

Chemical Variations Induced by Ozonization of 5% Glucose Solution and Evaluation of Generated Compounds

Review began 12/28/2023
Review ended 01/22/2024
Published 01/25/2024

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Abstract

The most commonly studied method of administering ozone therapy is systemic ozone therapy. However, there may be situations where this method is not feasible due to technical issues, such as poor vein condition or anemia. As an alternative method, pre-ozonized solutions, such as 0.9% saline solution, have been investigated for their ease of preparation and administration. However, concerns have been raised regarding the formation of chlorine compounds. Currently, there is no available literature on the treatment potential of pre-ozonized glucose solution. The objective of this study is to compare and evaluate the chemical changes induced by ozonization of a 5% glucose solution and determine if any toxic compounds are produced. Our findings indicate that the chemical alterations following ozone infusion are quantitatively minimal and pose a negligible risk in terms of safety.

Categories: Other, Integrative/Complementary Medicine, Therapeutics

Keywords: chemical risk assessment, oxygen-ozone therapy, ozone safety, glucose toxicity, ozone therapy

Introduction

Ozone therapy (OT) is a well-described medical practice based on official protocols and widely used as a complementary therapy [1-4]. OT can be administered locally or systemically. Local administration entails a direct injection of a well-defined mixture of oxygen-ozone gas into the targeted site, while systemic therapy requires intravenous infusion [3-5]. The most extensively researched method for systemic OT is systemic ozone therapy (SOT), which involves extracting a sample of 100-200 mL of the patient's blood. This blood sample is then combined with oxygen-ozone mixture gas at different concentrations, following specific protocols. The mixture is gently shaken for ten minutes and then infused back into the patient [4-7].

However, this approach may not always be feasible. Some factors can make this practice difficult to execute. In the event of poor vein assets, gaining venous access, withdrawing 100-200 mL of blood, and maintaining access for blood re-infusion can become challenging. Additionally, anemia can pose concerns when withdrawing such a significant amount of blood. To overcome these issues, pre-ozonized solutions have been studied as an alternative method of administration [8]. Following the "Russian school," there is a growing interest in ozonized saline solution (O3SS) as an alternative method of performing ozone therapy [9]. This mode of administration is easier to prepare and perform; however, many authors have expressed concerns about the formation of hypochlorous acid and other chlorine compounds such as chlorate [8,10-12]. Additionally, experimental evidence shows that Cl⁻ significantly accelerates ozone decomposition in water [10,13,14].

There is no literature available regarding the possibility of treating glucose solution with ozone. The 5% glucose solution consists solely of glucose (50 g/L) and water for injectable preparations, and this characteristic makes it simpler to study chemically as it involves analyzing the potential compounds resulting from the oxidation of a single molecule. Moreover, the solution is isotonic (278 mOsm/L of glucose 5% vs 275-295 mOsm/L of saline solution 0.9%) and, therefore, does not pose risks related to its infusion. This study aims to detect and measure the chemical variations induced by ozonization of a 5% glucose solution and determine if any toxic compounds are generated.

Materials And Methods

We conducted our tests on commonly used glucose solutions for clinical practice, under the same conditions. The solution was analyzed both before and after ozone treatment to have comparative data for analysis. This allowed us to avoid conducting a quantitative analysis of the formed compounds. The ozonized solution was prepared by dissolving a volume of 200 mL of an O₂-O₃ mixture at the concentration of 50 mcg/mL (10 mg of O₃) in 500 mL of 5% glucose solution. To establish variations in chemical composition caused by oxygen/ozone infusion, we made the following tests on treated and untreated solutions for comparison under the same conditions. The determination of the volatile component was performed using HSPME (Headspace solid phase microextraction) coupled with GC-MS both before and after specific derivatization

How to cite this article

Martinelli M, Romanello D (January 25, 2024) Chemical Variations Induced by Ozonization of 5% Glucose Solution and Evaluation of Generated Compounds. Cureus 16(1): e52946. DOI 10.7759/cureus.52946

for aldehydic compounds. Analysis for glucose and derived compounds was performed using derivatization and the GC-MS test.

Results

Volatile components

Detected volatile compounds are coming for the most part from butyl rubber stoppers (in this batch, traces of toluene and xylenes, alkyl aldehydes, etc.). Other brands of 5% glucose solutions did not show such contamination, which raises some concern about the quality of materials employed in medical supplies. Treatment with oxygen/ozone mixture does not alter in a significant way this composition, present in minimal quantities (micrograms per liter) anyway. The only compound attributable to the specific composition of the solution (glucose in water) is furfural, also present in minimal quantities. Furthermore, after oxygen/ozone mixture treatment, furfural is no longer detectable. There are no traces of smaller chain aldehydes (acetaldehyde, formaldehyde) (Figure 1). Furfural removal was confirmed after methoxime hydrochloride derivatization (Figure 2).

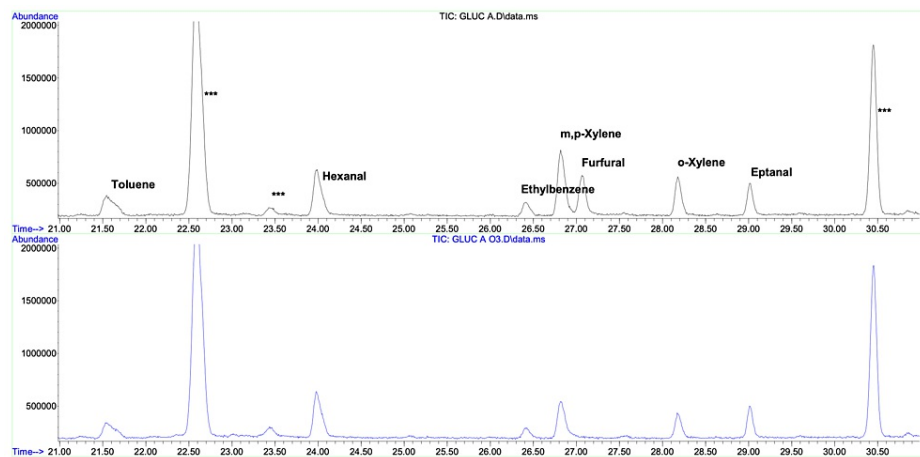


FIGURE 1: Volatile component

Upper Chromatogram: Untreated solution

Lower Chromatogram: Ozonized solution

***: Siloxanes derived from SPME fiber

The treatment with an oxygen/ozone mixture does not alter any compounds, except for furfural.

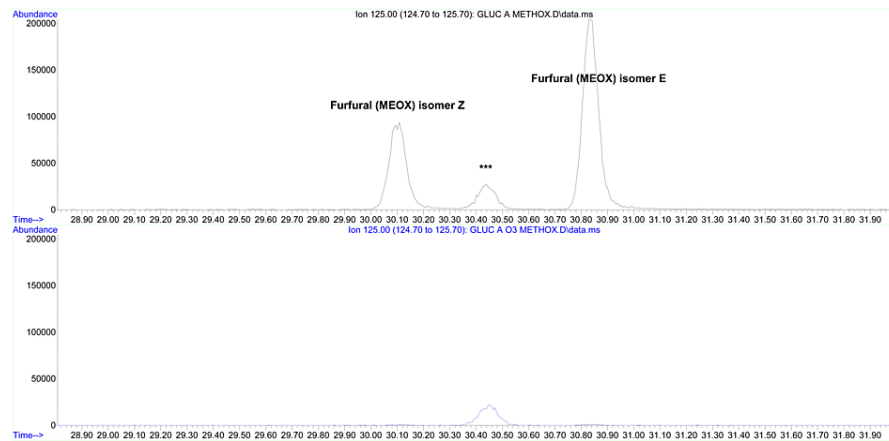


FIGURE 2: Methoxime hydrochloride derivatization

Upper Chromatogram: Untreated solution

Lower Chromatogram: Ozonized solution

The methoxime hydrochloride derivatization confirms an almost complete removal of furfural following ozonization.

Sugar analysis

The analysis of the variation of the composition following the oxygen/ozone mixture infusion has been made with a metabolomic protocol: 100 μ L were brought dry by freeze-drying, and the sample was derivatized with methoxime hydrochloride (40 mg/mL in anhydrous pyridine) at 70°C for 30 minutes and then silylated with 100 μ L of BSTFA 1% TMS. The sample is brought to 1 mL with hexane and then analyzed with GC-MS. Obviously, the variation in glucose concentration is minimal due to the low quantity of ozone. For clear statistical and kinetic reasons, glucose oxidation ends at first-stage products, glucuronic acid (A in Figure 3) and glucohexodialdose (B in Figure 3). These products are already present in the non-treated solution even if in lower quantities. Other glucose oxidation compounds are tetradialdoses resulting from the oxidative breakdown of the glucose ring (Figure 4).

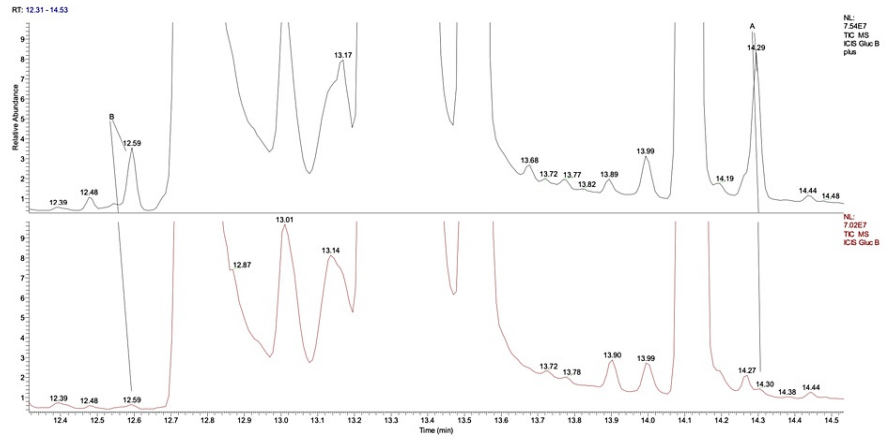


FIGURE 3: Glucose oxidation compounds: glucuronic acid and glucohexodialdose

Upper Chromatogram: Ozonized solution

Lower Chromatogram: Untreated solution

Minimal variation in glucuronic acid (A) levels. Glucohexodialdose (B) is present, albeit in smaller quantities, even in the untreated product. The quantity of these products is estimated to be in the mg/L range.

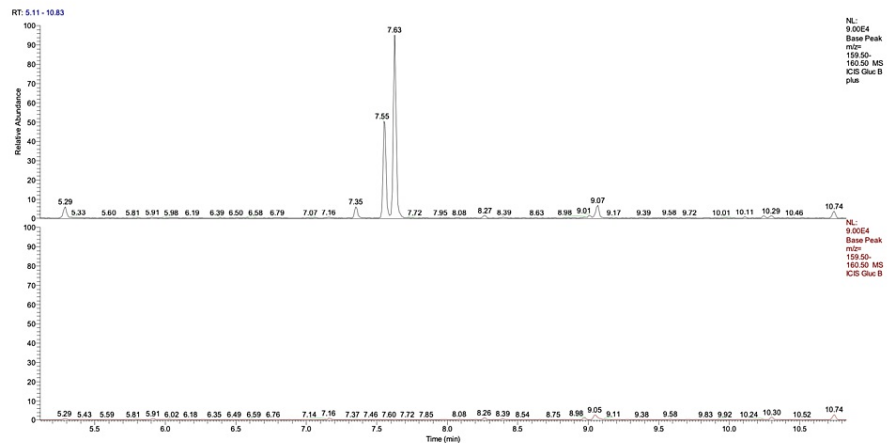


FIGURE 4: Glucose oxidation compounds: tetradialdoses

Upper Chromatogram: Ozonized solution

Lower Chromatogram: Untreated solution

They originate from the oxidative breakdown of the glucose ring. The quantity of these products is estimated to be in the microgram/L range.

Discussion

OT is a safe complementary therapy that can be practiced independently or in conjunction with other standard therapies [2,3,7,12]. However, the procedure required for the SOT can be complicated by certain conditions that may arise in patients, such as poor vein asset or anemia. In this regard, various alternative administration routes have been proposed to facilitate the procedure. Among these, a widely used alternative route is the infusion of ozonized saline solution. The gas is dissolved in the saline solution (0.9% sodium chloride) and adequately mixed. Subsequently, the solution is infused into the patient. Nevertheless, there are significant concerns about the potential formation of toxic compounds [4,8,12]. The literature provides limited data on alternative methods of administering ozone therapy, and there is a lack of

information regarding the safety of these techniques. Some authors attempted to determine the concentration of chlorite, hypochlorite, and perchlorate in the oxygen-ozone-treated saline solution, but the assessments at three, six, and 16 days are too distant from the therapeutic window [15]. As with any form of complementary therapy, safety is paramount for clinical practice. This study, following other long-term safety publications, aims to clarify the non-toxic nature of the therapy [16-18]. There are no studies regarding the possibility of using 5% ozonized glucose solutions as an alternative route for administering ozone therapy. The results of our study, while needing replication on a larger scale, demonstrate, as anticipated, the absence of toxic compounds generated from the ozonization of the 5% glucose solution. Specifically, the determination of the volatile component using HSPME coupled with GC-MS shows that the only alteration induced by the ozonization of the solution is the elimination of furfural, present in the naive solution and thus already at non-toxic concentrations. This result is confirmed by methoxime hydrochloride derivatization, which documents an almost complete removal of furfural. Naturally, levels of glucuronic acid and glucohexodialdose (glucose oxidation products already present in the untreated solution) are higher in ozonized solution. However, these levels are minimal (mg/L) and do not pose a clinical concern. In the same way, the levels of tetradialdoses (which originate from the oxidative breakdown of the glucose ring) are higher in the ozonized solution; however, the quantity of these products is estimated to be in the microgram/L range and holds no biological significance.

This research provides preliminary evidence of the safety of ozonated 5% glucose solution as an alternative in administering oxygen-ozone therapy. While our results are promising, it is crucial to note that further large-scale studies are necessary to confirm these findings. The positive outcomes of this study offer a solid foundation for future investigations and contribute to the growing knowledge in the field of OT.

Conclusions

The chemical changes observed following ozone infusion can be considered quantitatively minimal and essentially negligible in terms of chemical risk. Consequently, these alterations lead to the removal of furfural, which stands as the sole compound of significant toxic concern. However, to comprehensively understand the biological implications of this alternative method of administering the SOT, further studies are needed. These studies will be pivotal in elucidating the full spectrum of biological effects associated with this way of administration.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Acquisition, analysis, or interpretation of data: Daniele Romanello, Mauro Martinelli

Drafting of the manuscript: Daniele Romanello

Critical review of the manuscript for important intellectual content: Daniele Romanello, Mauro Martinelli

Concept and design: Mauro Martinelli

Supervision: Mauro Martinelli

Disclosures

Human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

References

1. Bocci V, Borrelli E, Travagli V, Zanardi I: The ozone paradox: ozone is a strong oxidant as well as a medical drug. *Med Res Rev.* 2009, 29:646-82. [10.1002/med.20150](https://doi.org/10.1002/med.20150)
2. Bocci V: Is ozone therapy therapeutic? . *Perspect Biol Med.* 1998, 42:131-43. [10.1353/pbm.1998.0056](https://doi.org/10.1353/pbm.1998.0056)
3. Chirumbolo S, Valdenassi L, Tirelli U, et al.: The oxygen-ozone adjunct medical treatment according to the protocols from the Italian Scientific Society of oxygen-ozone therapy: how ozone applications in the blood can influence clinical therapy success via the modulation of cell biology and immunity. *Biology (Basel).* 2023, 12:1512. [10.3390/biology12121512](https://doi.org/10.3390/biology12121512)

4. Bocci V: Is it true that ozone is always toxic? The end of a dogma . *Toxicol Appl Pharmacol*. 2006, 216:493-504. [10.1016/j.taap.2006.06.009](https://doi.org/10.1016/j.taap.2006.06.009)
5. Di Paolo N, Gaggiotti E, Galli F: Extracorporeal blood oxygenation and ozonation: clinical and biological implications of ozone therapy. *Redox Rep*. 2005, 10:121-30. [10.1179/135100005X38888](https://doi.org/10.1179/135100005X38888)
6. Sagai M, Bocci V: Mechanisms of action involved in ozone therapy: is healing induced via a mild oxidative stress?. *Med Gas Res*. 2011, 1:29. [10.1186/2045-9912-1-29](https://doi.org/10.1186/2045-9912-1-29)
7. Bocci VA: Scientific and medical aspects of ozone therapy. State of the art . *Arch Med Res*. 2006, 37:425-35. [10.1016/j.arcmed.2005.08.006](https://doi.org/10.1016/j.arcmed.2005.08.006)
8. Bocci V, Zanardi I, Borrelli E, Travagli V: Reliable and effective oxygen-ozone therapy at a crossroads with ozonated saline infusion and ozone rectal insufflation. *J Pharm Pharmacol*. 2012, 64:482-9. [10.1111/j.2042-7158.2011.01427.x](https://doi.org/10.1111/j.2042-7158.2011.01427.x)
9. Maslennikov OV, Kontorshchikova CN, Gribkova IA: Ozone Therapy in Practice Health Manual . Ministry of Health Service of the Russian Federation. The State Medical Academy of Nizhny Novgorod, Nizhny Novgorod, Russia; 2008.
10. Razumovskii SD, Konstantinova ML, Grinevich TV, Korovina GV, Zaitsev VY: Mechanism and kinetics of the reaction of ozone with sodium chloride in aqueous solutions. *Kinet Catal*. 2010, 51:492-6.
11. Martinelli M, Giovannangeli F, Rotunno S, Trombetta CM, Montomoli E: Water and air ozone treatment as an alternative sanitizing technology. *J Prev Med Hyg*. 2017, 58:E48-52.
12. Bocci V: Ozone as Janus: this controversial gas can be either toxic or medically useful . *Mediators Inflamm*. 2004, 13:3-11. [10.1080/0962935062000197083](https://doi.org/10.1080/0962935062000197083)
13. Levanov AV, Isaikina OY, Gasanova RB, Uzhel AS, Lunin VV: Kinetics of chlorate formation during ozonation of aqueous chloride solutions. *Chemosphere*. 2019, 229:68-76. [10.1016/j.chemosphere.2019.04.105](https://doi.org/10.1016/j.chemosphere.2019.04.105)
14. Egorova GV, Voblikova VA, Sabitova LV, Tkachenko IS, Tkachenko SN, Lunin VV: Ozone solubility in water. *Moscow Univ Chem Bull*. 2015, 70:207-10.
15. Ma L, Wen S, Yuan J, et al.: Detection of chlorite, chlorate and perchlorate in ozonated saline . *Exp Ther Med*. 2020, 20:2569-76. [10.3892/etm.2020.9005](https://doi.org/10.3892/etm.2020.9005)
16. Romanello D, Rotunno S, Martinelli M: Long-term safety of systemic ozone therapy in a patient with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). *Cureus*. 2023, 4:48261. [10.7759/cureus.48261](https://doi.org/10.7759/cureus.48261)
17. de Sire A, Agostini F, Lippi L, et al.: Oxygen-ozone therapy in the rehabilitation field: state of the art on mechanisms of action, safety and effectiveness in patients with musculoskeletal disorders. *Biomolecules*. 2021, 26:356. [10.3390/biom11030356](https://doi.org/10.3390/biom11030356)
18. Machado AU, Contri RV: Effectiveness and safety of ozone therapy for dermatological disorders: a literature review of clinical trials. *Indian J Dermatol*. 2022, 67:479.