

Chlorine Dioxide, Part 2

A Versatile, High-Value Sterilant for the Biopharmaceutical Industry

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Last month, Part 1 of this article discussed some proven applications of chlorine dioxide (CD) gas and solutions as cleaning/sterilizing agents for isolators, process vessels, filter membranes, water systems, hard surfaces, and cleanrooms. Potential applications were also described, including the production of sterilized water from USP-grade water; sanitization of chromatography columns; resins, and membranes; biowaste kill; and sterilization of disposable process systems. This month, Part 2 discusses economic and validation issues as well as methods of production.

APPLICATION VERSATILITY

CD has been demonstrated effective in both the aqueous and gas phases, which is highly advantageous for biopharmaceutical applications. It is easy to change CD from one state to the other. One benefit of the membrane sachet technology (see below) is that it can be used to generate an aqueous solution that can be stored until needed. To release the CD for gas-phase use, the stock solution is

sparged with air or nitrogen and resulting vapor directed to the work area. When an equipment generator is used to produce gaseous CD, aqueous solutions can be formed by exposing the gas to water in some type of contacting device (e.g., a packed column). For gaseous uses, depending on the volume and configuration of the treatment area, fans may be needed to circulate the CD vapor.

Information is extensive on using CD as an aqueous-solution sanitizer in food applications. CD is unique in that it attacks and erodes the structure of microbial plaques as well as individual microbes. So it can penetrate and breakdown microflora deposits and then assault the microflora themselves.

CD is easy to apply as an aqueous solution, fog, or foam. The best method will depend on the individual application. If timing is tight, gas is likely to provide the best results in pure sanitization of large spaces. There are no liquids or other chemicals to complicate and possibly interfere with the reactions. Fogging is a means of distributing a liquid uniformly over a large area and can be used to disinfect surfaces in air-handling systems and equipment. Foams require an active agent but can be valuable in the presence of small nooks or woven materials. CD liquid is best for hard surfaces, such as in many process applications in biopharmaceutical manufacturing. As stated, in a closed vessel the liquid phase need not contact all surfaces to accomplish sterilization. Liquid also provides enhanced cleaning capability. CD acts as an oxidizer for organic



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compounds and could displace caustic (NaOH) for certain clean-in-place (CIP) tasks. Time savings for a complete CIP-sterilization cycle could be significant. Liquids generally provide soil removal because of their convective energy and viscosity, which cannot be duplicated by low pressure gas.

COMMENTS ON VALIDATION

One concern when introducing novel methods and materials into a biopharmaceutical facility involves validation. In the case of CD, such concerns are minimal. Available literature indicates that standard methods of BI testing work well for both sanitization and sterilization applications. Traditional validation should be appropriate because of the simplicity of CD generation, the ability to detect CD at very low concentrations, and the ease of evacuating CD once disinfection is accomplished. Berry provides a complete outline for the pharmaceutical industry (19). BIs have received a great deal of attention in harmonization of international standards. Part one of ISO 1138 covers general requirements; part two

PRODUCT FOCUS: BIOPHARMACEUTICALS

PROCESS FOCUS: MANUFACTURING AND RESEARCH

WHO SHOULD READ: PROCESS DEVELOPMENT, MANUFACTURING, LABORATORY MANAGERS, AND QUALITY CONTROL

KEYWORDS: CLEANING VALIDATION, DISINFECTION/SANITATION, PRODUCTION, DOWNSTREAM PROCESSING, DISPOSABLES

LEVEL: INTERMEDIATE

is specific for BIs for EtO sterilization processes; and part three deals with BIs for moist-heat sterilization processes (20, 21). Table 4 summarizes the European standardization committee's standards series. The FDA's recent openness to process improvements that improve manufacturing economics to lower drug prices makes this an ideal time to transition to CD technologies.

THE VALUE PROPOSITION

The pharmaceutical industry has come under pressure in recent years to find ways to reduce drug prices. Expectations are that the situation will continue. Manufacturing is one area that has been targeted as having considerable potential for cost savings. CD use in biopharmaceutical applications identified herein should translate into economic advantages for implementers. The "Economic Advantages" box lists some of these.

Risk Minimization: Quality is the major issue for biopharmaceutical operations. The cost of contaminated product lots (whether or not they reach patients) far outweighs the costs associated with any sanitizing agent. Because of its effectiveness, speed of kill, and ability to enter the smallest spaces and quickly diffuse into liquid-filled deadlegs, CD could very well decrease instances of microbiologically contaminated batches. Potential benefits include elimination or mitigation of the following:

- Cost of rework and disposal of contaminated batches
- Loss of future revenues
- Legal costs and payouts to consumers
- Impact on corporate reputation and company valuation (stock price).

Economic Advantages Over Clean Steam: The value of a sanitizing agent depends on the specific problem it is intended to solve. Both operating factors and engineering factors come into play. Clean steam is not an inexpensive option if operating factors are taken into account. It must be prepared from WFI at the end of a complex water treatment sequence. In many biopharmaceutical processes, scheduling of sterilization steps becomes challenging because of a

Table 3: Disinfectant concentrations tested resulting in a 99.999% reduction in viable cell counts after a 60-second exposure; (SELECTIVE MICRO TECHNOLOGIES, WWW.SELECTIVEMICRO.COM)

Disinfectant	Test Organisms		
	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. cerevisiae</i>
Sodium hypochlorite 1 ¹	1,000	1,000	1,000
Sodium hypochlorite 2 ¹	820	820	1,600
Stabilized chlorine dioxide 1 ^{1,2}	310	1,200	640
Stabilized chlorine dioxide 2 ^{1,3}	48	93	95
Iodophor ¹	440	440	450
Peroxide ¹	36,000	68,000	270,000
Glutaraldehyde-phenol ¹	2,300	1,200	620
Acid glutaraldehyde ¹	6,600	2,200	18,000
Quat ¹	580	140	74
Acidified Quat ¹	150	1,200	300
Phenolic ¹	1,500	380	190
Peracetic Acid ⁴	300	400	800
Selective Micro Clean ⁵	5	5	—

¹ Tanner RS. *J. Ind. Microbiol.* 4, 1989: 145–154.

² Lactic acid activation

³ Citric acid activation

⁴ Reported by Vergagene Ltd. (Bolton, UK)

⁵ Testing performed by Microbiotest, Inc. (Sterling, VA)

desire to sterilize an entire train of equipment before feed operations begin. Long sequences and lengthy delays for equipment preparation must be included in the manufacturing cycle plan.

With CD, especially in a stock solution setup, equipment can be sterilized as it becomes available: The CD is prepared as an aqueous solution at several times its use strength. A portion of that liquid is then diverted to the system or vessel to be sterilized and mixed with water to provide the proper concentration. For vapor-phase treatment, the stock solution is sparged to produce the vapor, which is routed to an area for treatment. Effective at ambient temperature and atmospheric pressure, CD sterilization requires no heat-up or cool-down periods, nor must process equipment be pressurized as is needed for steam service.

Another challenge for steam sterilization is an inability to ensure that all surfaces are exposed to the appropriate temperature in a two-phase condensing system. This is especially true for deadlegs and small spaces. CD does not depend on temperature–time exposure (although its effectiveness depends on concentration–time). Doing its job as a liquid, gas in equilibrium with liquid, or as a totally gaseous input, CD can easily handle this challenge.

Disposal of steam after its use is also time consuming. Depending on the application, a vessel or system may

be vented or gas-purged—or vacuum and air may be applied in sequence to displace the steam. With CD in an aqueous-phase application, the system is merely drained and rinsed with one to 1.5 times the system volume of WFI. Drying may or may not be required. In a vapor-phase treatment, CD is purged to atmosphere with compressed air or nitrogen or by using normal vent or HVAC fans.

Finally, clean steam (especially clean steam condensate), can be very corrosive even to high-grade stainless steels. CD's high activity at ambient temperature eliminates the effects of repeated heating and cooling cycles. The resulting lower maintenance is another useful and valuable attribute.

Interrelated engineering factors revolve around time cycles. For a novel agent such as CD, the potential time

Table 4: Biological indicator (BI) standards for sterilization from the European Committee for Standardization (www.cenorm.be) (8)

Part	Topic
1, 2, 3	General requirements, EtO, and moist heat
4	Details BIs for irradiation sterilization
5	Specific to BIs for low-temperature steam and formaldehyde sterilization (LTSF) processes
6	BIs for dry-heat sterilization
7	Self-contained BI systems for moist-heat sterilization
8	Self-contained BI systems for EtO sterilization

savings could make a difference in process equipment scale, complexity, and cost. The simplicity of CD operation (over VHP) in sterilizing isolators reduces costs in time, labor, or both. Table 5 compares other important attributes of CD and clean steam.

CD PRODUCTION TECHNOLOGIES

If not handled properly, CD gas can become unstable and is potentially explosive. Aqueous solutions are much more stable. The US Department of Transportation will not permit manufactured CD to be transported. So generation must be performed on-site. This is a major reason CD has not been widely used in biopharmaceutical manufacturing until now.

Active CD can be generated safely in two ways: by equipment-based gas generators and membrane sachets for aqueous CD. Both share the common benefit of simplified waste disposal

because CD is environmentally friendly, dissipating rapidly in the gas or aqueous phase upon exposure to UV light.

Equipment-Based Generators:

Today's equipment-based CD gas generating processes have evolved from small chemical sub-processes involving several unit operations. Older versions were characterized by low-purity product, severe material-compatibility issues, and poor controls. Many improvements have been made over the years. Today gas-generation systems are based on several different chemical reaction routes that consistently produce high-purity CD. Each technology has advantages in certain applications.

In general, gas generators are appropriate for making large quantities. These generators have been offered to the pharmaceutical industry, but they may be a more appropriate fit for large, continuous users such as municipal water systems. The purity of CD produced is variable depending on the generator design: high for pharmaceutical units and moderate for municipal water units. Some technologies involve compressed, dilute chlorine as a raw material, which makes safety a consideration. ClorDiSys Solutions Inc. (www.clordisys.com) is a provider of CD gas generators appropriately sized and designed for the biopharmaceutical industry.

For biopharmaceutical companies, equipment-based gas generators offer

- semibatch CD generation
- self-contained systems including generator, controls, and monitoring provisions
- ability to generate larger quantities of CD.

When compared with membrane sachets, operating and capital costs favor the sachet method (below), as do space requirements. But based on cost per gram of CD produced, the gas generation raw material cost is lower.

Membrane Sachets: CD can be generated by several different chemical reaction routes through the reaction of dry chemicals with water. The quality (purity) and yield is highly variable across these technologies, so their individual utility to the

biopharmaceutical industry must be determined. Some products make CD from "stabilized chlorine dioxide" solutions. Such wet-chemical processes produce less effective and more corrosive form of CD (due to low pH and relatively high concentrations of undesirable byproducts) unsuitable for most biopharmaceutical applications. In some formats, CD precursors are supplied as a number of dry chemicals inside a reaction-controlling membrane sachet. These sachets are immersed in water to generate the CD.

Selective Micro Technologies, LLC (www.selectivemicro.com) uses a patented membrane system to generate CD in water at room temperature. With this approach, liquid water never contacts reactant material inside the sachet (called a microreactor) because the membrane is gas-permeable, allowing only water vapor inside. Only pure CD gas is transferred across the membrane and out of the sachet. This approach has advantages over other liquid and gas systems because it rapidly generates concentrated CD of the highest purity at neutral pH. With no impurities released, corrosion is minimal or nonexistent for stainless steel, plastics, and other materials commonly used in biopharmaceutical facilities.

Using that technology, a solution of CD is available after 1–10 hours, depending on the sachet size, number of sachets used, and desired concentration. These sachets can be used to make a concentrated stock solution that is diluted and added to substrate in a target vessel, or the solution can be used directly for applications such as laboratory surface decontamination. For gas-phase applications, the stock solution can be sparged to produce humidified CD gas. CD can be stripped from such aqueous solutions in a matter of minutes using modest gas sparge rates.

The microreactor approach is attractive because it offers

- Rapid cycles producing a >99% pure aqueous solution of CD
- Minimal capital cost and equipment space requirements
- Maximum flexibility of location and scale of generation
- Minimal storage requirements, low operating cost

ECONOMIC ADVANTAGES OF CD

Capital Cost Savings

Save time in CIP/SIP: smaller equipment for a specified capacity or higher capacities from existing equipment

Decrease WFI production

Eliminate or downsize clean steam

Reduce space requirements for equipment and storage

A single, plant-wide disinfectant

Operating Cost Savings

Lower materials cost than for other disinfectants with similar capabilities

Lower operating labor costs per unit of product (ease of preparation and application, especially with membrane-sachet CD production)

Lower CIP chemical costs

Reduced disposal costs

Lower operating costs for WFI and clean steam

Potential savings: fewer contaminated batches

Nontoxic, nonhazardous, nonflammable, environmentally friendly sanitizer/sterilizer use

Table 5: Comparison of chlorine dioxide and clean steam attributes

Attribute	ClO ₂ Gas	Steam Vapor
Sporicidal effectiveness	+	+
Effective through HEPA filters	+	–
Noncarcinogenic	+	+
Toxicity (TWA PEL ppm)	0.1	N/A
Nonexplosive (at normal use concentration)	+	+
Relative humidity requirement	65–90%	N/A
No residuals	+	+
Noncorrosive (to biopharm MOC)	+	+
Removal	Venting	Venting vacuum
Application development effort limited	+	–
Raw material cost	–	+
Capital cost	+	–

- Self-regulating CD generation without chlorine gas or dependence on control systems
- Nonhazardous waste products.

RELEVANT PROPERTIES

Although CD is soluble in organics and moderately soluble in water, it is easily transferred from aqueous solution to vapor phase because of weak hydrogen bonding. Its Henry's Law coefficient is sufficiently low to ensure a relatively high concentration in the liquid phase. The usual concentrations for CD application are at the mg/L level. Gas-phase sterilization applications are generally optimum at a relative humidity of about 70%.

With its fast kinetics against microbes, CD is remarkable for its stability and low interaction (22). Numerous investigators report D-values of a few minutes (23) across a wide range of microorganisms, even against virus and spore formers (3). The National Research Council has developed extensive documentation on chlorine dioxide for drinking water treatment (24).

Chlorine dioxide most likely inactivates microorganisms (as listed in the "Proven Effectiveness" box) through direct oxidation of tyrosine-, methionyl-, or cysteine-containing

portions of their proteins, interfering with key structural regions of sensitive metabolic enzymes or membrane components (2).

CD is classified as an irritant rather than a toxic agent. It can be mildly irritating to mucous membranes with direct exposure over time. Conventional carbon respirators have been effective. In a series of animal tests at certified laboratories, CD generated by microreactor sachets has been shown to be nontoxic for liquid ingestion and inhalation at concentrations well above those for recommended use.

AN EMERGING SOLUTION

Although it has been in industrial use for some time, CD's reputation as a sanitizer, disinfectant, and sterilant has improved considerably in recent years. One reason is that modern generation technologies can produce higher purity, "user-friendly" CD. Pricing (and profitability) pressures are likely to continue, forcing bioprocessors to continue searching for technologies that will improve manufacturing economics.

CD appears to have the potential to make a significant contribution in that regard. A number of biopharmaceutical industry applications have been targeted following successes in analogous applications elsewhere as well as some direct successes in applications unique to bioprocessing. Results to date have been impressive, with industry acceptance, implementation, and testing proceeding at a rapid rate.

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