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Measurement of the ozone concentration in liquids, at low doses

Carlos Fernández Yoldi
R&D Engineer, Healthcare-Ozone, Sedecal
Óscar Hidalgo
International Director, Healthcare-Ozone Line, Sedecal
José Francisco Ramos
Sales Spain, Healthcare-Ozone, Sedecal
Roberto Sánchez
Software Engineer, Healthcare-Ozone, Sedecal

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Abstract

One of the techniques used to transfer ozone to a patient consists of bubbling ozone at low concentrations in a saline solution and then, injecting the saline solution into the patient. Next, it is analyzed the efficiency of the indicated technique, in terms of the ozone dose that has been finally transferred to the patient via intravenous.
For this study, we have proceeded to bubble ozone at different standardized concentrations (1013.25 hPa and 273.15 K, id est, 1 atm and 0ºC) in a container with saline solution and then, we make the saline solution to pass through an ozone concentration analyzer in liquids, that will indicate the ozone retained in the saline solution. This amount of dissolved ozone was also compared with the amount of ozone dissolved in distilled and deionized water, under the same conditions.
After 10 minutes of ozone bubbling, a quarter of the programmed concentration (25%) remained in the liquid (approximately), which confirmed the recommendations of the ISCO3 [1]. This way, the user can know in advance the dose that will be applied to the patient by using this technique.
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Autor para correspondencia::Carlos Fernández Yoldi, R&D Engineer, Healthcare-Ozone, Sedecal.. Email: carlos.fernandez@sedecal.com

Introduction

One of the most studied and safest routes of application of ozone in patients is the major autohemotherapy.

According to the organization ISCO3 [1], a blood volume between 50 mL and 100 mL should be used, avoiding using over 200 mL so as not to compromise the hemodynamic stability of the patient. The ozone concentration must be between 10 mg/L and 40 mg/L (which implies a total dose of 1 mg to 4 mg, in 100 mL), concentrations higher than 70 mg/L increase the risk of hemolysis and other damages. The most advisable anticoagulant is the ACD-A (Anticoagulant solution of Citrate Dextrose A) in volume between 7 mL and 10 mL per 100 mL of blood. The session cycle varies between 15 and 20 applications (1).

The problem with this application method resides in the manipulation of the patient's own blood, since it is a laborious and delicate procedure. Also, we must to take into the account the local regulations of each country about blood products handling.

An alternative to this application way is the ozonation of the saline solution and its later transfusion to the patient.

According to a study carried out [2], introducing ozone into a NaCl solution at 0.9% does not produce hypochlorites or chlorates in any appreciable quantity, only the half-life of the dissolved ozone is reduced, which only produces oxygen when decomposed.

In any way of preparation (ISCO3 [1] suggests three ways), the proposed concentrations to be administered to the patient are 1 µg/kg, 2 µg/kg and 5 µg/kg (which implies a total dose of 75 µg, 150 µg and 375 µg respectively, for a 75 kg patient). According to the ozone concentration used to bubble in a volume of 250 mL of saline solution, it must be for the indicated situations:

- C 1= 75 □g / (250 mL * 25%) = 1.2 mg/L
- C 2= 150 □g / (250 mL * 25%) = 2.4 mg/L
- C 3= 375 □g / (250 mL * 25%) = 6 mg/L

The objective of the present study was to determine the real concentrations of ozone in saline solution, simulating the conditions of its clinical application, and comparing them with the pre-established theoretical values.

Materials and Methods

To measure the total amount of ozone dissolved in the saline solution, we proceeded as indicated in the second method described in the ISCO3 bulletin [1], that is, we saturated with ozone a volume of 250 mL, using the Bexen® system for saline solution Bexozone®, we kept it bubbling with a variable concentration, at a flow of 12L/h for 10 minutes and, the ozone concentration was measured inmediately, allowing the saline solution to flow by gravity.

The assembly of the experimental system indicated in Figure 1, corresponds to the image shown in Figure 2.

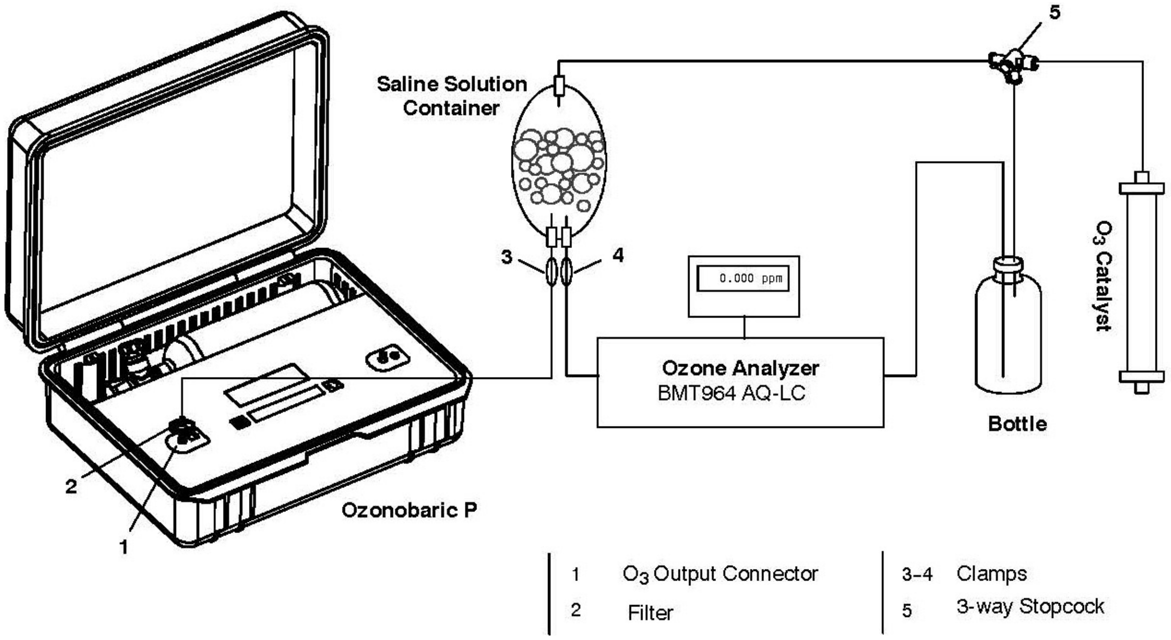

Figure 1. Connections Diagram of the devices used for the study.

Firstly, we measured the ozone concentration in distilled water without ozonation, secondly, we ozonated it with 2 mg/L standardized, a second sample with 5 mg/L standardized of gas. Then, we took samples of saline solution, and were also ozonated with 2 mg/L and 5 mg/L standardized. The data was collected in continuous operation mode, connecting the meter (BMT964 AQ-LC, Messtechnick GmbH, Germany) to a computer via serial port. Special care was taken with the artifacts introduced by bubbles (which were removed from the data).

The detailed procedure was the following:

1. Firstly, the liquid container was filled, to do so:
    a. The clamp (3) was closed,
    b. we connected the tube with the clamp (4) to a bottle of deionized distilled water, in order to perform a group of measurements, and to a standard saline bottle of 0.9 mg /100 mL for another group of measurements,
    c. vacuum was performed with a syringe connected to a 3-way stopcock (5) (previously the catalyst was disconnected) until obtaining the desired volume in the container (250 mL),
    d. the clamp (4) was closed,
    e. the tube of the clamp (4) was connected to the analyzer,
    f. the 3-way stopcock (5) was connected to the catalyst (with an open way from the container to the catalyst).
2. Then, with the connection shown in figure 2, the ozonation of the container content was performed, as next described:

a. The Ozonobaric P® was programmed with the desired concentration (0, 2 or 5 mg/L), flow of 12 L/h and 10 minutes of time,
b. the clamp (3) was opened, the gas was bubbled in the liquid and the remaining gas was being passed through the catalyst,
c. once finished the process, clamp (3) was closed,
d. the 3-way stopcock (5) was changed to connect the liquid collection bottle to the catalyst.

3. The measurement of ozone in the liquid was immediately performed, to do so:

a. The clamp (4) was opened, and the liquid was allowed to flow by gravity,
b. it was measured continuously, every second, until the container was emptied.

Once finished the process, we discarded the liquid from the bottle in order to repeat the procedure with another liquid volume.

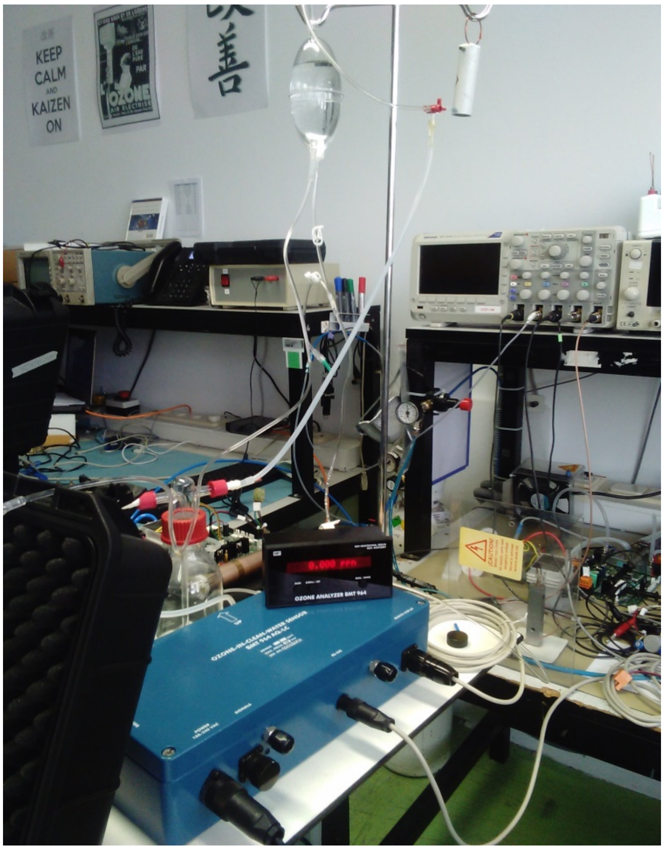

Figure 2. Image of the ensambly used for the study.

The generation and measurement equipment used was an ozone generator Ozonobaric P® from the company Sedecal®, and an ozone concentration meter for liquids from the company (BMT 964 AQ-LC, Messtechnick GmbH, Alemania) with ultraviolet light detector, with detection interval between 0.5 to 15 g/ m^3 and accuracy of 0.5%.

These measurements were made in triplicate for each medium (water and saline) and for each of the conditions studied without ozonation, or ozonating at 2 mg/mL or 5 mg/mL. The values of the kinetic behavior were represented and calculated by eliminating the base interference. The calculation was made from the theoretical point of view, and with the data, the percentage of ozone concentration, retained in the liquid solutions These values were compared with those referred for the method of administration of ozonated saline solution.

Results

The results of the measurements of the ozone concentration in the different liquid solutions are shown in the Figure 3. The result of the calculation of the concentration values, removing the blank values (concentration value determined in the non-ozonated solutions) are shown in the Table 1.

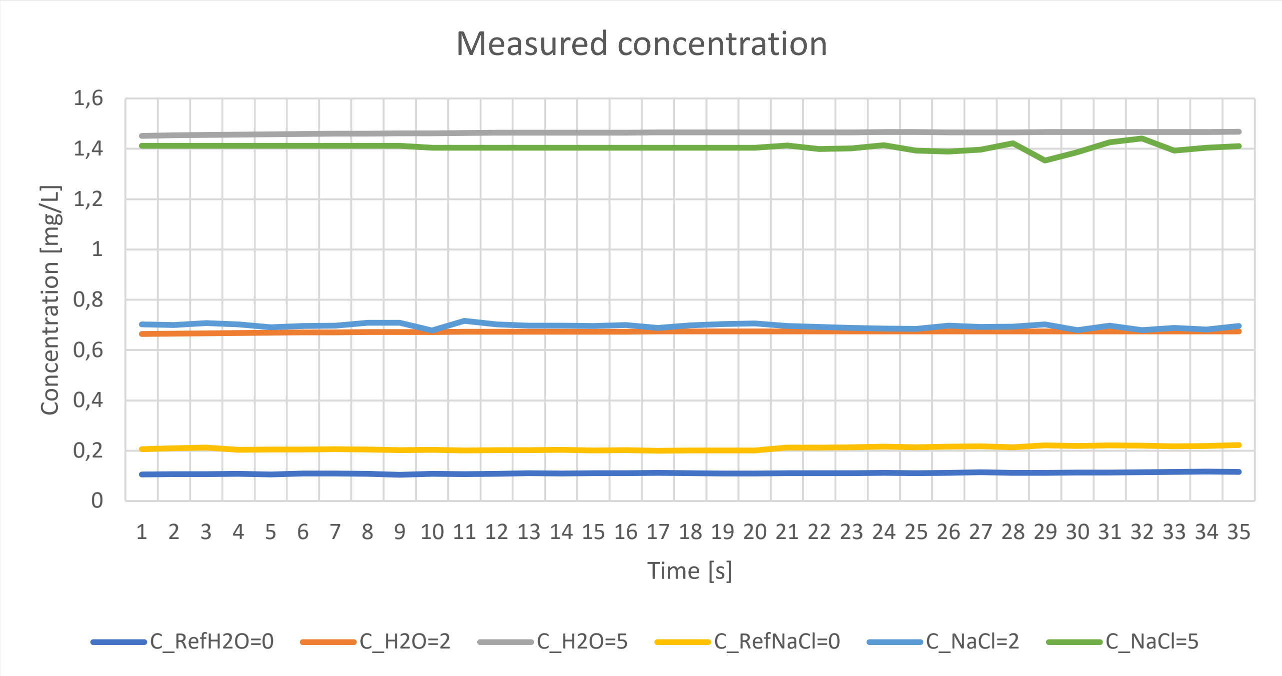

Figure 3. Kinetics of the behavior of ozone concentrations in each analyzed condition.

C_RefH2O=0: reference measure of distilled water, deionized without ozone.

C_H2O = 2: measure of distilled water, deionized, ozonated with 2 mg/L.

C_H2O = 5: measure of distilled water, deionized, ozonated with 5 mg/L.

C_RefNaCl = 0: reference measure of saline solution without ozone.

C_NaCl = 2: measure of ozonated saline solution with 2 mg/L.

C_NaCl = 5: measure of ozonated saline solution with 5 mg/L.

Table 1. Average concentration values of ozone, subtracting the values of the blanks from the corresponding non-ozonated solutions.

Standardized concentration applied to gas	Standardized concentration applied to H2O	Standardized concentration applied to saline solution 0,9 % NaCl
2 mg/L	0.56 mg/L	0.49 mg/L
5 mg/L	1.35 mg/L	1.20 mg/L

Discussion

There are several factors that affect the ozone transfer speed to the liquid but, after a certain time and keeping the liquid covered by a gas layer at constant concentration, the equilibrium may be considered reached when the amount of dissolved ozone is equal to the amount of ozone that is released. By bubbling with needle, the equilibrium would be considered to be reached after 15 minutes [6], although 10 minutes is the recommended time by ISCO3 [1] and it would be close enough to the equilibrium.

In the equilibrium situation, we can consider Henry's Law in order to calculate the amount of ozone dissolved in the water:

$H = Ca/Pr$ [mol/m³/Pa] (Ec. 1)

Ca: concentration in liquid phase [mol/m³]

Pr: partial gas pressure in equilibrium [Pa]

The partial ozone pressure is given by:

$Pr = Cg * R * T$ (Ec. 2)

Cg: concentration in the gas [mol/m³]

The most agreed parameter for ozone is [4]:

$Hc = 0.00011$ mol/m³/Pa @ $Tc = 298.15$ K

This parameter is very sensitive to temperature, but follows the van’t Hoff equation [4]:

$H(T) = Hc * \exp (d \ln H/d (1/T) . (1/T – 1/Tc)$

where [4]:

$d \ln H/d (1/T) = 2400$ K

although, for these calculations we will consider directly the working temperature of 298.15 K.

The measurement system provides the data under standard conditions at:

$P0 = 1013.25$ hPa

$T0 = 273.15$ K

so, to calculate the concentration at room temperature:

$Pc = 950$ hPa

$Tc = 298.15$ K

according to the equation:

$Cgc = Cg0 * T0/Tc * Pc/P0 = Cg0 * 0.977$ (Ec. 3)

Taking these equations into account, we can finally obtain the ozone concentration that results in the liquid (Ca) depending on the concentration of the standardized ozone (Cg0) in the gas:

$Ca = Cg0 * 0.977 * 0.011 * 0.08314459848 * 298.15 = Cg0 * 0.2664$

That is, the ozone concentration solubility is 26.64% with respect to the standardized one. To have a coherent reference, the concentration in the liquid will also be considered standardized; id est:

$Ca0 = Ca * Tc/T0 * P0/Pc = Ca/0.977$

Which what finally remains:

$Ca0 = Cg0 * 0.272685$

which indicates 27.27% of solubility. The values are indicated in table 2.

Table 2. Theoretical vaues of ozone concentrations dissolved in liquids.

Standardized concentration applied in gas	**Concentration applied in gas at 950 hPa and 25º C**	**Theoretical concentration dissolved in H2O at 950 hPa and 25º C**	**Standardized theoretical concentration dissolved in H2O**
2 mg/L (*)	1,95 mg/L	0,53 mg/L	0,55 mg/L (*)
5 mg/L (*)	4,9 mg/L	1,33 mg/L	1,36 mg/L (*)
15 mg/L (*)	14,7 mg/L	4,00 mg/L	4,1 mg/L (*)
40 mg/L (*)	39,1 mg/L	10,7 mg/L	10,9 mg/L (*)
60 mg/L (*)	58,6 mg/L	16,0 mg/L	16,4 mg/L (*)
80 mg/L (*)	78,2 mg/L	21,3 mg/L	21,8 mg/L (*)

Note: (*) Standardized ozone concentration (1013.25 hPa and 273.15 K, id est, 1 atm and 0ºC)

It was not considered that the presence of salts in water, generally, decreases the solubility according to the Sechenov equation [4]:

$\log (H0/H) = k * b$

although there is little documentation available about these coefficients.

Taking the actual measured data from Table 1 and comparing them with the theoretical results of Table 2, it can be seen that the deviation between them is in accordance with the theoretical reasoning presented (Table 3).

Table 3. Deviation between the measured and the theoretical results.

Standardized concentration applied in gas	**Deviation of the concentration in H2O (measured–theoretical)**	**Deviation of the concentration in Saline Solution 0,9% NaCl (measured–theoretical)**
2 mg/L	+0,01 mg/L => +1,8%	-0,06 mg/L => -11%
5 mg/L	-0,01 mg/L => -0,7%	-0,16 mg/L => -12%

Taking again Table 1 and presenting the data measured in percentage with respect to the gas concentration introduced, the actual amount of ozone absorbed by the liquids can be valuated.

Table 4. Ratio in percentage between bubbled ozone and dissolved ozone.

Standardized concentration applied in gas	**Standardized concentration measured in H2O, in %**	**Standardized concentration measured in saline solution 0,9 % NaCl, in %**
2 mg/L	28 %	24,5 %
5 mg/L	27 %	24 %

Conclusion

In conclusion, the 25% approximation presented by ISCO3 [1] to calculate the amount of ozone that dissolves in water, is in line with the calculations and measurements made and can be considered correct.

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Conflict of interests:

The authors of this work are affiliated to the company SEDECAL, producer of medical ozone generators.

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