Application Note #74- Chlorine Dioxide Gas Sterilization of Cold-Chain or Temperature Sensitive Devices

ClorDiSys' chlorine dioxide gas sterilization is an ideal sterilization method for cold-chain or temperature sensitive items due to its ambient temperature processing capabilities. Its gaseous nature allows for high efficacy in penetration and sterilization and can do so without elevating processing temperatures. This allows for high compatibility for pre-filled syringes or autoinjectors which may contain temperature sensitive drugs, however it also provides high material compatibility for a wide range of polymers, bioabsorbable products, and other materials.

Chlorine Dioxide Gas Properties

Chlorine dioxide gas (CD) 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 timeweighted 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 (Long, Battisti, Elyath 2000). In addition, chlorine dioxide gas breaks down into chlorite, chlorate, and chloride which have relatively low toxicities in humans and are not mutagenic.[1]

Chlorine Dioxide (CD) gas by ClorDiSys is generated with the CSI CD Cartridge (EPA label # 80802-1) and is registered with the US-Environmental Protection Association as a sterilant gas for porous and non-porous surfaces. Chlorine dioxide can be used to sterilize prefilled syringes, cold chain products, medical devices, as well as other applications. Prefilled syringes and medical devices are typically sterilized in an ambient temperature vacuum sterilizer.

Chlorine dioxide is not sufficiently stable to be stored, it must be generated onsite at the point of use. There are many ways to generate CD gas and one method is using, a low-level chlorine gas (2%) which is passed over solid sodium chlorite CD Generating Cartridges which convert the chlorine to pure chlorine dioxide (>99% yield).

The oxidation of sodium chlorite ($NaClO_2$) is the basis of the reaction:

$$2NaClO_2 + Cl_2 \text{ generating } 2ClO_2 + 2NaCl$$

The 2% chlorine gas is mixed with nitrogen. This stoichiometrically limits the chlorine dioxide gas concentration to 4% which is significantly below the 10% level which is potentially explosive. Chlorine purity levels should be greater than 99%. The CD Generating Cartridges have a 1-year shelf life and generate CD gas for up to 300 minutes before they require replacement. The shelf life of reagent gas tanks are manufacturer specific. The sterilizer control system automatically prevents the CD Cartridges from being used for more than the allowable capacity. This process produces a pure chlorine dioxide gas and is injected into the target chamber for a fixed time or until a dosage setpoint has been met. The chlorine dioxide flow rate is 20 liters per minute +/- 4 liters per minute. After the exposure is completed,

the gas is typically passed through a carbon scrubber to fully eliminate any chlorine dioxide gas. Alternatively, chlorine dioxide may be exhausted to the outside environment via house exhaust systems. Federal state and local regulations must be verified prior to exhausting CD gas. Direct venting is typically allowable.

Sterilization chambers for chlorine dioxide include ambient pressure chambers or vacuum pressure chambers. The target chamber choice depends upon the product requiring sterilization. If the product is simple in its geometry and has no small openings, then an ambient pressure chamber can be used. If the product is complex in its geometry and has many lumens or tubing, or small openings, then a vacuum process may be required. A vacuum sterilizer will remove most of the air particles from both the chamber as well as internal portions of the device itself, then replace the air with humidity and CD gas. This allows the moisture, as well as the CD, to penetrate tight areas of a device or packaging.

Chlorine dioxide gas sterilization is considered a true ambient temperature process since the temperature is not manipulated by the process. Chlorine dioxide gas has a yellow-green color, and this allows the concentration to be monitored by a UV-Vis spectrophotometer. Throughout the cycle, samples of gas are continuously pulled from the chamber and passed through the UV-Vis spectrophotometer to give a precise concentration reading. Since all critical process parameters are monitored, the system is set up to facilitate parametric of product. Chlorine dioxide gas also has a boiling point between -20˚C and -40˚C depending on use concentrations, thereby making it a true gas at room temperatures (15-25˚C). Being a true gas, it will not condense at use temperatures like a vapor would. Instead, a gaseous process is a more reliable and consistent process with better penetration. Chlorine dioxide has a molecular weight of 67.5 but stratification is not an issue with chlorine dioxide. Testing at ambient pressure was performed demonstrating that with minimal circulation, gas concentration is still evenly distributed throughout a chamber.

Chlorine dioxide gas is also flexible regarding product packaging considerations. The main criteria is that the product must be wrapped in suitable packaging to maintain sterility. Tyvek® packaging is often utilized to serve as the sterile primary packaging barrier since it allows CD gas to penetrate and does not allow organisms to re-contaminate the device inside. Primary packaging may also include foil or blister packaging or other packaging along with a gas permeable material, which includes Tyvek® or medical grade paper. Product to be sterilized may also be placed into secondary packaging which can include unit cartons, display boxes, pouches, trays, etc. Additionally, items may be placed within tertiary packaging or shippers. Chlorine dioxide gas is compatible with the sterilization of cellulosic materials which permits the incorporation of paper labels on devices as well as fiberboard or corrugated packaging. This allows for the simplification of the sterilization process and flexibility on the packaging of a device. For this product, the packaging consisted of the product filled syringe (PFS) placed within blister packaging and sealed with Tyvek® lidding. The blister packages are maintained within their secondary packaging that includes a unit carton and instructions for use (IFU) packet.

Below is a case study for the sterilization of a cold-chain, pre-filled syringe that was undergoing evaluation for what the optimal sterilization modality would be for the product.

Goal:
Compare sterilization modalities for a recombinant protein used for ophthalmologic use.

Restrictions:
Product must be maintained between 2-8° Celsius.

Chlorine Dioxide Gas Advantages Towards Product:
Chlorine dioxide gas sterilization cycles are processed at ambient temperature and generally last only between 4-6 hours on average. This allows for the product to not experience extreme temperatures during the cycle as well as it limits the Time out of Refrigeration (TOR) of the product. Unlike alternative, ambient temperature sterilizing agents, chlorine dioxide does have the ability to sterilize cellulose materials. This allows for the sterilization of Instructions for Use (IFU), unit cartons, paper labels, etc.

Feasibility Testing:
Preliminary testing by placing BI's in the perceived difficult locations within the PFS in order to determine the two most difficult locations and to develop the cycle parameters to obtain complete kill in those locations.

Ingress Testing:
One hundred forty (140) syringe components were assembled and filled with Type 1 HPLC Grade Distilled Water to the fill line of l 70µL. Seventy (70) filled syringes (176458-1) were used as blanks or controls and the remaining 70 (176468-3) were exposed to chlorine dioxide sterilization.

The determination made after ion chromatography testing of the Type 1 HPLC Grade Distilled Water was completed on the sterilized and unsterilized samples, was that Chlorine dioxide gas did not disrupt the recombinant protein drug.

Residual Testing:
Sixty (60) syringe components were assembled. Thirty (30) assembled and unfilled syringes (176458-2) were used as blanks or controls, and the remaining 30 (176458-4) were exposed to Chlorine Dioxide Sterilization.

The determination made after ion chromatography testing of the sterilized and unsterilized samples, was that Chlorine dioxide gas did not result in an increased occurrence of ions were found on the syringe.

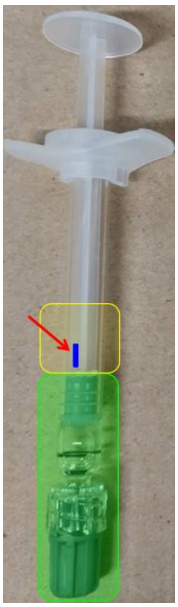
Validation:

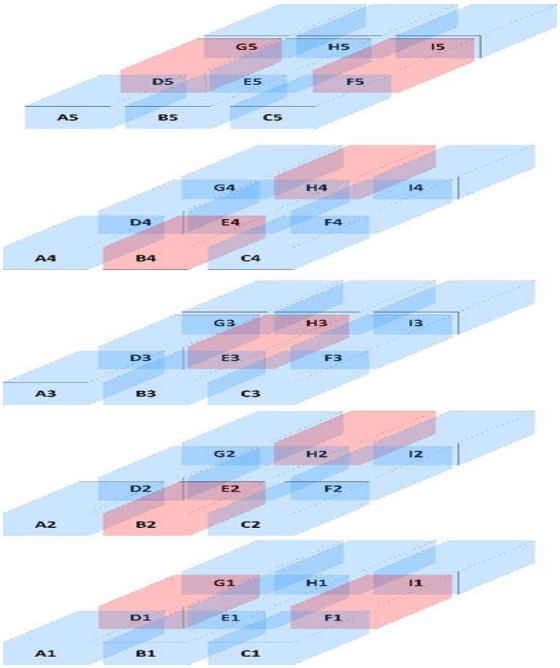
Validation of a medical device or combination product utilizing chlorine dioxide gas sterilization falls under ISO 14937:2009. The validation method utilized in this case study was the overkill method.

Materials used include-

- 1800 Product Filled Syringes to be used as a Maximum Load
- 9 Internal Process Challenge Devices (IPCDs)
- 9 External Process Challenge Devices (EPCDs)
- 11 Humidity and Temperature Monitors

Below: Biological indicator placements within IPCD.
Right: EPCD created for the sterilization cycle utilized.





Above: Product layout in chamber, red designates locations of IPCDs and EPCDs.

The validation process consisted of nineteen sterilization cycles to test a variety of load sizes and included a fractional cycle as well as nine half cycles and nine full cycles to provide an abundance of data. The fractional cycle established the lethality hierarchy between the product's natural bioburden, the IPCDs and the EPCDs. The half cycles indicate the cycle parameters needed to achieve lethality on the product's natural bioburden as well as complete kill of all IPCDs and all EPCDs.

For ClorDiSys' chlorine dioxide gas vacuum chamber sterilization consists of the following steps:

Step 1- Pull an initial vacuum to remove much of the air out of the chamber (typically 5-10 kPa).

Step 2- Perform a leak test to ensure that there is no significant air leakage into the chamber.

Step 3- Precondition. This step raises the relative humidity to the setpoint (generally 65-75%).

Step 4- Condition. This step holds the relative humidity at the setpoint for typically 30-90 min.

Step 5- Charge. This step raises the chlorine dioxide gas concentration to the setpoint (typically 1-30 mg/L).

Step 6- Exposure. This step holds the chlorine dioxide gas concentration at the desired CD concentration, expressed in mg/L, until either the dosage or the exposure time is attained. If the concentration drops

for any reason the Steridox-100 will inject more gas into the system until concentration returns to its desired set point.

Step 7- Aeration. This step removes the chlorine dioxide gas from the chamber by pulling vacuum and breaking with filtered air. Typically, 12 vacuum/break cycles are required to remove the gas to safe levels.

Step 8- Introduction of filtered air into the chamber to bring the chamber back up to ambient pressure (HEPA filtered air is an option).

The product had been previously validated with ethylene oxide and the cycle reached a maximum average temperature of 55 degrees Celsius and the duration of the cycle was over forty-six hours.

The validated chlorine dioxide cycle was performed at ambient temperature, allowing the product to not experience temperatures over 21 degrees Celsius. The average cycle had a duration of three hours and forty-five minutes, inclusive of pre-conditioning and aeration.

With ethylene oxide sterilization, not only was the sterilization elevated in temperature, but the time out of refrigeration (TOR) was greatly extended. The sterilization of the PFS utilizing ethylene oxide degraded the product to a significant degree and caused the drug to be unsellable in that format.

Ethylene Oxide Full Cycle		
Pre-Conditioning	38-49°C	18-24 hours
Conditioning	31-42°C	59-79 minutes
Gas Exposure	31-42°C	2.75-3 hours
Heated Aeration	38-55°C	12-18 hours
Clorine Dioxide Full Cycle		
Pre-Conditioning	21°C	12 minutes
Conditioning	20°C	60 minutes
Gas Exposure	21°C	2 hours
Aeration	20°C	30 minutes

The sterilization of the PFS utilizing chlorine dioxide gas was favorable to the PFS and maintained the quality of the drug. The supply chain can also be shortened due to the minimal cycle length and

opportunity for the manufacturer to sterilize the product in-house with the purchase of a chamber. The sterilization process is also much simpler than other alternative sterilization modalities because of the ability to sterilize in unit cartons, with IFUs, and other packaging. This streamlines the process and avoids additional logistical complexities. Chlorine dioxide also lessens environmental and health concerns as compared to ethylene oxide and other sterilization methodologies.

References:

1. Long, James, Diane L. Battisti, and A. Eylath. "STERILIZATION WITH GASEOUS CHLORINE DIOXIDE: APPLICATIONS AND OPPORTUNITIES.