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

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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]. It is therefore critical that robust development work is done to understand the CCI performance of any primary packaging components used for a product needing deep cold storage and transport temperatures.
EFFECTS OF DEEP COLD STORAGE ON STOPPERED VIALS

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.

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, twenty 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.

![Headspace CO\textsubscript{2}](image)

![Headspace Oxygen](image)

![Headspace Pressure](image)

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

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.
REFERENCES


2. Presentation ‘Ensuring container closure integrity of a gene therapy cancer vaccine needing deep cold storage’, Josine Wilmer, 2019 PDA Parenteral Packaging Conference, Venice, Italy

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