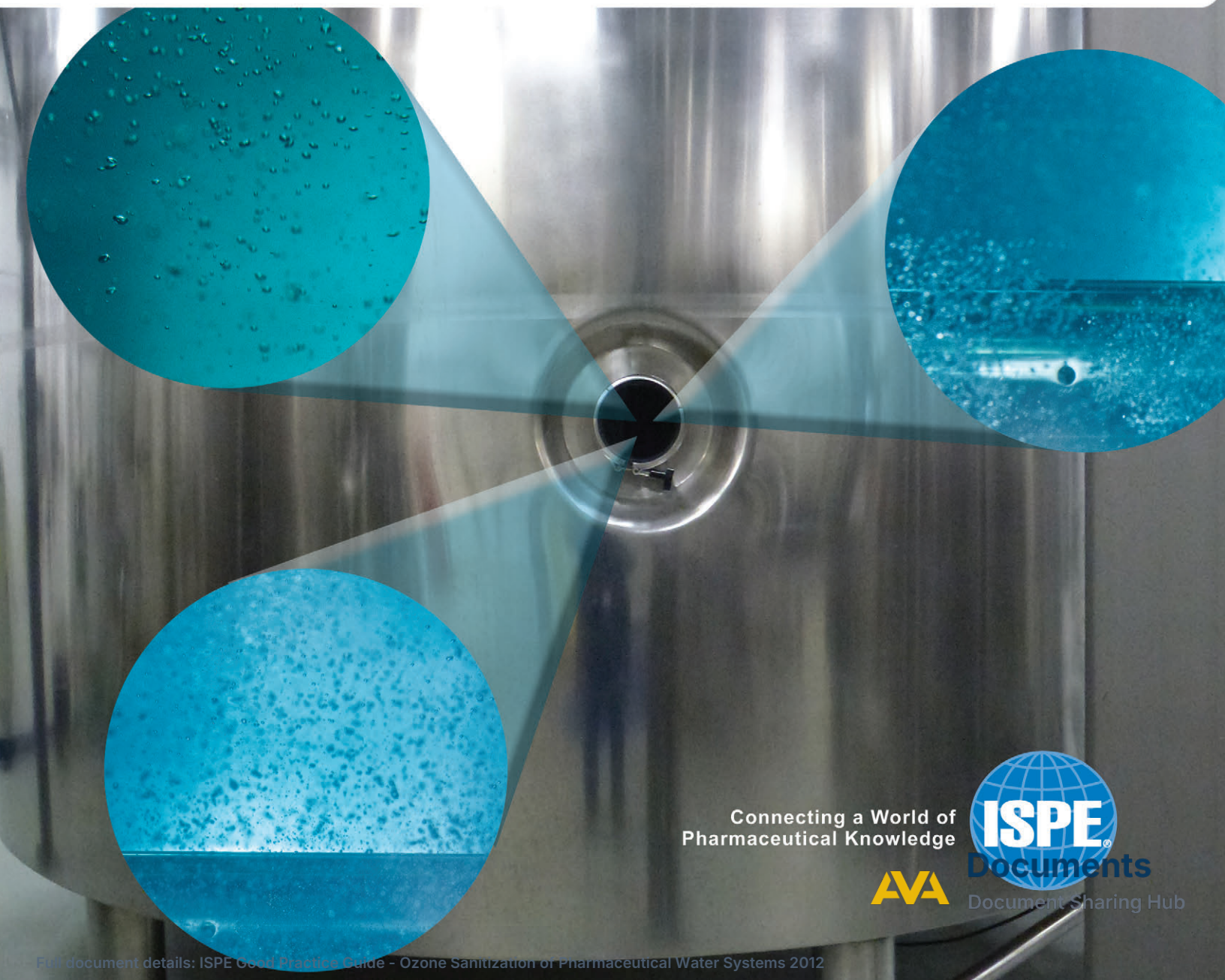




**Good  
Practice  
Guide**

# **Ozone Sanitization of Pharmaceutical Water Systems**



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## Good Practice Guide

# Ozone Sanitization of Pharmaceutical Water Systems

### Disclaimer:

This Guide aims to describe practices associated with ozone sanitization of high purity GMP pharmaceutical water storage and distribution systems. ISPE cannot ensure and does not warrant that a system managed in accordance with this Guide will be acceptable to regulatory authorities. Further, this Guide does not replace the need for hiring professional engineers or technicians.

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# Preface

The purpose of this document is to provide guidance when considering, designing, and operating an ozone system for the purpose of sanitizing a high purity GMP pharmaceutical water system. While the use of ozone as a sanitant in GMP high purity water storage and distribution systems is widely accepted, there is little design and operating guidance for such systems. It is the intent of this Good Practice Guide to provide such guidance.

This Guide includes a comprehensive assortment of design and operating considerations that system designers and owners should be aware of.

This is not a requirements or specification document. Based on user requirements and risk assessments design, teams will have to establish their own system requirements and specifications.

The reason for considering the use of ozone is that there may be a wide range of possible cost and operating advantages. The significance of these advantages may vary depending upon the site and project; however, as with all methods of sanitization, the use of ozone technology will place specific requirements on the system design and operation and also may impact the chemical attributes of the water. Some of these requirements and impacts might be considered disadvantages.

This Guide discusses these ozone-specific requirements, advantages, and disadvantages. It also steps the reader through system design, operation, and control. As with any system design, it is the responsibility of the design team and owner to evaluate these possible advantages and disadvantages, taking into consideration the design guidance outlined in this document and determine which method of sanitization, ozone or other, is best for their particular application.

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# 1 Introduction

The reason for considering the use of ozone as the primary sanitizing agent for a pharmaceutical water distribution system is that there may be a wide range of possible operating and cost advantages. The significance of these advantages may vary depending upon the site and project. However, as with all methods of sanitization, the use of ozone technology will place specific requirements on the system design and operation.

This Guide discusses these ozone-specific requirements, including associated advantages and disadvantages. It also steps the reader through system design, operation, and control. As with any system design, it is the responsibility of the owner and the design team to evaluate ozone's applicability taking into consideration the design guidance outlined in this Guide.

Ozone has been used extensively by many firms, while others have little or no experience with ozone as a sanitant. The reasons may include a lack of knowledge regarding system design and operation, concerns about compatible materials of construction, and safety and environmental issues. It has been reported that misunderstanding of the "added substance" issues with compendial waters also has been a potential deterrent. For this reason, there is also discussion of this issue in this Guide.

## 1.1 Purpose

The purpose of this Guide is to provide detailed guidance relating to design and operation of pharmaceutical water systems using ozone for sanitization as well as describing the principles that allow this sanitization approach to be effective.

## 1.2 Scope

The scope of this Guide includes designs and practices associated with ozone sanitization of high purity GMP pharmaceutical water storage and distribution systems. Other ozone applications are not considered (e.g., drinking water, cooling towers).

## 1.3 Structure of This Guidance

This ISPE Good Practice Guide: Ozone Sanitization of Pharmaceutical Water Systems is presented in the following sections:

- Introduction
- Use of ozone in the pharmaceutical industry
- Regulatory and industry guidance documents
- Ozone characteristics
- Effectiveness of ozone for microbial control
- Ozone generation
- Ultraviolet light for ozone destruction
- Ozone sensors

- System design
- System operation and control
- Commissioning and qualification
- Ozone and heat sanitization comparison
- Ozone and chemical sanitization comparison
- Appendix
- References
- Glossary

## 1.4 Background

This ISPE Good Practice Guide: Ozone Sanitization of Pharmaceutical Water Systems describes established design methodologies and practices to allow expanded use of ozone based on knowledge of the principles and benefits.

For more than 100 years, ozone has been used to sanitize municipal drinking water and reduce its organic content, and its use for pharmaceutical water systems extends beyond 30 years, yet its use is still to some extent limited in pharmaceutical applications [1].

Pharmaceutical water is used as a utility and ingredient for Good Manufacturing Practice (GMP) processes, and for the production of drug products and drug substances including Active Pharmaceutical Ingredients (APIs). Depending on the application, the purities of these waters are usually categorized as Purified Water (PW), Highly Purified Water (HPW), or Water for Injection (WFI) for which the world pharmacopoeias have monographs. In addition, water may be produced as a final product, such as USP packaged waters, including:

- Sterile water for injection
- Sterile water for irrigation
- Sterile water for inhalation
- Sterile purified water
- Bacteriostatic water for injection
- Water for hemodialysis (which also may be produced as a bulk water)

The USP monographs for bulk purified water and water for injection also provide for packaged options where microbial control within the package and inertness of the packaging are very important.

Because of the potential importance of the microbial content of water to the manufacturing of products, cleaning and some laboratory activities in pharmaceutical or biopharmaceutical facilities, water systems that produce and distribute these waters generally should be under a continuous state of control to minimize microbial content, biofilm buildup, and endotoxins.

Controlling microbial presence and proliferation within the storage and distribution sections of pharmaceutical water storage and distribution systems is typically accomplished by heat, sanitizing chemical solutions, or ozone. To heat sanitize, the storage and distribution systems are heated either continuously or on a periodic basis as validated. To sanitize using chemical solutions, these solutions are periodically circulated throughout the system followed by rinsing. This Guide focuses on the use of ozone as a sanitant.



## 2 Use of Ozone in the Pharmaceutical Industry

### 2.1 Introduction

This Chapter outlines design improvements that may contribute to considering the installation of an ozone sanitized pharmaceutical water system including:

- Water system design improvements
- Ozone advantages and disadvantages

#### Water System Design Improvements

Pharmaceutical water systems have become increasingly more reliable through improved design, operation, and maintenance. These general system design improvements also have improved ozone use reliability.

**System designs have been enhanced through improved:**

- Welding techniques
- Material composition control
- Inspection technologies
- Minimized dead leg piping designs
- Integrated valve and point of use design
- Improved ozone mixing designs
- Automation
- At-line measurement techniques

Ozone related technology also has improved. The combination of these water system design and operating improvements have resulted in many additional system design options to consider.

### 2.2 Overview of Ozone Use Advantages and Disadvantages

In order to judge whether or not to use ozone as a water system sanitant, either in a new water system or as a retrofit to an existing water system currently being sanitized by a different approach, the designer/owner must first weigh the advantages and disadvantages of using ozone.

As will be described later on, the use of ozone for sanitization is usually desirable; however, ozone is not a universal solution and situations are likely to exist where ozone may not be the best choice, e.g., where, an infrastructure with excess utility capacity such as steam and chilled water may already exist to support hot water sanitization. Utilizing this existing capacity at a relatively small incremental utility cost rather than investing in alternative capital and utilities may prove most beneficial for certain applications. Alternatively, water being fed to a distribution system may already be hot, such as from a still, so leveraging that energy investment from the purification process may be more cost effective than losing it to operate an ambient temperature ozonated system.

Elsewhere, one may be considering using ozone because of prior unsuccessful microbial control experience with another sanitization approach, one that the user urgently desires to be changed or should not be duplicated in a new water system. The user may not have the utility infrastructure for a heated water system (i.e., plant steam and chilled water), so the capital cost of using hot water sanitization could be significant compared to using ozone.

A balanced and thorough presentation of the advantages and disadvantages of using ozone is crucial for the decision process of whether or not ozonation makes the most economic and practical sense. Consideration should be given to the wide range of possible cost and operating advantages and disadvantages for using ozone to sanitize a water system, and the significance of each will vary depending upon the site and project. In addition, the use of ozone technology will place specific requirements on the system design and operation. This section of the Guide provides a high level summary of some of the main advantages and disadvantages of using ozone. Each of these issues will be discussed in more detail in subsequent sections.

### 2.2.1 Advantages

- Higher productivity – use of ozone may offer shorter sanitization cycles which can result in increased uptime and improved efficiencies for manufacturing operations. For example, sanitization of a distribution loop may be completed in one hour per day using ozonation, whereas heat and chemical sanitization may take many hours.
- Improved water quality reliability – ozonated water systems typically ozonate water in a tank continuously. This results in sustained microbial control and reduces Total Organic Carbon (TOC) concentrations in the water. The ease of relatively short loop sanitization cycles allow for more frequent sanitization and consistent microbial control.
- Capital avoidance – facilities may not have adequate steam capacity necessary to heat sanitize new water systems. Using ozone may avoid the need to install a new boiler (and a new chiller if coolant is required) and therefore, avoid the added capital cost. Potential additional capital costs for ozone generation and monitoring systems maybe outweighed by the savings, including by not having to use pipe insulation, as well as possible higher operating costs associated with heat sanitized water systems.
- Safety – the use of ozone may facilitate safer work conditions for operators and maintenance personnel by removing hazards associated with heated systems.
- Reduced energy costs – compared to hot water sanitization, ozone uses much less energy because little or no steam is needed; however, a small portion of this cost savings may be offset by added electrical costs from the generator and ozone destruct equipment.
- Reduced carbon foot print – ozone use can result in carbon footprint reductions in manufacturing facilities through energy conservation as a result of reducing the steam consumption needed for heated systems.
- Advantages over chemical sanitization – when the use of ozone is compared with the use of chemical sanitants, it has superior qualities when compatible with system materials and used appropriately:
  - Reduced water usage – compared with most other chemical sanitization approaches, ozone can be removed without water loss due to rinsing. Other chemical sanitants must be purged out with the loss of great quantities of costly treated water.
  - Reduced chemical disposal costs – some sanitants require additional costs for neutralization before they can be sewered. In areas where water discharge is costly or even prohibited, the zero-discharge associated with ozonation becomes quite appealing compared to other chemical sanitization options.
  - Reduced system downtime – since ozone can be easily neutralized in situ rather than by rinsing, a sanitization cycle can be completed more quickly than with conventional chemical sanitization's protracted rinse-out requirements and production downtime.

- Facilitated frequent use – the reduced water system downtime afforded by short ozone sanitization cycles allows its more frequent use and greater efficacy by interrupting even initial development of biofilm, if performed often enough.
- Reduced excursions and related cost avoidance – the improved microbial control resulting from frequent use of ozone as compared to less frequently used and less effective chemical sanitants results in fewer microbial limit excursions and avoidance of related investigation, potential remediation costs, and downtime. Though hard to quantify, it is nonetheless a tangible savings compared to using chemical sanitants.

The significance of each advantage is dependent upon the specific manufacturing facility, user requirements, and particular water system design being considered.

The primary economic advantages of ozone include improved water system up time (plant productivity), improved water quality, reduced utility consumption, and system flexibility. It is often difficult to quantify the related indirect savings though these may be some of the most important considerations. Return on investment calculations based on direct costs only, may not provide a complete comparison.

### 2.2.2 *Disadvantages*

- Cost of ozone generation equipment – the user should understand differences in methods of ozone generation, related costs of the oxygen source – if the ozone generation method selected requires an oxygen source, there are related equipment or raw material supply cost considerations.
- Ozone destruction equipment – the user should understand differences in methods of ozone destruction and related costs. This includes ozone destruction both in-line as well as (if necessary) at the tank vent.
- Ozone monitoring equipment – measurement of ozone levels is required prior to and after in-line ozone destruction equipment to control ozone introduction and to verify its removal, and also may be required at the end of the loop to verify sanitizing concentrations. This typically requires at least two sensor locations. The user should understand the sensor types and choose the ones most suited to their applications. Ozone monitoring equipment also has its unique maintenance and calibration requirements.
- Water quality –the conductivity of the water may gradually increase, in systems using corona discharge fed with low purity oxygen and that have little or no water use for extended periods of time (e.g., several days or more).
- Biofilm control – while ozone is excellent at controlling the onset of biofilm formation, once a biofilm has become well established, ozone may not be as effective as heat. Frequent ozone use (daily to weekly) prevents this from occurring, but where biofilm has been allowed to develop, increased ozone levels may be required or other sanitization methods may need to be employed to eliminate the biofilm before returning to ozone use only.
- Material compatibility – ozone compatible system materials of construction must be used. Generally, 316LSS has unlimited life with ozone; however, seals and other elastomers may have a limited life. Therefore, certain routine material replacement costs must be considered. Ozone incompatible materials or those left in service too long could degrade and fail and even enter the water as dissolved or particulate matter.
- Training – as with all water systems, operators must be trained on system operation. Ozonated systems may require unique training which may be different or more specialized; however, training is crucial for continuing safe and efficacious operation.
- Use with oxygen sensitive products – some oxygen is normally present in the water; however, the ozone generation and the degradation process can increase the amount of dissolved oxygen in the water. The increase over what might be dissolved from the air or remaining in the water after purification in ambient water systems is most likely more than would be present in systems utilizing heat due to oxygen solubility.

- Energy costs – some additional electricity may be needed to operate the ozone generator and UV light, as well as the oxygen generator if used for a corona discharge ozone generator.
- Safety – atmospheric release of ozone is a safety consideration in areas where ozone is generated external to the system (e.g., corona discharge), near vent filter operation, and when opening the system, particularly the tank. Proper safety precautions are needed as well as environmental ozone monitoring/alarming.

The significance of each advantage and disadvantage is dependent upon the specific alternative sanitization approach being compared to ozone. As mentioned above, each of these issues will be discussed in greater detail in subsequent chapters. After reviewing these more thorough discussions, a detailed worksheet for comparing these relative capital and operating costs and performance factors is provided in Appendix 1 to assist the decision process in choice of sanitization approach for new or retrofitted system. However, it must be reiterated that it is often difficult to quantify related indirect savings associated with improved quality and system control. Return on investment calculations based on direct costs only, may not provide a complete comparison.

## 3 Regulatory and Industry Guidance

### 3.1 Introduction

The acceptability of ozone for sanitization of water systems is well established within the pharmaceutical industry and has widespread approved use. Guidance on both the use of ozone and ozone sanitization may be found in:

- USP General Information Chapter <1231> Water for Pharmaceutical Purposes [1]
- ISPE Baseline® Guide on Water and Steam Systems (Second Edition) [2]
- FDA Guide to Inspections of High Purity Water Systems [3]
- FDA Recognized Consensus Standard for AAMI/ANSI RD62:2006 [4]

Along with this Guide, the USP General Chapter discussion and the ISPE Baseline® Guide discussion are authoritative and non-commercial discussions by industry experts of the current state of technology for ozone sanitization in high purity pharmaceutical water systems, and as such is the kind of information the FDA can draw upon as it enforces its evolving expectations for the “c” of cGMPs. Knowing the potential regulatory use of this information, efforts have been made to keep the text and tone of this Guide informational, because there are many approaches for using ozone to accomplish the goal of assuring water quality through successful microbial control.

### 3.2 USP General Information Chapter <1231>

In 2005, the USP recognized the need for authoritative, non-commercially motivated information about ozone and provided a brief commentary on its controversial association with the “no added substances” issues in its revised General Information Chapter <1231> “Water for Pharmaceutical Purposes” [1]. Chapter <1231> states:

*“Chemical additives are used in water systems (a) to control microorganisms by use of sanitants, such as chlorine compounds and ozone, (b) to enhance the removal of suspended solids by use of flocculating agents, (c) to remove chlorine compounds, (d) to avoid scaling on reverse osmosis membranes, and (e) to adjust pH for more effective removal of carbonate and ammonia compounds by reverse osmosis. These additives do not constitute “added substances” as long as they are either removed by subsequent processing steps or are otherwise absent from the finished water. Control of additives to ensure a continuously effective concentration and subsequent monitoring to ensure their removal should be designed into the system and included in the monitoring program.”*

Additional guidance on the use of ozone and some of its advantages and limitations are mentioned in the Sanitization discussion section within this same USP General Chapter:

*“These methods [of chemical sanitization] typically employ oxidizing agents, such as halogenated compounds, hydrogen peroxide, ozone, peracetic acid, or combinations thereof. Halogenated compounds are effective sanitizers, but are difficult to flush from the system and may leave biofilms intact. Compounds such as hydrogen peroxide, ozone, and peracetic acid oxidize bacteria and biofilms by forming reactive peroxides and free radicals (notably hydroxyl radicals). The short half-life of ozone in particular, and its limitation on achievable concentrations require that it be added continuously during the sanitization process. Hydrogen peroxide and ozone rapidly degrade to water and oxygen; peracetic acid degrades to acetic acid in the presence of UV light. In fact, ozone’s ease of degradation to oxygen using 254-nm UV lights at use points allows it to be most effectively used on a continuous basis to provide continuously sanitizing conditions.”<sup>1</sup>*

In the United States, the regulatory impact of references like USP, officially empowered by the FD&C act, allows FDA to utilize, at its discretion, any part of the USP as though it were enforceable law, or as a minimum, representing the “c” of cGMPs (current Good Manufacturing Practices). It should be noted that USP designates General Chapters (e.g., <1231>) [1] as informational and non-mandatory.

### 3.3 FDA and Other Regulatory Agencies

#### 3.3.1 FDA Guide to Inspections of High Purity Water Systems

The 1993 Guide to Inspections of High Purity Water Systems [3] contains several negative comments regarding ozone use. These comments date from a period when ozone sanitization of water systems in the US pharmaceutical industry was just evolving and many ozonated systems were either not being operated properly or were water systems retrofitted with ozone capability and not designed properly for ozone use. No standards or guidance for proper system design or use had been issued by the USP, the FDA, or ISPE at this time. Hence, this Inspection Guide contains much cautionary wording about examples where ozone did not work as intended. However, it is clear from these scenario descriptions that by current standards, the ozone was not being used properly. The industry has evolved since then and more user guidance is now available, including this Guide, making the ozone scenarios described in this Inspection Guide far less common.

#### 3.3.2 Other FDA Commentary

The FDA has not issued further guidance regarding the use of ozone in pharmaceutical water systems since that in 1993; however, the CDRH division of FDA has commented on ozone in the context of its use in sanitizing water systems associated with hemodialysis. This 2009 comment was made in FDA's Recognized Consensus Standard for the AAMI standard for water treatment equipment for hemodialysis applications [4]. In this document, the FDA reinforces the need to validate the efficacy of the ozone treatment:

“If a manufacturer [of a hemodialysis water treatment system/device] wishes to use an ozone disinfection system, adequate validation shall be provided ... to demonstrate that it can reduce biofilm in the water treatment system...”

Although water systems used for hemodialysis applications are distinct from the quality of design, operation, and microbial control expected by the FDA for pharmaceutical water systems regulated by CDER and CBER divisions, it is apparent that the FDA's validation concern in CDRH-regulated hemodialysis applications is focused on the efficacy of the ozone treatments used. By inference, this makes microbial control efficacy an essential validation element for ozonated pharmaceutical water systems. This validation concept and approach are discussed in Chapter 11.

### 3.4 EP, JP, and EMA

At time of publication, the use of ozone is not prescribed in the current European Pharmacopoeia (EP) 7.1 [5], the Japanese Pharmacopoeia (JP) 16 [6], or by the EMA. They do not preclude the use of ozone nor give guidance or reference on how it should be used. In Europe, however, the widespread use and familiarity with ozone for treating drinking water may be considered an indication that there are few concerns with its use in pharmaceutical water, as long as it is removed prior to using the water.

### 3.5 ISPE

The ISPE Baseline® Guide for Water and Steam Systems (Second Edition) [2] provides information regarding ozone in several of its chapters, but detail is focused in the Storage and Distribution Chapter and its sanitization efficacy issues are discussed in the Microbiological Considerations Chapter. Though these discussions constitute an excellent overview for ozonated system design and use, this Good Practice Guide details many of the issues mentioned in the Baseline® Guide as well as provides additional detailed guidance for issues not covered in the Baseline® Guide.

## 4 Ozone Characteristics

### 4.1 Introduction

This Chapter discusses topics which are fundamental to understanding the chemical nature of ozone and its degradation, including:

- What is ozone?
- Ozone and free radicals
- Degradants associated with ozone generation and destruction

### 4.2 What is Ozone?

Ozone is a gaseous triatomic form of oxygen ( $O_3$ ). It is created naturally in the atmosphere by ultraviolet irradiation (185 nm) of oxygen ( $O_2$ ); however, this method of production is generally considered too inefficient for most commercial purposes.

Though ozone is about 13 times more soluble in water than molecular  $O_2$ , it is still considered to be sparingly soluble.

Ozone is extremely unstable at atmospheric temperatures and pressures. However, its high redox potential makes it one of the most powerful oxidizing agents used in pharmaceutical water sanitization. It is more powerful than other commercially available oxidizing sanitants, such as chlorine or hydrogen peroxide. The comparative redox potentials of these sanitants are:

|                   |         |
|-------------------|---------|
| Ozone             | +2.07eV |
| Chlorine          | +1.36eV |
| Hydrogen Peroxide | +1.77eV |

Ozone decomposes readily to innocuous molecular  $O_2$ , unlike other sanitizers which need to be disposed of at the end of a sanitization process.

It is impractical to store ozone for subsequent use because of its unstable nature (it can be explosive at  $\geq 10\%$  v/v in air), aggressive reactivity, and short half-life. Ozone is usually generated at low concentrations for immediate action, relatively near to the point of application. The most common commercial methods for the manufacture of ozone are based on creating an electrical corona discharge in the presence of oxygen [7] (sometimes called “Silent Discharge”) and electrolysis of water.

Depending on the ozone concentration and contact time, its action on organic compounds in the water may generate small amounts of carbon dioxide, as the carbon portion of the organic compound is oxidized. Typically, organic compounds oxidized by ozone are only partially degraded to carboxylic acids or forms of smaller organics. The generation of these ionized degradants at levels of parts per billion (ppb) can eventually have a detectable impact on the pH and conductivity of the water, if not alleviated by deionization polishing or dilution with make-up water from water usage.

In high purity water with negligible levels of TOC, mostly oxygen will be formed as ozone decomposes.

### 4.3 Ozone and Free Radicals

Free radical formation is inherent and critical to the ozone sanitization process. A free radical is a molecule with an unpaired electron, and is highly reactive. Hydroxyl (OH•) free radicals are formed as ozone degrades. Though extremely transient (lasting only nano- to milli-seconds), hydroxyl free radicals have a redox potential (+2.80eV) even higher than that of ozone (+2.07eV). The combined oxidizing capability of hydroxyl free radicals and their parent ozone molecules is responsible for the highly aggressive and effective sanitization by ozone treatments.

There are three phases of reaction for free radicals, including those resulting from the presence of ozone:

1. Formation
2. Propagation
3. Termination

The time it takes to complete all three phases is extremely short [8 and 9].

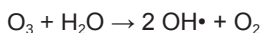
#### 4.3.1 Formation

Ozone (O<sub>3</sub>) is an allotrope of elemental oxygen formed by cleaving water or oxygen gas.

Similar chemical reactions with these two starting molecules produce the highly reactive:

- Oxygen free radical<sup>1</sup>
- Hydroxyl free radical
- Oxygen with an unpaired and non-bonded electron

Ozone either degrades naturally or through the action of ultraviolet light (UV<sub>254nm</sub>). As ozone degrades, free radicals are formed. This occurs through a series of reactions involving water, and results in the formation of molecular oxygen (O<sub>2</sub>) and two highly reactive hydroxyl free radicals. This reaction is nearly instantaneous (nanoseconds) in the presence of UV<sub>254nm</sub> but is much slower (seconds to minutes) with natural degradation. The half-life of ozone is about 20 minutes (assuming ambient temperature).



The formation of free radicals occurs regardless of whether the ozone was originally generated by corona discharge or by electrolysis of water.

#### 4.3.2 Propagation

Hydroxyl free radicals (OH•) (and other oxidants,) tend to extract electrons from the bonds of other molecules that are of lower energy than the oxidation potential of the free radical.

In the presence of dissolved oxygen, and where the target of a free radical is a covalent bond within an organic molecule (e.g., organic compounds in the water or a macromolecule on the surface of a bacterial cell) the bond will be oxidized by having an electron removed and an oxygen atom added. This either modifies or cleaves the target organic molecule.

<sup>1</sup> Free radicals are formed when the hydrogen-oxygen bonds of water or the oxygen-oxygen bonds of molecular oxygen (O<sub>2</sub>) are separated and covalent bonds do not reform.

Depending on the nature of the target organic molecule, this action may propagate (i.e., generate) another free radical until the reacting free radical is quenched by a termination reaction.

#### 4.3.3 Termination

Free radicals are terminated when two unpaired electrons react to form a covalent bond. This is a very quick reaction (half life  $10^{-7} \sim 10^{-9}$  seconds) and is difficult to characterize due to the transient nature of free radicals. Studies to observe free radical formation and reaction rates are indirect and they are performed using evaluations of the oxidation of target compounds.

Common donors of electrons for the termination of free radicals include:

- Organic compounds
- Other free radicals
- Carbonate ions
- Bicarbonate ions

Usually, there are at least trace levels (in the parts per billion (ppb) range) of organic compounds in the water. In addition, there also may be dissolved carbon dioxide ( $\text{CO}_2$ ) which equilibrates to bicarbonate ions in high purity water.

When termination reactions occur, the free radical is consumed (or quenched) in a covalent bond rather than being propagated. This free radical quenching process tends to dominate, resulting in the transient (but highly reactive) presence of free radicals.

#### 4.3.4 Dissipation of Free Radicals

The free radicals formed during the use of ozone contribute substantially to disinfection and microbiological control.

These free radicals dissipate extremely quickly (i.e., within nanoseconds) because of their rapid quenching by other molecules in the water. When the source of these free radicals (i.e., ozone) is removed or degraded, any residual free radicals also dissipate almost instantaneously; therefore, they do not constitute a concern relative to water quality.

### 4.4 Degradants Associated with Ozone Generation and Destruction

An “added substance” is prohibited by the pharmaceutical water monographs of USP and other major compendia.

Based partly on the discussion provided in Chapter <1231> of the USP [1], ozone is not an “added substance” if it is removed from the water prior to use.

However, some individuals are less confident about the completeness of its removal by  $\text{UV}_{254\text{nm}}$  as well as the interpretation as “added substances” and the by-products potentially created during the ozone’s generation, its action on organic compounds and microorganisms, and its destruction by ultraviolet light. The guidance provided below is intended to clarify these issues.

#### 4.4.1 Ozone Generation By-Products

When ozone is generated by the corona discharge approach, gaseous oxygen is required. When that oxygen source is air (which contains about 80% nitrogen) or oxygen-enriched air (which could contain up to 10% nitrogen), trace levels of nitrogen oxide gases can be co-generated along with ozone, especially if the gas is not extremely dry. When this ozone-containing gas is dissolved in water, any nitrogen oxide gas also dissolves and ultimately becomes nitrate ions. This results in a slight acidification of the water, along with an increase in conductivity.

When very dry oxygen-enriched air is used in corona discharge ozone generators, the amount of nitrate co-generated with ozone can be relatively low and detectable only as a slight conductivity increase in waters that have been highly purified.

Purified water, highly purified water, and water for injection are allowed to have specific low levels of ionic impurities.

They are not considered significant and should not be considered “added substances” as long as the relevant conductivity, nitrate, and pH requirements are met.

Ozone is commonly generated using high purity oxygen in corona discharge ozone generators or electrolytically to avoid trace levels of nitrates in the water from this source. For further information, see Chapter 6 of this Guide.

Interestingly, some corona discharge type ozone generators require at least trace (oxygen no purer than %99.9999) levels of nitrogen gas in the oxygen stream to most efficiently generate ozone (discussed in Chapter 6), so even under the most controlled conditions using corona discharge ozone generators, a tiny amount of nitrate can be added to the water along with ozone. However, these levels are so low as to be essentially undetectable and innocuous.

#### **4.4.2 Ozone Reaction/Degradation By-Products**

The degradation of ozone by contact with water impurities, e.g., organic compounds or the organic molecules on microorganism and biofilm surfaces is fundamental to its value.

When ozone degrades molecular O<sub>2</sub> and highly reactive, but very short-lived hydroxyl free radicals are liberated.

The study of ozone degradation and the fate of the various free radicals that may be formed during its life is difficult because of the speed of the steps in the process. Much of what is known about any intermediates is inferred from the resulting reaction products.

One of these intermediates is hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), formed when two hydroxyl free radicals interact with each other. This could be a concern as an “added substance” if the compound persisted, but intact ozone molecules degrade hydrogen peroxide back to hydroxyl free radicals, making its existence transient. This has been confirmed by ozone users being unable to detect measurable levels of hydrogen peroxide in ozone sanitized pharmaceutical water.

The hydroxyl free radicals tend to react rapidly with organic or inorganic molecules encountered. This interaction alters the structure of the organic (or inorganic) target compounds and may modify the organic compounds into branched carbon compounds or cleave them into shorter-chained carbon compounds. These resulting carbon compounds often terminate with carboxyl groups. The interaction also may generate carbon dioxide, along with the formation of dissolved oxygen and water.

The modified or cleaved organic compounds, the carbon dioxide and the oxygen, which are created through ozone's oxidative properties, are considered not to be “added substances” by the USP, because they satisfy at least one of the following criteria:

1. They are derived from compounds already present (and allowed) in the water.
2. They are identical to molecules already present and naturally found (and allowed) in these waters from other sources.

However, if these compounds are allowed to accumulate to levels that could cause any of the attribute tests to fail, the water could be unfit for use in pharmaceutical applications by virtue of failing purity specifications (not by virtue of being considered added substances).

### 4.4.3 Ozone Removal Confirmation

#### 4.4.3.1 Low Level Detection

Contaminant removal is critical to the success of any water system. Physical and chemical contaminants may be removed using any number of technologies which are suitable and proven. Elimination of viable microbial contamination can be accommodated primarily through the use of either heat or chemicals. When chemicals are employed, they must, except in rare instances, be removed prior to using the water. The verification of removal is often easier said than done. Few if any measuring and monitoring technologies (instruments) are capable of confirming a complete absence of a particular constituent simply because measuring zero is beyond most instruments capability based on their limit of detection.

Pharmaceutical compendial waters have the added burden of compliance with the “no added substance” rule which has on occasion resulted in elevated concern by some and which also has given rise to extended discussion of completeness of removal. This issue has not been documented to be problematic in the majority of instances; however, the following discussion is provided for those who remain concerned and/or for those with extremely ozone sensitive applications. These additional steps are most often not required and should not be considered as recommendations, but rather as optional for those who wish to consider ozone use but require additional assurances.

One of the concerns associated with the completeness of ozone removal/destruction by UV<sub>254nm</sub> is related to the Limit of Detection (LOD) of ozone analyses. Analyses cannot detect ozone levels below their LOD and none of the analyzers discussed in Chapter 8 of this Guide (or elsewhere), can detect “zero,” i.e., the complete absence of residual ozone.

In situations where the level of ozone may be below the LOD of the instrument and an assessment of this level is desired, a theoretical estimate may be made by following the approach outlined in Appendix 1.

The ozone destruction discussion is intended to provide assurance that the irradiation (UV<sub>254nm</sub>) intensity, when properly sized and selected, is more than adequate to completely destroy the levels of ozone that may be routinely present. See Chapter 7 of this Guide for the discussion regarding UV dose and UV destruct unit sizing.

Theoretically, if the absolute absence of ozone cannot be detected, then it cannot be known with certainty that ozone is absolutely destroyed. Although the detection of complete absence is not possible, this issue has been dealt with successfully in other scenarios, e.g., for impurity/related substances tests for APIs, cleaning validation, and even chemical sanitizer residue rinse out from water systems. At some point, the level of the analyte in question is so low that its effects are inconsequential. The philosophy behind ozone removal/destruction and examples of similar challenges are described in Appendix 1.

#### 4.4.3.2 Risk Minimization Considerations

For ozone, several common sense approaches may be used to assess its impact on a product or process, assuming the worst case residual levels that could remain even if this is at or below the LOD of the analysis. Factors in this assessment could include:

- The half-life of ozone of about 20 minutes (assuming ambient temperature and otherwise high water purity).
- The actual concentration of organic matter (which might immediately and innocuously consume the ozone) in the water or process where the trace ozone-containing water is used.
- The nature of the organic matter being potentially degraded, (is it background Total Organic Carbon (TOC) in the water, or excipient or primarily the active?)
- The minute amount of organic material that would be partially decomposed by the trace amount of ozone.

With a half-life estimated at 20 minutes and considering that the starting point is already at undetectable levels, it is not inappropriate to estimate the possible ozone levels based on actual production activities. For example, if 1200 gallons of water is charged into a process vessel at the rate of 20 GPM, it will take 60 minutes to fill the vessel. At that point, approximately 1/3 of the water will have had its ozone level reduced by a full two half-lives while another 1/3 will have had its level reduced by one full half-life. Under these circumstances, the ozone level in the vessel at the end of water dispensing, and prior to processing, could not be any greater than half of the original undetectable levels, and would most likely be far lower.

As an oxidant, ozone is capable of reacting with and degrading organic matter. Ozone that reacts with trace organics that may remain in the water will most likely reduce those organic levels while consuming some or all of any remaining undetectable residual ozone. Although immeasurable, these chemical reactions would be considered advantageous to water quality.

If the inability to analytically detect the absolute absence of ozone is of serious concern, it should be addressed using an appropriate risk assessment-based approach. This discussion along with Chapter 11 and the associated Appendix items are intended to provide possible considerations in the preparation of a risk assessment.

## 5 Effectiveness of Ozone for Microbial Control

### 5.1 Introduction

This Chapter discusses topics which are fundamental to understanding how ozone works against microorganisms, development of successful ozone sanitization approaches, and factors that compromise ozones efficacy, including:

- Ozone use in pharmaceutical water systems
- Mechanisms and targets of ozone attack
- Factors affecting ozone efficacy
- Ozone sanitization strategies
- Determining ozone sanitization frequency
- Summary elements of optimal microbiological control using ozone

### 5.2 Ozone Use in Pharmaceutical Water Systems

Ozone is an extremely rapid and efficient oxidizer frequently used as a microbial sanitant in pharmaceutical grade water systems. Ozone possesses a very high lethality coefficient as compared to other frequently used chemical sanitizers, such as chlorine, which must first diffuse into the bacterial cell to cause death. Ozone kills bacteria primarily by attacking the organic components of bacterial cell wall and membrane. This can cause the cell to rupture or “lyse” and is a much faster mechanism of destruction (by a factor of as much as several thousand) compared to chlorine.

### 5.3 Mechanisms and Targets of Ozone Attack

The oxidative effects of ozone, and the even more aggressive hydroxyl free radicals released by ozone decomposition to oxygen, alter molecular structures with which they interact, changing their properties. When these molecular structures are functional constituents of microorganisms, they permanently alter metabolic functions as well as essential cellular structures, affecting the ability of the microorganism to survive or proliferate. When it is the cell wall and cell membrane of bacteria that are affected, the cell loses its integrity against the external environment resulting in metabolic shutdown, possible lysis, and death.

Many chemical structures in biological macromolecules are susceptible to these strongly oxidative effects, including amines, amides, and sulfhydryl groups [8]. Ozone also can attack both saturated and unsaturated carbon double bonds that may be part of the molecule’s “backbone.” For instance, when the oxidative activity of ozone attacks the lipopolysaccharide (endotoxin) component of the cell, the unsaturated carbon bonds of the lipid portion of the molecule are degraded, making the molecule apyrogenic [7]. Water needs to be exposed to ozone for a prolonged period of time (perhaps many hours) before levels of specific organic compounds such as endotoxin can be seen to substantially decrease.

When this chemical degradation is allowed to progress to completion, ozone can convert the carbon backbone of most organic molecules to CO<sub>2</sub>, including the organic compounds found in high purity water. However, in practice, this process is relatively slow. It is not usually allowed to proceed to this level of completion when used as a pharmaceutical water system sanitant.

Usually, the organic molecules are broken into progressively smaller organic molecules, but only a small amount is converted to CO<sub>2</sub>, leading to only moderate TOC (or endotoxin) reductions, particularly in high purity water systems where both the TOC levels and amount of ozone may be quite low. During periods of low use, the accumulation of CO<sub>2</sub> may, over time (e.g., 12 to 48 hours), increase stored water conductivity. This increase can be counteracted by polishing or partial replenishment.

## 5.4 Factors Affecting Ozone Efficacy

Ozone's overall lethality against microbial contamination is a function of concentration and contact time. However, concentration can be affected by a number of factors. High temperature and high pH levels greatly reduce ozone's typical half-life (of about 20 minutes) to a matter of a few minutes or even seconds. In addition, high levels of dissolved organics, i.e., TOC, can consume a portion of the ozone before it has a chance to react with and kill microorganisms. Hence, both physical and chemical conditions should be considered when assessing proper water system ozone dosing and dosing locations [7].

Ozone's lethality rate is considered excellent against planktonic bacterial cells, by direct attack on unprotected cell walls and membranes. However, once cells are covered in thick protective layers of exuded organic macromolecules, (i.e., cells embedded within a biofilm and protected by its slimy exopolymer) ozone's effectiveness at killing these bacteria using the same dosage levels is significantly diminished. The pharmaceutical industry tends to prefer the use of the lowest levels of ozone which are capable of causing planktonic bacteria death, making the destruction of ozone by UV<sub>254nm</sub> quite easy and complete and the impact of ozone on polymeric system components less significant.

However, these low ozone concentrations (i.e., < 40 ppb) have difficulty in penetrating and destroying biofilms. Therefore, it is considered prudent to minimize biofilm formation within non-continuously ozonated portions of the system when such low ozone concentrations are used. This may be achieved through frequent (daily to weekly) system-wide sanitizations for perhaps just one to two hours each.

Depending on their thickness, biofilms can be destroyed by ozone if much higher ozone levels (> 200 ppb) and long contact times (many hours) are used. Using even higher ozone levels (> 1 ppm) could reduce the contact time needed to destroy the biofilms. Biofilms that have been allowed many weeks to develop can usually only be effectively killed by these high ozone levels (> 1 ppm). For this reason, ozone concentrations may be increased during distribution loop ozonations, or additional ozone injection points may be needed for complex or extensive distribution systems, to produce a "shocking dose" of ozone, particularly when such treatments are relatively infrequent (less often than weekly).

It is considered prudent to avoid biofilm control problems by using frequent loop ozonations rather than attempting remediation after they have formed due to infrequent loop ozonations. If biofilms are given too much time to develop, they will eventually colonize topographically protected locations such as weld surfaces, within narrow crevices or inside poorly turbulent side branches. Once this has occurred, all chemical sanitants, including ozone, are extremely challenged to kill these sequestered biofilms.

## 5.5 Ozone Sanitization Strategies

At low ozone levels, the protection afforded to biofilm microorganisms by the surrounding exopolymer slime is substantial. Therefore, for ozone to be effective at a relatively low concentration, the amount/thickness of exopolymer-coated biofilm that can be allowed to develop should be minimal. This avoids the need for ozone levels so high as to require significant oversizing of ozone generators with corresponding concern for effects on system materials, components, and facility operations.

For pharmaceutical grade water systems, the chemical impurity levels in the finished water (which act as food for biofilms) are usually low, fostering slower development of difficult-to-kill thicknesses of biofilm when compared to less pure waters.

### 5.5.1 *Routine Continuous and Intermittent Ozone Sanitization Approaches*

Any one of at least four distribution system ozone sanitization approaches can be used:

- Continuous storage tank and loop ozonation with UV<sub>254nm</sub> ozone destruction at each POU
- Continuous storage tank and loop ozonation with UV<sub>254nm</sub> ozone destruction for the entire loop upon demand at any POU
- Intermittent storage tank and loop ozonation
- Continuous storage tank ozonation with UV<sub>254nm</sub> ozone destruction for the entire loop except during intermittent loop ozonation

Regardless of the approach utilized, the need to flush POU outlets and sampling ports should be assessed. If needed, the frequency (may need to be daily or weekly) and method should be determined. Flushing with ozonated water to sanitize the downstream sides of all valves (which may otherwise never see sanitizing conditions) might be considered (using appropriate safety precautions).

For the best balance of adequate microbial control versus equipment cost and operational feasibility, the latter of the four options (continuous tank with intermittent loop ozonation) is by far the most widely used. This approach is often considered to be “continuous ozonation” even though only the tank portion of the system is continuously ozonated. Nevertheless, the ozone in the tank and the UV used to destroy the ozone as it exits the tank and enters the loop essentially sterilize the water entering the loop, precluding the introduction of biofilm-forming planktonic organisms that could have originated from biofilms in the water purification system. Nevertheless, care must be given in utilizing a short enough intermittency for the loop sanitization so that any sequestered biofilm-colonized location in the loop does not have a chance to proliferate and contaminate the distributed water.

### 5.5.2 *Initial Sanitization Issues*

The opportunity for biofilm development in a newly constructed or renovated water system may be significant if there are problems during commissioning or commissioning is not carried out properly. Biofilm growth can occur after system wetting (flooding) during construction or commissioning activities followed by:

- Inadequate or delayed cleaning or drying of the system
- Leaving the system stagnant, even for short periods of time
- The possible absence or improper-functioning of microbial controlling unit operations, e.g., filters, UV<sub>254nm</sub> sanitizers, or ozone equipment
- A delay in the execution of the first system-wide ozone sanitization after the system was initially wetted

Similarly, when a system is retrofitted from a chemically-sanitized to an ozonated water system, there may be biofilm already present due to a partially effective chemical sanitization approach (which may be the reason for converting to ozone sanitization).

Since well-developed biofilm is a challenge for ozone sanitization, this initial ozone sanitization cycle should be as extreme as possible in terms of ozone concentration and contact time, i.e., a “super-sanitization,” to better assure the complete removal of biofilm that may be present. This approach to “get it clean, then keep it clean” can reduce the number of manufacturing-disruptive system-wide ozone sanitizations that would otherwise be required.

In systems being retrofitted with ozone, care must be taken to not allow biofilm to become established prior to the ozone system becoming operable. Should biofilm become established very long or frequent (perhaps multiple daily) ozone treatments may be needed to achieve a near complete biofilm kill. If the biofilm development is substantial and the ozone levels used are at low levels (e.g., < 40 ppb), a thick biofilm, resulting from improper start-up, for example, could be essentially impervious to this ozone treatment, regardless of frequency or contact time. In such situations, this tenacious, established biofilm may need to be attacked with more extreme treatments, perhaps even alternative sanitizing agents, until it is eliminated.

## 5.6 Determining Ozone Sanitization Frequency

The frequency of ozone sanitizations should be sufficiently often to prevent or minimize exopolymer-coated biofilm development in storage and distribution systems. How often this system-wide treatment is needed can be empirically determined by post-sanitization microbial monitoring.

- Where the water distribution system loop is protected from planktonic re-inoculation, the ozone treatment and planktonic control are considered effective if water samples for microbial enumeration yield zero counts immediately after system-wide ozone sanitization and remain at zero for over a week thereafter. However, if levels rebound within a week, the loop ozonation probably killed the outer layers and not all of the biofilm thickness.

In this scenario, periodic ozone sanitizations should be more frequent (to catch the re-developing biofilm in a more susceptible form) and/or more stringent (longer exposure at higher ozone concentrations).

If the microbial counts immediately after the treatment are not zero, either the continuous planktonic control is not working properly or the system-wide ozonation treatment is not effective because there may be substantially thick biofilm present and/or dead legs somewhere in the system.

In this scenario, if longer exposure to higher ozone concentrations does not yield zero counts immediately after treatment, the system must be thoroughly inspected for the presence of dead legs and if found, they should be either removed or the dead leg condition corrected by routinely flushing during sanitization. Either hot water sanitization or prolonged sanitization with a much higher ozone concentration or some other strong sanitant should be considered to completely kill the existing biofilm, before resuming intermittent use of ozone.

Once the initial biofilm load in a retrofitted water system (or system started-up then left stagnant), is removed, a study to determine the frequency and ozone treatment conditions for routine microbial control could be undertaken using microbial testing.

However, rather than potentially compromising a pristine system to determine a less frequent treatment that yields an acceptable level of control, and in the process allow biofilm to develop, many users simply perform frequent system-wide sanitization (e.g., daily) with the added benefit of essentially avoiding biofilm re-development altogether. The longer the delay between ozone sanitizations, the greater the risk that problematic biofilm could begin to develop. In most cases, it has been found that ozone sanitizations should be performed weekly, as a minimum.

## 5.7 Summary Elements of Optimal Microbiological Control Using Ozone

If ozonated systems are properly designed and operated, significant biofilm development should never occur. When such systems are maintained biofilm-free or thoroughly cleaned of nearly all biofilm, routine ozonation of those systems will be able to keep them clean and free from essentially all microbial contamination thereafter.

### **5.7.1 The Importance of Proper System Design**

Important features include:

- Assuring that effective ozone levels are present at all locations in storage and distribution during system-wide sanitization.
- Assuring that there are no (or very few) crevices in the storage and distribution system where biofilm can grow and be physically protected from ozone exposure during system-wide sanitization.
- Assuring that all distribution piping branches including those associated with use point valves, gauges, and sensors are of minimal length and allow rapid water flow and ready mixing of ozonated water throughout the side legs during sanitization.
- Assuring that finished water purity is controlled to low TOC levels, in spite of potential seasonal and episodic variability in feed water quality. This should assure that the ozone demand from organic impurities stays relatively constant. Assuring that upstream treatment equipment is properly maintained to minimize biofilm development and subsequent inoculation of the storage and distribution system.

### **5.7.2 The Importance of Maintaining Hostility to Biofilm Development**

In order to maintain microbial control in the storage tank and distribution loop, microbially hostile ozone levels should be either continuously or intermittently present. The frequency of the sanitization intermittency and the minimum effective ozone concentration used are related to each other as well as to the amount of biofilm growth that has been allowed to develop during the period between ozonations. If ozone is used daily so almost no biofilm can develop, very little ozone is needed (e.g., 20 ppb to 50 ppb). If ozone is used weekly or less often so more substantial biofilm has been given the opportunity to develop, higher ozone levels may be needed (e.g., 200 ppb to > 1 ppm). Although ozonation frequencies of daily to weekly are the most common, longer periods, though riskier, have been occasionally employed.

### **5.7.3 The Importance of Initial or Remedial Sanitization of New or Retrofitted Systems**

As previously stated, proper start-up and sanitization of new systems is critical. As well, remedial sanitization of contaminated systems is particularly important where:

- An existing system is being retrofitted for ozone sanitization in place of a different previously used sanitant
- Start-up or commissioning operations have used water that may have allowed initial biofilm formation before the first sanitizations can be performed
- Substantial biofilm growth has been allowed to develop by a system upset or long-ineffective sanitization processes

Remedial biofilm removal in the portions of the system upstream of storage and distribution will typically not be addressed using ozone and hence is not discussed in this Guide. Methods for sanitizing these components should be provided by the manufacturer. For further information on other such sanitization approaches, see other ISPE guidance, such as the ISPE Baseline® Guide for Water and Steam Systems (Second Edition) [2].

If significant biofilm levels have been allowed to develop in storage and distribution, very high ozone concentrations could be needed (> 1 ppm) [10]. If the biofilm is particularly thick or if it is likely to have colonized in crevices where ozone penetrates poorly, alternative sanitization methods may be required such as a one-time heat sanitization (recirculating hot water at  $\geq 65^{\circ}\text{C}$ ) because it can penetrate and kill the biofilm in these crevices. If a one-time heat sanitization is not possible, other methods should be evaluated including the one-time application of an aggressive chemical sanitizer using:

- The highest tolerable temperature
- A high concentration
- A long contact time

Even though all chemical approaches can suffer from the same lack of penetration into biofilm-colonized crevices as ozone, most can be used at very high concentrations to improve penetration, killing, and removal of thick surface biofilms. For further information on other such sanitization approaches, see other ISPE guidance, such as the ISPE Baseline® Guide for Water and Steam Systems (Second Edition) [2].

Whenever extreme remedial sanitization approaches are used, system and component compatibility must be verified. Sensors or other components that might be incompatible with these extreme sanitization approaches should be removed during the sanitization process, and then reinstalled after sanitization in such a way as to minimize contamination reintroduction. It is also important for all system outlets to be flushed with the remedial sanitant to assure that they are also initially biofilm free.

Providing the distribution system is effectively biofilm-free and it is not recontaminated by upstream components, maintaining that condition (keeping it clean) using continuous tank ozonation and frequent, periodic system-wide loop ozonations should provide exceptionally high microbiological quality water at a low operating cost and with minimal manufacturing disruption.

## 6 Ozone Generation

### 6.1 Introduction

Corona discharge and electrolytic ozone generation are the two main technologies used for generating ozone for high purity water sanitization in the pharmaceutical industry. Both have been used successfully for many years. This Chapter discusses these two ozone generation technologies, as well as their sizing requirements, including:

- Corona discharge ozone generation
- Electrolytic ozone generation
- Ozone generator sizing

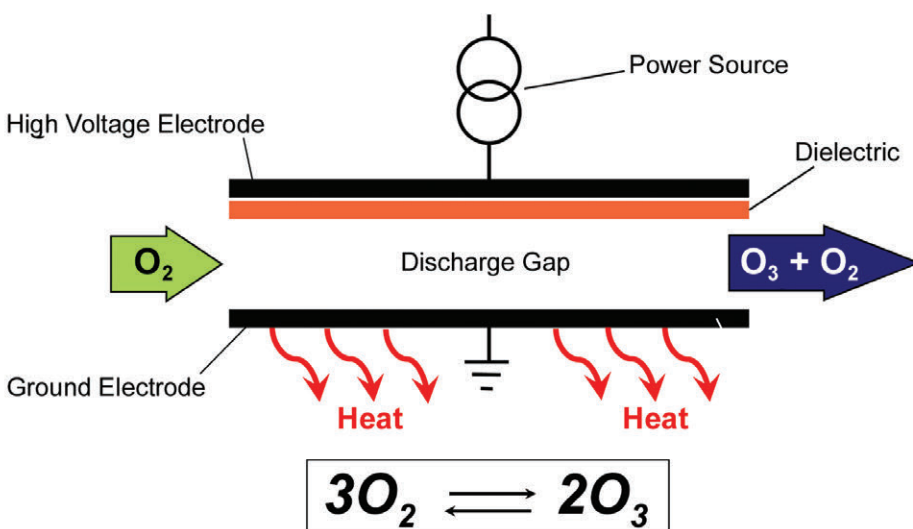
### 6.2 Corona Discharge Ozone Generation

In corona discharge ozone generation, dry oxygen or air is passed through an electrical field that causes oxygen molecules to split into their atomic components, which then recombine as ozone (O<sub>3</sub>).

Within the corona discharge ozone system is the dielectric, a solid material such as glass or ceramic, which is a poor conductor of electricity, but an efficient supporter of electrostatic fields. In an ozone generator, the electrical charge is diffused over the dielectric surface, creating an electrical field or “corona.”

Ozone generation starts with electron collision processes leading to free oxygen atoms. In general, the ozone yield is determined by the energy density within the discharge. Within the corona discharge system, there exists a wide range of proportionality of energy density and oxygen or ozone concentration. An increase of ozone production can be achieved by increasing the energy density, which may be achieved by increasing the number of charge pulses (frequency, voltage amplitude) or the amount of charge of single pulses [11].

Figure 6.1: Corona Discharge Ozone Generation



Frequency: there are several variations in corona discharge technology. The most widely used methods are:

- Low frequency (50 hertz [Hz] to 100 Hz)
- Medium frequency (100 Hz to 1,000 Hz)
- High frequency (1,000 Hz and greater)

Voltage: typically, a minimum of 5,000 volts of electricity is required to create the corona with most units operating between the minimum and 14,000 volts, which tends to be the maximum practical voltage.

For pharmaceutical water systems that require very low levels of ozone, a 60 Hz frequency range and 10,000 volts discharge typically are provided in factory constructed modules.

### 6.2.1 Feed Gas

Generally, corona discharge systems generate ozone using oxygen supplied from oxygen concentrators or directly from ambient air. The oxygen supplied could contain sufficient nitrogen to generate oxides of nitrogen (NO and NO<sub>2</sub>) which become nitric acid when dissolved in water and could impact water quality, e.g., by raising the conductivity. Under severe conditions, the nitric acid could cause corrosion, particularly within the corona discharge system.

Historically, a rise in conductivity may have been noticeable where a combination of conditions occurred simultaneously including:

- A long water residence time in the system (several days or more)
- High ozone concentrations (for example > 200 ppb)
- A corona discharge system fed with a low grade of oxygen (for example < 90% oxygen)

The potential for a rise in conductivity may be eliminated by using high purity oxygen as a gas source in corona discharge systems. The high purity oxygen can be supplied in bottles, cylinders, oxygen concentrators (which can achieve > 90% oxygen purity), or from another suitable source.

Ozone synthesis from high purity oxygen usually results in higher ozone concentrations and efficiencies than ozone synthesis from air.

An increase in frequency in the generator can increase the production rate, as long as cooling prevents an extreme temperature rise with an associated thermal decomposition of ozone. In commercial, well optimized corona discharge ozone generators, the ozone yield from high purity oxygen is about two to three-times higher than the ozone yield from air, e.g., increasing the oxygen content in the feed gas by a factor of three, results in an ozone output increase from 3% wt/wt to 9% wt/wt.

Proper feed gas preparation is considered essential to operating corona discharge ozone systems. The gas should be very dry with a dew point of -80°F/-62°C or lower, as the presence of moisture and nitrogen gas affects ozone production and can lead to the formation of nitric acid. Nitric acid corrosion of the internal components of a corona discharge generator can increase the frequency of maintenance or cause premature failure. [12]

#### Oxygen Purity

The higher the purity of oxygen, the cleaner the ozone stream. Oxygen purity of > 90 is typically sufficient. In extreme applications, the purity of oxygen is measured in 9s, e.g.:

- Grade 4 has four 9s (99.99% oxygen)

- Grade 6 has six 9s (99.9999% oxygen)

Some ozone generators which are fed with high purity oxygen (e.g., 99.9999%), may require the addition of small amounts of nitrogen (called dopant) to operate efficiently [12].

Guidance regarding the required oxygen purity and nitrogen content should be obtained from the generator manufacturer.

### Measuring Ozone Concentration

The concentration of ozone in the gas at the discharge of the ozone generator is measured as %wt/wt, i.e., the measurement of the mass of ozone per mass of carrier gas:

- The ozone concentration using clean dry air will typically be between 1 wt% and 3 wt%.
- The ozone concentration using oxygen as the feed gas will typically be between 5 wt% and 12 wt%.
- The remaining discharge from the ozone generator is feed gas.

Example: if 100 g/hour of oxygen is feeding the corona discharge generator and the ozone concentration measured in the discharge gas is 10%wt/wt, then 10 g/hour of ozone is being delivered.

### Feed Gas Flow Rate Considerations

The rate of oxygen consumption is dependent upon the concentration of ozone being delivered to the water system. Increasing the ozone concentration at the outlet of the ozone generator may be accomplished by:

- Holding the power constant and decreasing the flow

OR

- Holding the flow constant and increasing the power

### Oxygen Supply

A typical oxygen cylinder (8" D × 48" H) with 2640 psig pressure holds 9,500 liters of oxygen. At a consumption rate of one (1) liter per minute, the bottle will last about six days; however, changing the cylinder every few days may not be practical. Where bulk oxygen is not available in a facility, it may be more practical to use larger oxygen storage vessels or to use oxygen concentrators. Alternatively, several bottles can be connected with automatic switching to allow switching to the next bottle as one bottle empties.

Pressure Swing Adsorption (PSA) is the most common method for concentrating oxygen from air. The feed gas may be supplied from a suitable plant compressed air source, i.e., Clean Dry Air (CDA) or the PSA may have a dedicated compressor. PSAs usually concentrate oxygen to between 90% and 95% purity. PSAs are available in several sizes and should be selected according to the flow requirements.

Oxygen concentrators are usually specified based on their flow capability, i.e., in units of Standard Cubic Feet per Hour (SCFH) or Normalized Cubic Meter per Hour (Nm<sup>3</sup>/hr):

- A generator that can produce 2 to 3 SCFH should be sufficient for a 10 gm/hr system, with an ozone generator that produces 11 wt%. This would normally require a relatively small footprint.

PSAs normally utilize dual adsorption vessels which alternate allowing regeneration (desorption) of the off-line unit. An oxygen receiver is also normally included to minimize generator cycling. Accessories (such as regulators and relief devices) are usually required to assure proper operation. Plant compressed air may require several pretreatments, particularly if moisture or particulate is present.

Concentrators may be large and bulky, depending on the flow requirements. For very large systems, Vacuum Swing Adsorption (VSA) or liquid oxygen may be an alternative.

Liquid oxygen dewars are another method of providing a source of oxygen. Liquid oxygen dewars contain compressed liquid oxygen and are larger in capacity than standard compressed gas cylinders, allowing longer duration between cylinder replacement.

Vacuum Swing Adsorption (VSA), also known as Vacuum Pressure Swing Adsorption (VPSA) is similar to PSA; however, low pressure, high volume blowers are used for adsorption and vacuum blowers are used for desorption. Reduced air inlet pressure translates to lower oxygen output pressure, usually in the range of 3 to 5 psig, unless an oxygen booster or compressor is added. Oxygen produced is in the range of 88% to 94% pure with a dew point at -100°F. VSA/VPSA generators employ a 3:1 ratio of adsorption pressure to regeneration pressure although the regeneration pressure for VSA is below atmospheric pressure. VSA usually employs dual adsorbers and is considered suitable for large volume applications (not typical for pharmaceutical applications) where ambient air is drawn into the pressure blower with minimal prefiltration.

### 6.2.2 Cooling Requirements

Between 85% and 95% of the electrical energy supplied to a corona discharge ozone generator produces heat, so some method for removing heat for large units is required, and should be considered for smaller units. Proper cooling also affects the generator's energy efficiency; most corona discharge systems use either air or water, or both, for cooling.

As a rule of thumb, as the feed gas temperature increases, ozone production decreases, conversely as the generator feed gas temperature decreases, ozone production increases. In some systems a 1°C temperature change will cause a 1% change in output. Temperature should be considered during design as this can have a significant effect on generator performance. The manufacturer's recommendations for operation of the unit and heating or cooling needs should be considered.

The operating temperature or ambient conditions can have an effect on the ozone production. Ozone generators can use different cooling methods and may require:

- Air
- Deionized (DI) water
- Chilled water/glycol
- Tap water

If tap water is used the compatibility of the equipment with any chlorine/chloramine present should be confirmed. The manufacturer's recommendations for cooling should be considered. An external cooling source may be necessary in pharmaceutical applications.

The configuration of two typical water systems using corona discharge ozone generation are shown in Figures 6.2 and 6.3.

**Figure 6.2: Corona Discharge with Air Supply Configuration Example**

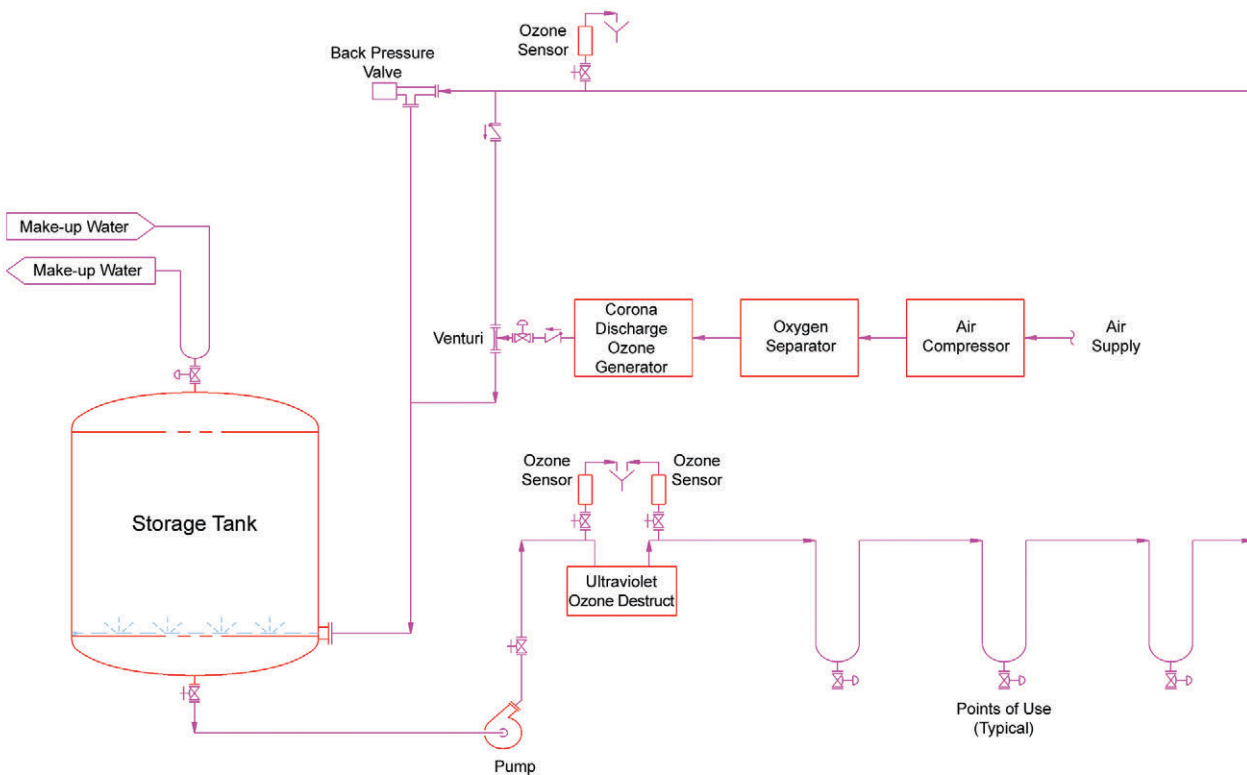
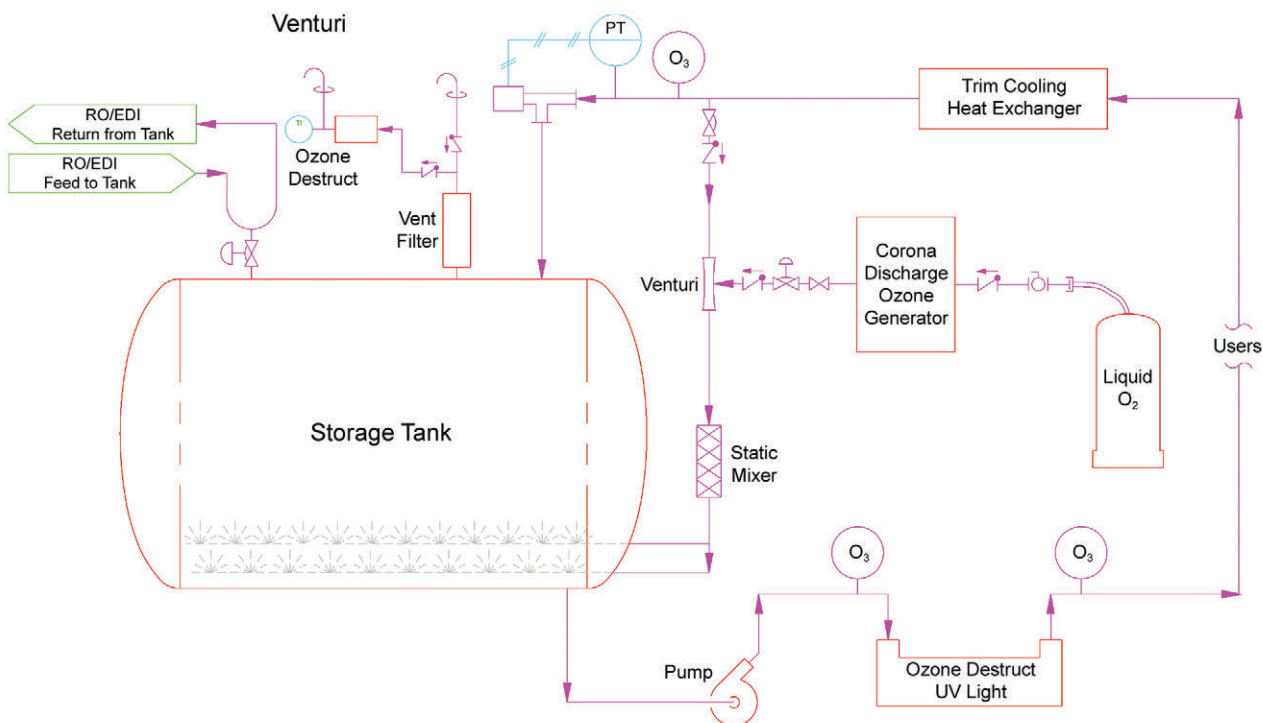


**Figure 6.3: Corona Discharge with Liquid Oxygen Source Configuration Example**



### 6.2.3 Corona Discharge Discussion Summary

For compendial water applications, the following should be considered:

- Oxygen fed corona discharge ozone generators generally have a higher output and the ozone produced is generally of higher purity.
- Feed gas must be high purity to avoid acid build-up in the water system.
- The efficiency of corona discharge systems is very temperature dependant so additional cooling requirements may be needed.
- Corona discharge systems typically have limited output pressure capability and this may necessitate ozone gas inducement into the system.
- Correct maintenance schedules should be established and maintained. Equipment should be operated and maintained according to the manufacturer's recommendations to ensure the ozone output is maintained at required levels, especially if the system uses oxygen as a feed gas. Extra care should be taken to ensure the oxygen supply is maintained at all times (particularly relevant with bottled oxygen).

## 6.3 Electrolytic Ozone Generation

Dissolved ozone produced through an electrolytic process utilizing using Proton Exchange Membrane (PEM) cell technology has been used successfully in the pharmaceutical industry [13]. PEM cells may be operated at high current densities, permitting the construction of compact units with high performance.

The PEM cells generate only oxygen and ozone with no water-borne by-products. A small amount of hydrogen gas is produced. In addition, the ozone is produced directly in the purified water which simplifies the devices for the injection of the ozone.

### 6.3.1 Construction and Principle Operation of a PEM Cell

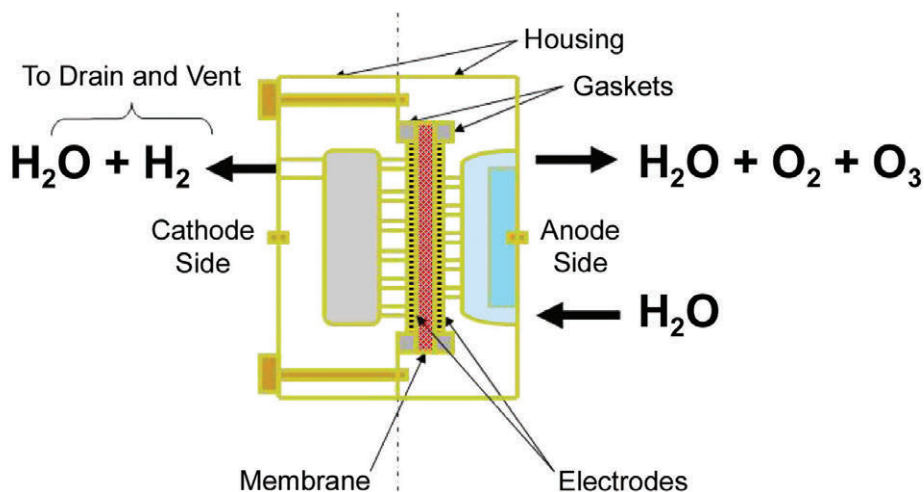
The proton-conducting electrolyte membrane is in direct contact with porous electrodes on both sides. The porosity ensures that any gases generated at the electrodes can be removed and that the water can reach the electrodes.

The voltage applied to the cell causes the water to become dissociated at the anode. The resulting electrons are removed through the electrode. The protons pass through the electrolyte membrane toward the cathode, where they recombine with the electrons supplied through the cathode to form hydrogen gas.

The reactions that form ozone and oxygen are competitive reactions. Their relative contributions to the total amount of released oxygen depend on various operating parameters and on the structure and composition of the boundary between the electrodes and the electrolyte membrane. At an optimum operating temperature of about 86°F/30°C, about 15% of the oxygen formed at the anode is released as ozone, and about 85% as oxygen. This corresponds to a current-efficiency of 15%.

The specific energy requirement to produce ozone (kWh per kilogram of ozone) using a PEM cell is typically much higher than that required to operate a corona discharge system, per gram of ozone generated. However, for the ozone quantities typically needed in purified water systems, which lie in the range of 0.3 to 12 g/hr, this higher power consumption may be compensated for by other advantages of this technology.

**Figure 6.4: Electrolytic Ozone Cell**



*Used with permission from Ozonia North America, [www.ozonia.com](http://www.ozonia.com)*

**Notes for Figure 6.4:** the porous electrodes ensure that the water can reach the active surface and that the gases resulting from the reaction are removed. The gas-tight, proton conducting electrolyte membrane, with a thickness of about 100  $\mu m$ , separates the anode, where the ozone is generated, from the cathode.

**Electrolytic ozone generation considerations include:**

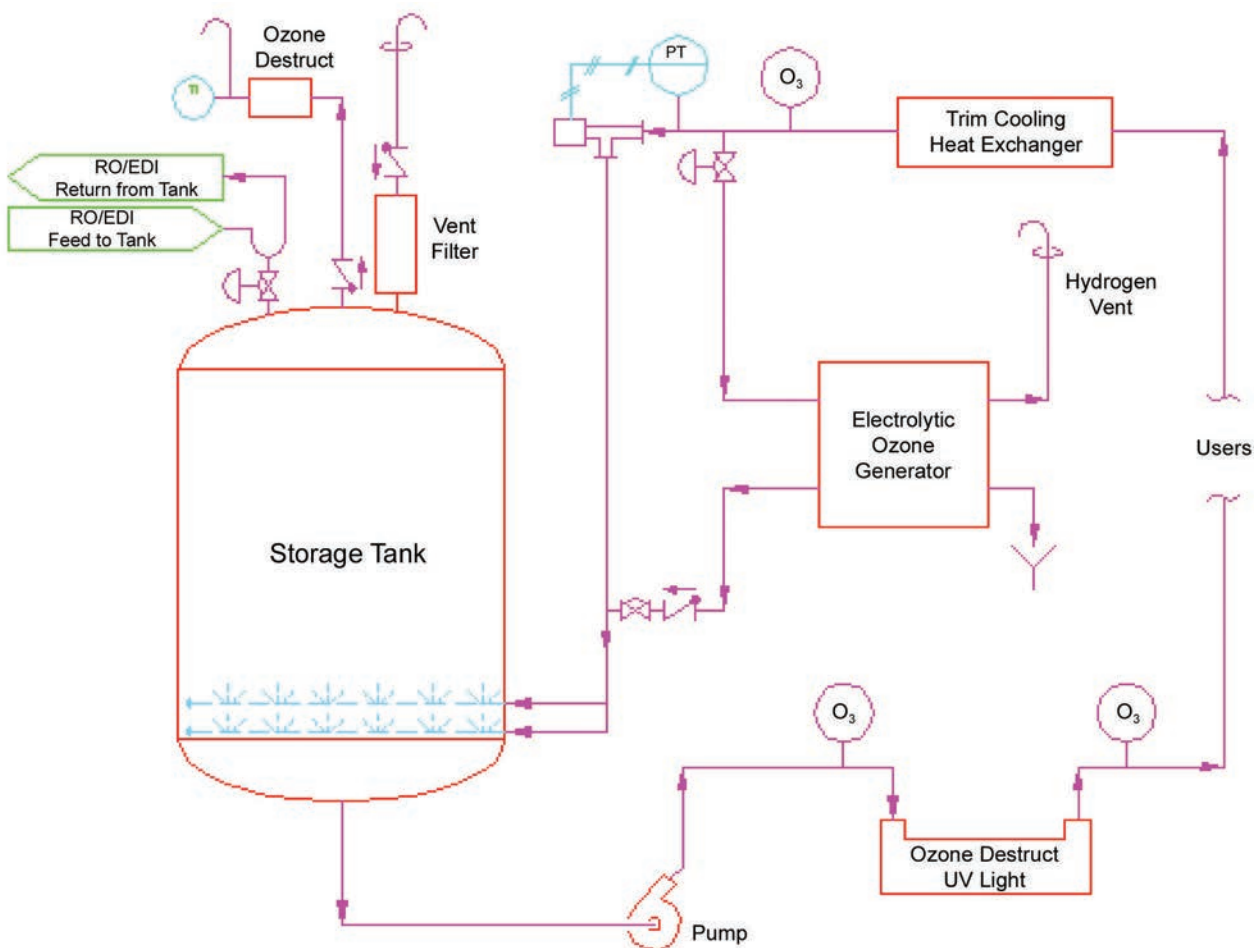
- The water undergoing treatment serves as the source of oxygen for the ozone production, no nitrogen oxides, foreign matter, or contamination is introduced.
- Ozone dissolves in the process water as soon as it forms, which minimizes the need for ancillary equipment and makes the generators extremely compact.
- The absence of oxygen and ozone gas external to the closed water system reduces the risk of ozone leaks with reduce risk to operators and the facility.
- The electrolytic process electricity consumption is typically in the low Watt range per hour.

Other considerations of this technology:

- Hydrogen (small quantity) is generated and needs to be discharged safely.
- Operating pressure limitations may require system design adjustment.
- Sizing should account for elevated concentrations needed (e.g., for system shock if necessary).
- The level of maintenance and interval for the replacement of components should be considered.
- On-off or intermittent operation of the cells may not be possible.
- Ramp up of ozone production may be slow.

An example of a water distribution system with an electrolytic ozone generator is shown in Figure 6.5.

Figure 6.5: Electrolytic Generation Configuration Example



## 6.4 Sizing an Ozone Generator

Ozone generator sizing calculations are specific for each system design, as they depend on:

1. The efficiency with which the ozone is dissolved into the water from the generator
2. The water storage tank and piping distribution system size
3. Flow rates and pressures

The concept for sizing ozone generators is that sufficient ozone should be supplied to ozonate continuously all water entering (loop return and make-up water) to the storage tank. Therefore, all flows, including water make-up to the tank and distribution loop pipe returns, should be added together. The ozone generator output needs to provide adequate ozone to this combined flow to maintain the target dissolved ozone concentration. Issues that should be considered include, but are not limited to:

- Ozone transfer efficiencies
- Ozone demand
- Water temperature

- Changes in water usage

Depending on the geographical location of the facility, there can be significant swings in water supply temperature due to changing seasonal conditions as well as other variables to consider including:

- Make-up water supply to the storage tank (quality and temperature)
- Tank turnover time
- Loop return flow rate
- Ozone half-life (half-life in this context is used to express the amount of time it takes for the ozone concentration present in the water to decay to half its original value and is dependent on several factors, including water temperature and pressure in the system, etc.)
- Ozone concentration in the storage tank water
- Safety factors
- Sanitization schedule and concentration
- Water quality (especially TOC)
- Tank venting
- Ambient air temperature (seasonal changes)
- Water system temperature

#### **6.4.1 Ozone Demand Calculation**

The following equation is used to calculate the ozone needed to achieve the desired water storage and distribution concentration level, as well as identify the necessary variables and safety factors.

$$\text{Ozone (grams/hr)} = \frac{[(\text{Make-up flow (m}^3\text{/hr)} + \text{Recirculation flow (m}^3\text{/hr)}) \times \text{Concentration (ppb)}]}{[\text{Design Factor}] \times (1/\text{transfer efficiency factor})}$$

This equation assumes that the water in the storage tank is continually ozonated.

#### **6.4.2 Design Factors**

Design factors may be needed to account for a variety of system conditions, including:

- Water temperature
- Imperfect mixing
- Ozone consumption in the water
- Ozone off-gassing in the tank
- Increased ozone for sanitization of the loop piping
- Location of ozone measurement instruments

- Ozone generation equipment operation

Examples of multipliers that should be determined in collaboration with the ozone generator supplier are:

- 2.5 to 5.0+ for normal conditions
- 4.0 to 5.0 for normal loss efficiencies and large tanks (lengthy turnover)
- 10 for extreme conditions (e.g., hot water temperatures or inadequate distribution design within tank)

A regular trend is for system designers and operators to maintain ozone levels in the storage tanks (with continuous ozonation) at low levels, e.g., < 30 ppb with some as low as 15 ppb. However, at these low levels, a large storage tank may encounter imperfect mixing; therefore, the proper introduction of ozone into the tank becomes a critical factor. A higher multiplier, in the range of 5.0, may give more operation flexibility, but this may incur significant additional cost in ozone generation equipment, ozone removal equipment (UV), and also additional equipment required to ensure the health and safety of the operators, i.e., lower ozone concentrations are inherently safer than larger concentrations.

Safety factors also may be applied in addition to the multipliers, but the two should not be confused.

#### **6.4.3 System Ozone Shock Capability**

Water distribution loops may include more challenging designs for ozone sanitization. These challenges may include pipe sections with lower flow rates, low utilization, or other design features that may require longer or higher ozone concentrations to ensure microbial control. For these situations, the ozone generator sizing calculation can be adjusted to allow for system shock capability by using a higher multiplier in the design, e.g., 5.0.

#### **6.4.4 Tank Turnover**

The time that it takes for the entire contents of the water to be turned over once is considered the tank turnover time. The half-life decay characteristics of ozone is approximately 20 minutes; therefore, if the turnover time exceeds 20 minutes, the multiplier should be large enough to take this into consideration. For example:

- Assume normal loss efficiencies and a large tank (5000 gal or 18.9 m<sup>3</sup>)
- Distribution loop return flow rate of 50 gpm or 11.4 m<sup>3</sup>/hr
- Tank turnover is 100 minutes (5000 gal / 50 gpm) or [(18.9 m<sup>3</sup> / 11.4 m<sup>3</sup>/hr) × 60 min/hr]
- At 20 min per ozone half-life: 100 min / 20 min = 5 half-lives
- Use multiplier of 5

#### **6.4.5 Transfer Efficiency**

Transfer efficiency of ozone from the generator into the water is a critical factor and is dependent on a number of design factors. Even significant over sizing of the ozone generators may not overcome poor design for ozone distribution and dissolution into the water.

##### **6.4.5.1 Electrolytic Source Transfer Efficiency**

Electrolytic ozone generators consist of cells. Each cell may typically produce up to 4 gms/hr of ozone. The number of cells required to meet the system ozone needs are dependent on the system design requirements.

The electrolytic system generates ozone directly from and in the water stream; therefore, the mass transfer rate typically is higher than the corona discharge method. For example, transfer efficiency factors for electrolytic systems may be as high as 95%.

An added benefit of this technique is that the ozone is created already dissolved in the water, not as a gas that needs to be dissolved, so there is no requirement for added equipment to mix the gas into the water stream although water mixing methods may still need to be employed. This can both save costs (especially in retrofit situations) and also avoid concerns that the eduction systems and some spargers used in corona discharge systems may not be suitable for use in pharmaceutical grade water systems.

#### 6.4.5.2 *Corona Discharge Transfer Efficiency*

Ozone transfer efficiencies for Corona Discharge (CD) systems will vary depending upon system design. Systems that use 90% to 95% oxygen and inject directly into the distribution loop return can achieve 90% transfer efficiency. Corona discharge systems that sparge or bubble the ozone gas into the water storage tank may achieve only 30% transfer efficiency; however, alternative designs, such as in-line mixing or eductors, can dramatically improve efficiency.

Transfer efficiency can be compromised severely if ozone distribution into the water is inadequate or unsuitable.

#### 6.4.6 *Generator Response Time*

Over sizing an ozone generator can provide the benefit of being able to achieve a higher sanitization ozone residual more quickly; however, the additional cost for this capability should be balanced against the benefit.

#### 6.4.7 *Example Generator Sizing Calculation*

This Guide recommends that for each application, system designers work closely with the ozone generator equipment supplier to identify the variables and determine the appropriate factors and facility costs.

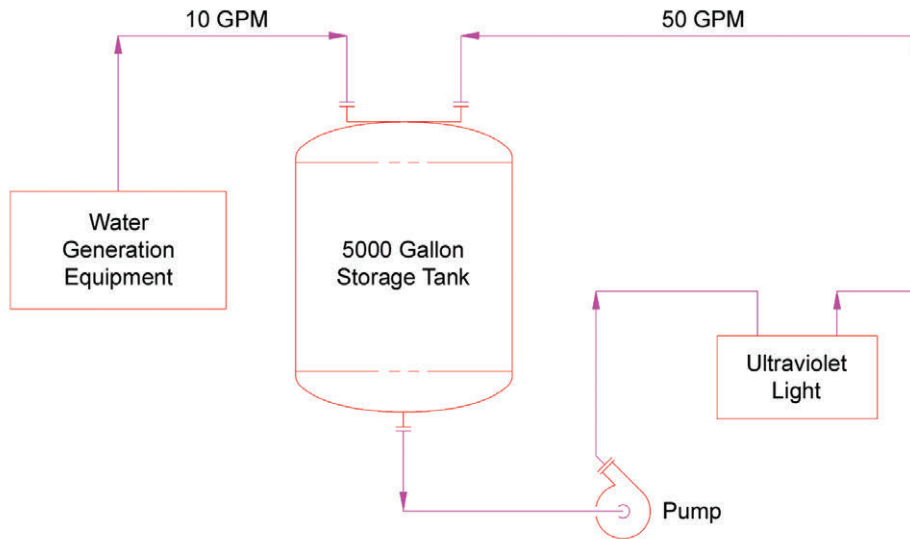
Example ozone generator design calculation<sup>2</sup>:

- 5000 gallon storage tank (18.9 m<sup>3</sup>)
- 10 gpm make-up water supply (2.3 m<sup>3</sup>/hr)
- 50 gpm loop return (11.4 m<sup>3</sup>/hr)
- 50 ppb normal ozone concentration in water storage tank

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<sup>2</sup> This is not adequate to be used as template for other calculations.

Figure 6.6: System Design for Example Ozone Generator Sizing Calculation



#### 6.4.8 Calculation

Ozone (grams/hr) =  $[(\text{Make-up flow (m}^3\text{/hr)} + \text{Recirculation flow (m}^3\text{/hr)}) \times \text{Concentration (ppb)}] \times (\text{Multiplier}) \times (1/\text{transfer efficiency factor})$

Ozone (grams/hr) =  $(2.27 \text{ m}^3\text{/hr} + 11.4 \text{ m}^3\text{/hr}) \times 50 \text{ mg/m}^3 \times 5 \times (1 / 0.95) \times (1 \text{ gm}/1000 \text{ mg})$

Ozone (grams/hr) = 3.59 gm/hr

**Note:** 50 ppb = 50 mg/m<sup>3</sup>  
 4.4 gpm = 1 m<sup>3</sup>/hr  
 10 gpm = 2.27 m<sup>3</sup>/hr  
 50 gpm = 11.4 m<sup>3</sup>/hr