Peritoneal dialysis (PD) solutions are currently sterilized in an autoclave using high-temperature saturated steam. Although thermal methods are an effective means of sterilization, the heating of PD solutions results in the formation of toxic glucose degradation products (GDPs). Here, we review basic concepts in the sterilization of PD solutions and discuss possible alternatives to steam sterilization, including filtration, ohmic heat, ionizing radiation, and pulsed ultraviolet light. Although the latter methods have several advantages, many also have prohibitive limitations or have not been adequately studied for use on PD solutions. Thus, in the absence of suitable alternatives, conventional heat sterilization, in combination with low-GDP manufacturing practices, remains the best option at the present time.

Key words
Peritoneal dialysis solutions, sterilization, glucose degradation products

Introduction
Sterility is most accurately defined as the complete absence of viable micro-organisms from a sample (1). To achieve sterility, manufacturers can use terminal sterilization methods or aseptic processing. During terminal sterilization, a finished product is sterilized after assembly has been completed. In contrast, during aseptic processing, the components of a product are sterilized separately and then assembled in an aseptic manner (1).

Terminal sterilization can take several forms that are classified as either traditional, nontraditional, or novel nontraditional (2). Traditional sterilization methods include dry heat, moist (steam) heat, ethylene oxide, and ionizing radiation. These forms of sterilization have been validated and recognized by the FDA, possess a long history of safety and efficacy, and are supported by a substantial amount of literature (2). Nontraditional forms of sterilization, such as hydrogen peroxide gas and ozone, do not have a long history of safe and effective use or FDA-recognized standards; however, the FDA has deemed them adequate because they are typically supported by medical literature (2). In contrast, novel nontraditional sterilization methods have little or no supportive literature and no recognized standards; moreover, they have not been deemed sufficient by the FDA. Examples of these methods include sound waves, chlorine dioxide, and high-intensity or pulsed light (2).

Currently, traditional thermal methods are used to sterilize PD solutions. Specifically, moist heat, in the form of saturated steam, is applied to the solutions under pressurized conditions in an autoclave. The high pressure is needed to prevent the solutions from boiling, thus maintaining product integrity. Although conditions vary, sterilization typically occurs at 121°C for 20 – 60 minutes (1,3).

After the solutions are heated, their sterility must be verified before they can be released into the market. Traditionally, large-volume liquids are tested by culturing random samples from the product batch. The number of samples needed can range from 4 to 20 depending on the size of the batch, and at least 10% of the volume in each container is required to be tested (4). However, traditional sterility testing is expensive and time consuming, and it cannot demonstrate the sterility of all finished articles. In fact, the tests are able to detect only gross contamination or major errors in the manufacturing process (1,5–7).
Because of those testing limitations, the FDA allows pharmaceutical manufacturers to forgo traditional sterility testing and instead to use a process known as parametric release (7). Under this strategy, manufacturers can release sterile products into the market if they can provide proof that certain critical parameters, such as temperature, were met during sterilization. Meeting these validated parameters ensures that the probability of contaminants surviving the sterilization process is low (7). For most pharmaceutical products, manufacturers must attain a sterility assurance level of $10^{-6}$, meaning that the chance that a viable micro-organism is present after sterilization is just 1 in 1,000,000 (7).

The use of parametric release requires FDA approval and can be applied only to well-defined and predicable sterilization techniques for which a sterility assurance level can be calculated, such as dry heat, steam heat, or ionizing radiation (6,7). Compared with traditional batch sterility testing, parametric release is desirable for manufacturers because it ensures a higher level of sterility and helps to bring products to market faster and at a lower cost.

Discussion

Steam heat sterilization

Steam sterilization of PD solutions is widely regarded as the industry standard, and its use has several advantages. For one, steam heat has been well studied and validated, and therefore allows manufacturers to qualify for parametric release (1,6,7). Steam is also an excellent carrier of thermal energy and can penetrate protective biofilms, making it an effective form of sterilization (8). Furthermore, steam sterilization is relatively inexpensive and easy to control.

However, the use of thermal sterilization techniques on PD solutions leads to the breakdown of glucose and the formation of toxic substances known as glucose degradation products (GDPs). These GDPs have been linked with several adverse outcomes, including fibrosis, thickening of the peritoneal membrane, inhibition of cell proliferation, and the formation of other toxic species known as advanced glycation endproducts (9–12). It is therefore important to examine other forms of sterilization that may avoid or reduce GDP production.

Sterile filtration

One alternative to thermal sterilization is filtration. Unlike heat sterilization, the process of filtration does not kill microbes, but rather removes them from the solution. Filtration can be nonsterile (using a filter membrane of 0.45 μm) or sterile (using a filter membrane of 0.22 μm) (1,13). Many large-volume parenteral manufacturers, including those who manufacture PD solutions, routinely use nonsterile filtration methods to reduce the bacterial bioburden before terminal heat sterilization. In contrast, sterile filtration is used to achieve solution sterility and is generally done as part of an aseptic process (13).

Several in vitro and animal studies have shown that sterile filtration can avoid the undesirable GDPs produced during high-temperature heating (3,10,14–17). Although few human studies exist, the feasibility of using sterile-filtered peritoneal dialysis (PD) solutions was demonstrated in two small prospective studies from the early 1990s (18,19). The authors created the solutions using a machine for the online generation of highly purified replacement fluids for hemofiltration. Each machine was modified to produce PD solutions with a pH of 7.1 that contained sodium, potassium, magnesium, chloride, bicarbonate or lactate, and glucose (18,19). Sterility was achieved by routing the fluids through four polyamide hollow-fiber ultrafilters with pore sizes ranging from 0.002 μm to 0.008 μm. The resulting solutions were collected in 15 sterile plastic bags, tested for common bacterial pathogens and endotoxins, and then instilled into 15 dialysis patients (18,19). The participants from both studies tolerated the solutions well, with no episodes of peritonitis, and their serum urea nitrogen and creatinine levels fell. The authors concluded that their method was a convenient and effective means of creating sterile PD solutions (18,19).

However, sterile filtration has several disadvantages that have prevented its widespread use for PD solutions. The process is expensive, and many more variables have to be considered when using filtration, including the fragility and degradation of the filter, product compatibility with the membrane, adherence to strict pressure and flow rate requirements, and other factors related to aseptic processing (1). Also, compared with destructive methods such as heat, the exclusion of microorganisms from a product by filtration also carries a higher risk of contamination (13,20). For those
reasons, the FDA will not generally allow filtration to be the sole means of sterilization unless no other alternatives exist. In addition, manufacturers who use this method may not qualify for parametric release because of the challenges of determining a sterility assurance level (6).

**Ohmic heat**

Another alternative to traditional steam sterilization is ohmic heating, also known as Joule or electrical resistance heating. During ohmic heating, an electrical current is passed through the product, thereby generating rapid (seconds in elapsed time) and uniform heat within the material (21). Unlike conventional thermal processing, which may damage the product because of the slow conduction and convection of heat, ohmic heating has been shown to preserve the quality of food and juice products (21–23). The principal mechanism of microbial inactivation during ohmic heating is thermal in nature. However, it has also been suggested that the low-frequency electrical charge may create pores in microbial cell membranes (21).

To date, only one study has examined the effects of ohmic sterilization on the formation of GDPs (24). The experiment was conducted using laboratory-made PD fluids with a pH of 6.6 and a glucose concentration of 4%. During the conventional sterilization phase, the sample solutions were placed into sealed glass jars and sterilized using an autoclave at 120°C for 20 minutes and for 40 minutes. Thermal treatments using an ohmic heating system were then performed on the remaining samples at three temperatures (105°C, 125°C, and 150°C), with resistance times varying from 0.84 s to 12 s. The results showed that the concentrations of 3-deoxyglucosone, glyoxal, and methylglyoxal were lower at all temperatures and time points of ohmic heating compared with conventional heat sterilization (24). The authors hypothesized that this effect might be attributable to the ultrarapid and uniform heating caused by ohmic sterilization, which results in less thermal abuse (24).

Although those results are promising, it is important to note that the authors did not validate the sterility of the PD solutions. The authors also did not measure all known GDPs, and it is possible that the kinetic parameters and temperature dependency of the other GDPs are different from those of the three that were studied. Thus, further testing is needed before widespread adoption of ohmic heat can be considered. Furthermore, while the parameters involved in ohmic heating seem to be predictable and measurable, the process may have difficulty qualifying for parametric release because it has not been well validated for use on pharmaceuticals.

**Ionizing radiation**

Ionizing radiation may be another sterilization method that can avoid the formation of GDPs. Radiation-based techniques are nonthermal in nature and work primarily by ionizing the chemical bonds within bacteria, leading to irreversible DNA damage (25,26). Many pharmaceutical companies now use radiation to sterilize medical devices and drugs, including ophthalmic preparations, topical ointments, and some parenterals. Although there are many types of ionizing radiation, gamma and electron-beam are the two types most commonly used for sterilization. In general, gamma radiation tends to take longer than electron-beam, but it has greater penetration and has been better studied for use on liquid pharmaceuticals (25).

Ionizing radiation holds many potential advantages because it provides uniform sterility, qualifies for parametric release, and is capable of sterilizing large batches of product (25). Although no studies have examined the use of ionizing radiation to terminally sterilize PD solutions, experiments have assessed the use of gamma radiation on total parental nutrition bags. In one study performed in the mid-1990s, the authors tested the effects of gamma radiation on batches of 50-mL total parental nutrition solutions, each containing various concentrations of glucose, amino acids, lipids, vitamins, and electrolytes (27). The batches were inoculated with varying concentrations of test organisms and were then exposed to gamma radiation at doses ranging from 1.5 kGy to 8.5 kGy (equivalent to 0.15 – 0.85 Mrad). After irradiation, all total parental nutrition bags were found to be sterile, and no changes in pH, glucose concentration, or amino-acid profile were noted (27).

However, gamma or electron-beam radiation have potential disadvantages that make them less feasible for use on PD solutions. Some studies have suggested that ionizing radiation may chemically alter carbohydrates and induce the formation of toxic substances such as malondialdehyde, formaldehyde, acetaldehyde, glyoxal, and furan (28–30). At certain doses, radiation may also cause chemical alterations in the polyvinylchloride used for packaging PD solutions,
leading to yellow discoloration and possibly odor development (31). Radiation, particularly electron-beam radiation, also has a low penetration depth into liquids and highly depends on the density of the solution (25).

Pulsed ultraviolet light
Another form of sterilizing radiation with potential applications for PD solutions is pulsed ultraviolet (UV) light, also called pulsed white light. The process uses intense flashes of broad-spectrum UV light at non-ionizing wavelengths of 200 – 1000 nm, which is thought to make it more effective than conventional UV sterilization (32). The sterilizing effect appears to be a result of both the high UV content, which leads to DNA damage, and the instantaneous heating effect, which results in disintegration of bacterial cell walls. Pulsed UV light is currently recognized by the FDA for the sterilization of foods (33); it also has potential applications for the terminal sterilization of pharmaceutical products provided that the packaging allows for the transmittal of light (32).

Although feasible for use on PD solutions, pulsed UV light is still too new for widespread adoption. Furthermore, it has not been recognized by the FDA as an acceptable form of sterilization for pharmaceuticals. There may also be technical issues to consider, because generation of the UV pulse requires a considerable amount of energy and some units require external cooling. In addition, there is the potential for drug or product degradation, and it is important to verify that the product container does not cause reflection or shading of light (25).

Summary
Moist heat is an effective means of sterilizing PD solutions, but it leads to the breakdown of glucose and the generation of toxic GDPs. Although several alternative forms of sterilization can avoid GDP formation, most have prohibitive limitations or have not been adequately assessed for use on PD solutions. Thus, in the absence of viable alternatives, steam sterilization will remain the technique of choice within the industry. It is therefore important to look toward alternative manufacturing practices and bag designs that will help minimize GDP formation.

Disclosures
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References


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