Chapter 3
Preparation of Ozonated Water and Oil for the Topical Therapy – Ozone as a Drinking Water Disinfectant: Ozone Disinfection to Prevent Nosocomial Infections

In the world, there are millions of people affected by dirty traumatic lesions, infected wounds, chronic torpid ulcers, bed sores, burns, herpetic lesions, fungal infections and insect stings, who suffer for a long time because the conventional topical treatments based on antibiotics and anti-inflammatory drugs are not sufficiently effective. Unfortunately, most physicians and nurses are not aware of the potency and efficacy of both ozonated water and oil. When possible, by enclosing a leg inside a polythene bag, we can also use the gas mixture: oxygen-ozone, but we must avoid the risk of breathing ozone and not all generators are equipped with a suction pump connected to an ozone destructor. On the other hand, it is easy to apply a gauze compress soaked with ozonated water or oil to any part of the body.

The preparation of ozonated water is carried out by using a glass cylinder about $^3/_4$ filled with deionized and bidistilled water through which the gas mixture (oxygen-ozone) has to be bubbled continuously for at least 5 min to achieve saturation. The unused ozone flows out via silicone tubings into a destructor and is converted to oxygen. Some ozone generators have already incorporated the system for ozonating water but, if it is not available, it can be simply built with a 500 ml glass bottle that we can fill with 250 ml water and 250 ml of the gas mixture containing the highest available ozone concentration and close with a silicone cork. Also with this rudimentary technique, a vigorous mixing for about 5 min insures a fairly good ozonation of pure water.

Solubilization of ozone in pure water occurs according to Henry's law (1803) that states that the saturation concentration of a gas in water is proportional to its pressure, concentration and temperature. This is correct only if the water is absolutely pure and the temperature and ozone pressure remain constant. Monodistilled water (or worse, tap water) is unsuitable because, by containing some ions, stimulate the chemical reactivity of ozone with the possible formation of toxic compounds. For this reason, **I recommend the use of pure water, which is commercially available. In such a case ozone is physically dissolved in water and its concentration, after 5–6 min of bubbling, is stable and equivalent to $^1/_4$ (about 25%) of the ozone concentration present in the gas mixture**. Thus, if we want a strong preparation of ozonated water, we must use an ozone concentration of 80–100 mcg/ml of gas that will yield a final ozone concentration of about 20–25 mcg/ml in the water. This solution is suitable for treating heavily infected wounds in order to eliminate

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pus, necrotic materials and bacteria. On the other hand, once the wound reaches the proliferation and remodelling stages, we must use a mild solution prepared with an ozone concentration of 20 mcg/ml of gas which will yield an ozone concentration of only about 5 mcg/ml water.

How stable is a preparation of ozonated pure water? Owing to the inherent ozone instability, this is a weak point. The ozonated water must be maintained in a glass bottle tightly closed with a silicone or Teflon cap, possibly in the refrigerator. If it is kept at 5°C, the ozone concentration is halved in some 110 h, but at 20°C the ozone half-life is only 9 h! This information has a practical importance because, if maintained properly, it can be used for a couple of days at the patient's home for domiciliary treatment. A recent patent by Yoshimura et al. (US 2006/0090473 A1) has presented a method and an apparatus for producing ice containing ozone useful for preserving fruits and vegetables for longer times. The relevance of maintaining a sufficient activity of ozonated water for several days at the patient's home cannot be neglected and we are evaluating several approaches hoping to find a useful one. In contrast, the half-life of ozone solubilised in monodistilled water is less than one h and therefore it must be used at once.

I cannot lose the opportunity of emphasizing the usefulness of ozonated water for removing thick pus from purulent abscesses, empyemas and osteomyelitic infections. After draining the water, the cavity, via a polypropylene cannula, can be insufflated with the oxygen-ozone gas mixture at least twice daily and soon the operator will be surprised to note a rapid healing. I know of several desperate cases where dedicated ozonetherapists were able to eliminate hopeless infections by only using the combination of ozonated water, gas and ozonated oil. Gas insufflation must be performed in a few minutes in a well ventilated room leaving the cannula clamped to prevent the exit of gas. Ozone will dissolve into the infected secretions, will sterilize them and will promote the reconstruction of tissues. Obviously one should use at first a high (70–80 mcg/ml) ozone concentration during the septic phase and then progressively lower it as soon as the infection subsides, for enhancing cell proliferation.

It is well known that decubitus and torpid ulcers (in diabetic, venous stasis and chronic limb ischemia patients) require a frustratingly prolonged treatment that often is a failure. Gas can only be used if the ulcer can be contained, with or without slight decompression, by using an ozone-resistant container, such as a polyethylene bag or a Teflon cup. Usually during the day it is more practical to use freshly ozonated water for cleansing, disinfection and stimulation of tissue granulation whereas, during the night, the application of ozonated oil is able to maintain sterile the lesion and enhance healing. In the last decade, there has been a growing interest in the application of ozonated oil: a particular merit goes to Dr. Renate Viebahn and Cuban scientists for developing it and today there are a great number of patents because of the oil cornucopia (Travagli et al., 2009a, b).

Probably owing to the scarce efficiency of conventional drugs, ozonated olive and sunflower oils have been employed in torpid ulcers, bacterial, fungal and parasitic infections not only with topical applications but also oral administrations.

As a natural preparation, ozonated oils are available in several countries but so far there is not a really standard preparation, which is urgently needed. Moreover also systematic and extensive clinical studies in different skin affections have not yet been performed for lack of funds thus really we do not yet know which is the oil that has the optimal cost/benefit.

Olive and sunflower oils have been the most utilized but they are not necessarily the best: indeed we must care not only about the degree of the number of unsaturated bonds per molecule but also on the pharmacological quality and we like very much sesame oil (Zanardi et al., 2008; Sega et al., 2010). Moreover it is important to qualify the degree of ozonation in terms of:

(1) **Iodine Value (IV)** that is a measure of the total number of double bond present in the sample. It represents the quantity of iodine (in grams) that will react with the double bonds in 100 g of sample. The IV is calculated by means of the following equation: $1.269 \times (n_1 – n_2)/m$, where n_1 is the volume in ml of thiosulphate solution used for carry out a blank test, n_2 is the volume in ml of thiosulphate solution used for the titration and m is the quantity in grams of the oil.
(2) **Acid Value (AV)** is the number that expresses in mg the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the oil. The AV is calculated by means of the equation: $5.610 \times n/m$, where n is the volume in ml of the titrant and m the quantity in grams of the oil.
(3) **Peroxide Value**. The *PV* represents the quantity of peroxide expressing in milliequivalents of active oxygen contained in 1 kg of the oil. The PV was calculated by means of the following equation: $1{,}000 \times (V_1 – V_0) \times c/m$ where V_1 is the volume in ml of thiosulphate solution used for the titration, V_0 is the volume in ml of thiosulphate solution used for carry out a blank, c is the thiosulphate concentration and m is the quantity of oil.
(4) **NMR** Spectroscopy, if it is available.
(5) **Viscosity** measurements measured before and after ozonation.

As an example, ozonation of sesame oil has been performed by using progressively increasing amounts of ozone from 0 up to 9,900 mg. In this case IV (g/100 g) decreases from 113.6 down to 13.9, AV (mg KOH/g) increases from 0.70 up to 12.6, PV (mEq/1,000 g) increases from 104 (control) up to 3,800. Finally the viscosity increases up to 69% indicating that the double bonds in the oil molecules have reacted with ozone to form a more bulky molecule. It is also important to monitor the stability of the ozonated oil for at least 1 year. No changes in the physico-chemical profile of the sesame oil have been observed when this is stored in a glass bottle in refrigerator.

Each preparation of ozonated oil must be certified and for the most appropriate use in relation to the state of the ulcer, the physician should have at hand a light, medium and strong grade of ozonated oil. The latter grade must be used with purulent, fetid, highly infected ulcers and then, as the ulcer improves, the medium and light preparations have to be used for preventing damage to the proliferating tissue, thus facilitating healing.

Ozonated sunflower oil (Oleozon) from Cuba was tested by Sechi et al. (2001) and it was found to have valuable antimicrobial activity against all the tested microorganisms. At our University Hospital, initially we made our own preparation by bubbling oxygen-ozone in pure olive oil for at least 60 min at room temperature but now we prefer to use a commercial preparation. At the IOA Congress in London (September 14–15, 2001), Miura et al. (2001) presented an interesting report on the elucidation of the structure of ozonated olive oil: ozonation was carried out for 2 days until the oil solidified and 1 g of oil could absorb up to 160 mg of ozone. A number of analyses led to the conclusion that the prolonged ozonation resulted in exclusive formation of triolein-triozonides, which remained stable in the refrigerator for 2 years. There is no real need to have a solid oil preparation, except for commercial purposes and long stability. In practice, the pathological situations are so variable to require great flexibility so that the very viscous oil can be either warmed or diluted with pure oil, or with pharmaceutical *Vaselinum album* (at 50%) when the wound is aseptic.

How ozonated oil acts remains an open question. Probably, when the stable triozonide comes into contact with the warm exudate of the wound, it slowly decomposes and generates Reactive Oxygen Species (ROS) and Lipid Oxidation Products (LOPs) that can explain the prolonged disinfectant and stimulatory activity. This reasoning implies the usefulness of having titrated preparations with high, medium or low triozonide concentrations to be used during the inflammatory septic phase I, regenerating phase II or remodelling phase III, respectively. These phases have been related to the rapidly changing cell types and to the release of cytokines and growth factors that modulate the complex healing process (Section 9.1).

In the Department of Surgery, Chiba-Tokushkai Hospital in Japan, Matsumoto et al. (2001) tested the efficacy of the oil prepared by Miura et al., in intractable fistulae and wounds after surgical operations (acute appendicitis with peritonitis, intrapelvic, abdominal and perianal abscesses, etc.). In a series of 28 patients, the ozonated oil was fully effective in 27 cases, without adverse side effects. Between the period 2001–2004, I treated several desperate cases in old people (prevalently bed sores) with a great success so that I can fully confirm Matsumoto's results. I just learnt that patients with radiotherapy skin reactions, treated with ozone, perceived a benefit in terms of pain relief (Jordan et al., 2002). Surprisingly, these results were obtained in Manchester (UK), with an unsuitable method and therefore the use of ozonated oil will likely yield even better results. Ozonated oil has also proved to be very effective in burns and it would be interesting to compare this treatment with the Moist-Exposed Burns therapy and the Moist-Exposed Burns Ointment invented by Xu in China (2004).

Moreover I will mention that there are several pharmaceutical vehicles for the administration of ozonated oil, such as gastro-resistant capsules, pessaries, suppositories and even collyriums, to be used in intestinal, vaginal, anal-rectal and ocular infections. As one can imagine, ozonated oil smells of rancid fat but capsules ingested by mouth have been tolerated by Cuban children. Silvia Menendez et al. (1995) treated 222 children affected by infantile giardiasis, a parasitic disease, obtaining a remarkable cure without toxicity in 76% of children.

Today, we are still using ozonated oil in a very empirical fashion and, when I report these informations, people do not disguise their incredulity and only results obtained after controlled studies will be convincing. However, **once physicians and nurses will realise the therapeutic potential of ozonated water and oil, these products will become a very useful and inexpensive medical treatment**.

In spite of a large use of chlorine, 2.4 billion people or **40% of the world's population do not have access to adequate sanitation**. Unfortunately chlorine has unsatisfactory organoleptic characteristics and it is being widely substituted by ozone all over the world. Ozone is possibly an even more potent drinking water disinfectant able to inactivate several human pathogens, e.g. as many as 63 different bacteria (*Salmonella, Shigella, Vibrio, Campylobacter jejuni, Yersinia enterocolitica, Legionella*, etc.), some 15 viruses (polio-, echo-, Coxsackie viruses, etc.), some 25 fungi and mould spores (*Aspergillus, Penicillium, Trichoderma*, etc.), several yeast varieties, and up to 13 fungal pathogens (*Alternaria, Monilinia, Rhizopus*, etc.). More recently, due to contamination of groundwater with faecal material, the problem of disinfection has become more complex, since encysted protozoa, such as *Giardia lamblia, Cryptosporidium parvum* oocysts and helminth eggs (*Ascaris suum and Ascaris lumbricoides*), require a much longer time of contact with ozone than bacteria and viruses. Every year Cryptosporidium causes outbreaks of sickness, which can be fatal for elderly and very ill patients (AIDS).

Water is rapidly becoming a precious commodity and wastewater from cities, animal breeding (particularly cattle, sheep, and swine) and industrial plants must be reused for irrigation in order to increase agricultural production. This happens most frequently in underdeveloped countries, but also in the USA and Italy, and poses a health risk by causing serious gastro-intestinal diseases (Stein and Schwartzbrod, 1990; Ayres et al., 1992; Johnson et al., 1998; Orta de Velasquez et al., 2001; Liou et al., 2002). Toze (1999) has reported that, **in countries with poor sanitation systems, about 250 million people are infected each year by waterborne pathogens, with about 10 million deaths**.

The oxidation of organic and inorganic materials during ozonation (gas to water phase) occurs via a combination of molecular ozone and hydroxyl radicals. Water companies throughout the world are evaluating several methods to optimize the various steps of the water-treatment process, which varies in different countries depending on the quality of the water, concentration of organic matter, turbidity and salt content (Kadokawa et al., 2001; Evans et al., 2001; Courbat et al., 2001; Hijnen et al., 2001). Ozone appears very effective in inactivating most bacteria and viruses, while protozoan cysts and helminth eggs are far more resistant; only by using realistic ozonation conditions can one achieve a moderate degree of inactivation (Graham and Paraskeva, 2001; Lewin et al., 2001). This is an important problem that requires more intensive sanitation of wastewater, particularly from animal breeding.

Another aspect for prevention of outbreaks of intestinal infections is the possibility of using ozone as an antimicrobial agent in direct contact with food and fruits. On June 26, 2001, the US Food and Drug Administration (FDA) formally approved

the use of ozone, in the gaseous and aqueous phase, as an antimicrobial agent for the treatment, storage and processing of foods (Rice, 2001). It must be mentioned that, in addition to the disinfection of drinking water, the use of ozone can also improve its organoleptic properties. In fact, it enhances the coagulation and flocculation process, oxidizes bad taste and odour compounds (as well as iron and manganese), and improves particle removal in filters or through bioactive granular activated carbon. **The efficacy of ozone has now been validated by more than 3,000 municipal water treatment plants around the world**.

During the last decade nosocomial infections have become common because the resistance of pathogens to antibiotics has increased to a point where we no longer have an effective drug for some strains. This is a complex story, partly due to the extensive use of antibiotics in animal food and the improper use in patients. The result is dramatic because almost every month, we hear of a series of deaths due to incontrollable infections (mostly due to methicillin-resistant *Staphylococcus aureus*) breaking out in hospitals after more or less complex operations and in intensive therapy units. With some approximation, it seems that several thousand deaths could be avoided each year if we could eliminate the resistant bacteria. The problem is so important that some 1,000 papers per year report relevant data (Aitken and Jeffries, 2001; Guerrero et al., 2001; Kollef and Fraser, 2001; Olsen et al., 2001; Shiomori et al., 2001; Slonim and Singh, 2001; Stephan et al., 2001; Stover et al., 2001; Wenzel and Edmond, 2001).

Applications for ozone can be divided into two phase:

(1) The gas to gas phase,
(2) The gas to water phase (liquid phase-ozone).

The first phase is widely used to remove as many as 272 organic odours and pollutants: these range from acrolein to bathroom smells, body odours, cigarette smoke, decaying substances, ether, exhaust fumes, faecal and female odours, hospital odours, medicinal odours, mould, putrefying substances, sewer odours, toilet odours, waste products, etc. Ozone is proficiently used in hospital wards and nursing homes to get rid of the smells caused by incontinent patients. In air conditioning systems (cooling towers, etc.), a small amount of ozone rids the recirculating air of odours, bacteria (Legionella pneumophila, etc.) and viruses. Moreover, ozone is providential for fumigation of bedding, bedclothes and treatment of air in operating rooms. Ozone is effective but it is necessary to take precautions:

(a) To allow enough time, even days if necessary, for the ozone gas (which is less active and slower than aqueous solubilized ozone) to be in contact with the contaminants to be oxidized and destroyed.
(b) When confined spaces are treated with gaseous ozone, people must not be present. The ozone generator must be regulated by a timer, which can be operated by every user. Ozone release must stop well before people re-enter the facility.

(c) Prior to returning the air mixed with ozone into the atmosphere, the gas mixture must pass through an ozone destructor. Personnel can usually re-enter an area treated with ozone, after appropriate de-aeration, after a short while.
(d) To prevent lung toxicity, an ozone monitor must be installed to check for any residual ozone concentration.

Ozone fumigation of bedding, bedclothes and any other object can be carried out according to the instructions given by Inui and Ichiyanagi (2001). Ozone is used in conjunction with a negative ion generator and, if necessary, a heater to control mites and ticks.

Several pharmaceutical firms in the USA have recently started to package pharmaceutical products in an ozone-containing atmosphere to maintain a sterile packaged product line.

The gas to water phase has been adopted in the USA by a number of laundries to effectively launder and sterilize various linens used in health care facilities. It seems that, although this process is not energy efficient, it does extend linen life by 25–50%. Moreover, ozone washing provides a good alternative to conventional linen processing, since it is more effective in preserving the environment from contaminated water. All these innovative technologies increase health care costs, but the quality of service is improved and, more importantly, nosocomial infections can be minimized.

A full report informing about how to improve safety in hospitals can be found online at http://www.ahrq.gov/making health care safer: a critical analysis of patient safety

3.1 Conclusions

Ozonation of either bidistilled water or vegetable oils is performed by bubbling the gas mixture (O_2–O_3) for either 5 min or up to 2 days, respectively. Air should not be used because it can lead to the generation of toxic compounds. The ozone concentration in pure water, due to solubilised ozone, corresponds to 25% of the used ozone concentration, which is more than enough for an optimal disinfection. One gram of oil can bind up to 160 mg of ozone. While ozonated water remains efficacious for 1–2 days, the oil remains stable for 2 years if it is kept in the refrigerator. Both acts as potent disinfectants and enhance healing by stimulating cell proliferation. As soon as the medical community will appreciate their efficacy, both ozonated water and oil will become indispensable tools in chronic wound healing units. *I would like to predict that the application of ozonated oil, a simple and inexpensive remedy, will become far more useful than expensive pharmaceutical creams and will herald a medical revolution for the topical treatment of torpid ulcers and wounds. Under these terms, it is not exaggerated to proclaim ozone as "the wonder drug of the twenty-first century".*

The problems of the disinfection of drinking water and the prevention of nosocomial infections have become of primary importance because their solution means life ore death for many people. In comparison to chlorine, the versatility and efficacy of ozone is widely acknowledged.