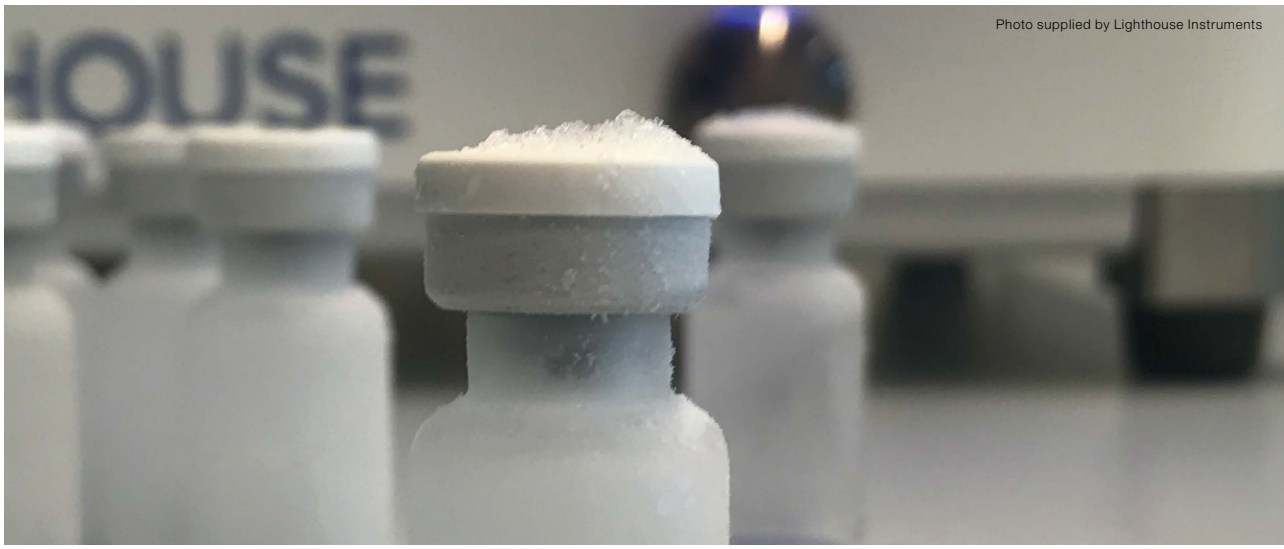
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Container Closure Integrity Test Method Development for Vials Stored at -80°C

Allison Alix

STERILE PHARMACEUTICAL PRODUCTS SUCH AS LIVE VIRAL VACCINES, GENE THERAPIES, AND PRODUCTS THAT CONTAIN ACTIVE CELLS OFTEN REQUIRE DEEP COLD STORAGE, EITHER AT -80°C OR CRYOGENIC TEMPERATURES (-196°C) TO MAINTAIN STABILITY AND/OR ACTIVITY. THESE STORAGE CONDITIONS POSE A CHALLENGE TO THE PACKAGING COMPONENTS; IN PARTICULAR TO THE SEALING PERFORMANCE OF THE VIAL/RUBBER STOPPER COMBINATIONS TRADITIONALLY USED TO PACKAGE THESE PRODUCTS. IT IS THEREFORE ESSENTIAL TO GENERATE ROBUST DATA THAT DEMONSTRATES THE MAINTENANCE OF SEAL INTEGRITY DURING DEEP COLD STORAGE AND/OR TRANSPORT WHICH, IN TURN, REQUIRES A ROBUST CONTAINER CLOSURE INTEGRITY (CCI) TEST METHOD. THIS ARTICLE DESCRIBES THE DEVELOPMENT OF SUCH A TEST METHOD FOR VIALS STORED AT -80°C.

The glass transition temperature (T_g) of rubber stoppers used to package many pharmaceutical products ranges from -55 to -70°C. When the rubber stopper is exposed to temperatures below its T_g, it runs the risk of losing its elasticity and becoming brittle.

In addition, the differing coefficients of thermal expansion of the packaging material (glass, rubber, metal crimp) means that the packaging components are shrinking at different rates, potentially leading to gaps at the material interfaces. The combined effects of these phenomena can result in potential breaches in CCI. The leaks that are created in this process are often transient, meaning they can reseal when the vial is brought up to room temperature, at which point the rubber stopper regains its elastic properties and the packaging components regain their initial forms. This makes these types of defects difficult to find and/or monitor (1, 2).

One method for detecting these defects is to use a gas ingress approach in which a tracer gas, such as carbon dioxide, is present in the freezer where the vials are stored. Carbon dioxide exists in very low concentrations at ambient conditions and is rarely used as a headspace purge gas, making it a good candidate for a tracer gas. Note that often times, vials stored at cold temperatures are shipped on dry ice, so they may be exposed to carbon dioxide during the transport process. When the vials are initially brought down to -80°C, a partial vacuum within the headspace develops due to the decrease in temperature. If a leak is present, the carbon dioxide enriched atmosphere of the cold storage environment will flow into the vial headspace. As previously mentioned, if the vial is brought up to room temperature (i.e., for patient administration), the leak may reseal, trapping the cold carbon dioxide present in the storage environment in the vial headspace while also creating an increase in pressure as the vial warms up.

"If the vial is brought up to room temperature (i.e., for patient administration), the leak may reseal, trapping the cold carbon dioxide present in the storage environment in the vial headspace while also creating an increase in pressure as the vial warms up."

A leaking vial can therefore be identified by measuring elevated carbon dioxide levels in vials after they are removed from the cold storage environment.

This headspace gas ingress method for CCI testing can be used during the development phases of the product lifecycle, including during initial selection of the packaging components to determine if a particular stopper and vial combination are appropriate for cold storage. As an example, **TABLE 1** summarizes a sample set for choosing between two different vials, a 2R glass vial and a 2mL polymer vial, both using the same injection stopper and crimp cap. It includes unmodified "samples" (empty intact vials) as well as three different sets of positive controls: vials with laser-drilled defects in the glass body of the vial, vials with tungsten micro-wires at the stopper seal interface, and vials with a needle through the stopper. The positive controls should be chosen based on where the defects are expected to be and should be included in various sizes of interest, to confirm the limit of detection of the method for a particular vial/stopper configuration.

TABLE 1: Sample Set Summary

Group	Vial Type	Defect Size* (µm)
Unmodified Sample	2R Glass Vial & 2mL Polymer Vial	none
Laser-Drilled Defect Positive Controls	2R Glass Vial	2
Tungsten Micro-Wire Positive Controls	2R Glass Vial	5
	2R Glass Vial	10
	2R Glass Vial & 2mL Polymer Vial	20
	2R Glass Vial & 2mL Polymer Vial	41
	2R Glass Vial & 2mL Polymer Vial	64
	2R Glass Vial & 2mL Polymer Vial	80
Gross Positive Controls	2R Glass Vial	159

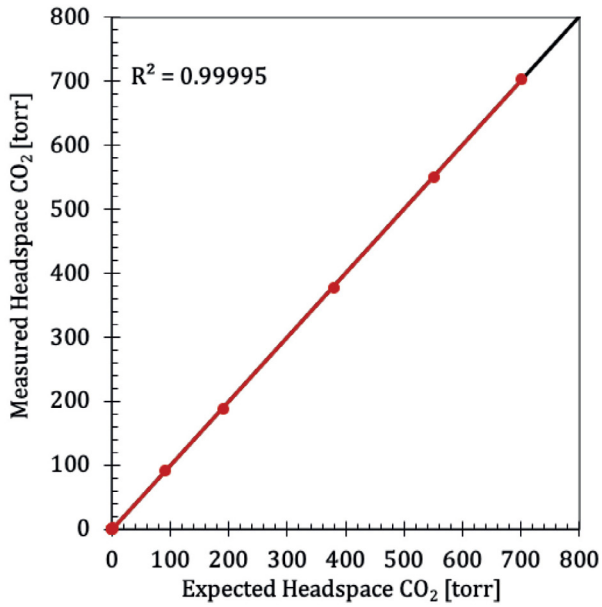
Case Studies

A study was performed using the sample set described in TABLE 1. Two identical sample sets were created, one to be stored for 7 days and one to be stored for 30 days, both in a carbon dioxide enriched -80°C freezer. These two different time points can allow for observation of the time dependency of the method (i.e. which leaks can be detected at 7 days, if more leaks are created the longer the vials are stored, etc.). The -80°C freezer in which the samples were stored included a box of dry ice to establish a carbon-dioxide enriched environment prior to placing any of the vials inside. An FMS-Carbon Dioxide Headspace Analyzer was used to acquire all measurements. This instrument utilizes a tunable diode laser absorption spectroscopy technique to measure the amount of carbon dioxide in the vial headspace. Prior to each measurement session, a set of flame-sealed carbon dioxide standards containing NIST-traceable gas mixtures were measured to verify performance of the instrument. FIGURE 1 shows linearity of the instrument over 0 to ~700.5 Torr. Initial T_0 measurements were acquired on all vials prior to storage to determine a baseline carbon dioxide partial pressure.

FIGURE 2 displays the change in headspace carbon dioxide content from the initial T_0 measurement for both the set stored for 7 days and the set stored for 30 days. The initial headspace carbon dioxide content for all vials was consistent with ambient atmospheric conditions (~1 Torr). Once removed from the freezer at the specified storage period, all vials were left to thaw at ambient conditions for approximately 1 hour before being measured. Referring to FIGURE 2, during both storage periods, the gross positive controls confirmed the carbon dioxide enriched environment of the -80°C freezer. Additionally, all positive controls prepared with the wires showed an increase

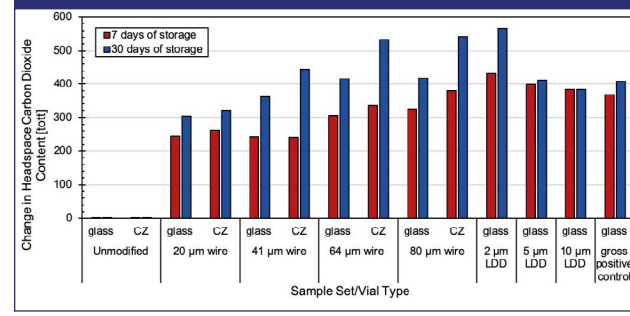
CCI TEST METHOD DEVELOPMENT FOR VIALS STORED AT -80°C

FIGURE 1: Plot of the measured carbon dioxide partial pressure versus the expected carbon dioxide partial pressure for flame-sealed standards fabricated with NIST- traceable gas compositions at known total pressures. A linear fit of the data confirms the linearity of the system response over carbon dioxide partial pressures ranging from 0 to 700 Torr (linear fit coefficient $R^2 > 0.9999$).



in carbon dioxide content, confirming this method is capable of detecting defects at the stopper seal interface for these vial configurations in as little as 7 days of storage. Similarly, all vials with laser-drilled defects contained carbon dioxide down to 2 µm (the smallest defect tested). Finally, all unmodified samples for both vial types maintained container closure integrity and neither the vial type nor the storage time correlated to an increase in leaks.

FIGURE 2: Change in headspace carbon dioxide content for two vial types after 7 days or 30 days of storage in a carbon dioxide enriched - 80°C freezer.

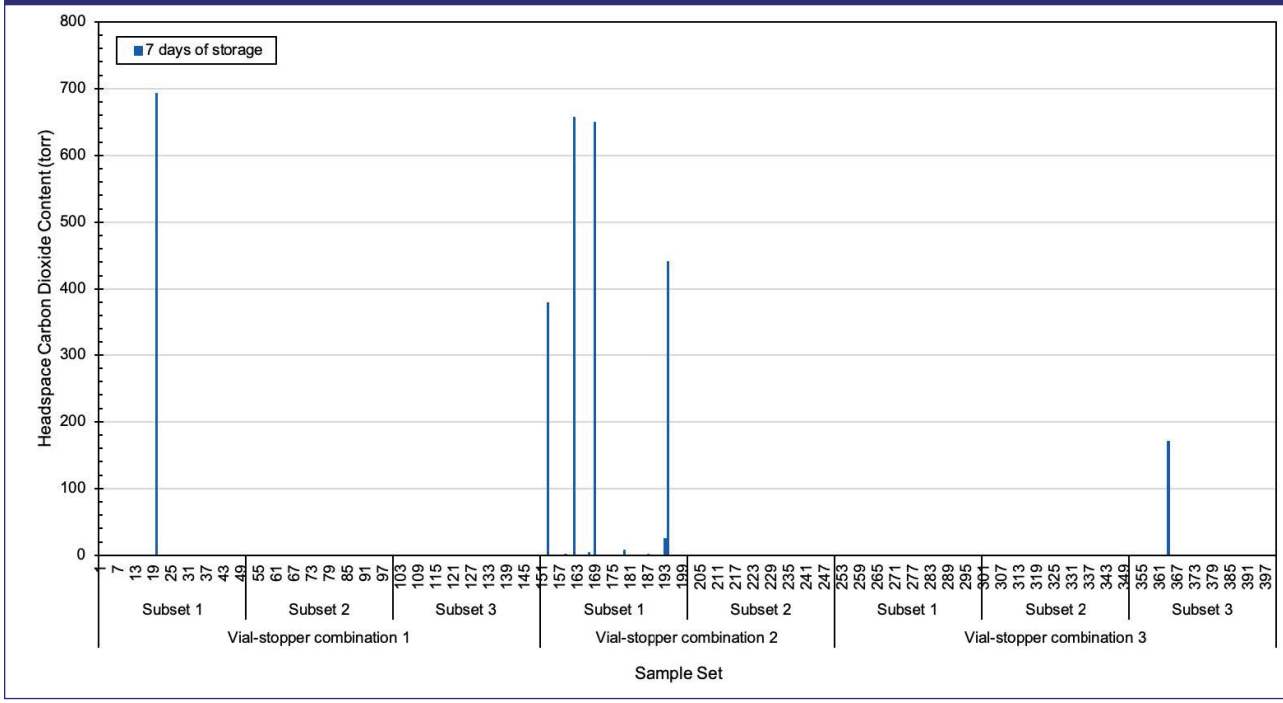


The results of an additional packaging development study for a product requiring -80°C storage is summarized in FIGURE 3. Several hundred samples of three different vial-stopper combinations were tested for their CCI after one week of storage in a -80°C freezer having a carbon dioxide enriched environment. As in the previous study, leaking vials were identified as those measuring elevated headspace carbon dioxide levels after the one-week storage period. The results for this study, however, did indicate the presence of several leaks within unmodified samples. In particular, the vial-stopper combination 2 sample set had multiple CCI failures and the remaining two vial- stopper combinations each had a single CCI failure during the -80°C storage. Both of the case studies presented here emphasize that each vial-stopper combination intended to be used for cold storage should be tested for CCI.

Recognizing that it may not be feasible to flood a storage freezer with a tracer gas or thaw the vials as described in the first case study, the CCI test method could instead include transferring the frozen vials to a chest filled with dry ice, and storing for

CCI TEST METHOD DEVELOPMENT FOR VIALS STORED AT -80°C

FIGURE 3: Example data from a packaging development study using headspace carbon dioxide gas ingress method for CCIT.



several days. This set-up relies on diffusive flow (no total pressure difference) of carbon dioxide into the leaking vial, so it may take longer to detect leaks, but it will not require flooding the storage freezer with carbon dioxide. After any number of hours/days, the vials can then be measured for their headspace carbon dioxide content. This set-up could be used to test product samples taken from the GMP freezer and to study the effects of transport on dry ice.

To summarize, headspace gas ingress testing can be used to evaluate the CCI of vials stored at -80°C. Carbon dioxide can be used as a tracer gas by filling a freezer with dry ice, and analyzing the headspace carbon dioxide content after a specific storage period.

"After any number of hours/days, the vials can then be measured for their headspace carbon dioxide content."

Alternatively, vials can be stored in a -80°C freezer and transferred to a chest filled with dry ice as a way to mimic the shipping process. These tests can be performed on a wide variety of vial configurations and can be used throughout the product lifecycle.

The benefits of CCI gas ingress testing of vials stored at -80°C using laser-based headspace analysis include:

- The measurement is analytical, rapid, and non-destructive.

- The ability to detect vials that have (temporarily) leaked during deep cold storage.
- The measurements can be performed on frozen product.
- The measurement technique enables the definition of a robust validated container closure integrity test method.

References

1. Brigitte Zuleger, Uwe Werner, Alexander Kort, Rene Glowienka, Engelbert Wehnes, and Derek Duncan. (2012). Container/Closure Integrity Testing and Identification of a Suitable Vial/ Stopper Combination for Low-Temperature Storage at -80°C. *PDA J Pharm Sci and Tech*, 66(5), 453–465.

2. Alejandra Nieto, Holger Roehl, Helen Brown, Jonas Nikoloff, Michael Adler, and Hanns-Christian Mahler. (2016). Evaluation of Container Closure System Integrity for Frozen Storage Drug Products. *PDA J Pharm Sci and Tech*, 70(2), 120–133.

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Case Study: Ensuring Container Closure Integrity of a Gene Therapy Cancer Vaccine Needing Deep Cold Storage

Marjolein Olthof

Introduction

The following case study describes how a gene therapy manufacturer handled a possible Container Closure Integrity (CCI) issue due to deep cold storage at -80°C. The product was in clinical trial when the manufacturer discovered potential breaches in CCI. The identified issue occurred when a product vial from a stability study was punctured with a syringe and apparent overpressure in the vial moved the syringe plunger upwards. Referral to previous work involving a live viral vaccine stored at -80°C lead to investigation of CCI (1). Scheduled clinical trial was halted and product batches were put under quarantine. Discussions with the national regulator were started and a root cause analysis was initiated with the objective to characterize the problem and propose corrective actions. The regulator would approve restart of the trial once suitable corrective actions were implemented.

Study Objective

The objectives for this study are summarized as follows:

1) Investigate if a fundamental CCI issue exists with the specific vial-stopper combination at deep cold storage.
2) Demonstrate the feasibility of using Headspace CO_2 Analysis as a basis for CCI testing.
3) Perform Residual Seal Force (RSF) testing to gain insight into the sealing process quality and capping/crimping process.

An extensive amount of development studies were performed, including method development for CCI testing at cold temperatures. In this particular case, the manufacturer required all products to be stored in a GMP cold storage environment and product samples were not allowed to thaw during CCI testing. Critical CCI testing requirements were that the test be non-destructive and that the sample excursion time from deep cold storage should remain below 60 seconds.

Headspace Carbon Dioxide Analysis

Several product batches were made available for headspace analysis as part of initial testing: Batch 1 (engineering batch), Batch 2 (GMP batch used in clinical trial), Batch 3 and 4 (GMP batches prepared for use in clinical trial). In total, 986 product samples stored at -80°C were selected and transferred to dry ice for more than 120 hours. In addition, positive control samples were added to the -80°C storage and were transferred to dry ice together with the product samples. The positive controls consisted of empty stoppered vials with a 10 micron capillary inserted in the robber stopper. After storage on dry ice, headspace CO_2 analysis was performed. A leaking vial was identified by the detection of CO_2 ingress into the container. For each measurement, samples were transferred from dry ice for less than 60 seconds.

FIGURE 1: Headspace CO_2 levels of Batches 1-4. Increased levels of CO_2 suggests a failure in CCI at deep cold storage.

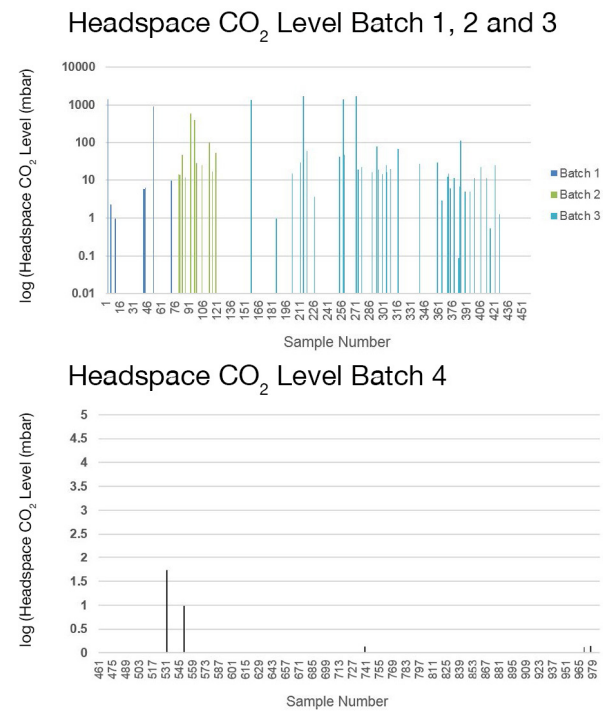
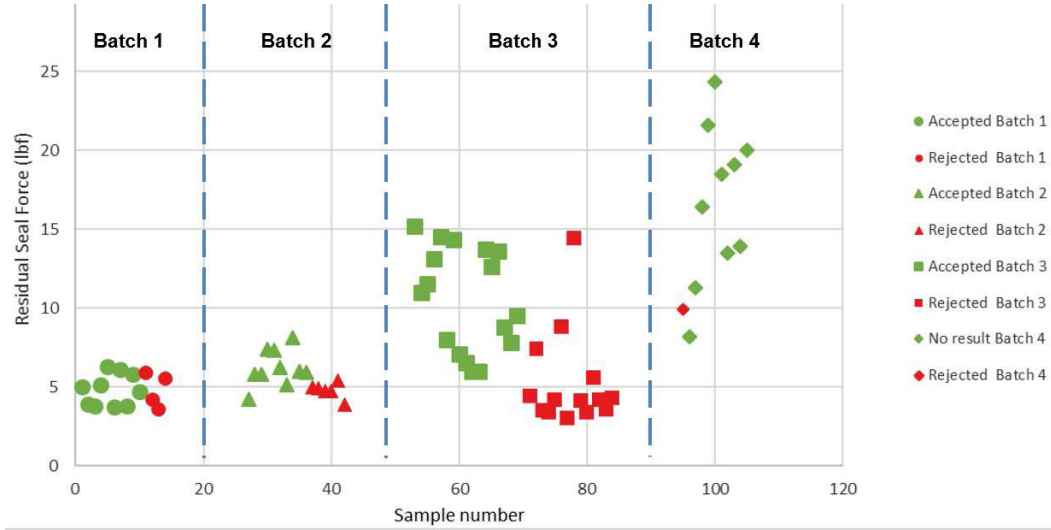


FIGURE 1 gives an overview of headspace CO_2 levels in all batches. Batch 4 (bottom graph), the most recently manufactured batch, had only slightly elevated headspace CO_2 levels when compared to the other three batches (top graph).

Residual Seal Force Testing

RSF testing was performed as part of a root cause analysis to investigate the

FIGURE 2: Residual Seal Force measurements of Batches 1-4. Red data points indicate rejected (leaking vials), green data points indicate accepted (non-leaking) vials.



potential correlation of sealing quality (capping & crimping) to the CCI results determined by using headspace analysis. The RSF measurement is associated with the stopper compression force, and can therefore be used to quantitatively characterize seal quality. FIGURE 2 compares RSF measurements of product samples in the different batches, Batches 1 and 2 had lower RSF values than Batches 3 and 4 suggesting that an inconsistent capping and crimping process, resulting in low RSF values, correlates to an increased risk to CCI during deep cold storage.

The red data points in FIGURE 2 belong to product samples that had been identified as leaking and were rejected by the CCI test. The green data points belong to vials that did not leak. These results show a clear trend: leaking vials tend to have low RSF values suggesting that vials which are more loosely crimped have a higher risk to lose CCI at deep cold storage temperatures.

Corrective Actions

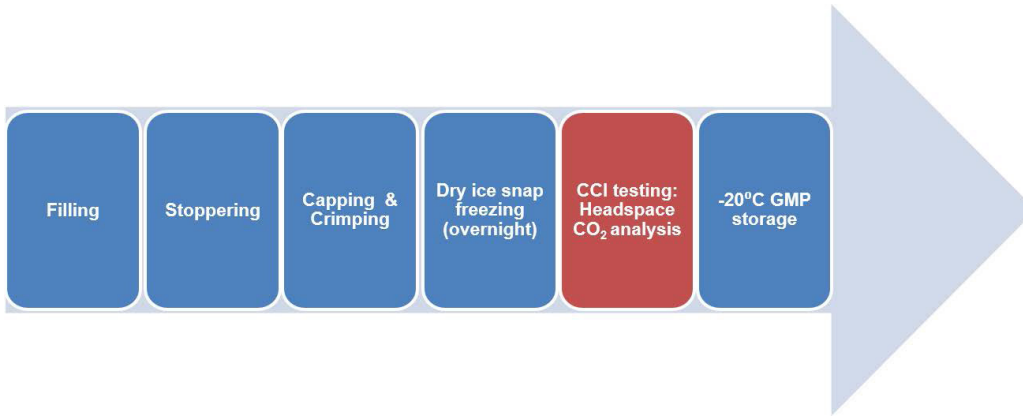
By performing both headspace analysis and RSF testing, it was concluded that these product batches were suffering from (temporary) leaking vials due to the -80°C storage as well as variation in the capping and crimping process. Two options were defined for potential corrective actions:

- Option 1: Adjust the storage temperature conditions to a warmer temperature. In this case, the manufacturer already had stability data for storage at -20°C for up to one year.
- Option 2: Adjust and/or improve the packaging components.

Additional CCI studies performed with the packaging components at -20°C revealed no breaches in CCI at this temperature.

CASE STUDY: ENSURING CCI OF A GENE THERAPY CANCER VACCINE NEEDING DEEP COLD STORAGE

FIGURE 3: Overview of the final production process, including an In-Process Control CCI test using Headspace CO_2 Analysis (red) after the dry ice snap freezing step.



It was therefore decided to increase the storage temperature to -20°C and focus on generating more stability data. Furthermore, additional RSF studies were performed to further optimize and qualify the capping and crimping process.

In addition to these corrective actions, an additional issue needed to be addressed. Product vials were still temporarily exposed to deep cold temperatures due to a snap freezing step after capping and crimping in the production process (see FIGURE 3). Lighthouse supported the manufacturer with performing additional CCI studies which demonstrated that any vials losing CCI in the snap freezing step could be detected with a headspace CO_2 ingress measurement. The manufacturer therefore implemented an in-process control test performing 100% CCI testing after the quick-freezing step and before storage at -20°C.

Case Study Conclusions

A gene therapy manufacturer identified a potential CCI issue in product vials needing deep cold storage at -80°C. A CCI test method was developed that enabled non-destructive CCI testing of product vials at these cold temperatures. The capability to non-destructively test clinical product in storage enabled troubleshooting studies. These studies confirmed product vials could temporarily lose CCI during deep cold storage. The vials resealed at room temperature resulting in an overpressure in the stoppered vial observed during administration. A root cause investigation showed that the risk of losing CCI correlated to low stopper compression and the quality of the capping and crimping process as measured by RSF. The corrective actions included changing the product storage temperature to -20°C, improving the capping and crimping process based on RSF characterization, and implementing a 100% IPC CCI test. The corrective actions were approved by the regulator allowing the manufacturer to rapidly restart clinical trials (FIGURE 4).

CASE STUDY: ENSURING CCI OF A GENE THERAPY CANCER VACCINE NEEDING DEEP COLD STORAGE

FIGURE 4: Case study timeline.

Reference

1. Zuleger, B. et al. "Container/Closure Integrity Testing and the Identification of a Suitable Vial/Stopper Combination for Low-Temperature Storage at -80°C", *PDA Journal of Pharmaceutical Science and Technology*, 2012, 66 p. 453-465.

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