Application Note

2008-0424

Sterilization – Ethylene Oxide, Gamma Radiation

Single-use systems offer the promise of faster process turn-around times, higher efficiency and utilization of equipment, improved batch-to-batch sterility, and simplified cleaning validation. The elimination of glass vessel autoclaving or stainless steel reactor SIP/CIP is a significant benefit as both methods consume time and operator resources, energy and water, and require specific liquid handling and disposal systems. Furthermore, traditional cleaning methods often use hazardous chemicals that pose a safety risk to the operators who handle them.

This application note discusses the benefits of single-use sterilization methods when compared to stainless steel reactor sterilization. It also describes the two primary methods are used for the sterilization of disposable components, namely exposure to ethylene oxide and gamma radiation. An excellent reference with numerous useful links for additional information about both ethylene oxide and gamma radiation sterilization can be found at: http://www.isomedix.com/TechTeam/Resources.html.

Stainless Steel Reactor Sterilization

Stainless steel systems are sterilized either by autoclaving (smaller systems) or by steam-in-place (SIP) and clean-in-place (CIP) procedures (larger systems). The cleaning process usually involves dismantling the reactor and removing sensors, housings, filters, and other components that cannot survive the temperatures or caustic chemicals of the cleaning process. After CIP sterilization, stainless steel hard-piped systems must be rinsed with sufficiently enough purified water to ensure the removal of any residual cleaning fluid. Often, significant amounts of water for injection (WFI) are required for the system flush. The system must be then be re-assembled with sterilized components, and sometimes re-sterilized a second time (e.g., SIP), which adds further time to the operation as well as increases labor costs.

Comparable to time and materials costs, is the often hidden validation cost of the sterilization process. Complex sterilization processes involve detailed process documentation, but are also very hard to validate since the inspector must prove that all traces of the cleaning chemicals have been removed from the bioreactor system. Gaps in the resulting validation documentation can cause regulatory scrutiny, and in the worst case, product production can become delayed. Specifically, any failure to execute the documented cleaning standard operating procedures (SOPs) is considered a non-compliance, leading to the validity of the cleaning process being questioned. Such errors cost additional time and effort.

Single-use systems can be purchased pre-sterilized by ethylene oxide or gamma irradiation, thereby eliminating the need for sterilization and associated sterilization validation procedures. The end user can essentially

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remove the product from its package and install it in the process – in many cases, several disposable components can be pre-assembled by the manufacturer and gamma sterilized, thereby ensuring sterile connections and further reducing operator labor. In addition, disposable product manufacturers often provide validation documentation, thereby eliminating the need for the end user to duplicate this effort, once the vendor's process has been qualified and approved.

While the 'gross' sterilization cost is comparable between disposable component sterilization methods (ethylene oxide and gamma radiation) and traditional steam sterilization methods, the net cost of single-use systems is lower, because the sterilization methods reduce labor, and circumvent many potential issues associated with in-house sterilization procedures. For example, if SIP-condensate has not been properly drained from a vent filter, the filter membrane can become damaged and malfunction in the actual process owing to excessive pressure in the one direction caused by the left-over condensate.

Figure 1 **Stainless steel bioreactor with SIP/CIP**

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Ethylene Oxide Sterilization

Ethylene oxide (EO) is an industrial chemical compound often used as a sterilant for medical devices (http://en.wikipedia.org/wiki/Ethylene_oxide). About half of all medical devices are sterilized using EO, whereas the other half are sterilized using radiation. About 17 billion pounds are used worldwide.

EO sterilizes by alkylation, namely, it substitutes for hydrogen atoms on molecules and disrupts the chemical processes required to sustain life. Specifically, EO can damage proteins and DNA. Under low-temperature sterilization processes, an excess of EO is used to maximize the damage and prove lethal to most micro-organisms. Typical operating temperatures are 120°F to 135°F, and the products are exposed to EO for 2 to 3 hours.

Note that unlike with radiation, products sterilized with EO must be directly exposed to the gas, so that they cannot be packaged prior to sterilization. Once sterilized, the products must be handled in a clean environment so as not to be contaminated during final packaging. EO, however, can kill micro-organisms in devices having physical complexity (hard-to-reach places) without damage to the device, because it is a gas, and can diffused through the device.

Because EO is a hazardous substance to humans and a carcinogen, a lengthy aeration time must follow each sterilization cycle, in order to ensure that the products and packaging do not have residual EO. An EO sterilization unit typically requires both vacuum and air lines. Therefore, EO cycles can be lengthy (up to 15 hours) to ensure sterilization and maximize operator safety.

EO is also flammable, so appropriate precautions must be taken. Often, EO is diluted with other gases into non-flammable form. In all cases, the sterilization service provider must manage the toxic and reactive hazards of OE and comply with the following standards:

- OSHA 29 CFR 1910.134 Respiratory Protection
- OSHA 29 CFR 1910.1200 Hazardous Communications
- NIOSH Pocket Guide to Chemical Hazards (guideline)
- ACGIH Threshold Limit Values for Chemical Substances (guideline)

The primary benefit of using EO is that exposure times can be set to maximize the sterility assurance level (SAL). The efficacy of EO to kill micro-organisms and penetrate certain types of barriers in a device is usually not know and must be tested. Biological indicators are placed in sealed containers within the devices being sterilized and are then removed and tested for the kill factor. EO can sterilize to a 10^{-6} level, i.e., the probability that a live micro-organism exists in the sterilized device is one in a million. Many EO sterilizers expose the load for longer periods to provide an “overkill” option.

Overall, EO processing provides some advantages and some disadvantages for single-use component sterilization. Disadvantages include long processing times, the requirement of performing sterility tests associated

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with biological indicators, inventory quarantine and the potential for re-processing as well as the safety hazards involved in handling EO gas. Benefits of EO sterilization include low cost and system flexibility.

Figure 2 **Ethylene Oxide sterilization system**

Radiation Sterilization

Radiation has been used as a safe and cost-effective sterilization method for healthcare products and medical devices for over forty years. More recently, the food and cosmetic industries have also begun to use radiation for sterilization of products, in order to reduce waste and improve throughput. For example, salmonella can be eliminated from recycled egg trays, and feathers can be irradiated before being packaged in bedding to remove the risk of avian flu.

Radiation sterilization can be very effective, because the radiation can non-invasively penetrate the product packaging and pass through the product itself, even if the product has a complex physical shape. Radiation does not leave unwanted residue or mechanically affect the product (such as heat sterilization). Moreover, the product is available for use immediately after irradiation.

Gamma rays (like X-rays) are electromagnetic radiation, and result from natural radioactivity of atoms, such as cobalt-60 or iridium-192. Natural radioactivity can also produce two other types of radiation, alpha and beta, which are particulate in nature. Gamma rays have the highest energy of all electromagnetic radiation, and have a very short wavelength (< 0.1 nm). Gamma rays are referred to as *indirectly* ionizing radiation since, having no charge, they mostly pass through matter until they interact with a particle. Consequently, gamma rays have a large penetration depth of about 80 cm for radiation processing.

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When gamma radiation interacts with matter, such as during a collision with a particle, it transfers energy to the particle, such as an electron. The excited electron can then directly interact with other electrons or atoms and cause ionization, which is why the process is called indirect ionization.

Ionization is the basis for sterilization, because it disrupts the chemical bonds in DNA molecules and prevents cellular division, As a result, the cells of a micro-organism exposed to a large enough dose of gamma radiation will not be able to produce critical proteins or enzymes, will not be able to propagate and the micro-organism will die. Note that although gamma rays are highly effective in killing micro-organisms, they do not have sufficient energy to impart radioactivity, nor do they create residues. However, ionization can initiate cross-linking in polymeric materials, which must be accounted for in the design of single-use components for bio-processing.

Gamma radiation processing is therefore generally characterized by its deep penetration, low dose rates, and effective destruction with minimal temperature effect of micro-organisms and bacteria in products already sealed inside their final packaging. The irradiated product then remains sterile until the packaging is removed for final use. One advantage of using gamma radiation with a large penetration depth is that a single radiation source can be used to sterilize multiple layers of a product on a pallet, thereby reducing the overall sterilization cost per component. Another advantage is that no area of the product, its components, or packaging is left with uncertain sterility after treatment, so that even high-density products can be processed with confidence. In addition, because the sterilization chamber does not require pressure or vacuum, package seals will not be stressed and the packaging will remain intact.

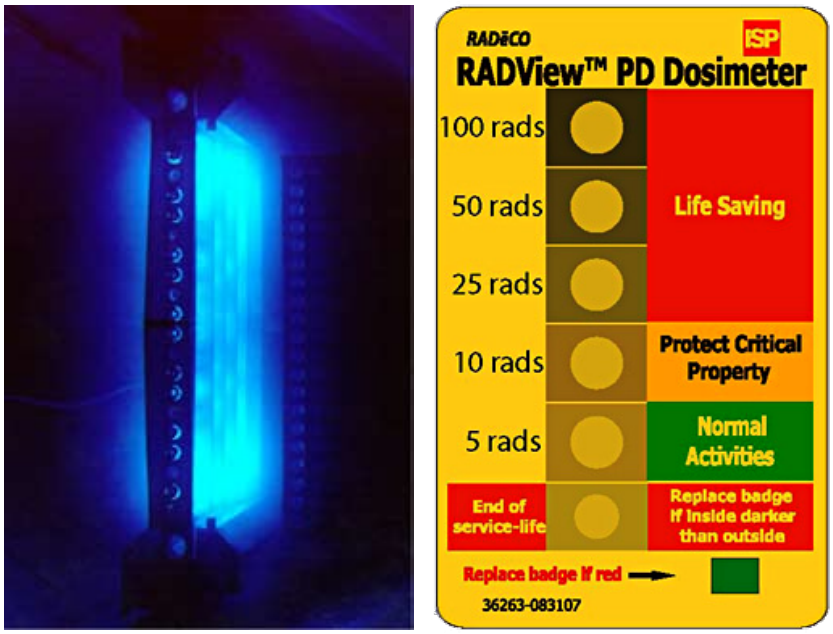

Figure 3 **Gamma radiation dosimeter (left) in chamber, (right) badge**

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Although sterility is a relative term, it can be used to represent the likelihood (or probability) of a micro-organism surviving irradiation. Using this definition, gamma radiation processing is a highly reliable process, owing to its inherent simplicity. Because the does is adjusted by exposure time, and time is the only variable requiring control, the possibility of deviation from the approved process is minimized. Moreover, once a product is irradiated and remains in its sealed container, there is no further risk of contamination. Because the product never becomes radioactive, there is no need for employees handling the product to take special precautions. The product can also be used immediately after sterilization.

Colored labels that are sensitive to radiation and change color during the irradiation process are sometimes used to indicate the radiation dose. Dosimetry is a used to measure the exact amount of radiation absorbed by the product being sterilized, is based solely on the dosage of radiation delivered to the product, and is usually reported in units of kiloGrays (kGy). Dosimetry is measured by radiation monitors placed on the product containers that are irradiated with the product. The monitors are then read using a special instrument (and often compared to calibrated standards) to verify the minimum and maximum radiation to which the product was exposed. Once the delivered does is verified, the products can be released for shipment and use. Proof of radiation treatment is therefore fully traceable and can be archived for several years after the product is released to the marketplace. Some products have a “radura” on the label and indicate that they were “treated with radiation”.

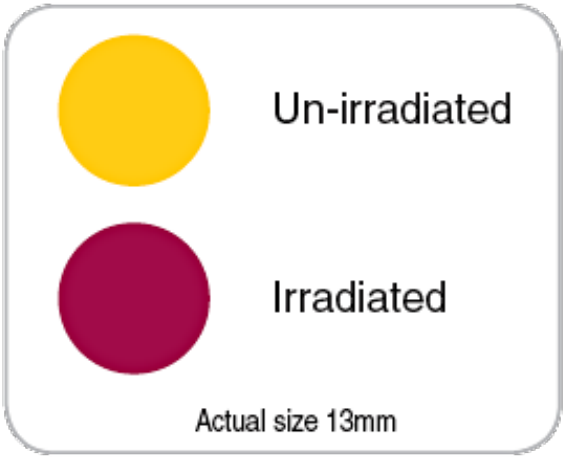

Figure 4 **Typical label indicator for single-use products sterilized using radiation**

Overall, gamma radiation processing provides fast, flexible and highly cost-effective sterilization. Cost savings can be largely attributed to the elimination of sterility tests associated with biological indicators, or BIs, (due to the FDA’s acceptance of dosimetric release), as well as the elimination of inventory quarantine and the potential for re-processing. Benefits of gamma radiation include precise dosing, rapid processing, uniform dose distribution, system flexibility and immediate availability of product after processing.

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Table 1 below provides a summary comparison of gamma radiation with ethylene oxide.

	Gamma Radiation	Ethylene Oxide (EO)
Process Methodology	Continuous batch	Batch
Product Release	Immediate, no post-sterilization testing is required.	Biological indicators are required to verify sterility assurance levels (SAL).
Penetration	Complete penetration	Complete penetration with the use of gas-permeable packaging.
Material Compatibility	Most materials are satisfactory. Considered somewhat incompatible with PVC, PTFE and Acetal	Nearly all materials are compatible
Residuals	None	Ethylene Chlorohydrin, requiring an aeration period following processing. With advanced CyclEOne technology, separate aeration is not required.

Table 1

References

1 Scholla, M.H. and Wells, M.E. “Tracking Trends in Industrial Sterilization.” Medical Device and Diagnostic Industry, September 1997, pp. 92-95.

2 American National Standard, ANSI/AAMI/ISO 11137-1994, Sterilization of health care products–Requirements for validation and routine control–Radiation sterilization,1994

3 American National Standard, ANSI/AAMI ST32-1991, Guidelines for Gamma Radiation Sterilization, 1991.

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