Nitrogen Dioxide Sterilization: Maintains Prefilled Syringe Integrity

Noxilizer has developed an optimal sterilization process specifically for prefilled syringes and other drug-delivery devices. The low temperature NO\textsubscript{2} sterilization process will effectively sterilize the external, gas accessible surfaces of the syringe with minimal to no impact on the contents of the syringe. The process is carried out between 10°C and 30°C, without the need for a deep vacuum, and typically lasts 2 to 3 hours depending upon the product design and packaging materials. The low temperatures and short processing times allow manufacturers to maintain an acceptable time-temperature budget during the manufacturing process. The ability to process without a deep vacuum minimizes the risk of plunger movement on the syringes.

One of the main benefits to the NO\textsubscript{2} sterilization process is that the integrity of the container closure system is maintained for prefilled syringes. This is also true for filled vials and cartridges. The container closure integrity can be demonstrated by testing for NO\textsubscript{2} ingress after a prefilled syringe has been exposed to the NO\textsubscript{2} sterilization process. The NO\textsubscript{2} ingress test is typically performed on syringes that have been filled with water for injection (WFI), deionized (DI) water, or saline solution. If NO\textsubscript{2} were to penetrate the prefilled syringe, either through the rubber plunger, a tip cap or needle shield, it would be absorbed into the aqueous content of the syringe. Upon contact with the liquid water, NO\textsubscript{2} undergoes an equilibrium reaction to form both nitric (HNO\textsubscript{3}) and nitrous (HNO\textsubscript{2}) acids:

\[
2 \text{NO}_2 (g) + \text{H}_2\text{O (l)} \rightarrow \text{HNO}_2 (aq) + \text{HNO}_3 (aq)
\]

\[
\text{HNO}_2 (aq) \rightarrow \text{H}^+ (aq) + \text{NO}_2^- (aq)
\]

\[
\text{HNO}_3 (aq) \rightarrow \text{H}^+ (aq) + \text{NO}_3^- (aq)
\]

The ingress of NO\textsubscript{2} past the container closure system can be detected by testing for the presence of the anionic components of the acid products, specifically the nitrate anion (NO\textsubscript{3}\textsuperscript{−}). Noxilizer has recently validated a colorimetric assay for NO\textsubscript{3} and nitrite (NO\textsubscript{2}\textsuperscript{−}) anions in solution in order to detect any HNO\textsubscript{3} or HNO\textsubscript{2} products of NO\textsubscript{2} ingress. The assay converts NO\textsubscript{2}\textsuperscript{−} into NO\textsubscript{3}\textsuperscript{−}, and both components are reported as ppm NO\textsubscript{3}\textsuperscript{−}. An example of the chromogenic reaction used to detect NO\textsubscript{2} ingress products is shown in Figure 1, where the DI water blank is colorless, and the sample containing NO\textsubscript{2} exhibits a bright pink hue. The intensity of the absorbance in the red region, as determined by visible light spectroscopy, is correlated to the NO\textsubscript{3}\textsuperscript{−} concentration in the analyte.

The European Pharmacopoeia (EP) specifies a limit of 0.2 ppm NO\textsubscript{3}\textsuperscript{−} for WFI\textsuperscript{2}. Since the US Pharmacopeia does not have a requirement for NO\textsubscript{3}\textsuperscript{−} concentration in WFI, Noxilizer uses the EP limit as a guideline for the acceptability of WFI after the NO\textsubscript{2} ingress test. The limit of detection for the assay is 0.024 ppm NO\textsubscript{3}\textsuperscript{−}, which is an order of magnitude lower than the EP limit. The standard curve showing the response of the assay is shown in Figure 2. Therefore, the assay is sensitive enough to determine if NO\textsubscript{2} ingress has caused the WFI to fall out of EP specification.
Nitrogen Dioxide Sterilization: Maintains Prefilled Syringe Integrity

Noxilizer recently demonstrated the integrity of the container closure system in the NO$_2$ sterilization process using Type 1 glass, 1-mL long syringes with bonded needles. The plunger, which was fitted to a polypropylene rod, was gray pharmaceutical rubber; as was the liner of the rigid needle shield. The syringes were filled with 1 mL of DI water, prior to being packaged in 1073B Tyvek®-Mylar® pouches. The syringes were subsequently exposed to a sterilization process that had been defined following the “overkill approach” in ISO 14937:2009. The process parameters, representative of a typical sterilization process for prefilled syringes, are shown in Table 1.

Table 1. The cycle parameters for the sterilization process are shown. Note that the vacuum refers to the minimum absolute pressure experienced in the sterilization chamber during the process. At sea level the typical ambient pressure is 760 Torr.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Vacuum (Torr)</th>
<th>Relative Humidity (%RH)</th>
<th>NO$_2$ Dose (mg/L)</th>
<th>Exposure Time (min)</th>
<th>Aeration Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 (+/- 3)</td>
<td>700 (+/- 10)</td>
<td>&gt;75</td>
<td>10 (+/- 1)</td>
<td>60 (+/- 1)</td>
<td>90 (+/- 1)</td>
</tr>
</tbody>
</table>

The DI-filled syringes were tested 1 day after exposure to the NO$_2$ sterilization process. The results of the test for NO$_2$ ingress are shown in Figure 3. Both the unexposed controls and the exposed syringes remained under the limit of quantification for the assay, demonstrating that the NO$_2$ sterilization process did not impact the quality of the prefilled syringe contents.
The results of the colorimetric assay demonstrate that the NO₂ sterilization process does not affect the quality of the WFI contents of the syringe.

Noxilizer provides the assay for NO₂ as a means of demonstrating container closure integrity during validation of a sterilization process. It is one piece of information that prefilled syringe manufacturers can use to bolster their regulatory submissions. The data from the assay may be used to supplement any product quality tests, such as purity, activity, or stability tests, that are required for the actual drug or biologic product.

**SUMMARY:**

NO₂ sterilization provides unique benefits for prefilled syringe and drug-delivery products administered in the operating room or to address other concerns through the manufacturing process. Studies have demonstrated that NO₂ can sterilize the surface of the delivery device without compromising the contents. Further, Noxilizer provides an assay for NO₂ that demonstrates container closure integrity during validation of a sterilization process, supplementing manufacturers’ product quality tests and bolstering regulatory submissions.

---

**For more information, please contact:**

Mike Valentine  
Vice President, North American Sales  
Noxilizer, Inc.  
mvalentine@noxilizer.com  
(443) 842-4421

**REFERENCES:**

