ARTICLE

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

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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.

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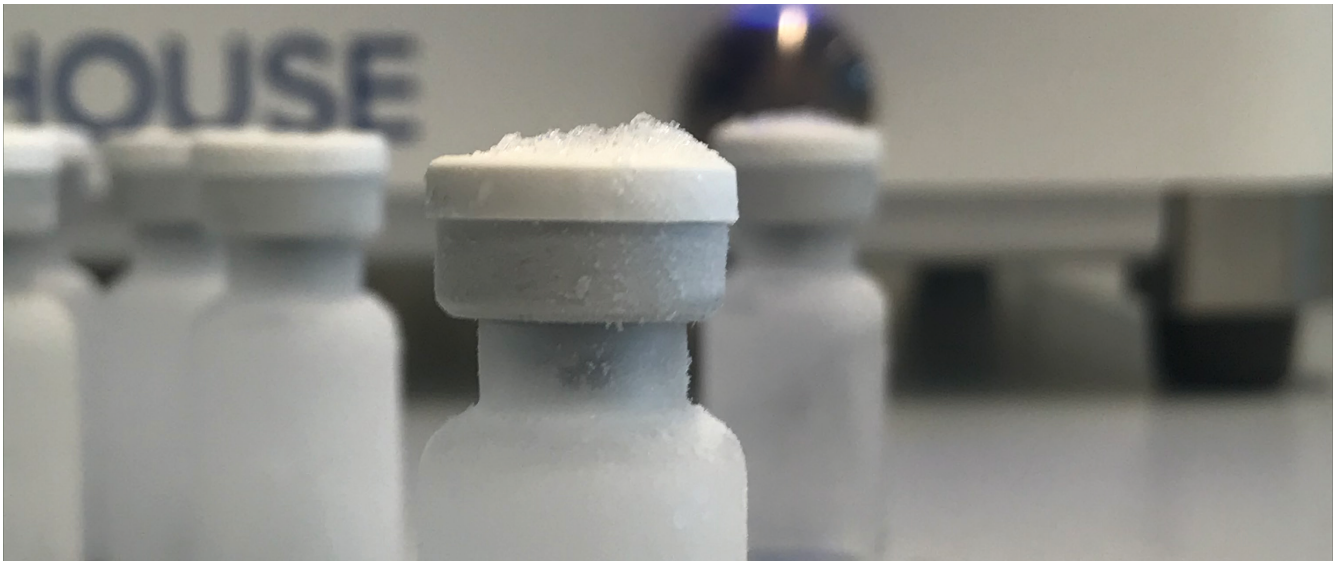
The glass transition temperature (T_g) of rubber stoppers used to package many pharmaceutical products ranges from -55 to -70°C. When the rubber stopper is exposed to temperatures below its T_g, it runs the risk of losing its elasticity and becoming brittle. In addition, the differing coefficients of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



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.

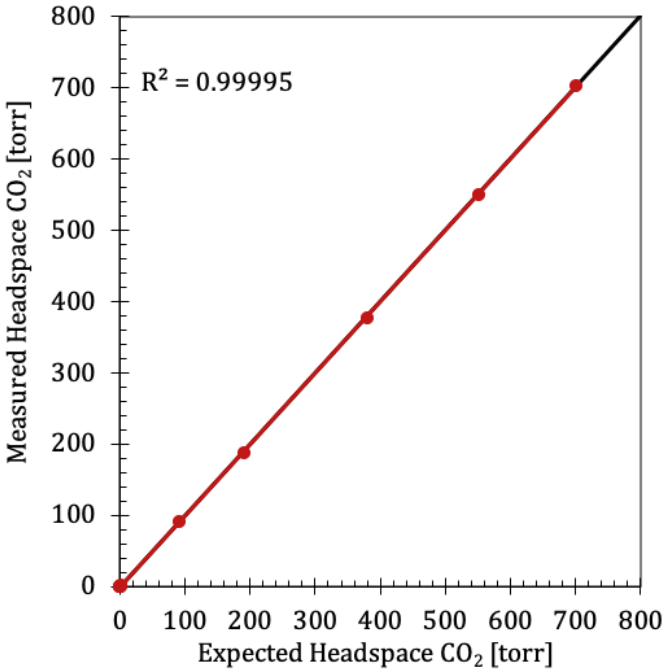


Figure 1. Plot of the measured carbon dioxide partial pressure versus the expected carbon dioxide partial pressure for flame-sealed standards fabricated with NIST-traceable gas compositions at known total pressures. A linear fit of the data confirms the linearity of the system response over carbon dioxide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

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

**Sizes listed for the tungsten micro-wire positive controls are not representative of the actual defect size. Defects created from the wires are two channels on either side of the wire.*

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 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.

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.

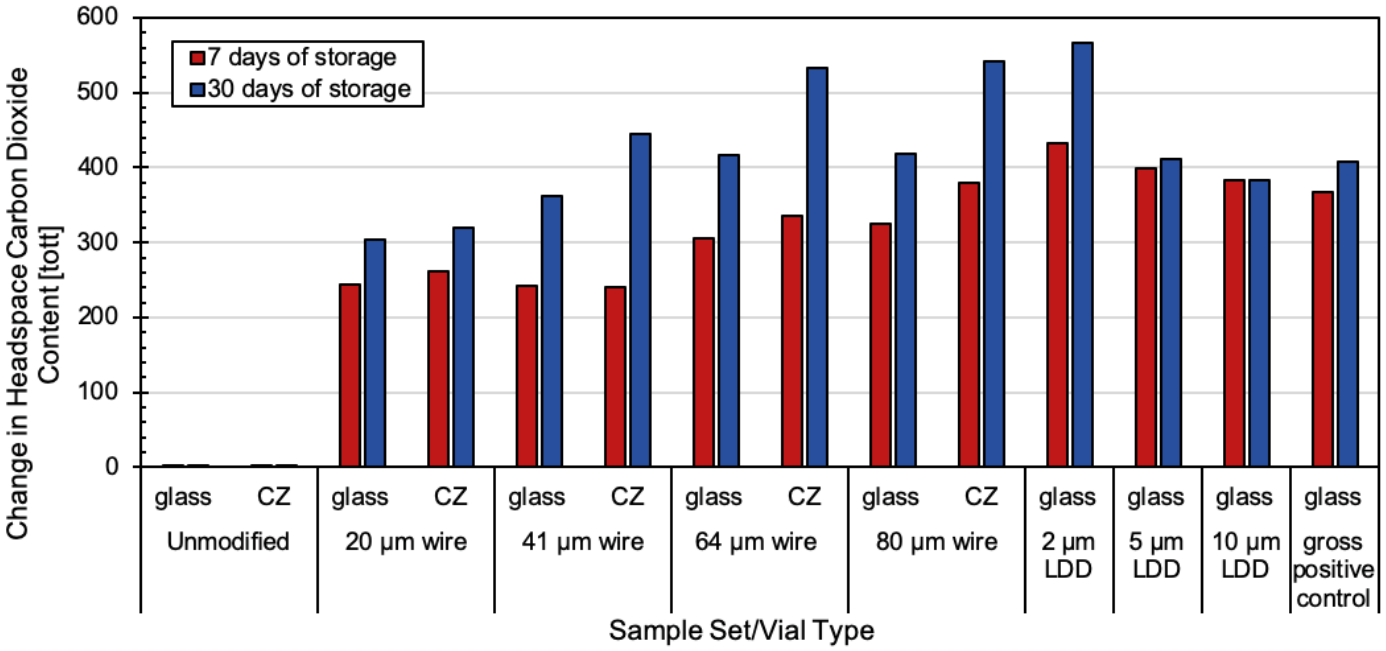


Figure 2. Change in headspace carbon dioxide content for two vial types after 7 days or 30 days of storage in a carbon dioxide enriched - 80°C freezer.

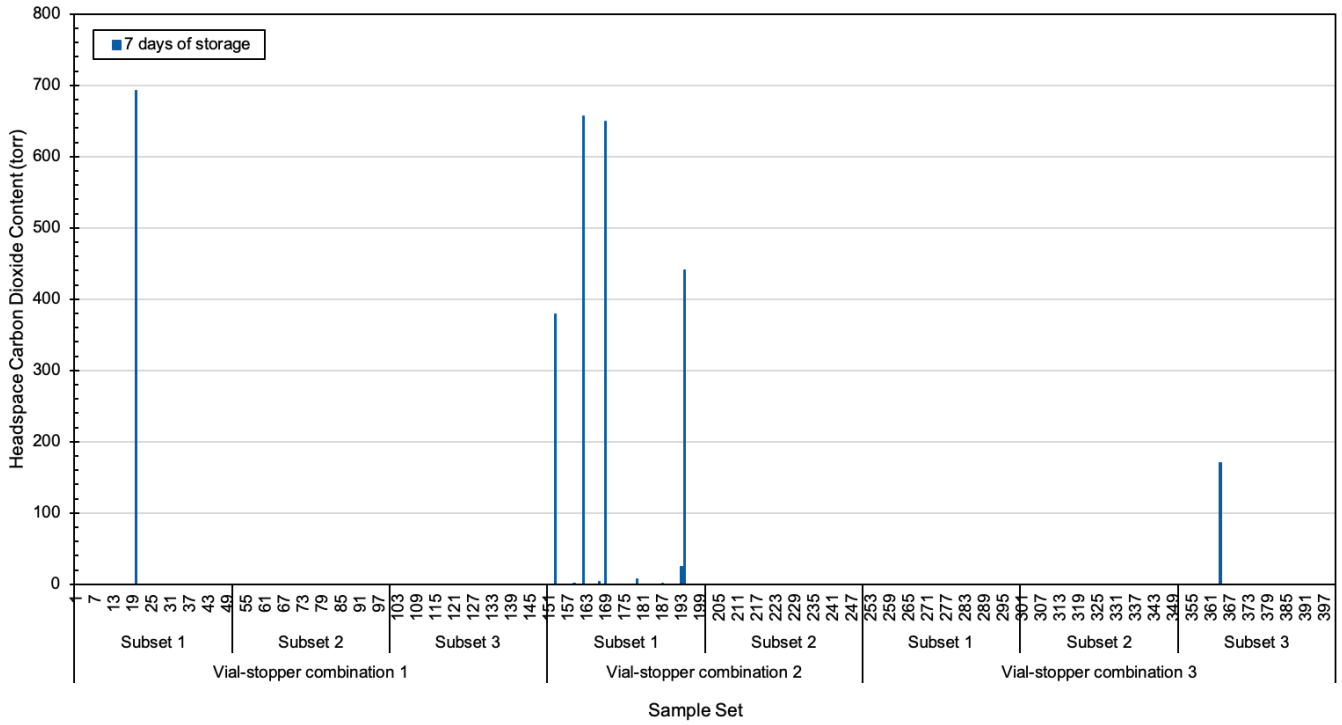


Figure 3. Example data from a packaging development study using headspace carbon dioxide gas ingress method for CCIT.

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 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.

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

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2. Alejandra Nieto, Holger Roehl, H. B., 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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