NO_2 Sterilization Residues and Nitrosamines
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Abstract

The conditions needed for nitrosamine formation is unlikely to occur in primary containers (e.g., pre-filled syringes, cartridges, etc.) during or after the NO_2 sterilization process. Nitrosamine formation requires the presence of the precursor amines and conditions that cause nitrite to form nitrosonium cation ($N{=}O^+$). Such reactions require heat, radiation or low pH. NO_2 sterilization typically is done at low temperatures, does not involve radiation and uses water at a neutral pH. This leads to the conclusion that nitrosamines will not form. There are two options for measuring whether nitrosamines formed in primary containers due to the NO_2 sterilization process. One option is to assay for the presence of nitrosamines using gas chromatographic techniques. A second option is to assay the contents of primary containers to evaluate for sterilant residue permeated through closures (called ingress), followed by assessment of conditions that can lead to nitrosation of amines.

NO_2 Sterilization Process

Figure 1 illustrates the essential design of a sterilization system configured for vacuum cycles using the NO_2. A graph of the chamber pressure versus time for a vacuum cycle is shown in Figure 2. The graph in Figure 2 shows the pressure within the sterilization chamber as a function of elapsed time. The first step is the evacuation of the chamber to remove the atmosphere in the chamber. Next, the pre-chamber is filled to the appropriate pressure of sterilant gas. Then, the pre-chamber is opened to the evacuated sterilization chamber allowing the NO_2 to flow into the sterilization chamber. Pressure transducers, recording the pressure rise associated with the sterilant gas addition, or optical NO_2 detectors can be used to confirm delivery of the sterilant to the sterilization chamber. After the sterilant is added to the chamber, humidified air may be added to the sterilization chamber until the target relative humidity is achieved, and dry air may be added until the target pressure for the exposure dwell is reached. The chamber pressure during the exposure dwell is typically between 500 mmHg and 600 mmHg (roughly between 65 kPa and 80 kPa). These steps can be repeated, as needed, to achieve the intended sterilization process. Figure 2 shows two exposure dwells, representing the first half-cycle and second half-cycle of an overkill approach, described in Annex D of ISO 14937. After the final exposure dwell is completed, the sterilant gas is removed from the sterilization chamber, and the load therein, using repeated evacuation and rinsing with air or inert gas (e.g., nitrogen).

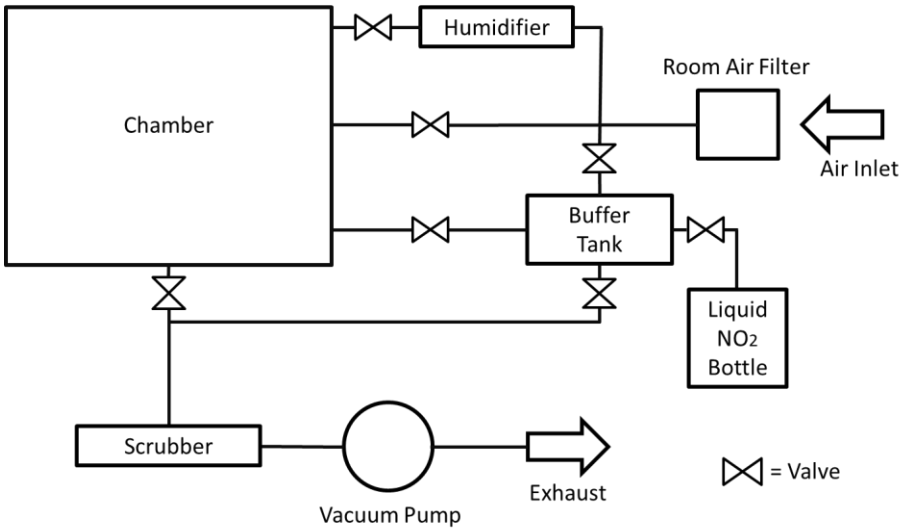


Figure 1. Block Diagram of a NO_2 sterilization system configured for vacuum cycles. When using a scrubber, the air pumped from the chamber can be safely vented.

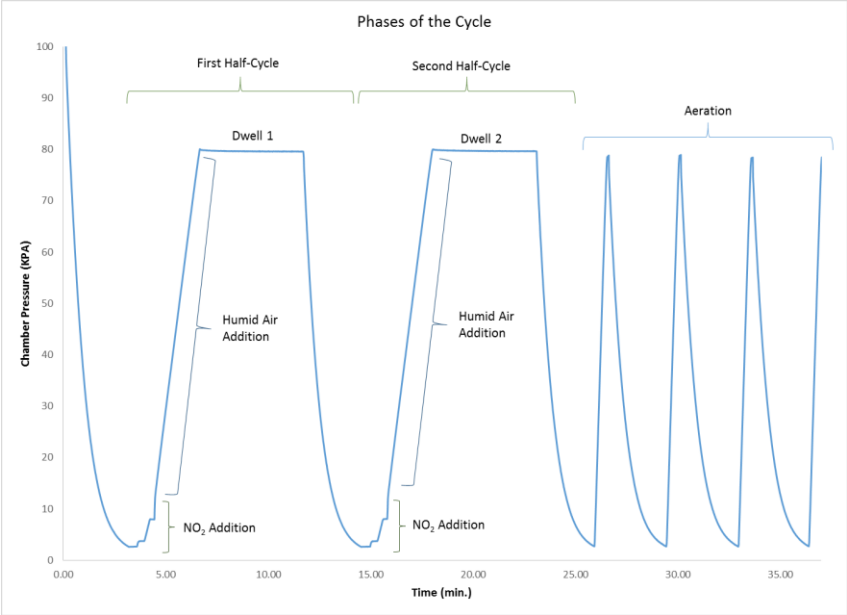


Figure 2. Pressure versus time for a typical NO_2 cycle with vacuum. The sterilant exposure phase has an evacuation step, a gas filling step, and an exposure dwell. These phases can be repeated as needed to complete the sterilization cycle. The exposure phases are followed by an aeration phase.

The sterilant in the chamber exhaust can be scrubbed by chemical means. The scrubbing process removes unsafe levels of NO_2 from the exhaust. The chemical removal of sterilant from the air stream may use a solid chemical scrubber media (e.g., sodium permanganate) or water scrubber systems. The sodium permanganate (solid media) scrubber material neutralizes and captures the NO_2 in a non-hazardous, solid material. The spent scrubber material may be disposed of as non-hazardous solid waste with no special handling measures required.

Chemical Reactions and Sterilant Residuals

Reactions that occur in the sterilization chamber convert a fraction of the NO_2 sterilant to other oxides of nitrogen. The literature suggests that gaseous NO and HONO will be formed. The formation of the NO and HONO was confirmed in NO_2 sterilization chambers using FTIR spectroscopy. A measurement of the HONO and NO formed in the sterilization chamber is shown in Figure 3. The data were acquired during the dwell phase of a cycle consisting of a 10 mg/L (about 5800 ppm) NO_2 dose added to a chamber that had been humidified to 75 %RH. After 6 minutes, the amount of HONO was found to be approximately 390 ppm, and the amount of NO in gas was 502 ppm.

Sterilant residues on surfaces contain nitrates and nitrites. NO_2 reacting with water (humidity in the chamber) produces trace amounts of nitrous acid (HNO_2) and nitric acid (HNO_3). The acids are measurable as the associated anions nitrate (NO_3^-) and nitrite (NO_2^-), respectively. The sterilization process ends with an aeration phase the includes repeated chamber evacuation pulses. This vacuum portion of the aeration phase dries the water, leaving residuals comprised of un-dissociated HNO_3 and HNO_2 (and its isomer, HONO), which may remain attached to binding sights on the material surface (Saliba, 2001). Therefore, the residue of the sterilant will be mostly HNO_3 and HNO_2. Measurements of the residues recovered from medical devices and material samples confirm that the residue from the NO_2 process is more than 90 % nitrate (NO_3^-) and less than 10 % nitrite (NO_2^-).

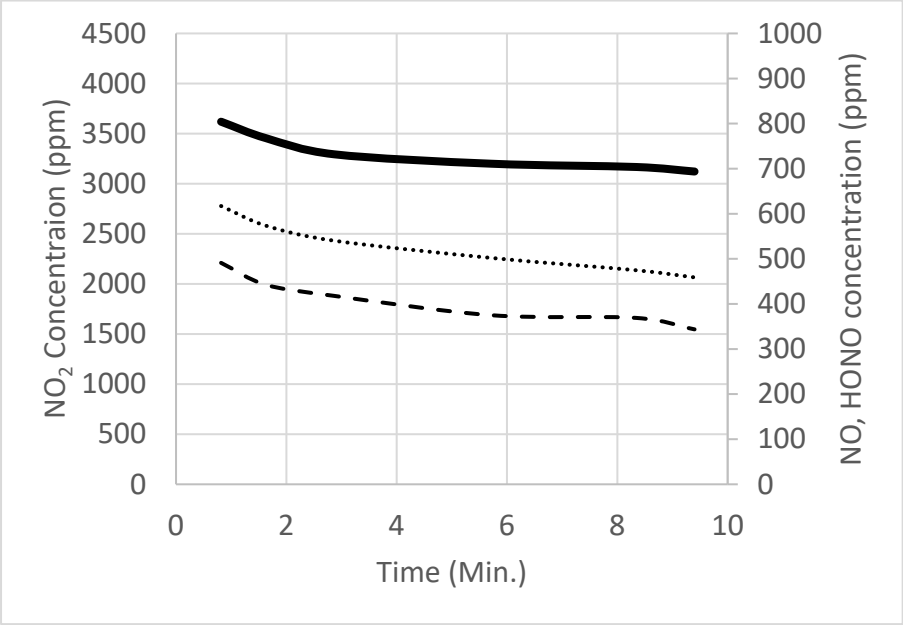


Figure 3. The gas concentration measured during the sterilant exposure dwell shows NO and HONO are formed. The solid black line is the NO_2 concentration (left vertical axis), HONO concentration is represented by the dashed line (right vertical axis), NO is represented by the dotted line (right vertical axis).

Primary Container Ingress

In studying the ingress of sterilant into primary containers, we do not know whether the ratio of nitrate and nitrite that might permeate container closures would be different from the nitrate-dominated surface residues. This can be studied during future testing.

Under the assumption that both nitrate and nitrite are found in a closed primary container, then a subsequent reaction is required, in which the oxides of nitrogen react with amines to form nitrosamines. However, nitrosamines are not directly formed from reactions between nitrate or nitrite and aromatic amines. Specifically, neither nitrite nor nitrous acid are nitrosating agents but are intermediates in the formation of nitrosating agents that includes dinitrogen trioxide (N_2O_3), dinitrogen tetraoxide (N_2O_4) and nitrous acidium ion (H_2O+ NO) (Tricker and Kubacki, 1992). Furthermore, the literature indicates that the reactions that form nitrosamines are reactions with intermediate nitrogenous species and are facilitated by heat, radiation, acidic conditions, dissolved oxygen and other similar factors.

Heat – One example of endogenous nitrosamine formation comes from cooking food. The typical diet in most countries contains nitrates and nitrites. Nitrates and nitrites occur naturally in fruit and vegetables and are often used as food additives in processed meats such as ham, bacon, sausages, and hot dogs, to retard microbial spoilage, and preserve appearance. When meats are heated at high temperatures, the nitrites of which can also turn into nitrosamines (Peng Song, 2014)

Radiation – N-Nitrosamines are also formed at ambient temperatures by the gamma-radiolysis of neutral aqueous solutions of either $NaNO_2$ or $NaNO_3$ and secondary amines (Challis, 1980).

Acid – Nitrite under acidic condition is converted to nitrous acid and other reactive nitrogen species, which can cause nitration of aromatic compounds such as tyrosine, de-amination of DNA bases, and nitrosation of amines (Pannala, 2003). For example, the conversion of nitrates to nitrites in the oral cavities followed by the formation of nitrosamine in the stomach under acidic pH (Ohshima, 1984). In acidic conditions, nitrite reactions that can result in NO:

$$NO_2^- + H^+ \rightarrow HNO_2$$

$$2HNO_2 \rightarrow H_2O + N_2O_3$$

$$N_2O_3 \rightarrow NO + NO_2$$

$$3HNO_2 \rightarrow HNO_3 + 2NO + H_2O$$

$$HNO_2 + 2HNO_3 \rightarrow H_3O^+ + 2NO_3^- + NO^+$$

The formation of nitrosamines can be inhibited by some chemicals and catalyzed by others. Nitrosation reactions can be inhibited by redox compounds such as ascorbate and vitamin E, or accelerated by catalysts such as metal ions, carbonyl compounds and nucelophilic anions including chlorine and iodine (Tricker and Preussmann, 1991). In support of this observation, the yield of nitroso compounds formed endogenously was shown to be linked to the intake of precursors including nitrate and nitrite, but

several inhibitors and catalysts, either as pure substances or occurring in complex mixtures, were shown to modify the nitrosation reaction *in vivo* (Ohshima, 1984).

Prior to 2016, The European Pharmacopoeia requirement for WFI (Monograph 0169) lists the maximum amount of nitrate as 0.2 ppm. However, only Korea and China keep this requirement in their pharmacopeial standards. Regards, this limit can be used as a benchmark when testing ingress in the primary containers. No acceptable level of nitrite is listed in standard or pharmacopeial monographs.

Conclusion

It is not clear that nitrosamines can form in primary containers exposed to the NO_2 sterilization process. The conditions cited in the literature necessary for nitrosamine formation (heat, radiation, or low pH) are not part of the NO_2 sterilization process. Direct testing of primary container contents and analysis of the nitrogenous species found with can support the claim that nitrosamines are not formed.

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