INTERPHEX

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Sterilization with Gaseous Chlorine Dioxide: Applications and Opportunities

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STERILIZATION WITH GASEOUS CHLORINE DIOXIDE: APPLICATIONS AND OPPORTUNITIES

by

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Abstract

A process utilizing gaseous chlorine dioxide (CD), a unique oxidizing agent with broad-spectrum antimicrobial activity, has been developed and proven efficacious for the sterilization of a wide variety of sealed enclosures and chambers. Efficacy was demonstrated in evaluations with flexible and rigid isolation systems ranging in size from 25 to 250 ft^3 and with a 6000-ft^3 sealed room. Successful results were also obtained with sterilization chambers containing medical devices or pharmaceutical components. Efficacy was demonstrated at low concentrations of CD (1 - 10 mg/L), ambient temperature, and gas exposure phase pressure ranging from deep vacuum to atmospheric. The continuous real-time CD concentration monitoring documents the excellent reproducibility needed for parametric release. Residual CD was extremely low due to minimal absorption for most materials tested which resulted in rapid aeration of sterilized materials and enclosures. This paper will review the results of studies with gaseous CD and discuss other potential applications such as lyophilization chambers and storage tanks.

Introduction

The increasing use of isolation technology has spurred interest in new methods and technologies for the high-level disinfection (HLD) or sterilization of these enclosures. Vaporized hydrogen peroxide, formaldehyde, and peracetic acid have been used for this purpose but each of these agents and/or its associated process has an undesirable aspect with respect to reproducible efficacy, toxicity, or ease of use. Investigations with CD for the sterilization of medical devices suggested that its wide spectrum of antimicrobial activity, coupled with its existence as a true gas at ambient conditions, antimicrobial activity at relatively low concentration, and ease of measurement would be advantageous when applied to the HLD or sterilization of

isolation systems and other sealed enclosures.

CD Properties and Chemistry

CD was first prepared in 1802, it is a greenish yellow gas with a molecular weight of 67.5. It is a unique single-electron-transfer-oxidizing agent and has been termed a "stable free radical". CD has the microbiocidal properties of chlorine and is highly soluble in water, but unlike chlorine, its reaction chemistry does not lead to the formation of chlorinated organic products (trihalomethane and chloramine). CD is a respiratory irritant with an 8-hour time-weighted average exposure limit of 0.1 ppm; the 15-minute short-term exposure limit is 0.3 ppm. At use concentrations, CD is not flammable nor is it carcinogenic or ozone depleting.

CD Gas Generation

CD gas cannot be compressed and stored in high-pressure cylinders; it is generated at the point of use with a cartridge-based, solid-phase reaction system.
The oxidation of sodium chlorite ($NaClO_2$) is the basis of the reaction:

$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl$$

Solid, flaked $NaClO_2$ is contained in plastic cartridges. A gas mixture composed of 2% chlorine (Cl_2) and 98% nitrogen (diluent) is passed through the cartridges to yield high purity CD gas and sodium chloride (NaCl). The CD gas (~ 100 mg/L) in nitrogen is piped to the desired enclosure yielding a final concentration that is generally between 1 and 30 mg/L.

CD Generation Equipment

A CD gas generation system schematic diagram is shown in Figure 1. The system is modular in design with four main elements:

- Gas generation module
- Gas injection module
- Sensor module
- Humidity control module (optional)

A single gas generation module can be connected, in a manner similar to a utility, to four separate "target" enclosures. The targets can be sterilized individually in any desired sequence, but not simultaneously. Targets that can be sterilized include isolators, sealed rooms, "pass-throughs", mixing tanks, lyophilizers, and sealable biosafety cabinets. A conventional sterilization chamber, such as an autoclave or EtO chamber, can also be a target.

Each target to be sterilized requires a gas injection and a sensing module to provide the necessary monitoring and control functions. If the target does not have provisions for moisture monitoring and control, the humidity control module is also required.

The gas generation module utilizes two high-pressure cylinders of 2% Cl_2, 98% nitrogen mixture and three $NaClO_2$ gas generation cartridges. This module also contains the operator control

interface, system monitoring, and report generation functions. The control system is comprised of a PLC for internal system functions and an industrial PC for the operator interface. Each of the satellite modules, which are located at or near the target(s) are interfaced electrically to the PLC in the gas generation module.

The gas inject module consists of a stainless steel enclosure mounted closely to each target(s). Its major component is valving that is monitored and controlled by the gas generation module. Also in close proximity to each target(s) is the sensor module, which extracts a small portion of the atmosphere of the target via a sampling loop. The sensor module monitors CD concentration, temperature, humidity, and pressure. A pressure relief function is also provided.

The humidity control module uses dry steam to maintain the required level of humidity for optimal antimicrobial activity for CD gas. The monitoring and control functions for this module are again provided by the gas generation module.

CD Gas Sterilization Process

For isolation systems and other enclosures at or near atmospheric pressure, the CD gas sterilization process has these basic steps:

- The chamber is loaded, sealed and a leak test is performed according to the recommendation of the manufacturer of the chamber.
- Establishment of ~70% relative humidity(RH)in the target, typically for 30 minutes.
- Injection of CD gas to achieve the target concentration by displacement of a small portion of the atmosphere.
- Exposure at the desired CD gas concentration for the required time period.
- Aeration of the target by the introduction of fresh air (filtered) and exhaust to atmosphere or recovery device.

Applications Testing

The CD sterilization process was successfully evaluated with the following targets:

- 25-ft^3 flexible-wall (PVC) isolator
- 100-ft^3 rigid-wall workstation-type isolator with half-suit
- 250-ft^3 rigid stainless steel and polycarbonate, aseptic fill isolator with HEPA filtration system and two half-suits
- 6000-ft^3 sealed room with integrated air recirculation system
- 2- and 32-ft^3 stainless steel sterilization chambers

During these evaluations, the following observations were made and results obtained:

- The humidity control module rapidly raised the RH to the desired level, generally in less than 5 minutes, with no observable wetness or "puddling".
- The RH held constant during the 30-minute dwell period.
- The CD gas injection time was relatively rapid (< 20 minutes) due to the differential between the generated versus use concentration (~100 mg/L

at generation, diluted to 1 to 10 mg/L for isolator/room applications).

- The CD gas concentration held constant during the gas exposure step. A typical CD gas concentration curve for a 25-ft^3 sterility testing isolator is shown in Figure 2.
- Aeration time was dependent upon target volume and the turnover rate of the air recirculation system. Due to the low chemical affinity of most materials for CD, aeration was rapid, with processing times as low as 15 minutes with one air change per minute.
- Uniform gas distribution was obtained in all test systems based upon homogeneous CD gas color and/or spectrophotometric assay of CD gas concentration and/or microbiological testing.
- Isolator and room materials withstood numerous exposures to CD gas without significant deleterious effects.

Microbiological Testing

Initial studies with gaseous CD were performed in a stainless steel vacuum chamber and focused on the effect of gas concentration on the rate of inactivation of *Bacillus subtilis* spores inoculated onto paper-strips. *B*. *subtilis* has been shown to be the biological indicator (BI) of choice for gaseous CD being more resistant than the other common indicator organisms. The BIs were placed throughout the sterilization load and exposed to CD at 10, 20, and 40 mg/L; the results of these studies are shown in Table 1. As expected, the time to achieve sterilization of all of the BIs decreased with increasing CD concentration. With this test system, less than 30 minutes were required at 40 mg/L of CD to sterilize 10^6 *B. subtilis* spores. Considering that the exposure temperature was slightly above ambient (30 to 32°C) and the gas concentration was only 40 mg/L, rapid sterilization kinetics were observed when compared to ethylene oxide (EtO) at much higher concentrations.

Initial studies with isolation systems were performed with a 25-ft^3 flexible-wall sterility testing isolator. CD gas was used at 10 mg/L due to the ease of gas access to the isolator surfaces compared to the highly dense sterilization loads used in the study described above. BIs (10^6) were placed in petri dishes on the floor of the isolator and the RH was raised to ~70% as described previously. After a 30-minute dwell period at this RH, CD gas was introduced and the BIs exposed for selected time periods. The results of this study were as follows:

Exposure Minutes	Fraction Nonsterile
5	8/10
10	1/10
15	0/10

These results clearly demonstrated the potential application of gaseous CD for the HLD or sterilization of isolators and other enclosures at a gas concentration of 10 mg/L.

The visually uniform distribution of the CD gas within the isolator suggested that all areas were being equivalently exposed. To test this hypothesis, studies were performed with an isolator configured as shown in Figure 3. Stands

fabricated from rigid polyvinyl chloride (PVC, known not to bind significant amounts of CD) rods were placed in the isolator as depicted in Figure 3. Unwrapped paper strip BIs (10^6) were suspended from the ends of the rods and exposed to CD gas at 10 mg/L for 7 minutes. At this exposure time, a fraction of the BIs would be expected to be nonsterile. The following results were obtained:

BI Position	Fraction Nonsterile
1	2/6
2	2/6
3	2/6
4	2/6
5	4/6
6	2/6
7	0/6
8	4/6
9	1/6
10	3/6

In repetitions of experiments of this type, no BI position(s) could be identified that consistently differed in the observed fraction of nonsterile BIs. When the gas exposure time was raised from 7 to 15 minutes, all sterile BIs were observed. These results support the visual observation that there was uniform distribution of CD gas within the isolator.

Initial studies on the efficacy of gaseous CD for the HLD or sterilization of isolation systems used a gas concentration of 10 mg/L. At this concentration, greater than six log reductions of resistant spores were observed in $\leq$ 15 minutes. In order to more accurately determine D_{10} values, an experimental procedure was developed to ensure that "square wave" exposure conditions were simulated as closely as possible. Using this procedure, D_{10} values were determined at 10, 20, and 30 mg/L of CD in an empty isolator. The following results were obtained:

CD Concentration (mg/L)	D_{10} value (minutes)
10	.75
20	.27
30	.12

As expected, the D_{10} values decreased as the CD concentration increased. To evaluate the lower range of CD efficacy, studies were conducted at concentrations of 5, 3 and 1 mg/L. In a 250-ft^3 rigid-wall isolation system a 5 mg/L concentration of CD was tested in a 30-minute exposure and a 10^{-6} SAL (sterility assurance level) was achieved. In a 6000-ft^3 room, CD concentrations of 3 and 1 mg/L were evaluated by suspending a total of 36 BIs (with a population of 10^6 *B. subtilis* spores) throughout the room. In a 15-minute cycle, at 3 mg/L, a 10^{-6} SAL was achieved and the same results obtained at a concentration of 1 mg/L in a 120-minute exposure. D_{10} values were determined at 3 and 5 mg/L as follows:

CD Concentration (mg/L)	D_{10} value (minutes)
3	4.88
5	3.56

In all cases, the inactivation rate was very rapid, even at extremely low concentrations. These results indicate

that efficient, short duration, high concentration exposures and low concentration sterilization processes can be developed with CD.

Application Examples

Rapid sterilization of a 25-ft^3 Sterility Test Transfer Isolator has been previously described using *B. subtilis* as the preferred BI. The sterility test transfer isolator application is straight forward because all chamber surfaces are readily accessible, the chamber volume is minimal and the sterilent can be easily distributed throughout the chamber. Similar work in the same lab using a small rigid wall one-suit workstation demonstrated efficacy in the larger chamber (100 ft^3). Total cycle time was only slightly increased to accommodate the larger volume chamber.

The additional time for scale up is consistent with the chamber volume increase and affects only the humidification and sterilent charge times. The added time is minimal to achieve the target humidity level because of the small quantity of steam required to move the ambient humidity from 30-40% to 70% RH. Charge time for sterilent is likewise minimally impacted because the stock concentration from the cartridge is supplied at approximately 100 mg/L. Using a concentration of 10 mg/L then requires only that 10% of the chamber volume be displaced. In practice slightly more is displaced due to loss of some sterilent out the vent valve during the charge phase. These chamber volume factors, supply capacity (for steam and sterilent) and connection locations are critical considerations for scale up and will be further discussed later.

The final phase of the cycle, aeration, is rapid with CD because of the minimal absorption into polymer matrices. As a result, aeration typically requires 11-15 air exchanges depending on the mixing pattern within the isolator.

Testing with a larger isolation chamber was performed as a cooperative investigation. A manual prototype CD Generating unit was interfaced to a custom made Aseptic Fill Isolator with a total internal volume of 250 ft^3. The control system of the Aseptic Fill Isolator controlled pressure within the isolator throughout the sterilization process by controlling fan speed of the HEPA-blower units with pressure relief through a vent valve. In addition, the isolator controls operated the humidity control module to achieve and maintain consistent humidity levels. The isolator included two half-suits and a removable hatch for access to the fill equipment. Laminar airflow during normal operation was provided by six independently controllable HEPA/blowers. During the sterilization process only one of the six HEPA-blower units was in operation at a time.

Distribution was achieved by cycling operation of the six HEPA-blower units sequentially. Process steps were as follows:

- Placement of 20 *B. subtilis* BIs throughout the chamber, isolator door sealed and unit powered
- Isolator controls performed an automated leak test

- Isolator established desired pressure differential
- Humidification to programmed level by Humidity Control Module
- Humidity maintained for 30 minutes above 55% RH
- CD injected from CD prototype
- CD mixed in chamber by HEPA-blower units and monitored with spectrophotometer in sample loop
- CD concentration continuously monitored for defined exposure time frame
- At end of exposure phase exhaust vents opened for direct to atmosphere air wash of the isolator with HEPA filtered air. Aeration was continued until chamber concentration was below 0.1 ppm.

With this testing configuration, a number of cycle designs were shown to provide a six log reduction of *B. subtilis* with CD concentrations as low as 5.0 mg/L. Allowing for mixing and distribution, exposure times were consistent with previously discussed D-values.

Conclusion

Finally, connection to chambers such as lyophilizers or other vacuum chambers is also possible using the same CD gas generation system as that used for isolator and room sterilization applications. Each isolator and vacuum chamber will have a dedicated gas injection and sensor module connected to the common gas generation module. Remotely located isolators and vacuum chambers can then be processed in sequence without the need to make and break connections or purchase separate sterilent generators.

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