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Knowledge Brief

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Understanding Dissolved Ozone and Its Use in Pharmaceutical Water Systems

by Nissan Cohen

Introduction

Charles W. Eliot was quoted as saying that "all business proceeds on beliefs or judgments of probabilities; and not on certainties." This is also largely true of what we call science today. The use and analysis of dissolved ozone in USP or general pharmaceutical waters is no exception. There are risks and rewards for using the ambient chemical sanitizer. This article will seek to establish a fundamental understanding of dissolved ozone and its use in a pharmaceutical water plant.

What is Dissolved Ozone?

Ozone is a naturally occurring triatomic form of oxygen (O_3) and exists in the gas form in nature. Familiar sources of ozone are generated from lightning in the atmosphere, the sun's ultraviolet (UV) in the upper stratosphere creating the infamous "ozone layer," and copy machines, or laser printers. Ozone forms when oxygen comes in contact with ultraviolet energy wavelength of 185 nm or shorter. The UV energy splits the oxygen molecule, which then reattaches to another oxygen molecule (see Equation 1 below).

Equation 1:

$$3O_2 + UV^{185nm} \rightarrow O + O + 2O_2 \rightarrow 2O_3$$

The resulting unstable ozone gas molecule wants to revert back to the stable diatomic oxygen molecule (O_2). In order to do this, it must react with another compound or transfer energy through another source. This makes ozone an oxidizer. In fact, it is one of the strongest oxidizers known to man (see table below). Dissolving this gas into water makes for a very potent antimicrobial solution, which can then be used as a sanitizing agent. Ozone is different than most sanitizers because it is a gas and it remains a dissolved gas during the sanitization process for water systems. It does not metamorph into an ionic form like chlorine, therefore is much harder to stabilize in water.

Why Use Dissolved Ozone?

There are many types of systemic sanitizers: heat (> 65 °C), chemicals (acids/bases), oxidizers (ozone, chlorines, peroxides). Each has its peculiar advantages

Oxidant	Oxidation Potential eV
Fluorine (F_2)	3.0
Hydroxyl radical (OH^-)	2.8
Ozone (O_3)	2.1
Hydrogen peroxide (H_2O_2)	1.8
Potassium permanganate ($KMnO_4$)	1.7
Chlorine dioxide (ClO_2)	1.5
Chlorine (Cl_2)	1.4

and risks. Heat has been proven over the years to produce high quality, low microbial growth water, yet it can be expensive to maintain and difficult to work around. Chemical sanitizers can be effective for removing biofilms, but must be rinsed out with excessive amounts of high quality water and involve hazardous chemical handling and disposal.

Dissolved ozone has the advantage of being able to reach into every part of the water system and then be easily removed. Ozone will revert naturally to oxygen without outside inducement in approximately 15 minutes. This "half-life" reduces the effect of ozone and produces oxygen. UV at 254 nm wavelength is typically used insitu to destruct the ozone to oxygen in a pharmaceutical water system. There is no "handling" required of ozone as it is generated from air or compressed gas and automatically injected. The ability to operate at room temperature obviates the need for expensive complex heating systems, and heat tracing on pipes.

The dissolved ozone mechanism is different than dissolved chlorine, the world's most popular potable water sanitizing agent. Ozone attacks (oxidizes) all organic (carbon-carbon) bonds; these organic species make up the cell walls and external structures of bacteria,[1] spores, and cysts. Since most biological structures are organic, ozone is an equal opportunity biocide. If properly dosed, dissolved ozone can cause complete destruction of the biological entity.

Ozone is also extremely fast at eliminating microbiological activity in the water at relatively low doses. One source has shown that 0.1 mg/L of ozone will destroy 60,000 cfu E. coli in one minute; whereas the same dose of chlorine will take up to 400 hours.[2] As one USP water user and system designer related in confidence, "zero counts over three years are hard to argue with."

Comparatively, chlorine is an oxidizer that works through the mechanism of diffusion into the cell. It attaches to and denatures the protein structures that comprise the enzymes of a cell. This ultimately inactivates the organism inhibiting reproduction and proper functioning. Chlorine destroys from the inside out. The diffusion required to enter the cell is a delay that the ozone mechanism does not endure.[3]

Some of ozone's additional benefits:

- Adds no residual chlorine compounds
- Is easily removed by exposing to UV light (wavelength 254 nm) or by degasification
- Is an antistatic agent loosening particulates from vessel walls
- Can oxidize inorganic materials such as nitrites, sulfides, etc.[4]
- Acts as a clarifier actually removing color[5]
- Reduces THM, TOC,[6] endotoxin[7] and endocrine disruptor[8] levels
- Deactivates Cryptosporidium and Guardia cysts[9]

The strength of ozone and the associated advantages lead to the conclusion that ozone use for sanitization can offer increased product quality and lower the risk of water-borne contraindicative components.

Why Measure Dissolved Ozone?

Microbiological analysis is a requirement for all grades of USP and EP water and there are clearly defined limits for each type.[10,11] Microbiological testing is a time consuming process. It can take from six hours to two weeks to perform impinging product acceptability. Since real-time microbiological monitors do not currently exist, one solution is to correlate another more easily adaptable real-time measurement to the disinfection required. Ozone disinfection can be defined by the term "contact time" (CT). CT is the residual ozone quantity in a storage tank or loop multiplied by the time the ozone is in contact with the water. Therefore, a direct dissolved measurement of ozone can be validated to disinfection efficacy. In an ozone-sanitized system, the measurement of dissolved ozone by a real-time instrument is a risk mitigation tool, as the values can be correlated to assured ingredient quality.

Equal in concern for the user is the verification that ozone has been removed from the water prior to use. Ozone is strong oxidant. It must be removed before it comes in contact with other excipients or active ingredients, or else there is a risk of product alteration or stability issues. It is best to choose a measurement technology based on its ability to measure at the very lowest level. Most users can adjust the residual confidence limits for just about any technology used to measure dissolved ozone. For instance, an analysis with a +/- 10 ppb accuracy would mean that the user who needed a 50 ppb residual could set its target value at 60 ppb and still have some room for variation. This is not so with post destruct measurement. For example, a value of 10 ppb with this same technology +/- 10 ppb could be zero or it could be 20 ppb. These are vastly different values. The assurance of removal is a concern because of ozone's speed and power. Therefore, in some systems, redundant destruct mechanisms and measurement systems are in place to ensure destruction before product is released.

How is Dissolved Ozone Made?

Ozone is made onsite at all facilities. In most US-based pharmaceutical companies, ozone is generated by passing air or oxygen between two electrically charged plates, commonly known as the corona discharge method (also known as dielectric barrier discharge), which simply means generating a high voltage electrical field and passing air through it. Passing air through the corona converts approximately 2% of the oxygen into ozone. The gas must then be dissolved in the water. Usually via a venturi injector12 system, the ozone is administered into a moving stream. Sometimes injection is administered by bubbling into a filled water tank or reservoir.

In some European pharmaceutical companies, the preference is to create dissolved ozone electrolytically from the water itself. In this case, a strong potential is applied to a set of electrodes and either the water is split or dissolved oxygen is converted and ozone made. The ozone is created as a dissolved species. There are, of course, advantages and disadvantages to each method. The traditional difference seems to be that the corona discharge method produces a higher concentration than the electrolytic method. For applications requiring higher levels of dissolved ozone, corona discharge would be the generation method of choice.

What Are the Operating Costs?

The main operating expenses are electricity and equipment maintenance. In some cases, liquid oxygen (LOX) (in only very large water systems when generation of over 200 grams to kilos of ozone is needed per day) is used with the corona discharge method to gain a higher ozone weight. Electricity is by far the largest and most predictable expense. Wasted or unnecessary ozone production increases operating costs and also reduces system component longevity. Excess levels of ozone will rapidly degrade non-resistant seal materials. Therefore, monitoring dissolved ozone levels can be used as a feedback control to adjust the generator output. This is cost effective and important when designing a system with variable water usage.

A quick review of potentially negative impacts:

- Ozone is electrically expensive to produce. It is therefore important to consider a feedback control mechanism involving the dissolved monitor.
- Dissolved ozone is aggressive on materials and has been known to destroy piping, seals, and components that have been poorly chosen. A small materials selection chart is below, aiding the user in deciding on common materials of manufacture.
- Because dissolved ozone desires to be in its gaseous state and can be a human health hazard[13] it is advisable to have ambient monitors with alarms in the vicinity of the water system and ozone generator.

Are There Regulatory Concerns?

The US FDA's early reluctance to endorse ozone usage is changing as ozone has been shown to reduce the risk of microbial contamination. This is evidenced by the FDA approval of dissolved ozone as food contact disinfectant in 2001 (21 CFR Part 173). It is also consistent with the recent 21st Century Initiatives including the move toward risk-based decisions[14] and assuring production quality versus testing for it.[15]

For users making USP water, USP states Water For Injection (WFI) will contain "no added substance."[16] The FDA, depending on the auditor, may consider ozone to be an "added substance." Therefore, it must be shown to be removed.

For Purified Water (PW), the manufacturer is required to label ozone as an added ingredient or prove that the ozone has been removed from the water. USP users should remove ozone before formulation of the final product. The user should then verify that ozone has been removed.

Some exceptions would be sterile bottled waters, dialysate buffers and saline preparations where the ozone actually acts as a package sanitizer. In these cases, packaging studies performed would need to show the ozone had naturally dissipated or a small residual was intact.

Development of Dissolved Ozone Analysis

Initially, the common way to measure dissolved ozone was by titration. A colored titrant of known stochiometric properties could be dripped into the water sample until the color no longer disappeared because the ozone was consumed. The titrant volume used would be indicative of how much dissolved ozone was present.

Material	Durability Grade
Silicone	D
EPDM/EPR	C
Buna N (Nitrile)	D
Viton	B
Kalrez/Simrez	AA
Teflon (PTFE)	A
PFA	A
PEEK	A
PVC	B
Delrin	D
Brass	B
Carbon Steel	C
316L SS	A
Hasteloy C	A
Monel	A
Titanium	AA

Legend:
AA = exceptional – never replace due to ozone damage.
A = excellent – replace only as needed or very seldom.
B = good – replace at defined time intervals.
C = susceptible to damage, replace at short time intervals or requires monitoring.
D = generally not acceptable, short term exposure only.

Dissolved Ozone Materials Compatibility Chart (@ 20°C).

As later instrumentation methods were developed, UV spectroscopy became the dominant measuring technology. Later the usage of electrochemical technology was employed to measure ozone. Ozone, in its gas form, has a very distinctive UV absorption band and can be measured directly in water.

UV absorption of ozone using optics and detection at 254 nm wavelength offers many advantages over other techniques. The measurement is based on the Beer-Lambert equation. A 254 UV lamp is

installed in the unit. The lamp is not a laser and various spectra wavelengths are contained in the lamp with 254 nm being the primary emitted wavelength. Ozone absorption ensues due to the UV lamp irradiation, a detection system determines the absorption, and a microprocessor calculates the concentration of the ozone in the sample. This method is very effective in very small concentrations of ozone below 1 mg/L (ppm), as the limit of detection is three magnitudes lower at 0.4 µg/L (ppb).

Conclusion

Dissolved ozone is a powerful and effective ambient water loop sanitizer gaining in popularity with pharmaceutical companies. The properties of this dissolved gas are unique and may be somewhat unfamiliar to the pharmaceutical professionals who normally deal with temperature or liquid chemical sanitation methods. Dissolved ozone measurement is an essential indicator for quality control use.

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13. OSHA temporary and permissible exposure limits are detailed at www.osha.gov reference 29 CFR 1910.1000.
14. FDA CDER's Risk Based cGMPs for the 21st Century, www.fda.gov/cder/gmp/
15. FDA CDER's PAT Initiative, www.fda.gov/cder/OPS/PAT.htm
16. United States Pharmacopoeia, Volume 35 Supplement 2, official December 1, 2012 (or current), Water for Injection and Purified Water Monographs.

For Further Information

For more detailed information, the following ISPE resources are available:

Guidance Document

- *ISPE Good Practice Guide: Ozone Sanitization of Pharmaceutical Water Systems*, First Edition, July 2012. http://www.ispe.org/ispe-good-practice-guides/ozone-sanitization-pharmaceutical-water-systems

Knowledge Brief

- "Ozone Sanitized Pharmaceutical Water Systems: Tank Venting Concerns," Manfredi, J., KB-0022-Jun10. http://www.ispe.org/publications/knowledge-briefs/ozone-sanitized-water-tank-venting

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Nissan Cohen is a worldwide expert in Total Organic Carbon (TOC) and in high purity, ultrapure, reclaim, and recycle water systems with expertise in instrumentation, automation, and organic contamination oxidation systems using ozone, UV, ion exchange, and catalysts. He is a member of ISPE, Technical Editor of the *Journal of the Institute of Environmental and Science Technology* (IEST), Technical Reviewer of *Pharmaceutical Engineering*, Chairman of the ISPE Water and Steam Forum, Founder/Chair of the ISPE Discussion Forums, and a former member of the Technical Advisory Board of *A2C2* magazine. Cohen was a contestant on *Jeopardy!* that aired 14 April 2006. His education includes a degree in agriculture and genetics from the University of Wisconsin and Ruppin Institute. He is a member of International Standards Committees: ASTM E-55, IEST (WG 14644 Contamination Control), ASTM D-19.03, as well as ISPE Steering Committee member for the ISPE Critical Utilities Community of Practice (COP). Cohen is the former Chairman of several ISPE Committees, including Membership Services, Publications, *Pharmaceutical Engineering* and website, and several ISPE COPs, including HVAC and PAT. He is currently a member of the North American Chapter Council.