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Analysis of Ozone Stability in Saline Solution –Vs- Double-Distilled Water Both Under Nano/Microbubbling

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ABSTRACT

Ozone therapy is a technology currently used to treat a wide variety of diseases, with increasing interest in the medical field.

One of the most efficient and safe techniques for delivering ozone to a patient involves bubbling ozone through a saline solution at customized concentrations.

Objective: To analyze the efficiency and safety of ozonation using a saline solution and double-distilled water under nanobubbling at different concentrations in a closed glass device, establish its stability over time, compare its advantages over microbubbling, and its benefits in medical and dental applications. To determine if there is an increase in the production of hydroxyl radicals and the formation of bromates in the saline solution.

Materials and Methods: For this study, the Ozonobaric P® Sedecal® classification IIb, ozone generator (Spain) and two glass devices for liquid ozonation were used. One device had a plate for nanobubbling (less than 1 nm) and the other a plate for microbubbling (20 nm). The study was conducted at CSI ANALITICA S.L., Tres Cantos, Madrid, Spain.

This is a descriptive observational study. Ozone was nanobubbled at different standardized concentrations (3 and 5 µg/NmL) in a specially designed, sealed glass device, ASSO3®, using 250 mL of saline solution (0.9% NaCl). The solution was then tested using a BMT964 AQ-LC ozone concentration analyzer (Messtechnick GmbH, Germany), an Anseros® OZONE MONITOR GM-RTI® spectrophotometer (Germany), a Merck ozone kit, and a GC-MS to verify the stability of the ozone concentration over time in saline solution and double-distilled water. Simultaneously, the study was conducted to determine if there was an increase in trace amounts of bromine and hydroxyl radicals in saline solution and double-distilled water under ozonation at low concentrations. The same procedure was performed with double-distilled water at two concentrations: 250 mL (20 and 80 µg/NmL).

Results: Although the saline solution was bubbled for 5 minutes to saturate it, it was observed that in 3.45 min of nanobubbling, both the saline solution and the double-distilled water became saturated with ozone.
In contrast, with microbubbling of the saline solution, ozone saturation was achieved in 5 min.

Despite de fact ozone concentrations under nanobubbling in both saline solution and double-distilled water decreased slowly over time, this did not compromise their therapeutic use, which was the ozone concentrations under nanobubbling, in both saline solution and double-distilled water, decreased slowly over the different time periods, this did not compromise their therapeutic uses, which were maintained for up to 72 h. This was not the case with microbubbling, where concentrations were maintained for 30 min without refrigeration.

The ozone concentration in double-distilled water was more intense than in the saline solution, which is understandable. Double-distilled water does not contain elements or residues that prevent ozone dissolution, as occurs in saline solution. At the same time, it has been shown that, when normalized with respect to the amount of ozone transferred to the water, no increase in hydroxyl radical production or bromate formation was observed.

By maintaining a stable ozone concentration in the post-saturation saline solution under nanobubbling, it can be administered to the patient without bubbling for the following 24–48 hours, provided it is kept refrigerated at 8°C. In the case of double-distilled water, it can be used for one week without bubbling (kept refrigerated). This frees up the generator, saves time and oxygen, and optimizes treatments.

Keywords: Ozone, Ozonated Saline Solution, Nanobubbling, Microbubbling, Double-Distilled Water, Ozone Generator, Asso3® Device, Liquid Saturation, Bromates, Biocompatibility

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Introduction

In accordance with scientific advancements, ozone science is poised to achieve significant breakthroughs in medicine, and the nanobubbling of saline solutions is one such major application.

Currently, the use of ozone for water treatment is increasing worldwide, both for drinking water and for industrial and wastewater treatment, due to its excellent bactericidal, virucidal, and fungicidal properties, as well as its deodorizing and bleaching effects [1-3].

Consequently, the organoleptic (odor, color, and taste) and microbiological quality of water after ozone treatment ensures that it is completely safe for public health.

Over the last decade, micro/nanobubble technology has become a promising avenue for improving various processes that utilize bubbles, such as plant growth, ripening and removal of residual pesticides in fruit products, fish aeration, biodiesel production, and gas-liquid contact processes for water and wastewater treatment [4-7].

Ozonation is particularly useful in removing color, aromatic organic compounds, pharmaceuticals, pesticides, and a wide variety of other micro-contaminants [8-12].

Ozonated water also has a wide range of applications in dentistry, as its use during dental procedures achieves a high degree of asepsis, even surpassing that of chlorhexidine, which stains tooth enamel, among many other benefits [13-17].

The mechanism of action of Ozonated Saline Solution (O3SS) is genomic, meaning that the response depends on the activation of the nuclear signal transduction mechanisms of Nrf2 (erythroid nuclear factor 2), a powerful protein located within every cell in the body. Nrf2 is activated by the Nrf2 activator, which induces the synthesis of antioxidant proteins such as SOD (superoxide dismutase), CAT (catalase), and HO1 (heme oxygenase 1), among others [18-22].

Behavior of Ozone in Liquids

The rate of ozone decomposition in saline solution is 5-8 times greater than in the gaseous phase. Ozone's water solubility is 10 times greater than that of oxygen: 49.0 mL of O3 per 100 mL of water, compared to 4.89 mL of O2 per 100 mL of water. In general, ozone solubility in water follows Henry's Law (1803), which states that the saturation level of a gas in water is proportional to its concentration and saturation decreases with increasing salt concentration [23].

In aqueous media, ozone decomposition depends largely on water quality, temperature, and pH. An increase in pH accelerates ozone decomposition, thereby decreasing its concentration in the water. Analogous processes occur with increasing temperature [24,25].

When high concentrations of ozone are used in an ozonated saline solution (O3SS), ozone can oxidize chloride ions in the saline solution to form chlorate. It is well known that higher ozone concentrations result in higher levels of chlorate, which are highly toxic to the body.

It is important to note that ozone can react with its container and, therefore, increase the levels of toxic substances in the ozonanted solution. Therefore, the saline solution bottle must be made of ozone-resistant material (glass). If it is made of plastic or polypropylene, it can oxidize due to the action of ozone, resulting in the release of phthalates and an increase in chlorate content, both of which have multiple toxic effects on the body [26,27].

When high concentrations of ozone are used in ozonated saline solution (O3SS), the ozone can oxidize chloride ions in the saline solution to form chlorate. It is well known that higher ozone concentrations result in higher levels of chlorate, which are highly toxic to the body [28].

The half-life of ozone in double-distilled water is 10 hours; in demineralized water, it is 80 minutes; and in distilled water, it is 120 minutes [29-32].

Until the advent of nano/microbubbles, the maximum amount of ozone in a water sample was observed for 8-15 minutes; after one hour, only oxygen free radicals were detected in the solution. The most important of these is the hydroxyl radical (.OH), something that must be taken into account when using ozonated water for therapeutic purposes.

Regarding bromide. Although bromide is present as an impurity in salt (sodium chloride), pharmaceutical manufacturing processes minimize its conversion to bromate. There is no indication in the results that bromate is a significant component of standard 0.9% saline solutions. pH range: 4.5–7.0

Russian chemists demonstrated that sodium hypochlorite concentrations in saline solution were less than 0.001 μg/mL. Chemical analyses of H_2O_2 in O3SS and water samples also revealed no hydrogen peroxide accumulation greater than 0.002%. No bromates were detected in the solution when ozonated at low concentrations.

All of this has changed considerably with the advent of the nanobubble. The size of an ozone gas bubble in saline solution or double-distilled water is a determining factor in understanding its properties, since a nanoscale size distribution is associated with improved stability and mass transfer, significantly influencing its behavior and other physicochemical and electrical characteristics within a liquid such as a saline solution [33].

In general, the observed improvement in performance can be attributed to an increase in the volumetric mass transfer coefficient, driven by a combination of higher specific interfacial area and lower mass transfer coefficient.

Dr. Alexander's 2023 study established that the increased surface area outweighed the decrease in the mass transfer coefficient, resulting in an overall improvement factor of 1.6 for microbubbles compared to conventional bubbles. All other differences were due to the increased mass transfer, resulting in higher concentrations of dissolved ozone when operating with a fixed input dose [34]. Thanks to the inhibition of bubble coalescence under nanobubbling, this results in greater greater

ozone exposure of the treated tissue surface, thus optimizing, streamlining, and shortening treatments [35].

Unlike larger bubbles, micro/nanobubbles possess unique characteristics, such as their surface charge, which gives them a half-life of up to 6 months under in vitro conditions [36].

Among the properties of bubbles is their ability to retain gases compared to the occupancy of the dissolved gas in solution. This is explained by the greater surface area that nanobubbles offer per unit area, making them up to 200 times more efficient than a normal bubble [37].

It has also been shown that when normalized the amount of ozone transferred to the water, the production of hydroxyl radicals, the formation of bromate, or the impact of background components does not increase [38].

Larger bubbles tend to rise, exhibiting greater buoyancy, while smaller bubbles remain in the liquid medium more easily and for a longer period due to a random motion pattern or Brownian motion [39].

The International Organization for Standardization (ISO) has established various definitions regarding bubbles, determining that a bubble is a gas contained in a medium surrounded by an interface (ISO 20480-1:2017). According to their diameter, bubbles can be classified as fine (less than 100 μm), microbubbles (greater than 1 μm, between 10 and 50 μm), and finally nanobubbles (less than 1 μm). The diameter of the bubble and the viscosity of the liquid determine the speed of ascent, being greater for macrobubbles and less for microbubbles and almost negligible for nanobubbles.

The micro/nanobubbles remain suspended in the water or saline solution for an extended period, acting like a battery that continuously supplies ozone to the water. As the ozone is consumed, the micro/nanobubbles diffuse further, maintaining the level of dissolved ozone (the concentration). The micro/nanobubbles do not rise to the surface but are distributed homogeneously throughout the water.

The therapeutic indications for ozone are based on the understanding that the use of low, physiological, hormetic doses of ozone plays a significant role within the cell. This is particularly important in the case of saline ozonation. The doses used in this technique are low and calculated per kilogram of the patient's weight, making it safe and effective.

As shown, when applied to ozonation, the nanobubble technique has demonstrated significant improvements in mass transfer, residual dissolved ozone, and the speed and extent of compound removal compared to the application of ozone via coalescing bubbling.

Nanobubble technology represents a promising advance in optimizing the process of producing ozonated saline solution.

Use Of Ozonated Saline Solution (O3ss) in Medicine

The application of O3SS under nano/microbubbling in medicine is broad. O3SS under nano/microbubbling ozonizes a greater quantity of blood [44,45].

In a single procedure, O3SS ozonizes between 5-6 liters of blood, substantially optimizing and shortening treatments, thus saving the patient time, discomfort, and money. Results are obtained with fewer sessions (1 to 2). This is one of the most crucial factors in O3SS effectiveness under nanobubbling [46].

Another advantage is that the doses are calculated per kilogram of the patient's weight, making it personalized, safe, and effective [47].

Data is available on the effects of O3SS under microbubbling in renal failure patients undergoing hemodialysis, where renal function (all renal biomarkers) improved significantly compared with markers obtained with O3SS using coalescing bubbles [48].

Studies conducted with O3SS to treat COVID-19 in a Madrid hospital during the peak of the pandemic in 2020 yielded exceptional results [49]. PCR curves plummeted within 24 hours. Oxygen saturation normalized within 72 hours. Patients began to be discharged after five sessions. By the tenth day, the entire COVID ward had been discharged. No deaths were recorded.

Thanks to the anti-inflammatory and antibacterial action of ozone, bladder irrigation with ozonated double-distilled water under nano/microbubbling is a priority in the treatment of recurrent and resistant urinary tract infections [50,51].

In cardiology, the use of ozonated saline solution (O3SS) is already proven. Research by B.A. Korolev et al., between 1980 and 1983, demonstrated that ozone, in addition to the bactericidal and virucidal action of parenteral administration of ozonated solutions, provides more effective protection of the myocardium against ischemia [52-54].

Another major application of this technology is in dentistry. The application of ozone in dentistry has evolved and is now becoming safe thanks to the promising effects of intraoral irrigation with ozonated saline solution or double-distilled water under nanobubbling. Subgingival irrigation with ozonated double-distilled water causes rapid inactivation in dental biofilm by disrupting the cell wall membrane of the organisms associated with the lesion [55].

The intraoral use of ozone in gaseous form has long been the most critical method of ozone application in dentistry, due to the difficulty of its administration caused by the inadvertent or accidental aspiration of the ozone gas, given its close proximity to the patient's upper respiratory tract. O3SS under nanobubbling has solved this major problem. It is much safer to use saline solution or ozonated double-distilled water irrigation than to apply dry gas. Since O3SS is hemostatic and bactericidal, it is a valuable tool in surgical procedures.

Irrigation with O3SS or double-distilled water under nanobubbling is more biocompatible and less irritating to epithelial cells than dry ozone gas. Ideal for oral irrigation. The

ozone nanobubbles cause deeper perfusion due to their minute size, and their cavitation effect releases ozone and kinetic energy, while simultaneously disrupting biofilms and destroying microorganisms [56].

Furthermore, it reduces the number of bacterial species associated with oral biofilm, mitigating dependence on antibiotics and the resulting resistance, which is becoming increasingly evident and thus contributing to periodontitis and dental caries [57]. Therefore, the development of these new technologies is very necessary and useful.

In different studies, its antimicrobial capacity and cytocompatibility properties have been demonstrated when compared to other types of mouthwashes, such as chlorhexidine, which is currently considered the gold standard [58-60].

Clinical disinfectants are often irritating and can cause skin problems. Ozonated water is unique among disinfectants. It does not damage skin cells and readily decomposes into oxygen without generating harmful residues. On the other hand, it quickly loses its disinfectant activity. Ozonated water with nanobubbles, a recently developed technology, can maintain its disinfectant activity for much longer.

One study aimed to examine the microbicidal effects of O3SS under nanobubbles after prolonged storage. The ozone concentration in the saline solution ozonated under nanobubbles was examined by measuring their redox potential. Microbicidal activity was assessed using colony-forming assays after incubating bacteria with nanobubbles for specific periods of time.

The nanobubble lost its microbicidal activity after one year of storage at 4°C. Stored frozen, the nanobubble maintained appreciable microbicidal activity after one year of storage. Mycobacterium smegmatis, one of the most disinfectant-resistant bacteria, was eliminated in 15 minutes. The nanobubble was resistant to freezing and thawing.

The stored and frozen nanobubble showed sufficient microbicidal activity to eliminate bacteria even after one year of storage [61].

O3SS under nanobubbling remineralizes tooth enamel, relieves pain when scaling and root planning, and stops bleeding; therefore, any surgeon can work in an aseptic and blood-free area [62]. Finally, O3SS under nano/microbubbling allows for the dissolution of tartar, resulting in reduced working time and less pain for the patient [63,64].

Materials and Methods

The Ozonobaric P® Sedecal® ozone generator (Spain) classification IIb was used for the study (Figure 1). The ASSO3® closed-glass nano- and microbubble devices were used for nano- and microbubble generation (Figure 2). The study was conducted at CSI ANALITICA SL, Tres Cantos, Madrid, Spain, https://www.csianalitica.com between November 2025 and February 2026.

Figure 1: Ozonobaric P generator

Figure 2A: Nanobubble device

Figure 2B: Microbubble device

Sample Preparation and Analytical Conditions

The analytical strategies, instrumentation and materials used, sample preparations, and analytical methods were developed to achieve the objective and are described in CSI Analítica SL's own documents. It should be noted that ozone standards are not available from CSI Analítica's usual chemical suppliers due to their inherent physicochemical properties.

To verify the functionality of the Merck ozone kit, two saline solutions were measured. One sample was treated with ozone, and the other without. A stronger absorption signal at 550 nm was observed in the sample containing ozone compared to the one without. This indicates that the protocol is valid for measuring ozone in this type of sample.

250 mL of 0.9% saline solution was nanobubbled into at concentrations of 3 and 5 µg/NmL with a flow rate of 200 mL/min for 5 minutes. However, it was observed that saturation occurs before that time, at 3.45 min. The Merck ozone kit protocol was applied for ozone analysis, and absorbance measurements were taken by first introducing the blank and then the samples. A gas-mass spectrophotometer (GC-MS) was used to detect the presence of bromine and hydroxyl radicals.

Results

Table 1 presents the results obtained along with the percentage of residuals.

First phase: Analysis of Saline Serum Samples with Ozone Under Nanobubbling

The first two samples were ozonated with nanobubbles and analyzed in situ without refrigeration at concentrations of 3 and 5 µg/NmL. A specially designed, sealed glass device, ASSO3®, containing 250 mL of saline solution (0.9% NaCl), was used for this purpose. Five additional samples were then ozonated at a concentration of 3 µg/NmL for subsequent analysis; these were refrigerated at 8°C.

The ozonated samples were analyzed using a BMT964 AQ-LC ozone analyzer (Messtechnick GmbH, Germany), an Anseros® OZONE MONITOR GM-RTI® spectrophotometer (Germany), a Merck ozone kit, and a GC-MS. The same exercise was performed with 250 mL of double-distilled water under two concentrations (20 and 80) µg/NmL

It was observed that 3.45 min of nanobubbling were sufficient to saturate the saline solution with ozone.

An equation was generated from the data obtained for sample quantification.

The equation is **Y = 0.0339X,** where Y is the absorbance signal and X is the ozone concentration in the sample.

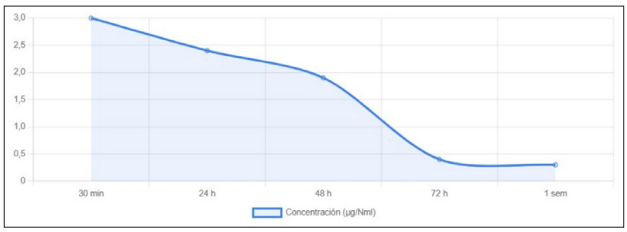
A 250 mL sample of 0.9% saline solution was prepared by bubbling ozone at 3 µg/NmL through it at a flow rate of 200 mL/min for 5 min. Five 10 mL vials of the ozone-saturated saline sample were prepared and stored under refrigeration. The samples were measured at different times: 30 min, 24 h, 48 h, 72 h, and one week. After applying the Merck protocol, the absorbance results obtained from these measurements were quantified using the equation calculated above in Table 1. The results are presented in Table 2.

Table 1: Saline solution 0,9% response factor

SALINE SOLUTION RESPONSE FACTOR 0,9%					
#	Added concentration in µg/NmL	Abs.	Concentration found in µg/NmL	Residuals in %	Minimum saturation time in minutes
1	3	0,099	3,0	97	5
2	5	0,171	5,0	101	5

Table 2: Data obtained in saline serum under nanobubbles.

SALINE SOLUTION 0,9%			
#	SAMPLE CODE 3 µg/NmL	Absorbency	Concentration µg/NmL
1	CS 30 min	0,099	3,0
2	CS 24 h	0,079	2,4
3	CS 48 h	0,063	1,9
4	CS 72 h	0,013	0,4
5	CS 1 week	0,009	0,3



Graphic 1: Graph of the stability of ozone concentration in saline solution under nanobubbling

Second phase: Analysis of Samples of Double-Distilled Water with Ozone Under Nanobubbling with Double-Distilled Water

250 mL of double-distilled water was ozonated under nanobubling at (20 and 80) µg/NmL concentration with a flow rate of 200 ml/min for 5 min. The Merck ozone kit protocol is used for ozone analysis, and absorbance measurements are taken by first introducing the blank and then the samples. Table 3 presents the results obtained along with the percentage of residuals.

Table 3: Response factor of double-distilled water.

RESPONSE FACTOR OF DOUBLE-DISTILLED WATER					
#	SAMPLE CODE	Added concentration in µg/NmL	Abs	Concentration found in µg/NmL	Residuals in %
1	CD20	20	0,326	19,1	95
2	CD80	80	1,421	83,1	104

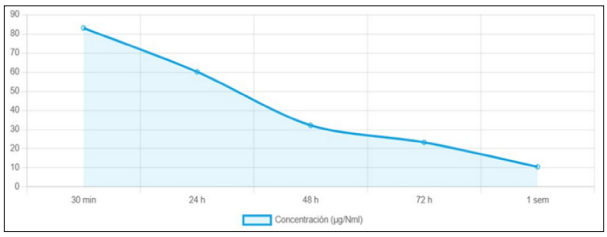
An equation is generated from the data obtained for the quantification of the samples.

The equation is Y = 0.0171X, where Y is the absorbance signal and X is the ozone concentration in the sample.

A 250 mL sample of double-distilled water was prepared by bubbling ozone at 80 µg/NmL through it at a flow rate of 200 mL/min for 5 min. Five 10 mL vials of the double-distilled ozone water sample were prepared and refrigerated. The samples were measured at different time points: 30 min, 24 h, 48 h, 72 h, and one week. After applying the Merck protocol, the absorbance results obtained from these measurements were quantified using the equation calculated earlier in Table 3. The results are presented in Table 4.

Table 4: Results in double-distilled water

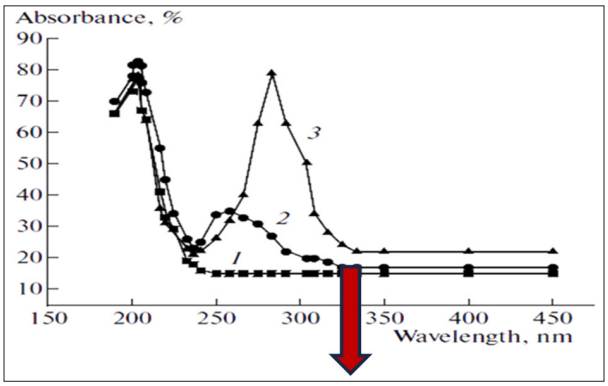
RESPONSE FACTOR OF DOUBLE-DISTILLED WATER			
#	SAMPLE CODE	Absorbency	Concentration µg/NmL
1	CD 30 min	1,368	80,0
2	CD 24 h	1,315	76,9
3	CD 48 h	1,046	61,2
4	CD 72 h	0,621	36,3
5	CD 1 week	0,011	0,6



Graphic 2: Stability of ozone concentration in double-distilled water under nanobubbling

Presence of Bromine in O3SS

Regarding the presence of bromine in saline solution, it was observed that during the first 5-8 minutes of ozonation at low ozone concentrations, the bromine level dropped and was no longer detectable. If the wavelength of hypobromite (330 nm) is added to the Razumovski, S. D. graph, it is observed that the hypobromite disappears and there is no increase in hydroxyl radicals. A wavelength of 330 nm for the detection or spectroscopy of the hypobromite ion in water is a normal, very reasonable, and characteristic value.



Species	BrO^-	HOBr	BrO^-	HOBr
λ (nm)	330	261	254	254
Absorption coefficient (L mol^{-1} cm^{-1})	340	93	90	22

Although bromide is present as an impurity in salt (sodium chloride), pharmaceutical manufacturing processes minimize its conversion to bromate. The results do not indicate that bromate is a significant component in standard 0.9% saline solutions. On the other hand, it has already been established that as the pH of water ozonation increases, the rate of bromate formation also increases. Furthermore, the formation of hydroxyl radicals is also promoted at high pH due to the higher concentration of hydroxyl ions present and the lower stability of ozone at high pH. The pH range of the saline solution is 4.5–7.0.

Temperature also plays a role. It has been shown that higher temperatures increase the rate of bromate formation. With a pH of 4.5–7.0, the presence of hydroxyl ions, the use of low ozone concentrations, and the use of low temperatures in ozonation, bromate formation is significantly inhibited.

Russian chemists demonstrated that sodium hypochlorite concentrations in saline solution were less than 0.001 µg/mL. Chemical analyses of H2O2 in the O3SS and water samples also revealed no hydrogen peroxide accumulation greater than 0.002%. No bromates were detected in the solution when ozonated at low concentrations [66].

The same cannot be said for drinking water. Bromate formation is an important operational factor when considering the implementation of ozone, as it is controlled in drinking water at levels of around 0.08 µmol L−1 [67].

Bromate has been identified as a possible human carcinogen, and its levels in drinking water are strictly controlled at 10 µg/L in most developed countries. For this reason, the World Health Organization (WHO) has established a provisional guideline concentration of 10 µg/L of bromate in drinking water (WHO, 2004). European Union legislation also specifies that all member states must apply this maximum bromate concentration (European Drinking Water Directive, 2008) [68]. United States regulations also specify a maximum value of 10 µg/L (United States Environmental Protection Agency (USEPA) regulations).

The factors that affect the presence of bromide in drinking water depend on several factors:

The Concentration of Bromide in Drinking Water

Amy et al. (1994) suggested that up to 30 µg/L of bromate can form from an average bromide concentration of 100 µg/L, significantly above the target bromate concentration of 10 µg/L. Studies by von Gunten (2003b) concluded that waters with <20 µg/L of bromide do not present problems for bromine-derived disinfection byproducts, while waters with >100 µg/L of bromide are likely to cause significant bromate problems. Therefore, the propensity to form bromate from bromide depends largely on the quality and origin of the raw water, as well as the disinfection objectives [69,70].

The pH

As the pH of water ozonation increases, the rate of bromate formation also increases [71]. Furthermore, hydroxyl radical

formation is promoted at high pH due to the higher concentration of hydroxyl ions present and the lower stability of ozone at high pH [72]. Bromate formation has been shown to increase from 10 µg/L at pH 6.5 to 50 µg/L at pH 8.2 (Legube et al., 2004), while Krasner et al. (1994) observed a 60% decrease in bromate formation for each unit decrease in pH. pH ozonation is widely considered the most effective strategy for bromate control in wastewater treatment plants and should be considered the best available treatment for its management [73-75].

It is worth remembering that the pH range in saline solution is 4.5–7.0, which is low enough to produce bromates under ozonation.

Ozone Concentration Applied and Contact Time

An important consideration in bromide-to-bromate conversion is the specific disinfection objective [76]. For example, if the target is bacteria and viruses, the bromide-to-bromate conversion is low. However, if the target is Cryptosporidium parvum oocysts, the bromide-to-bromate conversion is high.

Concentration and Nature of Organic Matter in Water

Both the concentration and nature of organic matter in water can affect bromate formation [44]. During ozonation, naturally occurring organic matter (NOM) present in water generally reduces bromate formation. This is because ozone and hydroxyl radicals are consumed in the oxidation of organic molecules and are therefore removed from the bromate formation pathways. This same phenomenon occurs when O3SS comes into contact with blood.

Temperature

It has been shown that higher temperatures increase the rate of bromate formation. It has also been shown that the effect of temperature is more pronounced at higher ozone doses [78].

Conclusions

- The Merck ozone kit is suitable for measuring dissolved ozone in aqueous liquids. The same applies to the BMT964 AQ-LC ozone concentration analyzer for liquids, Messtechnick GmbH, (Germany), and the Anseros® OZONE MONITOR GM-RTI® spectrophotometer (Germany).
- It was observed that 3.45 minutes was sufficient to saturate both double-distilled water and saline solution with ozone.
- The ozone concentration remained stable in saline solution and double-distilled water during the study period, although it decreased over time, without compromising its therapeutic efficacy.
- Since the ozone concentration remained stable in the saline solution after saturation under nanobubbling, it could be administered to the patient without bubbling for the following 24-48 hours, thus avoiding the risk of introducing bubbles into the patient and causing an accidental embolism. 5. In the case of double-distilled water, it can be used for a week without fizzing. If kept refrigerated, the water will last even longer. This allows the water to be stored in the refrigerator and given to the patient for home rinses.
- The ozone concentration in double-distilled water was higher than in the saline solution, which is understandable. Double-distilled water does not contain elements or residues that prevent ozone dissolution, as occurs in the saline solution.
- No increase in the production of hydroxyl radicals or the formation of bromates was observed.
- At the same time, it has been shown that, when normalized with respect to the amount of ozone transferred to the water, no increase in the production of hydroxyl radicals or the formation of bromates was observed.
- O3SS under nanobubbling has greater biocompatibility and tissue penetration capacity.
- Clinical disinfectants are often irritating and cause skin problems. Ozonated water under nanobubbling is unique among disinfectants. It does not damage skin cells and decomposes easily into oxygen without generating harmful residues.
- In dentistry, it eliminates the risk of accidental ozone aspiration by the patient.
- It has broad and proven therapeutic utility in medicine (urology, cardiology, neurology, endocrinology, wound and ulcer treatment) and dentistry.
- Using a closed glass device eliminates the risk of toxicity associated with plastic.
- Microbubbling maintained concentrations for 30 minutes, and 5 minutes of bubbling were needed to achieve saturation. The nanobubble proved to be more stable.
- Because the device is enclosed and made of glass, it is reusable (it can be sterilized in an autoclave) and environmentally friendly. It reduces costs for both the doctor and the patient.

Ethical Considerations

The study was approved by the ethics committee of CSI ANALITICA S.L. from Madrid and Clínica Fiorela S.L.

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Conflict of Interest

The author declares no conflict of interest related to this work.

Author's Contribution

The author is responsible for the study conception, methodological design, literature review, and manuscript writing.

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