LIGHTHOUSE
The Science of Pharmaceutical Manufacturing

Customer note **102**

Case Study: Container Closure Integrity Testing for Sterile Vial Products in Deep Cold Storage

Introduction

Storage of sterile pharmaceutical product vials at -80°C is sometimes practiced to increase shelf life, especially for live viral vaccines. In addition, vials are sometimes stored on dry ice during transport to achieve a cold storage shipment. Storing vial product samples at such low temperatures can, however, result in practical problems such as (temporary) closure integrity failures.

Industry Case Study

A vaccine-focused biotechnology company approached LIGHTHOUSE to help investigate a phenomenon observed in liquid vaccine product stored at -80°C. During QC testing, a number of vials were found to have an overpressure. The vaccine samples, frozen in glass vials with rubber stoppers, were thawed to room temperature prior to QC testing. When the rubber stopper was punctured with a syringe needle, the syringe pistons in some of the samples moved backwards. After removal of the needle, vaccine product shot out of the needle insertion holes indicating a considerable overpressure in these vials. This phenomenon represented a serious safety risk and LIGHTHOUSE was asked to support an investigation to identify the root cause. The working hypothesis was a potential container closure issue during cold storage at -80°C. Working closely with the client, LIGHTHOUSE carried out a series of studies to investigate this hypothesis using headspace analysis systems. These laser-based platforms enable rapid non-destructive characterization of the headspace gas conditions inside a sealed container.

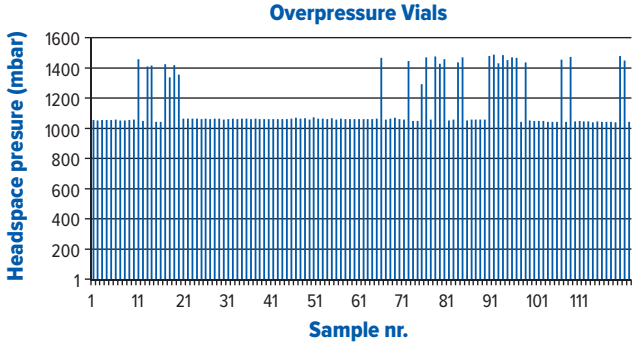

Figure 1: The headspace pressure measured in stoppered viral vaccine vials stored at -80°C. The measurements show that approximately 25% of the vials display overpressure, sometimes measuring as high as 1500 mbar.

A LIGHTHOUSE headspace system made quantitative non-destructive measurements of the headspace pressure in live viral vaccine samples after storage at -80°C. Figure 1 plots the measured headspace pressure levels per sample. A clear distinction can be made between vials

with an overpressure and vials that remained at atmospheric pressure. The overpressure phenomenon was linked to a seal integrity failure during deep cold storage.

Commonly used rubber butyl stoppers have glass transition temperatures (T_g's) that lie between -55°C and -70°C. At a temperature below the T_g, the rubber stopper loses its elastic properties potentially risking a seal integrity failure at these low temperatures. If the seal integrity is indeed lost, surrounding cold dense gas from the cold storage environment leaks into the vial. When the sample is taken out of cold storage and allowed to warm up to room temperature, the rubber stopper regains its elastic properties resealing the vial and trapping the cold dense gas inside. The cold dense gas will expand during warm-up, thereby creating an overpressure in the vial.

In Figure 2 another measurable change in headspace conditions caused by temporary closure integrity failure during deep cold storage is illustrated. In this part of the study, buffer samples prepared with ten different vial/stopper combinations were stored on dry ice (-78.5°C). A leak during cold storage allowed carbon dioxide gas of the dry ice storage environment to ingress into the vial resulting in a decrease of the original 20.9% oxygen content (atmospheric air) in the vial headspace.

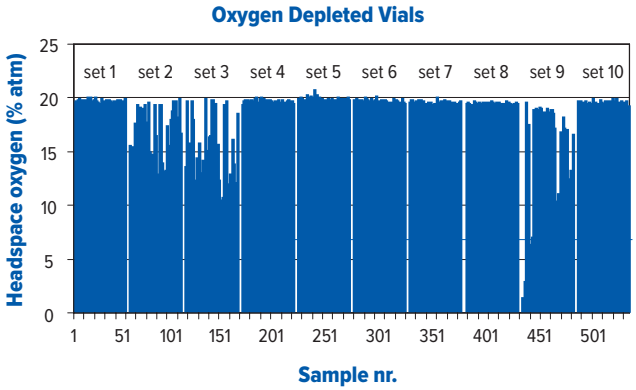

Figure 2: Headspace oxygen measured in buffer vials stored on dry ice (-78.5°C). Ten different vial/stopper combinations were used, 50 samples in each set. A large number of samples in specific vial/stopper combinations (Sets 2, 3, and 9) display headspace oxygen depletion. These samples suffered from closure integrity failures during storage, allowing carbon dioxide to ingress and displace the original atmospheric levels of oxygen in the vials.

Conclusions

Close collaboration with the client enabled the described studies which successfully identified the root cause of an overpressure phenomenon observed in viral vaccine vials stored at -80°C. Laser-based headspace analysis provided insight by quantifying the headspace conditions.Eventual solutions for the issue included an appropriate choice of vial/stopper combination as well as appropriate settings for the capping and crimping process (for further details, see Ref [1]).

[1] Zuleger, B.; Werner, U.; Kort, A.; et al. Container/Closure Integrity Testing and the Identification of a Suitable Vial/Stopper Combination for Low-Temperature Storage at -80°C. PDA J. Pharm. Sci. Technol. 2012, 66, 453–465.