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An Introduction to Gas Diffusion Sterilization

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In recent years, in large part because of stringent new environmental regulations, the use of ethylene oxide (EtO) to sterilize medical devices has become increasingly complicated and expensive. As a result, many device manufacturers have closed their in-house sterilization operations and shifted to contract EtO sterilizers. Those who have retained in-house operations have had to surmount many regulatory hurdles.

However, by adopting gas-diffusion technology, a fundamentally different approach to in-house EtO sterilization, some manufacturers have been able to comply more economically with regulatory requirements. Compared to sterilization by means of a large vacuum/pressure vessel, this technology more effectively controls emissions and reduces gas use.

Like traditional EtO systems, gas-diffusion systems can be validated in accordance with AAMI/ANSI/ISO 11135, the industrial EtO standard.[1] Although the language of ISO 11135 refers only to traditional chamber sterilizers, the principles of process validation it describes can be applied to gas-diffusion technology by analogy.

The gas-diffusion system injects EtO directly into the product packaging to eliminate the need for an industrial vessel.

SYSTEM FUNDAMENTALS

There are two gas-diffusion systems currently available, both manufactured by H.W. Andersen Products, Inc. (Haw River, NC). One, the Sterijet system, is designed for large-scale production; the other, the EOGas system, is best suited for health-care and small-batch manufacturing.

The Sterijet processor combines a package sealer with an EtO-injection machine. Both the injection system and the sealer mechanism are validated to deliver an ethylene oxide shot and package seal of consistent and known parameters. The processor can be located near the device manufacturing area and operated continuously, sterilizing product as it comes out of final assembly.

Products to be sterilized must first either be packaged in a breathable sterile barrier package or simply wrapped in a permeable material. The size of the process lot is not determined by the volume of a chamber, as it would be in an industrial vessel. With a gas-diffusion system, the manufacturer can either wait to build standard size product lots, sterilize individual devices or small batches, or process continuously. The steel vacuum/pressure vessel is replaced by a plastic bag, typically of low-density polyethylene (LDPE) film. The bag, by analogy, represents the chamber as defined in ISO 11135.

Figure 1. An LDPE bag shrinks around the inner sterile product as EtO diffuses out of the package.

Forced preconditioning of the products through storage in heated rooms under conditions of high humidity is unnecessary with gas-diffusion technology. Facilities need only keep workspace humidity and temperature above 40% and 70°F to allow continuous processing of the products as they leave the assembly line. Workspace temperature and humidity control can be achieved through a combination of steam injection, when necessary, and HVAC treatment.

In contrast to the natural prehumidification described above, product sterilized inside vacuum/pressure vessels is usually preconditioned at extreme conditions that differ markedly from those of the manufacturing environment. The potential danger is that secondary packaging can absorb elevated quantities of humidity during such preconditioning, and later during chamber steam injection, to the point that the moisture actually interferes with the final sterility assurance level (SAL) achieved by the cycle.[2] Since gas-diffusion sterilization can be integrated into the manufacturing environment, the products can be continuously processed at the same conditions under which they were manufactured, thus avoiding climatic variations that cause condensation on product and packaging.

The sterilization process begins with placement of the finished product inside the LDPE bag. One LDPE bag can contain a single product, such as a large custom surgical kit, or small multiple units, such as sutures. Multipacks can be double wrapped, with each product individually sealed into its own primary packaging. The quality of the packaging is strictly controlled to ensure consistent gas permeation characteristics. The size of the LDPE bag is tailored to the physical characteristics of the product to be sterilized *(Figure 1)*.

The mouth of the LDPE bag is placed onto the injector processor between the jaws of the processor. After the jaws close, the processor removes the excess air from the LDPE bag. The processor then injects a preprogrammed amount, or gas shot, of vaporized EtO. The nozzle then automatically withdraws and the LDPE bag is heat sealed. The size of each gas shot is measured by a mass flowmeter and verified by recording the critical operating parameters of gas pressure, temperature, and inject time. The time, processing sequence number, and EtO mass of each gas shot is recorded on a printed label that is used to track individual packages.

As they are filled, evacuated, and injected with sterilant, the bags are transferred to a temperature-controlled room with engineered ventilation. The sealed bags contain only air trapped within the product and packaging, so that the contents are not flammable. Moreover, each bag contains very little EtO gas, typically less than 10 g. At this point, the hot cell fulfills the function of the traditional sterilizer chamber by providing heat for both the diffusion and sterilization process. Through forced ventilation, the hot cell eliminates the EtO as it elutes from the LDPE bag. As aeration progresses and EtO diffuses out of the package, the LDPE bag begins to exhibit a skintight, vacuum-packed appearance as it shrinks around the inner sterile product *(Figure 1)*. The vacuum-tight package not only offers more protection against damage due to shifting during shipment, but also offers the user a visual assurance of package integrity. Loose fit of the LDPE bag after processing would indicate that the package seal had been violated.

THE GAS-DIFFUSION PROCESS

The transmission of EtO across a permeable membrane depends upon several factors, including the following:

- The type, thickness, composition, and crystallinity of the barrier.
- The area exposed to the transmission.
- The concentration gradient that exists across the barrier.
- The pressure gradient across the barrier.[3]

When EtO gas is injected between the inside of the LDPE bag and the outside of the porous packaging containing the product, it gradually diffuses through the porous membrane and contacts all accessible surfaces of the product. Two important physical forces drive the sterilant across the packaging and allow the process gases to contact the product's bioburden. The first is the slight pressure gradient created by the gas injection. The second and most important factor is the concentration gradient across the barrier. In the open sterilizer bag, the inside pressure is the same as the atmospheric pressure of the workplace. When excess air is removed by the Sterijet processor, the effective pressure inside the bag is lowered. The injection of EtO into the LDPE bag introduces a pressure gradient across the sterile barrier package, thus driving EtO toward the product. This is analogous to the chamber dynamics of an industrial vacuum/pressure vessel. As the chamber headspace is evacuated, air is drawn out of each individual product package. A negative pressure equilibrium between individual product and the headspace follows. When the vaporized EtO is added, the chamber headspace pressure is greater than that of each product. This pressure gradient drives the gas into the product.

During the sterilization phase of the cycle, the hot cells are precisely maintained at a temperature validated to deliver a 106 SAL at the worst-case location within the product package. Since the permeability of the LDPE bag is far lower than the permeability of the inner product packaging, initial gas loss to the hot cell is insignificant and predictable, while the immediate tendency of the gas is to diffuse inward across the packaging layer, contacting the product.

During the aeration phase of the cycle, the concentration of EtO in the LDPE bag decreases, and the primary direction of gas diffusion reverses. The concentration gradient now favors diffusion out of the bag into the hot cell environment. The hot cell, first responsible for aiding the inward diffusion of the gas and promoting the chemical inactivation of product bioburden, now causes outward diffusion. Because of ventilation, the hot cell EtO concentration is near zero, thus aiding diffusion of the gas from the product and through the walls of the LDPE bag. This is analogous to the heat supplied by the wall jackets of an industrial vacuum/pressure vessel. Initially the heat serves to increase gas diffusion across the multiple layers of packaging and to promote uniform chemical reaction on the products. Later it helps drive the gas back out of all layers of packaging, where it can be evacuated to the emission control system.

GAS PERMEATION CONSIDERATIONS

Those who have had the chance to follow an entire EtO process validation for an industrial vessel will have noticed that as the size of the product load increases, the sterilization process becomes less efficient. To compensate, the following cycle parameters must be boosted:

- *Load heat-up time.*
- *Humidity addition.*
- *Humidity penetration time.*
- *EtO addition.*
- *EtO penetration time.*
- *Sterilant dwell time.*

The gas sterilization process loses efficiency as product lot size increases not because the vessel hardware is insufficient, but as a result of the progressive increase in package interference and load density. By way of comparison, product sterilized in a biological indicator evaluation resistometer (BIER vessel) is handled singly, and has only one layer of protective barrier separating the product from the critical process elements (temperature, humidity, and EtO concentration). In a hospital-size vessel, we see a comparative increase in package-related interference. In an industrial vessel, where several more layers of packaging are added (master cartons and shelf cartons, for example), the minimum sterilant concentration that is required increases still more.

Users of industrial-size vessels are forced to compensate for process interference caused by load characteristics by using levels of humidification and concentrations of EtO greatly in excess of those actually needed at the microbial site. While Kaye and Phillips determined that moisture levels of 2040% would allow EtO to inactivate spore samples,[4] these levels are rarely employed by the sterilization industry. The original Kaye and Phillips experiment was performed directly on the microbial site and did not include multiple layers of packaging to hinder gas permeation. Even in so-called low-concentration industrial cycles, excess moisture and EtO are needed to fill the empty space around each pallet and between products. When an industrial vacuum/pressure vessel is used, external preconditioning ranging from 12 to 24 hours is commonly carried out to bring the load to a uniform temperature and humidity level. Subsequently, a series of multiple vacuum cycles and nitrogen washes are needed to displace oxygen from the load. Only then is steam injected into the vessel and the load allowed to condition. A modern, industrial-size vessel typically injects steam into the headspace until a relative humidity of 5080% is achieved. Additional steam is used to maintain the high level of humidity throughout conditioning dwell. The same holds true for EtO concentration. While a BIER vessel may inactivate spores with a sterilant concentration of 350 mg/L, industrial vessels use 6001000 mg/L, injecting 510 lb of EtO per pallet of product. Thus process obstruction caused by packaging and load density never allows an industrial-size vessel to achieve the performance of a BIER vessel.

The dynamics of EtO sterilization can be explained by reference to Fick's first law of diffusion, $J = D\,dc/dx$, where the rate of diffusion (J) is directly proportional to the concentration gradient. In this equation, D is the diffusion coefficient and dc/dx is the gradient concentration in the direction of diffusion.[5]

In the sterilization system, the rate of diffusion toward the microbial sites increases as the gas concentration in the sterilizer is raised. Stated differently, as the resistance of the system to diffusion increases, the gas concentration must be increased to achieve the same rate of penetration. Absorption of EtO and moisture by packaging results in depletion of the headspace levels, thus contributing to the inefficiency of permeation dynamics in an industrial EtO chamber. Each level of porous corrugated packaging and paper is capable of absorbing up to 10% EtO w/w (100,000 mg/L on a weight/volume basis).[6] As the gas permeates the first level of cardboard master cartons, the amount remaining that can continue permeating across the second layer of shelf cartons is already significantly reduced. The slowest permeation rate, therefore, will occur where penetration is most critical: at the primary packaging level. Unless a sufficient quantity of moisture and EtO contacts the product, the proper SAL will not be achieved. For a system where the product is separated from the sterilizer headspace gases by three layers of packaging, the overall permeation time can be represented by the sum of the individual permeation times for each layer (the value of k may vary from 2 to 6, L is the packaging layer thickness in centimeters, and D the diffusion coefficient in square centimeters per minute):[6]

Total Permeation Time = k L2/D(3)+ k L2/D(2)+ k L2/D(1)

The gas-diffusion method largely circumvents the problem of permeation obstruction by delivering precisely metered doses of both moisture and EtO right to the primary packaging--mere centimeters from the microbial site. In a sterilizer bag, the permeation time of EtO depends on only one layer of porous packaging; consequently, the gas absorption and depletion by multiple layers of corrugated packaging is avoided. In designing a gas diffusion validation, the user must consider only two sources of resistance to the process: product bioburden and one layer of porous packaging. The permeation time is much more efficient as represented by:

Total Gas Diffusion Permeation Time = k L2/D

By avoiding the dramatic overgassing that takes place inside an industrial chamber, gas-diffusion technology cuts the time and energy required to aerate the final product to FDA-mandated EtO residual levels and reduces the demand on the facility's emission control system.7 We estimate that devices sterilized with the system use 30 to 80% less EtO than industrial vacuum/pressure vessels achieving the same SAL.

PROCESS VALIDATION OF A GAS-DIFFUSION SYSTEM

Validation of the Sterijet system has been carried out according to a protocol based on ISO 11135. Following the installation and operational qualifications, a series of fractional cycles were performed to verify the relative humidity (RH), EtO concentration, and sterilization phase temperature required to deliver the needed degree of lethality at the most resistant location in a specific product. For each product family, at least two fractional cycles were performed, one at minimum, one at maximum sterilization temperature. In this way a temperature window was determined within which the required level of delivered lethality could be ensured. Preconditioning parameters and injected EtO weight were validated at below the minimum specification. Minimum workplace environmental conditions were simulated by preconditioning the product at 3539% RH and at a temperature below 65ºF. Temperature of the product load was monitored throughout the preconditioning, gas injection, sterilization, and aeration. Product humidity was monitored through preconditioning up until gas injection. Temperature and humidity probes were placed within the product as close to the worst-case biological indicator (BI) site as possible.

The processor was then programmed and calibrated to inject a mass of EtO below the minimum level routinely used in production. Because the sterilization bag material has a very low permeability to water vapor, the absolute amount of water sealed in the product package is assumed to remain constant during sterilization. Sterilization of the BIs under worst-case processing conditions confirms that the humidification of the load is adequate to achieve the intended SAL.

For validation purposes, the products sterilized were divided into several product families. We will examine here in greater detail the validation testing carried out for the product family represented by a wound drain consisting of a double-lumen PVC tube about 17 in. long. Product characteristics that might impede EtO penetration include an antimicrobial filter on the vent lumen. The tube is double wrapped, first within a polyethylene primary package, then into a polyethylene peel-open pouch. Ten individually packed tubes constitute the multipack that is sterilized as a unit in the LDPE bag.

To carry out the fractionals, 30 multipacks of 10 tubes each were inoculated with BI spore strips or disks with certified B. subtilis var. niger populations of at least 1.0 * 106 and resistance of 34 minutes. The tube located at the worst-case location within the multipack (the center) was inoculated with three BIs. Two BIs were placed at positions within the product where diffusion of EtO would theoretically be restricted (within the aspiration lumen and under the filter assembly). The third was to function as a reference and was placed within the primary packaging, adjacent to the tube. Additional multipacks were probed for measurement of temperature and RH during preconditioning and temperature during the sterilization phase.

Each of the multipacks was then injected with a preprogrammed mass of vaporized EtO and placed into the hot cell. Multipacks were removed from the hot cell at eight graded time periods between 0 and 12 hours. The BIs were aseptically removed, macerated, and cultured for enumeration of survivors. The log10 of the survivors was plotted against time to yield an inactivation curve for the three test sites, and regression analysis was used to determine the decimal reduction time (D-value) for each curve.[8] The D-value curves revealed a classical pattern of microbial inactivation: the interior of the single product package is more resistant than the exterior and the least accessible location within the product (BI in the aspiration lumen) is the most resistant location of the three tested. At the minimum sterilization temperature (82ºF), D-values of 1.46, 1.10, and 0.67 hours were obtained for the lumen of catheter, the filter assembly, and the primary package, respectively. The fractional study carried out at the higher temperature revealed an approximately 30% reduction of the D-values for each of the three sites. The reduction in D-value is predicted by Q10 and again demonstrates that established patterns of sterilization kinetics are observed in the sterilization system.[9]

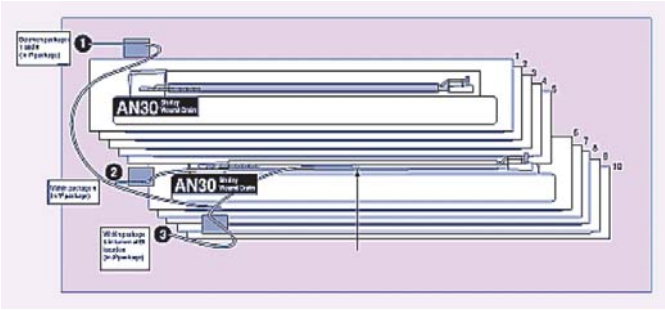

Figure 2. Temperature and humidity probes were placed in three product multipacks. The arrow indicates the worst-case biological indicator site.

In addition to the BI and temperature/RH sensor test packs, three product multipacks were probed for EtO concentration analysis. The three product multipacks were each equipped with three capillary tubes leading to sampling ports outside the LDPE bag. One of the sampling tubes was located at the worst-case location within the aspiration lumen. The second was positioned to sample gas within the primary package, and the third was located within the LDPE bag *(Figure 2)*. The sampling tubes were led to the outside of the hot cell, so that sampling could be accomplished without opening the door. (While this approach to probing gas concentration would be impractical in an industrial vessel, it is a relatively simple procedure in a gas-diffusion system).

After clearing the gas chromatograph syringe t equalize the gas concentration within the package with the sampling tubes, gas samples were withdrawn from the multipack via a syringe and analyzed on a gas chromatograph (GC). The EtO concentration within the product package was measured at 1- and, later, 2-hour intervals over the course of 24 hours. In this way, EtO diffusion kinetics were mapped for the three sites over the standard (24-hour) full cycle time. Like the BI inactivation curves, the GC mapping studies revealed the expected relationship between product, packaging layers, and EtO concentration. The GC curves demonstrate that EtO diffuses from the LDPE bag through the packaging to reach the most restrictive sites of the product. The relationship between the EtO concentration and lethality delivered at the worst-case site within the product is further elucidated by graphing the corresponding GC curve together with BI inactivation data *(Figure 3)*.

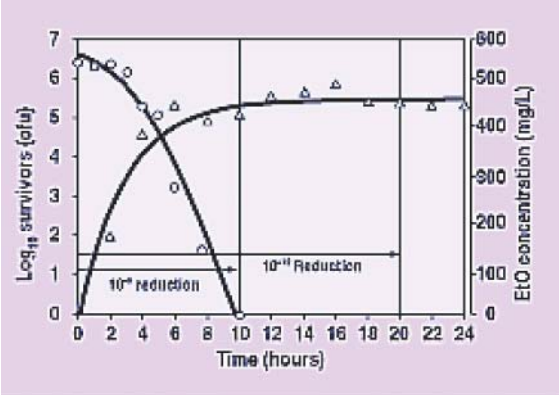

Figure 3. EtO concentration was mapped over a 24-hour cycle time. This graph depicts 10 degrees of lethality correlated to product level gas concentration and time at a constant process temperature.

This graph shows 10 degrees of lethality correlated simultaneously to both product level gas concentration and time at a constant process temperature. During the first 5 hours after EtO is injected into the LDPE bag, the gas concentration is increasing and the lethality curve is nonlinear as the system pushes to achieve a concentration equilibrium across all layers of inner packaging. Once EtO has penetrated into each product package and a diffusion equilibrium across the packaging is achieved at approximately 450500 mg/L, the linearity of the curve becomes evident. Inactivation of the BI at about 10 hours indicates delivery of an initial six logs of lethality and completion of the theoretical sterilization half-cycle. Maintenance of the EtO concentration level around 450500 mg/L for hours 1020 confirms that an adequate amount of EtO is present during the second half-cycle to deliver an additional six logs of lethality.

To verify that BI inactivation was achieved at the end of the first half-cycle, a series of three half-cycle runs were subsequently completed. Product was preconditioned at subminimum conditions and 28 product packages were prepared, each with a BI placed in the worst-case location identified through fractional testing. (As per ISO 11135, section B4, the number of BIs used for fractional testing is based on sterilizer volume.) Additional multipacks were probed for measurement of temperature and RH during preconditioning and of temperature during the sterilization phase. Each of the product multipacks was injected with a subminimum mass of EtO and then held in a hot cell at subminimum temperature for the half-cycle time. (The half-cycle time was calculated from the D-value at the worst-case location to give a six-log reduction plus a safety factor of two D-values.) At the conclusion of the half-cycle, the BIs were removed from the product multipacks and aseptically transferred to media for culturing. For successful completion of the half-cycle test, no positives were permitted at seven days' incubation for any of the three consecutive half-cycles.

To complete the suite of validation tests, three full cycles were performed for EtO, ethylene glycol (EG), and ethylene chlorohydrin (EC) residual determination and product functionality testing. During this phase, preconditioning was pushed to its maximum limits for temperature and relative humidity and the Sterijet processor was programmed to inject a quantity of EtO in excess of the maximum parameter. Following the sterilization phase, samples to be tested for residuals were then aerated at a subminimum temperature (below 90°F), while product functionality samples were aerated at a temperature exceeding the maximum production limit (100°F). For the full-cycle runs, BIs were placed at the worst-case location within the product, as identified through fractional testing. The number of BI-inoculated product packs (10) was based on the volume of product sterilized, as per ISO 11135, section C.3.2. The BIs were retrieved immediately after the sterilization period was finished and were incubated as the load continued to aerate.

The sterilization cycle validated here was run isothermally--that is, sterilization and aeration occurred at the same temperature without interruption. To decrease product aeration times, cycle temperatures can also be ramped--the aeration phase being run at a higher temperature than the sterilization phase. Once the maximum load size has been validated during full-cycle testing, routine lot sizes may differ depending on the production volume.

CONCLUSION

Gas-diffusion technology is a safety-conscious, effective, and efficient way for device manufacturers to routinely perform terminal EtO sterilization of medical devices. It is based on the well-known chemical and physical laws of gas diffusion across permeable barriers. By controlling temperature and humidity in the manufacturing area and by selecting packaging materials with compatible EtO permeation properties, a gas diffusion sterilization system can be installed, validated, and operated to deliver an SAL of 106.

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